



# Planktonic processes contribute significantly to the organic carbon budget of a coastal fish-culturing area

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**ABSTRACT:** We assessed the role of planktonic processes, in comparison to allochthonous input from fish cages and sedimentary loss, in the organic carbon (OC) budget of the water column in a semi-enclosed fish-culturing area (culturing red sea bream *Pagrus major* and yellow tail *Seriola quinqueradiata*). The sedimentation rate of particulate organic carbon (POC) at the fish-cage station was an average of 1.5 times that at non-cage stations. There was no significant difference in photosynthesis or respiration rates between fish-cage and non-cage stations. Annual allochthonous OC input in the form of leftover feed and fish feces was estimated to be 5 or 10 times that of autochthonous OC input by planktonic photosynthesis. In contrast, POC derived from phytoplankton accounted for a significant part (8 to 61 %) of total POC sedimentation. As to sinks of OC in the water column, annual planktonic respiration was twice as high as sedimentary loss at the fish-cage station. The plankton community tended to act as a source of OC in spring and summer and as an OC sink in fall and winter. The present study shows that a significant part of allochthonous and autochthonous OC input is respired by plankton and that the remaining OC input is deposited on the seafloor of fish-culturing areas.

**KEY WORDS:** Organic carbon budget · Planktonic photosynthesis · Planktonic respiration · Sedimentation · Fish farming impact

## INTRODUCTION

In a coastal fish-culturing area, deposition of organic wastes on the seafloor and subsequent oxygen consumption through the mineralization process sometimes result in formation of an anoxic water mass, a decline in benthos density, and occasional mass mortality of cultured fish (Brown et al. 1987, Gowen & Bradbury 1987, Wu 1995). Phytoplankton abundance often increases as a result of nitrogen and phosphorus release from fish cages (Eloranta & Palomaeki 1986, Gowen & Bradbury 1987); therefore, it is possible that phytoplankton photosynthesis is another important source of organic matter in fish-

culturing areas. Moreover, it has been suggested that this allochthonous and autochthonous organic matter is actively respired within the water column before it is deposited on the seafloor (Yoshikawa et al. 2012).

Thus, in order to develop an effective countermeasure for organic pollution at fish aquaculture sites, it is necessary to investigate the importance of planktonic processes as a source or sink of organic carbon (OC) relative to organic wastes from fish cages and sedimentary loss. Most previous studies have focused on the direct impact of organic waste from fish cages and the process of waste sedimentation onto the seafloor (e.g. Findlay & Watling 1997, Tsutsumi et al. 2006, Yokoyama et al. 2006). However,

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few studies have simultaneously evaluated the relative importance of planktonic metabolism and fish-cage waste on material cycles in a fish culture area (cf. Alongi et al. 2003, á Norði et al. 2011).

The present study aims to investigate the importance of planktonic processes as a source or sink of OC relative to organic wastes from fish cages and sedimentary loss, in order to develop an effective countermeasure for organic pollution at fish aquaculture sites. In addition, the causes of seasonal and regional variations in the relative importance of planktonic metabolism on the OC budget are discussed.

## MATERIALS AND METHODS

### Study site

All observations and measurements were conducted in the innermost part of Tanabe Bay, Kogaura Inlet (33°41'N, 135°21'E; Fig. 1), during early summer (15 to 16 June), midsummer (17 to 18 August), fall (19 to 20 October), and winter (7 to 8 December) in 2004, and during spring (18 to 19 May), summer (9 to 10 August), fall (18 to 19 October), and winter (6 to 7

December) in 2005. Tanabe Bay is located on the west coast of the Kii Peninsula, Wakayama, Japan, and opens to the Northwest Pacific Ocean. Its tidal range varies from 1.4 to 2.0 m. Yellow tail *Seriola quinqueradiata* and red sea bream *Pagrus major* are intensively cultivated in net-pen cages in Tanabe Bay, including Kogaura Inlet. These aquaculture activities cause moderate eutrophication of the bay, which results in occasional algal blooms and anoxic water mass formation at the bottom of the bay during summer (Takeuchi 1994). These types of environmental degradation were particularly severe in the 1970s and 1980s when fish production was very high; however, since the 1990s, the environmental conditions have improved somewhat (Uede 2003).

Four stations were established during each observation period; the first station was a cage station located near a fish cage (Stn F at 16 m in depth; Fig. 1, closed star), and the remaining 3 stations were non-cage stations (Stns N1, N2, and N3; Fig. 1, open stars) located at 10s (N1) and 100s of meters (N2 and N3) from the fish cages. The location of the non-cage stations varied between sampling periods (N1, June 2004, 13 m depth; N2, August and October 2004, 14 m depth; N3, December 2004 and all periods in 2005, 16 m depth) because some fish cages in other

locations of Kogaura Inlet were moved to the non-cage stations temporarily when there was an occurrence of fish disease. There were fish cages at Stn F during all sampling periods.

### Planktonic photosynthesis and respiration

Planktonic photosynthesis and respiration rates were measured using the light-and-dark-bottles method (Strickland & Parsons 1972). A Van Dorn water sampler was used to collect seawater samples for incubation experiments from 3 depths (0, 5, and bottom – 2 m, a depth of 2 m above the seafloor) at both fish-cage and non-cage stations in 2004, and from 5 depths (0, 2, 5, 7, and bottom – 2 m at only the fish-cage station in 2005. The seawater samples were carefully distributed between 3 clear 100 ml BOD bottles (light bottles), 3 light-shielded dark bottles, and 3 zero-

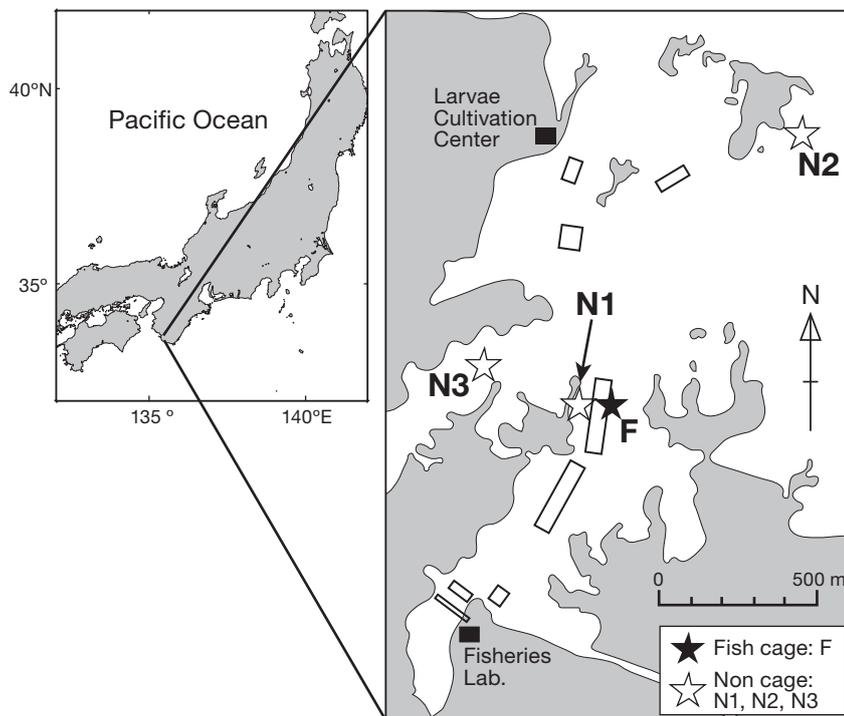


Fig. 1. Location of the sampling sites in the Kogaura Inlet of Tanabe Bay. Fish-cage station (F, all periods; closed star) and non-cage stations (N1, June 2004; N2, August and October 2004; N3, December 2004 and all periods in 2005; open stars). Rectangles: fish cages

time blanks. The light and dark bottles were incubated for 24 h in an on-land, flow-through incubator ( $\pm 1^\circ\text{C}$  of *in situ* surface temperature). The light levels at the sampled depths were calculated based on the attenuation coefficient estimated from the Secchi transparency and were reproduced using neutral density screening of individual light bottles. Dissolved oxygen (DO) concentrations in the BOD bottles were measured by the diaphragm galvanic battery method using a DO meter (OM-51, Horiba) with a resolution of  $0.01\text{ mg l}^{-1}$ . The seawater was stirred with a magnetic stirrer at 500 to 1000 rpm to prevent the occurrence of eddies. For each bottle, triplicate DO measurements were performed, and in most cases the difference between measurements was within  $0.01\text{ mg l}^{-1}$ . The values of photosynthesis and respiration were calculated as the differences in DO between the light bottles and the zero-time blank and the dark bottles and the zero-time blank, respectively. Photosynthesis and respiration in terms of oxygen were converted to the equivalent carbon values, assuming a photosynthetic quotient (PQ) of 1.0 and a respiratory quotient (RQ) of 1.0.

#### Chemical and biological properties of the seawater

Seawater samples for chemical and biological analyses were collected from 3 depths (0, 5, and bottom – 2 m) at both stations during all observation periods using a Van Dorn water sampler.

For particulate organic carbon (POC) analysis, the suspended particles in the seawater samples were filtered through 25 mm Whatman GF/F filters that were precombusted at  $450^\circ\text{C}$  for 1 h and stored below  $-20^\circ\text{C}$  until analysis. The filters were dried at  $110^\circ\text{C}$  for 24 h and fumed overnight with concentrated HCl to remove any inorganic carbon. The POC content of these samples was then determined using a CHN analyzer (MT-6, Yanako).

Dissolved organic carbon (DOC) was analyzed only for samples collected near the bottom (bottom – 2 m). Bottom water samples were passed through precombusted 25 mm Whatman GF/F filters. The DOC of the filtrate was analyzed using a total organic carbon (TOC) analyzer (TOC-500, Shimadzu) after HCl acidification and 10 min of bubbling with  $0.22\text{ }\mu\text{m}$ -filtered  $\text{N}_2$  gas.

Macronutrients (nitrate, nitrite, phosphate, and silicate) in seawater samples that had been stored at  $-20^\circ\text{C}$  were determined using an autoanalyzer (TRAACS 2000, Bran+Luebbe). Ammonium was determined manually using a standard method (Strick-

land & Parsons 1972). Temperature and salinity profiles were obtained from CTD casts (Alec Electronics).

The chlorophyll *a* (chl *a*) and its phaeopigment (phaeo *a*) content of particles collected on 25 mm Whatman GF/F filters were determined fluorometrically (TD-700, Turner Design) after extraction by *N,N*-dimethylformamide (Suzuki & Ishimaru 1990).

Seawater samples for bacterial cell counts were immediately preserved by the addition of neutralized formaldehyde solution to a final concentration of 2% and stored at  $4^\circ\text{C}$  until microscopic analysis. Bacterial cells in the seawater samples were stained with DAPI (4',6-diamidino-2-phenylindole; final concentration,  $0.5\text{ }\mu\text{g ml}^{-1}$ ), collected on a black polycarbonate membrane filter ( $0.2\text{ }\mu\text{m}$  pore size), and counted under ultraviolet radiation using an epifluorescence microscope (Porter & Feig 1980).

#### Sedimentation rates of POC and phytoplankton pigments

In order to obtain the sedimentation rate of total POC and that of POC derived from phytoplankton, sinking particle samples were collected for 24 h using cylindrical sediment traps that were positioned 2 m above the seafloor (bottom – 2 m) at each station. The cylindrical sediment traps had a small diameter and a high aspect ratio (67 mm diameter, 600 mm height, and aspect ratio of 9), as recommended by Bloesch & Burns (1980). No preservatives were used in the traps. Immediately after collection, the particles present in each trap were gently transferred to a plastic bottle along with 0.5 l of seawater from the trap. Total POC content and pigment concentration of the sinking particle samples were then determined following the same procedures indicated above. POC derived from phytoplankton was estimated from pigment concentrations. It is known that the carbon:chl *a* ratio (C:chl *a*) varies depending on taxonomic composition and physiological status (Strickland 1960); therefore, a minimum C:chl *a* value of 30 was applied in the present study. In this calculation, we included phaeo *a* as chl *a* because phaeo *a* are derivatives of chl *a* and were abundant in sinking phytoplankton cells.

#### Organic carbon derived from aquaculture activity

OC waste derived directly from aquaculture activity was calculated according to the following procedure. In Kogaura Inlet, fish aquaculture is conducted by the Fisheries Laboratory, Kinki University. Fish stocking

densities in the fish cages were  $<8$  kg wet weight  $m^{-3}$  for the yellow tail and  $<10$  kg wet weight  $m^{-3}$  for the red sea bream. The total fish-cage area was  $2.20 \times 10^4$   $m^2$ , and the entire fish-culturing area (Kogaura Inlet) was  $1.47 \times 10^6$   $m^2$ . The total amount of feed used in Kogaura Inlet was calculated from the 2004 monthly records of the Fisheries Laboratory, and fish production in the 2005 season was assumed to be the same as in the 2004 season. Aquaculture fish feed primarily consists of dry pellets that have an average carbon content of 40% (dry weight), according to elemental analysis using the CHN analyzer. The amount of feed (kg dry weight) used in each month was therefore multiplied by 0.4 to determine the amount of carbon added to the system as feed (kg C).

In terms of OC, most of the organic waste is released as POC (leftover feed and fish feces) at first, then some parts of released POC are converted into DOC through physicochemical processes, before they are mineralized into dissolved inorganic carbon (DIC). For instance, Wang et al. (2012) reported that 15% of the sum of the POC in leftover feed and fish feces (= 3% of POC in total feed) is reported to finally be converted to DOC. Organic waste as leftover feed and fish feces may be 1 to 38% of the total feed in terms of OC, depending on feed type, culture method, fish species, and other factors (Gowen & Bradbury 1987, Wang et al. 2012). For salmonid fishes in tanks and pond farms, 1 to 5% of dry pellets remain as leftover feed (Warrer-Hansen 1982). The percentage of feed that is excreted as fish feces is generally reported to be 20 to 30% for salmonid fishes (Gowen & Bradbury 1987). In the present study, the maximum waste percentages were assumed (5% for leftover feed and 30% for fish feces) in order to avoid underestimation

of the direct impact of aquaculture activity on the carbon budget. OC waste derived from aquaculture activity was therefore calculated by multiplying the feed amount (kg C) by 0.35.

### Carbon budgets at fish-cage and non-cage stations

Each process involved in carbon flow is expressed as an areal rate. Areal rates of planktonic photosynthesis and respiration ( $mmol\ C\ m^{-2}\ d^{-1}$ ) were calculated from volumetric rates ( $mmol\ C\ m^{-2}\ d^{-1}$ ) at each depth by integrating them over the water column from the sea surface to the bottom and applying linear interpolation with depth. Depth-integrated POC and DOC pools within the water column were calculated using the same procedure. The sedimentation rate and OC waste impact were determined as areal rates.

### Statistical analysis

The differences between the averaged values of environmental and biological parameters between the fish-cage and non-cage stations were tested using the 1-sample, paired *t*-test.

## RESULTS

### Hydrographic conditions of the water column

Fig. 2 shows vertical distributions of water temperature and salinity at the fish-cage station in 2005. Water temperature at 0 m depth ranged from 19.2°C

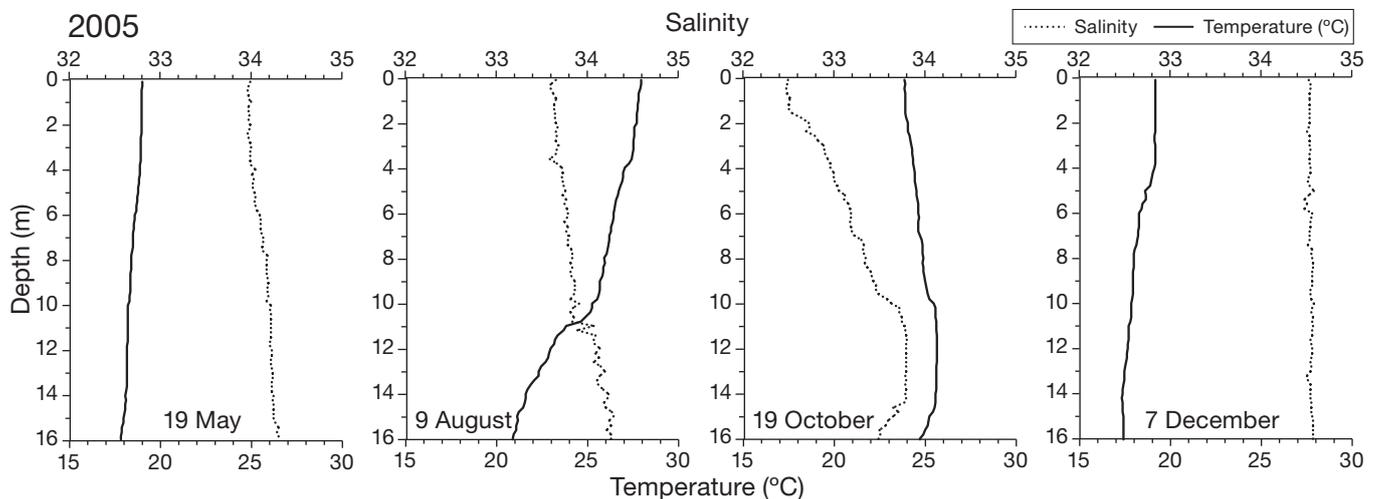


Fig. 2. Vertical distributions of salinity and temperature at the fish-cage station in May, August, October, and December 2005

Table 1. Comparisons of chemical water properties (particulate organic carbon [POC], dissolved organic carbon [DOC], dissolved oxygen [DO], nitrate, and phosphate) between fish-cage and non-cage stations (mean  $\pm$  SD, ranges in parentheses). Asterisks denote significant differences ( $p < 0.05$ )

	POC ( $\text{mg C m}^{-3}$ ) (n = 24)	DOC ( $\text{mmol C m}^{-3}$ ) (n = 8)	DO ( $\text{mg l}^{-1}$ ) (n = 21)	Nitrate ( $\mu\text{M}$ ) (n = 21)	Phosphate ( $\mu\text{M}$ ) (n = 21)
Fish cage	415 $\pm$ 133 (160–697)	3.0 $\pm$ 2.0 (1.2–7.2)	6.3 $\pm$ 1.3* (4.5–10.5)	1.28 $\pm$ 0.97* (0.01–4.09)	0.41 $\pm$ 0.19* (0.19–0.87)
Non-cage	427 $\pm$ 117 (263–708)	2.8 $\pm$ 2.3 (0.8–7.4)	6.7 $\pm$ 1.3* (3.8–10.1)	1.07 $\pm$ 0.97* (0.00–3.59)	0.30 $\pm$ 0.16* (0.10–0.65)

Table 2. Comparisons of biological parameters (chlorophyll *a* [chl *a*], bacterial abundance, planktonic photosynthetic, and respiration rates) and sedimentation rate of total particulate organic carbon between fish-cage and non-cage stations (mean  $\pm$  SD, ranges in parentheses). Asterisks denote significant differences ( $p < 0.05$ )

	Chl <i>a</i> ( $\text{mg m}^{-3}$ ) (n = 24)	Bacterial abundances ( $\times 10^6$ cells $\text{cm}^{-3}$ ) (n = 24)	Planktonic photosynthesis ( $\text{mmol C m}^{-3} \text{d}^{-1}$ ) (n = 12)	Planktonic respiration ( $\text{mmol C m}^{-3} \text{d}^{-1}$ ) (n = 12)	Sedimentation ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ) (n = 8)
Fish cage	3.6 $\pm$ 2.8 (0.6–9.0)	1.4 $\pm$ 1.1 (0.4–4.2)	17 $\pm$ 27 (0.0–74)	11 $\pm$ 8.2 (0.17–22)	116 $\pm$ 56* (48–213)
Non-cage	3.3 $\pm$ 2.4 (0.4–7.7)	1.2 $\pm$ 1.1 (0.4–4.8)	13 $\pm$ 20 (0.0–58)	7.5 $\pm$ 6.2 (0.00–21)	79 $\pm$ 36* (43–131)

on 7 December to 27.9°C on 9 August. Salinity at 0 m depth ranged between 32.5 and 34.5. Density stratification was observed on 9 August and 19 October.

### Comparisons between fish-cage and non-cage stations

There was no significant difference in POC and DOC concentrations in the seawater collected at fish-cage and non-cage stations (Table 1). DO was lower, while nitrate and phosphate were higher, at the fish-cage station than at the non-cage stations, but these differences were small.

There was no significant difference in chl *a* or bacterial abundance between the fish-cage and non-cage stations (Table 2). The sedimentation rate of total POC at the fish-cage station was an average of 1.5 times that at the non-cage stations. Photosynthesis and respiration rates tended to be higher at the fish-cage station than at non-cage stations; however, these differences were not statistically significant.

### Organic carbon derived from aquaculture activity

OC waste per unit area of the fish cage ranged from 610 to 1519  $\text{mmol C m}^{-2} \text{d}^{-1}$  and was 9 to 23  $\text{mmol C m}^{-2} \text{d}^{-1}$  for the entire fish-culturing area (Table 3). OC waste tended to be high during spring

and summer, during production of red sea bream *Pagrus major* seedlings.

### Planktonic photosynthesis and respiration rates

Because there was no significant difference in planktonic metabolism between fish-cage and non-cage stations, we describe only the results at the fish-cage station. Photosynthesis (*P*) was highest at 0 m depth and decreased with depth (Figs. 3 & 4). Respiration (*R*) was relatively stable within the water column, as it is not directly controlled by light intensity. As a result, the *P*:*R* ratio was  $> 1$  at the surface; except on 19 to 20 October 2004 and on 7 to 8 December

Table 3. Estimated values of organic carbon (OC) waste derived from aquaculture activity per unit area of the fish cage and that per unit area of the entire fish-culturing area (Kogaura Inlet). Data from the 2004 season were also used for the 2005 season

	May	Jun	Aug	Oct	Dec
OC waste per unit area of fish cage ( $\text{mmol C m}^{-2} \text{d}^{-1}$ )	1519	1168	1303	610	698
OC waste per unit area of Kogaura Inlet ( $\text{mmol C m}^{-2} \text{d}^{-1}$ )	23	18	20	9	11

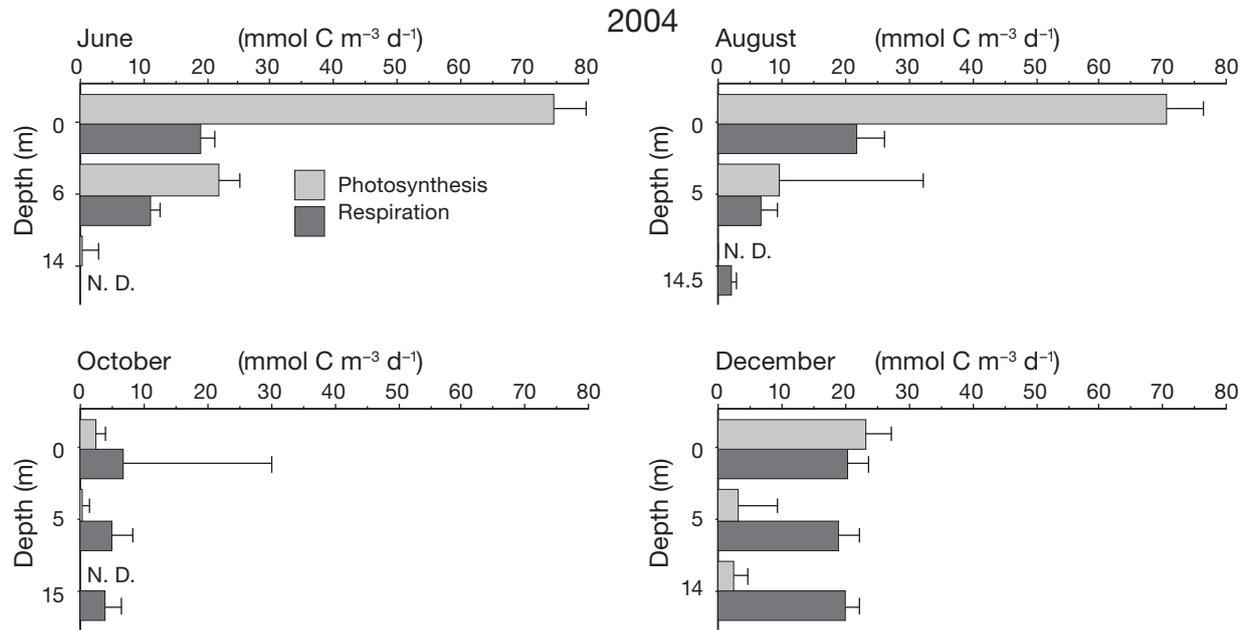


Fig. 3. Vertical distributions of planktonic photosynthesis and respiration (mean  $\pm$  SD; mmol C m<sup>-3</sup> d<sup>-1</sup>) at the fish-cage station in June, August, October, and December 2004. N.D.: not detected

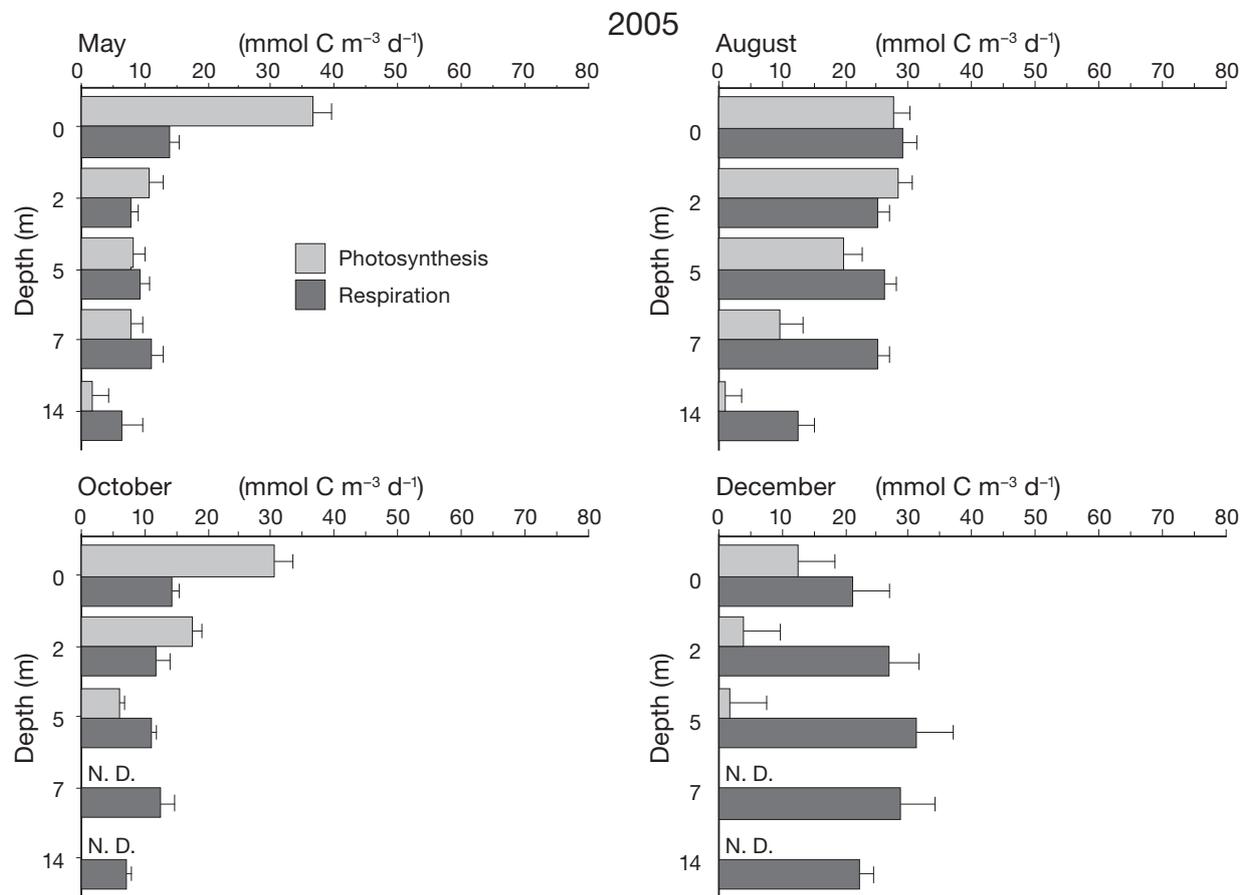


Fig. 4. Vertical distributions of planktonic photosynthesis and respiration (mean  $\pm$  SD; mmol C m<sup>-3</sup> d<sup>-1</sup>) at the fish-cage station in May, August, October, and December 2005. N.D.: not detected

2005, but was  $<1$  at the bottom layer. Photosynthesis was low in the entire water column on 19 to 20 October 2004 and 9 to 10 August 2005, when the weather was overcast.

### Sedimentation rate of organic carbon

The sedimentation rate of total POC ranged from 42 to 215  $\text{mmol C m}^{-2} \text{d}^{-1}$  (Fig. 5). The sedimentation rate of total POC at the fish-cage station was higher than, or equal to, that at the non-cage stations. There was no obvious seasonal trend for sedimentation rate.

POC derived from phytoplankton accounted for 8 to 61% of the total POC sedimentation (Fig. 5). It was lowest in December in both 2004 and 2005 at both fish-cage and non-cage stations and accounted for only 8 to 19% of total POC sedimentation during this period. Both allochthonous and autochthonous sources of POC sedimentation at the fish-cage station tended to be higher than those at the non-cage stations.

### Carbon budget

Figs. 6 & 7 show the OC budgets at the 2 stations in the 2004 and 2005 seasons, respectively, and Fig. 8 shows a summary of the budgets. Horizontal advection and resuspension were not estimated in the present study but are shown by arrows without values in Figs. 6 to 8, taking account of their possible importance in the OC budget.

The OC from planktonic photosynthesis was 1 to 2 orders of magnitude lower than that from aquaculture activity at the fish-cage station (Fig. 6). Planktonic respiration was comparable to, or higher than, the sedimentation rate. The ratio of respiration to sedimentation rates varied from 0.4 to 7.

Planktonic photosynthesis was 2 to 3 times higher than respiration at both fish-cage and non-cage stations in June and at the fish-cage station in August 2004 (Fig. 6), i.e. the total plankton community was autotrophic and acted as a source of OC. In October and December, respiratory loss at both stations was up to 9 times higher than photosynthetic input, and plankton communities acted as a sink of OC.

The OC contribution of planktonic photosynthesis was 1 order of magnitude lower than that of aquaculture activity at the fish-cage station during each observation period (Fig. 7). Planktonic respiratory loss at the fish-cage station was higher than sedimentary loss during all observation periods. The total plankton community at the fish-cage station was heterotrophic and acted as a sink of OC during all observation periods. In December, the amount of OC lost due to respiration was 12 times the amount of OC added due to photosynthetic input.

Fig. 8 shows a summary of the OC budget averaged over the 2004 and 2005 seasons. The mean value of aquaculture activity was 6 times higher than that of photosynthesis at the fish-cage station. On average, the total plankton community acted as a sink of OC. The mean quantity of OC sequestered by planktonic respiration was 2 times greater than that lost due to sedimentation at the fish-cage station.

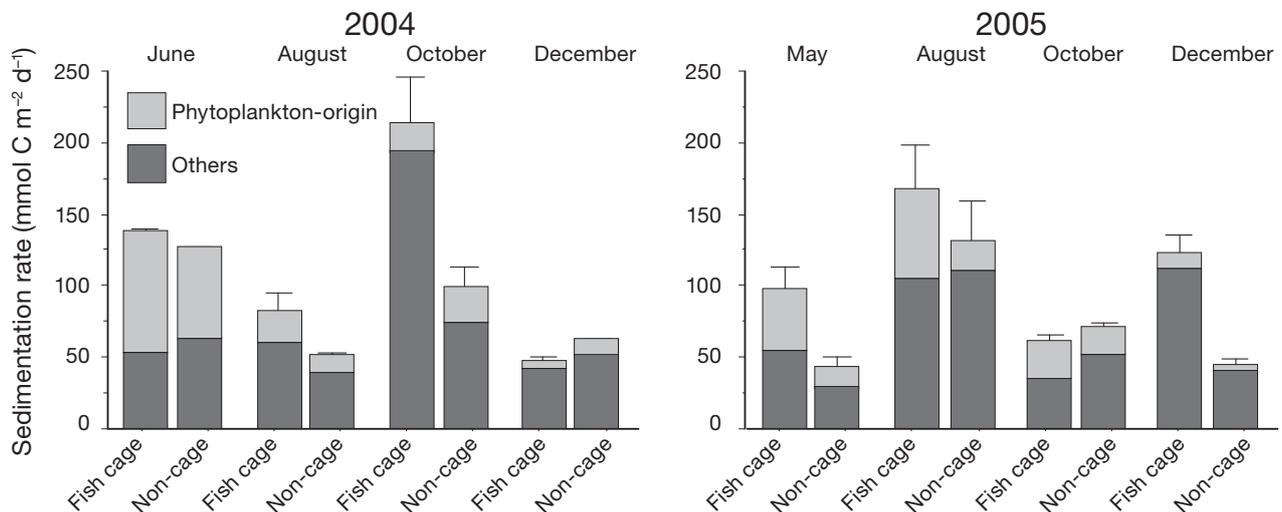


Fig. 5. Sedimentation rates of total organic carbon ('phytoplankton-origin' + 'others'; mean  $\pm$  SD;  $\text{mmol C m}^{-2} \text{d}^{-1}$ ) at the fish-cage and non-cage stations in 2004 and 2005

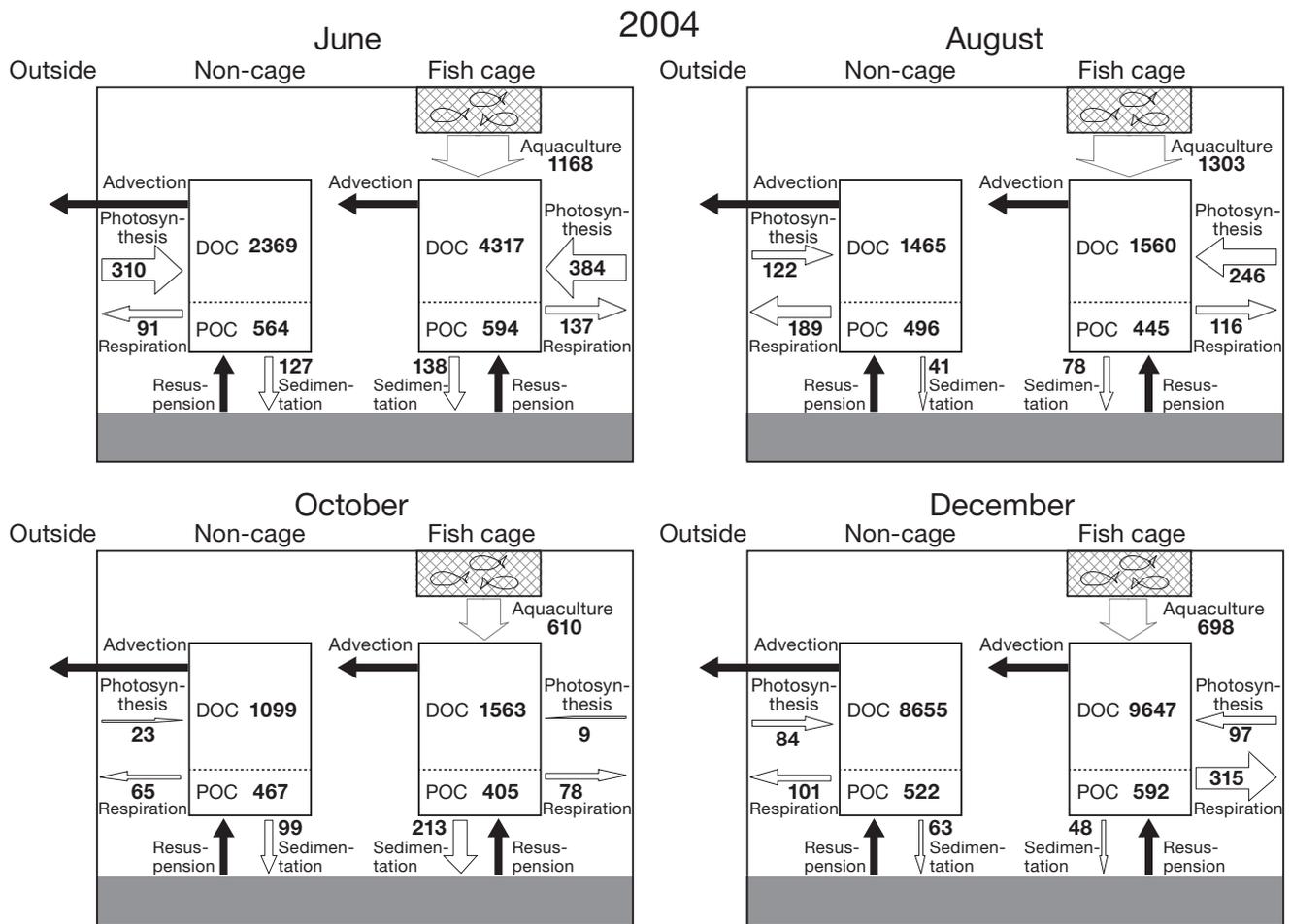


Fig. 6. Organic carbon budgets at the fish-cage and non-cage stations in June, August, October, and December 2004. Values beside arrows indicate the rate of each process ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ). DOC: dissolved organic carbon; POC: particulate organic carbon

## DISCUSSION

### Aquaculture activity versus planktonic photosynthesis as a source of organic carbon

The present results suggest that allochthonous input of OC through aquaculture activity was several times higher than that of autochthonous input by planktonic photosynthesis (Figs. 6–8). In contrast, a significant part (8 to 61%) of OC sedimentation was ascribed to phytoplankton photosynthesis in spite of a conservative estimate of its contribution (Fig. 5), which implies that fluctuation in planktonic photosynthesis may influence the quality and quantity of OC deposited on the bottom sediments. In the same fish-culturing area examined in this study, previous analysis of the sugar composition of bottom sediments indicated that a large amount of phytoplankton may have been deposited on the seafloor (Kitada et al. 1987). Thus, the relative contribution of phytoplankton as a

source of OC was small in the water column, but large in the bottom sediments. á Norði et al. (2011) conducted one of the few studies in which planktonic photosynthesis as a source of OC in a fish-culturing area was evaluated. According to their data, sedimentation of OC from photosynthesis constituted two-thirds of the total OC sedimentation beneath a trout farming cage in a high-latitude fjord. Alongi et al. (2003) reported that, in low-latitude mangrove areas, the amount of C added by trash fish in a sea bass farm was only 16% of that added by phytoplankton photosynthesis. However, the amounts of N and P added by the trash fish were 32 and 99% of the amounts added by phytoplankton photosynthesis, respectively. In contrast, McKinnon et al. (2010) reported that the C and N input of artificial feed was 4 times that of the input by phytoplankton photosynthesis.

There are 2 possible explanations for the relatively high contribution of photosynthesis to OC sedimentation, despite its minor contribution as an OC

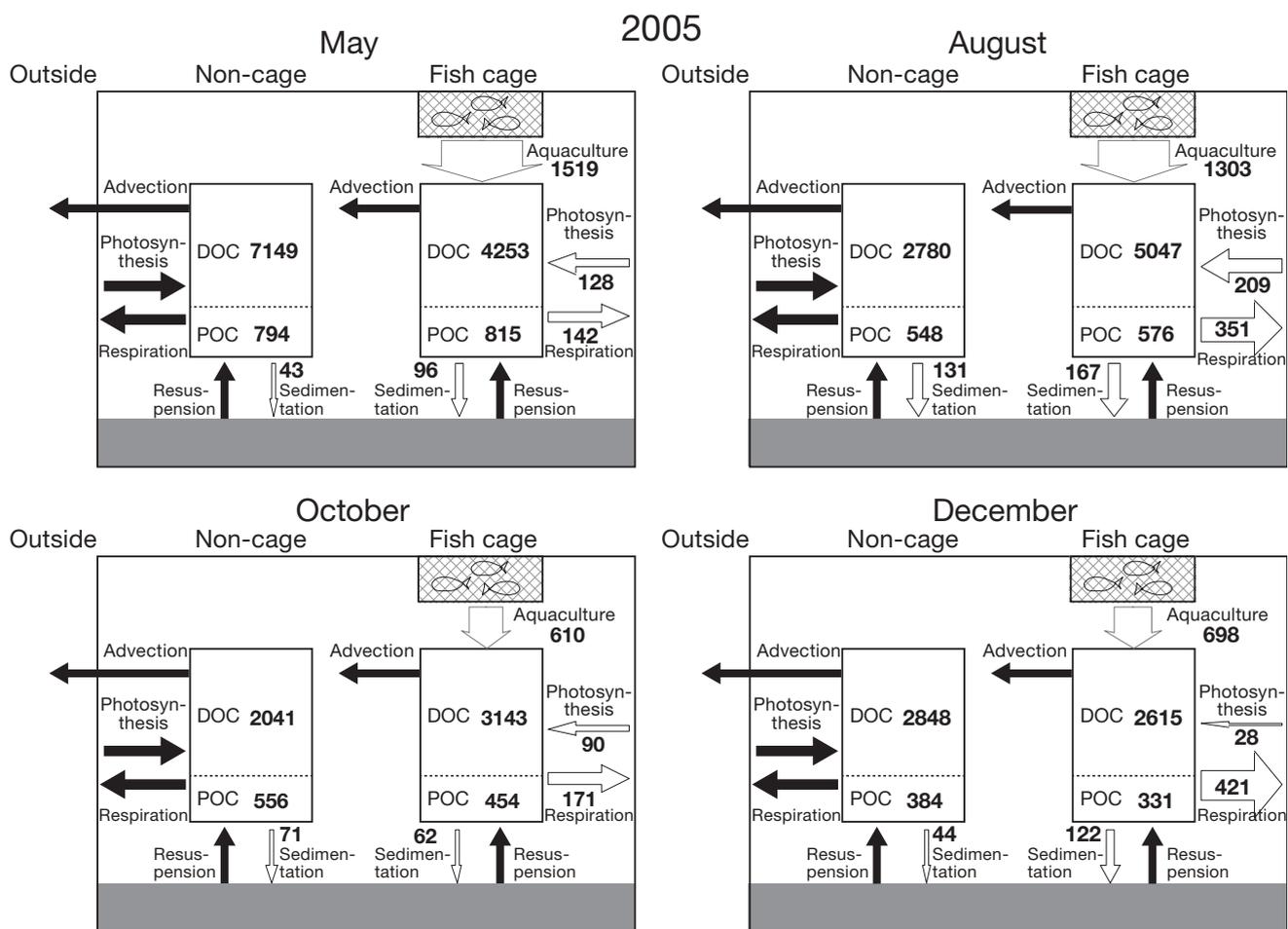


Fig. 7. Organic carbon budgets at the fish-cage and non-cage stations in May, August, October, and December 2005. Values beside arrows indicate the rate of each process ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ). Data from the 2004 season were used to estimate aquaculture activity. DOC: dissolved organic carbon; POC: particulate organic carbon

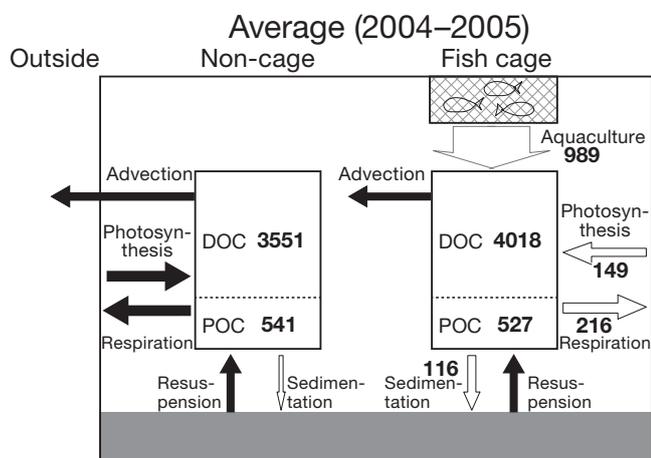


Fig. 8. Summary of organic carbon budgets at the fish-cage and non-cage stations in the 2004 and 2005 seasons. Values beside arrows indicate the rate of each process ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ), averaged over time ( $n = 8$ ). DOC: dissolved organic carbon; POC: particulate organic carbon

source: (1) the degradation and sedimentation processes of the 2 types of OC (derived from aquaculture and photosynthesis) differ from one another and (2) OC waste due to aquaculture activity is overestimated. As to the former reason, *Heterosigma akashiwo* (Raphidophyceae) dominated the phytoplankton assemblages in June 2004, when the sedimentation rate of OC that was derived from phytoplankton was highest. *H. akashiwo* is easily lysed by viral infection and aggregates, which results in rapid settling onto the seafloor (Nagasaki et al. 1994). In addition, the packaging of phytoplankton cells into rapidly falling zooplankton fecal pellets might contribute to the high sedimentation rate of phytoplankton-origin OC in some areas (Turner & Ferrante 1979).

It is important to note that in the present study, the estimation of OC input from aquaculture activity was at the upper end of the range of uncertainty. This was done to avoid overestimation of the rela-

tive importance of phytoplankton. We applied the previously reported maximum conversion factor (0.35) to calculate the leftover feed and feces after feeding dry pellets to cultured fish. In addition, we assumed that all parts of the leftover feed and fish feces were incorporated into the OC pool just below the fish cage, and we neglected to consider losses due to horizontal advection. In general, POC flux decreases exponentially with distance from fish cages, but aquaculture waste extends to areas 100s of meters away from farming sites (Yokoyama et al. 2006, á Norði et al. 2011). á Norði et al. (2011) estimated that a third of the OC waste accumulated directly below a trout cage farm in a fjord. Tsutsumi et al. (2006) reported that the sedimentation rate of OC at a fish cage was 2.5 times that at a control point 400 m away from any fish-cage areas. In the present study, the sedimentation rate of OC at the fish cage was 1.5 times that found at the non-cage stations, which were >300 m from any fish-cage areas, except in June 2004, when the non-cage site was 50 m from the nearest fish-cage area (Table 2). Thus, a significant part of aquaculture-derived OC appeared to be deposited on the seafloor just below the fish cage. However, there was no significant difference in OC pool size or planktonic metabolism between fish-cage and non-cage stations. It might be more appropriate to assume that aquaculture impact was uniform over the fish-culturing area. OC waste for the entire fish-culturing area was 11 to 29 mmol C m<sup>-2</sup> d<sup>-1</sup> (the lower rows of Table 3) and was 1 order of magnitude lower than OC input due to planktonic photosynthesis. The actual OC sedimentation rate was considered to be intermediate between the value calculated per unit of fish-cage area and the value calculated for the entire fish-culturing area (Table 3).

#### **Sedimentary loss versus planktonic respiration as a sink of organic carbon**

The mean sedimentation rate of OC at the fish-cage station during the 2004 and 2005 seasons was 116 mmol C m<sup>-2</sup> d<sup>-1</sup> (Fig. 8). This value was near the lower end of previously reported ranges (100 to 1000 mmol C m<sup>-2</sup> d<sup>-1</sup>; Findlay & Watling 1997, Tsutsumi et al. 2006, á Norði et al. 2011). In general, the percentage of leftover dry pellets (1 to 5%) is much lower than the percentage of leftover moist pellets (5 to 10%) or trash fish (10 to 30%) (Warrer-Hansen 1982). In the present study, the relatively low OC sedimentation rate seems to be due to the fact that

dry pellets were used as the primary fish feed. Fish stocking densities in the present study (<8 kg m<sup>-3</sup> for the yellow tail *Seriola quinqueradiata* and <10 kg m<sup>-3</sup> for the red sea bream *Pagrus major*) were moderate in comparison to other studies, such as the 4 to 17 kg m<sup>-3</sup> for red sea bream reported by Tsutsumi et al. (2006).

Planktonic respiratory loss of OC was similar to, or higher than, sedimentary loss (Figs. 6 & 7). This suggests that a significant portion of the OC was respired before arriving at the seafloor. Particularly in winter, planktonic respiration was high, despite low photosynthesis, and plankton community metabolism acted as a sink of OC. The relatively high respiration rate in winter appeared to be due to high DO levels and a high cell-specific mineralization rate of amino acids and monosaccharides (Yoshikawa et al. 2012). This is the first study that clearly describes the possible importance of planktonic respiration as a sink of OC even in a mid-latitude fish-culturing area where planktonic metabolism is not expected to be as highly active as that in a low-latitude area. According to the data reported by Trott et al. (2004), the planktonic respiration rate was approximately half the rate of OC sedimentation and was 1.6 times that of benthic respiration in a mangrove creek that received shrimp farm effluent (as recalculated by the authors of the present study). On the basis of data reported by McKinnon et al. (2010), the rate of planktonic respiration per unit area was twice that of benthic respiration in a barramundi farm in a mangrove area.

#### **Planktonic metabolism as source and sink of organic carbon**

In fall and winter, net planktonic metabolism was negative and the plankton community acted as a sink of OC (Figs. 6 & 7). On the other hand, in spring and summer, net planktonic metabolism tended to be positive or near zero and the plankton community acted as a source of OC. The mean annual net planktonic metabolism at the fish-cage station varied from year to year; it was near zero in 2004 and negative in 2005. We cannot determine whether planktonic metabolism acted as an overall source or a sink of OC, as the sampling interval in the present study was not short enough to evaluate annual photosynthesis. A previous study by Yoshikawa et al. (2007) that was conducted in the same area as the present study showed that DO at the surface layer fluctuated markedly throughout the day, reflecting changes in

planktonic photosynthesis due to changes in solar radiation.

Most previous studies of the environmental impact of aquaculture have focused on organic pollution and anoxication at the bottom layer and assumed that the total plankton community at aquaculture sites is heterotrophic. However, dissolved inorganic nitrogen and phosphate are also released from fish cages, which can stimulate phytoplankton photosynthesis and drive the total plankton community into autotrophic status. Some models have shown little effect of aquaculture on primary production in high-latitude areas (e.g. Skogen et al. 2009), while others have indicated a significant impact of aquaculture on primary production in oligotrophic waters, particularly during the stratified season (e.g. Wild-Allen et al. 2010).

### Physical processes influencing the OC budget

The present results were obtained from a semi-enclosed fish aquaculture site (cf. Takeuchi 1994), and therefore they cannot be directly applied to open bays. The relative importance of planktonic versus physical processes with regard to the OC budget in a fish-culturing site is determined by the topographic condition of the site. Inoue (1977) estimated that planktonic photosynthesis, inflow of external water, and flux from the atmosphere explained 70, 20, and 10% of the total supply of DO in a semi-enclosed aquaculture area of the yellow tail *Seriola quinqueradiata*, based on measurements of spatial variation in DO. On the other hand, the contribution of external water inflow on the supply of DO was >98% in open coastal areas.

In the same area investigated in the present study, Yoshikawa et al. (2007) showed marked short-term covariation between DO and phytoplankton photosynthesis during stratified seasons by mooring various types of sensors, including current meters. The covariation between DO and phytoplankton photosynthesis was masked by the diffusion of DO and inflow of external water when the current velocity was high. Their results also indicated that intrusion of external water was more important than tidal currents in terms of water movements and the dynamics of DO during fall. Furthermore, Tanaka et al. (1999) found that the coupling of coastal upwelling and internal tidal flow may cause an intermittent intrusion of ocean water resulting in the thorough exchange of water between the outside and inside of Tanabe Bay during summer. These previous studies

suggest that occasional events such as internal tidal flow and wind-driven currents rather than tidal currents are the dominant physical processes in the present study area.

Horizontal advection and resuspension of sediments are potentially important physical processes with respect to the OC budget, although their values were not estimated in the present study. The fact that there was no significant difference in OC pool size between the fish-cage and non-cage stations implies the possible effect of horizontal advection. There are unpublished data for the present study indicating that active microbial mineralization of OC occurs in the resuspendable sediments (Yoshikawa et al. unpubl. data), which is probably owing to a relatively higher DO level compared with that in the bottom sediments.

### Concluding remarks

In conclusion, the present study clearly demonstrates the importance of planktonic processes on the carbon budget of a semi-enclosed fish aquaculture site, based on field measurements. Autochthonous production of OC by photosynthesis was estimated to be much smaller than allochthonous input of OC through aquaculture activity. However, POC derived from phytoplankton accounted for a significant part of total POC sedimentation, particularly in spring and summer. As to sinks of OC, the annual mean planktonic respiration rate was twice the rate of sedimentary loss at the fish-cage station. The traditional concept of the impact of aquaculture activity is that organic wastes are deposited on the seafloor, diffuse horizontally, and are mineralized there. However, the results of the present study urge us to change this concept to a more complicated one, in which a significant part of allochthonous and autochthonous OC input is respired by plankton and the remaining OC is deposited on the seafloor.

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