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Seasonal variation of primary productivity in the Shinkawa River estuary, eastern Seto Inland Sea, Japan

Keigo HIGASHIZONO[1)], Hitomi YAMAGUCHI[1)], Kuninao TADA[1)] and Kazuhiko ICHIMI[2)]

Abstract: Seasonal variations in the increase rate of chlorophyll (Chl.) *a* and the primary production rate were measured by *in situ* seawater incubation in the Shinkawa River estuary in the Seto Inland Sea, Japan. Nutrient concentrations in the seawater were always sufficient for the growth of phytoplankton. The study site was very shallow, but photo-inhibition, which occurs at high light intensities, such as > 1,000 μmol/m^2/s, was not observed in photosynthesis test samples. Therefore, light intensity was a minor factor to explain primary production in this area throughout the year. Chl. *a* concentration reduced a little or was almost constant at low water temperatures. In contrast, Chl. *a* concentration increased significantly in the period of high water temperature (July to September), showing a maximum specific increase rate of 0.3–0.4/h. The primary production rate was also high while water temperature was high. Monthly primary production rates, estimated from water temperature and PAR, varied from 0.01 ± 0.00 to 1.13 ± 0.24 gC/m^2/day, which was similar to that reported for other estuaries and coastal seas.

The most influential factor in primary productivity in this study area was water temperature, with peak temperatures associated with extremely high primary productivity, with up to 460 μgC/l/h recorded in summer.

Keywords: *Estuary, Primary production, Chl. a, Nutrient, Light*

1. Introduction

Large amounts of inorganic nutrients, such as nitrogen and phosphorus, are supplied from rivers into estuarine tidal flats. In addition, physical environmental factors, such as temperature, salinity, and nutrient concentrations, vary widely in the short term because of the tidal cycle. Many secondary producers, which feed on organic matter on the sediment surface or suspended organic matter, inhabit tidal flats (COLIJN and DIJKEMA 1981; COLIJN and de JONGE 1984). Primary producers, such as pelagic and benthic microalgae, support the biological production of higher trophic level organisms.

Benthic microalgae inhabiting the sediment surface are major primary producers in tidal flat ecosystems (ADMIRAAL, 1977), and primary production in the surface sediment has been measured in various regions (JOINT, 1978; COLIJN and de JONGE, 1984; BARRANGUET *et al*., 1998). On the other hand, higher trophic level organisms in tidal flats are supported by pelagic phytoplank-

1) Faculty of Agriculture, Kagawa University, Miki, Kita, Kagawa 761–0795, Japan
2) Seto Inland Sea Regional Research Center, Kagawa University, Kamano, Aji, Takamatsu, Kagawa 761–0130, Japan
*Corresponding author:
E-mail:ichimi@ag.kagawa-u.ac.jp

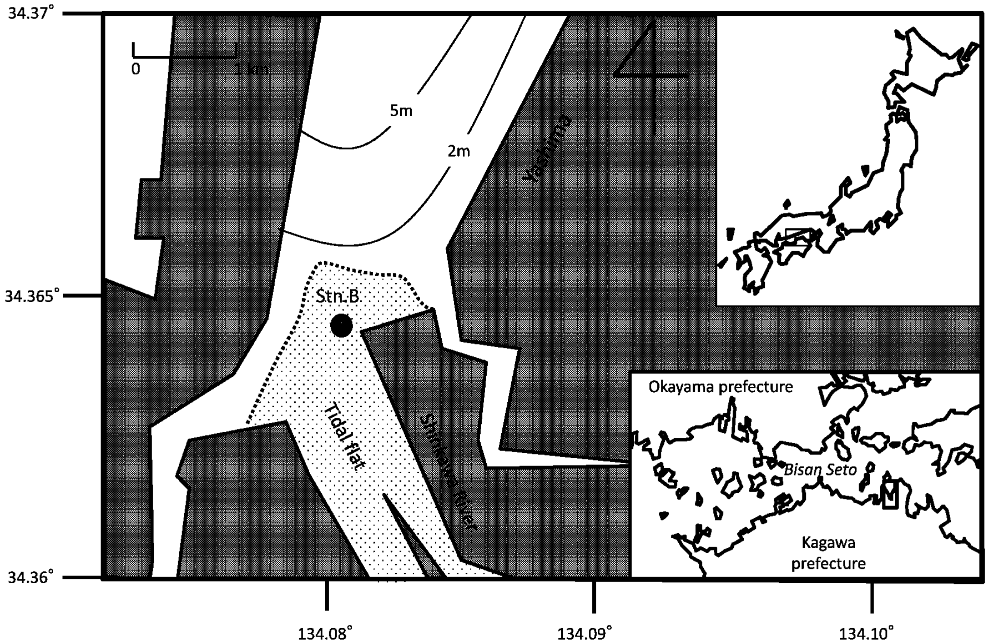

Fig. 1. Study site and sampling station.

ton in the water column and are also major primary producers. CLOERN *et al.* (2014) previously summarized the features of primary productivity in coastal seas. For example, primary productivity was reported as 300–400 gC/m^2/year in Chesapeake Bay and 200–300 gC/m^2/year in Tampa Bay, both in the United States, and 400–500 gC/m^2/year in Tokyo Bay and 100–200 gC/m^2/year in the Seto Inland Sea, both in Japan. BOYNTON *et al.* (1982) also summarized primary production rates in the water column for 45 estuaries with the wide range of 19–547 gC/m^2/year. In addition, CLOERN *et al.* (2014) reported that the primary production rates in the Swan Estuary, Australia and the Cienaga Glande Estuary, Colombia were apparently higher (1,000 gC/m^2/year) than in coastal areas. Many reports about primary production rates have focused on European and North/South American estuaries, which were located on large rivers (For example, FLEMER, 1970; GAMEIRO *et al.*, 2011). On the other hand, have been few reports on the small-scale estuaries, as seen around the Japanese coast, where environmental parameters, such as temperature, light intensity and nutrient concentrations, change drastically with the tidal cycle.

In this study, we examined the effects of the water temperature, light intensity, and nutrient concentrations on the primary production rate and characteristics of productivity in the Shinkawa River estuary, Japan.

2. Materials and Methods

2.1 In *situ* incubation of seawater samples

The Shinkawa River estuary, located in Takamatsu City, Kagawa Prefecture, Japan, has a tidal flat (< 600,000 m^2) that appears at low tide. Low saline water flow into the estuary from Shinkawa River is observed up to several kilometers from the edge of the intertidal zone (Fig. 1). Seawater sampling and incubation experiments were carried out from January to December 2006, April

2007 to March 2008, August to September 2011, April 2012 to March 2013, May to September 2014 and April to August 2015 at Stn. B, at which the average water depth at high tide was about 1.5 m.

Surface seawater was collected using a plastic bucket when the water depth was ca. 70 cm during flood tide. Water temperature and salinity were measured by CTD (AAQ1186S-Pro, manufactured by ALEC Inc.). Large zooplankton in the collected seawater was removed using a plankton net (mesh size 300 μm), and transferred into a 500 ml clear polycarbonate bottle. These bottles were placed in the sea surface in the field and were incubated for 4–9 hours during the day (08:00 to 17:00). In 2015, the incubation experiments were carried out in 100% light (L-100%) and three other light intensities. 0%, 10%, and 50%, by shading with a plastic net (L-0%, L-10%, and L-50%, respectively).

Solar radiation levels for the study area (Takamatsu City) were referenced from data recorded by the Japan Meteorological Agency (http://www.jma.go.jp/jma/). Photosynthetically available radiation (PAR) was calculated as follows. Because the wavelength of solar radiation is 290–3,000 nm, it was multiplied by a factor of 0.48 to convert to the wavelength of PAR (390–770 nm) (MURAMATSU *et al.*, 2008). This value was then multiplied by 4.57 to obtain photon flux density (PFD) in order to convert the units from $MJ/m^2/d$ to $\mu mol/m^2/s$ (THIMIJAN and HEINS, 1983). Because 15% of light is reflected by sea surface (TAKAHASHI *et al.*, 1996), subsurface PAR was obtained by multiplying by 0.85.

2.2 Concentrations of Chl. *a* and nutrients

Seawater samples (200 ml) at the start and end of the incubation experiment were filtered using Whatman GF/F. Chl. pigment was extracted for 24 hours with 90% acetone. Chl. *a* concentration was determined by the fluorescence method (HOLM-HANSEN *et al.*, 1965) using a fluorometer (10AU, Turner Design Co., Ltd.).

The specific increase rate of Chl. *a* during the experimental incubation (μ: /h) was calculated using the following equation:

$$\mu\ (/h) = \ln\ (Ce / Ci)\ /\ t \qquad (1)$$

Ci, Ce, and t indicate the Chl. *a* concentration at the start and end of incubation and incubation time (hour), respectively.

Incubated water samples were also filtered through a disc filter (Pore size 0.45 μm; Advantech Co., Dismic Filter) to measure nutrient concentrations. DIN ($NH_4 + NO_{2+3}$), PO_4 and $Si(OH)_4$ levels were determined using a nutrient automatic analyzer (Auto Analyzer III, BL-TEC Co., Ltd.).

2.3 Primary production rates

Primary production was measured by the ^{13}C method (HAMA *et al.*, 1983) using ^{13}C solution (0.2 mM). Water samples (200 ml) were filtered using Whatman GF/F, which was then baked at 470℃ to remove organic matter. After the addition of several milliliters of 1 N HCl to avoid inorganic carbon, the filters were stored at −20℃ until analysis. To determine the particulate organic carbon (POC) concentration in the seawater, water samples was also treated using the same procedure. The stable carbon isotope ratio was measured using a stable isotope analyzer (Model-FlashEA1112-Deltav Advantage ConFlo IV System, Thermo Fisher Scientific). Primary production rates were calculated using the following equations (HAMA *et al.*, 1983).

$$a_{ic} = \{({}^{13}C_{cont} \times 0.011 + {}^{13}C_{add}) / (DIC + {}^{13}C_{add})\} \times 100 \qquad (2)$$

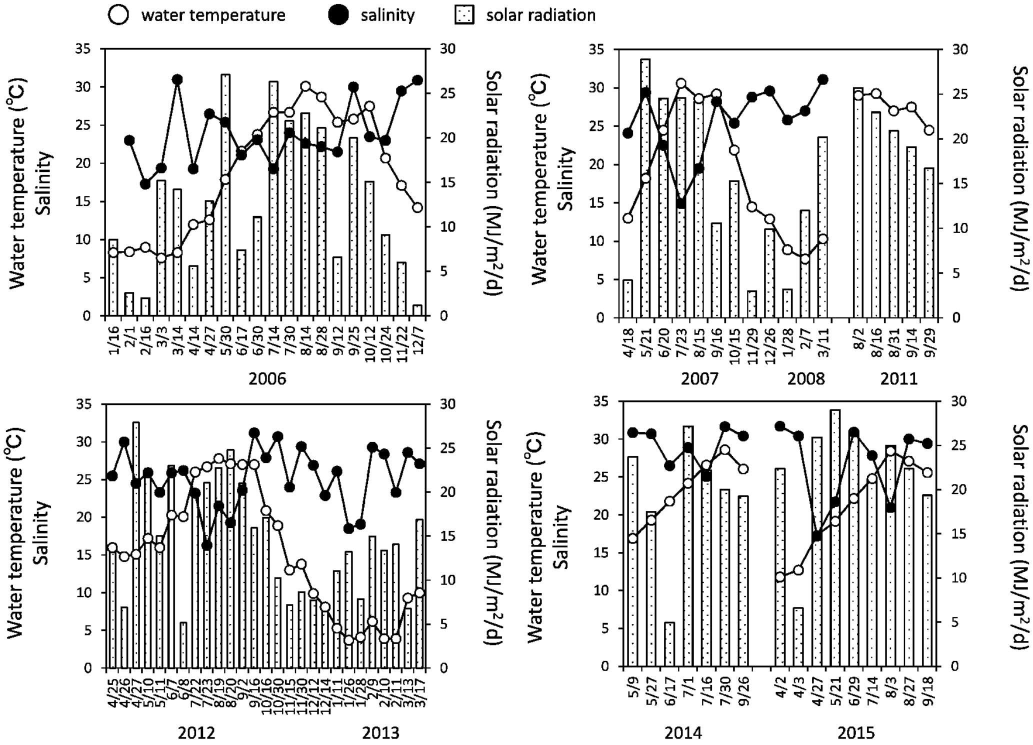

Fig. 2. Temporal variations in water temperature, salinity, and PFD.

$$\text{Primary production } (\mu\text{gC/l/h}) = \{\{\text{POC concentration} \times (a_{is} - a_{ns}) / (a_{ic} - a_{is})\} \times f/h\} \quad (3)$$

$^{13}C_{cont}$ and $^{13}C_{add}$ show ^{13}C content in DIC and added ^{13}C, respectively; a_{ic}, a_{is}, and a_{ns} show ^{13}C atom% in DIC concentration, ^{13}C atom% in the POC at the end of incubation, and ^{13}C atom% in the initial POC concentration, respectively; f / h is 1.025, which was the ^{13}C isotopic fractionation correction coefficient (HAMA *et al.*, 1983) and incubation time, respectively. DIC concentration was determined based on carbonate alkalinity and pH, as described in STRICKLAND and PARSONS (1968). POC concentration was measured using a CHN Coder (JM10, J Science Labo.).

The primary production rates for L-50% and L-100% were similar, as described later in the results. In addition, about 60% of the annual light at the sea surface reached to the bottom even at high tide at the experimental station (HIGASHIZONO *et al.* in preparation). Therefore, the integrated primary production rate ($mgC/m^2/h$) was converted using the average water depth (0.75 m) and the primary production rates ($\mu gC/l/h$) obtained for L-100%. The primary production rate ($mgC/m^2/h$) was converted to a daily primary production rate ($gC/m^2/day$) by multiplying by the ratio of solar radiation during incubation (h)/daily total solar radiation (h).

3. Results

3.1 Water temperature, salinity, and solar radiation

Water temperature varied in the range of 3.7-30.6℃ (Fig. 2). Water temperature was generally lower than 15℃ from November to early April, and 15-25℃ from late April to June or

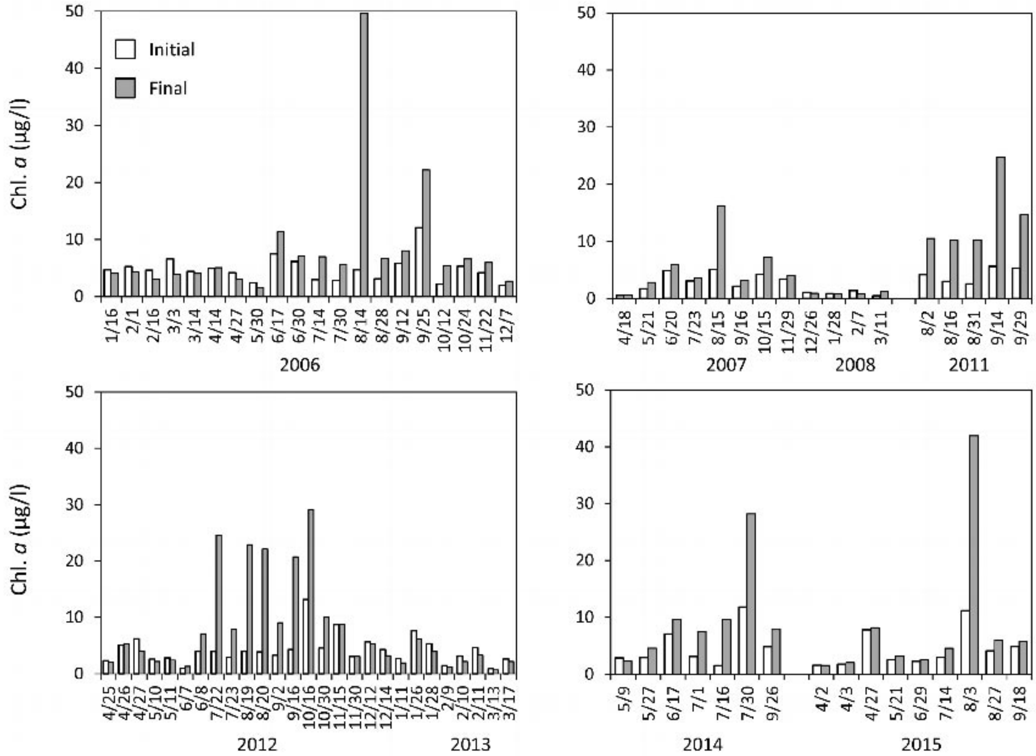

Fig. 3. Temporal variations in initial and final concentrations of Chl. *a*.

late September to October (Fig. 2). It generally exceeded 25℃ in July to early September (Fig. 2). Salinity varied in the range of 20–30, with a lowest recorded level of 14.9 on 23th July 2007 (Fig. 2). Solar radiation varied in the range of 1.20–29.0 $MJ/m^2/d$ (Fig. 2). It was lower in June (rainy season) and October to March, and higher in April to September (Fig. 2).

3.2 Chl. *a* and nutrient concentrations in seawater

Initial Chl. *a* concentration in the incubation bottle ranged from a minimum of 0.50 μg/l to and a maximum of 13.2 μg/l (Fig. 3). The Chl. *a* concentration was higher in July to September (average 4.73 μg/l; Fig. 3). The specific increase rate of Chl. *a* in L-100% light was in the range −0.08–0.34 μg/l/h (Fig. 4). A higher increase rate, 0.1–0.3 /h, was observed in July to September when water temperature exceeded 25℃ (Fig. 4). The highest increase rates, 0.34 /h and 0.33 /h, were recorded on 14th August 2006 and on 3rd August 2015, respectively (Fig. 4). On the other hand, the increase rates in November to April, when the water temperature was below 15℃, were quite low −0.08–0.05 /h (Fig. 4). The increase rates showed a good correlation with water temperature (Fig. 5).

The increase rate of Chl. *a* tended to decrease with increasing light intensity (Fig. 6). The average increase rate was 0.01 /h with L-0% (−0.04–0.05 /h), 0.14 /h (0.03–0.39 /h) with L-10%, 0.11 /h (0.00–0.42 /h) with L-50%, and 0.08 /h (−0.01–0.33 /h) with L-100%, showing that the increase rates with L-10% and L-50% were higher than with L-100% (Fig. 6). On 3rd April,

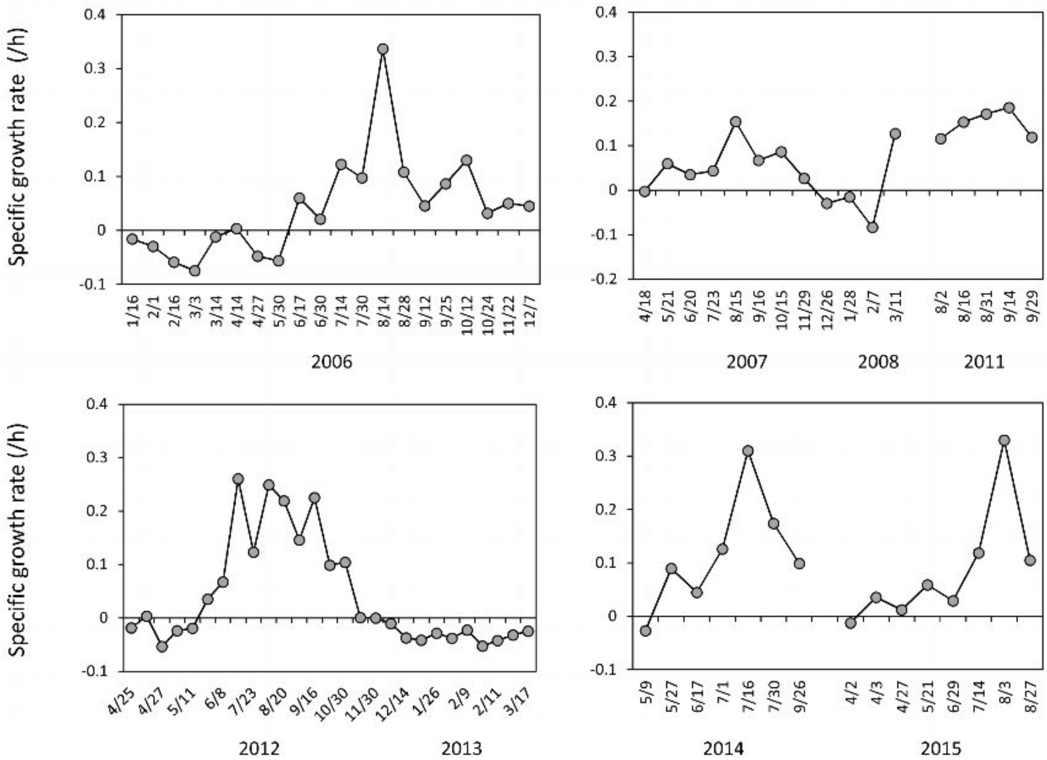

Fig. 4. Temporal variations in increases in Chl. *a* concentration.

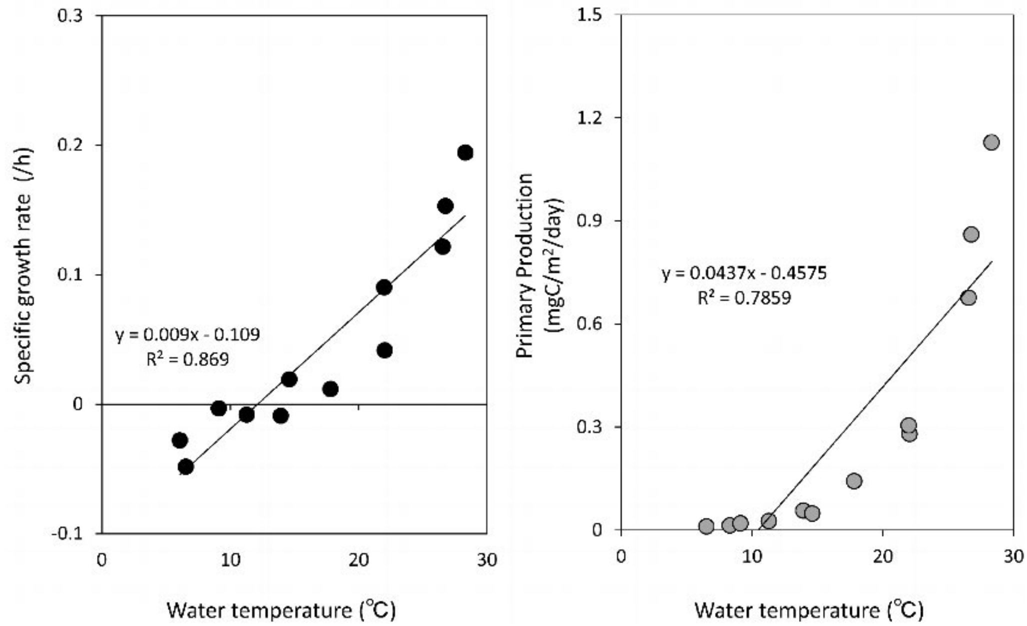

Fig. 5. Relationship to water temperature and specific growth rate of Chl. *a* concentration, primary production.

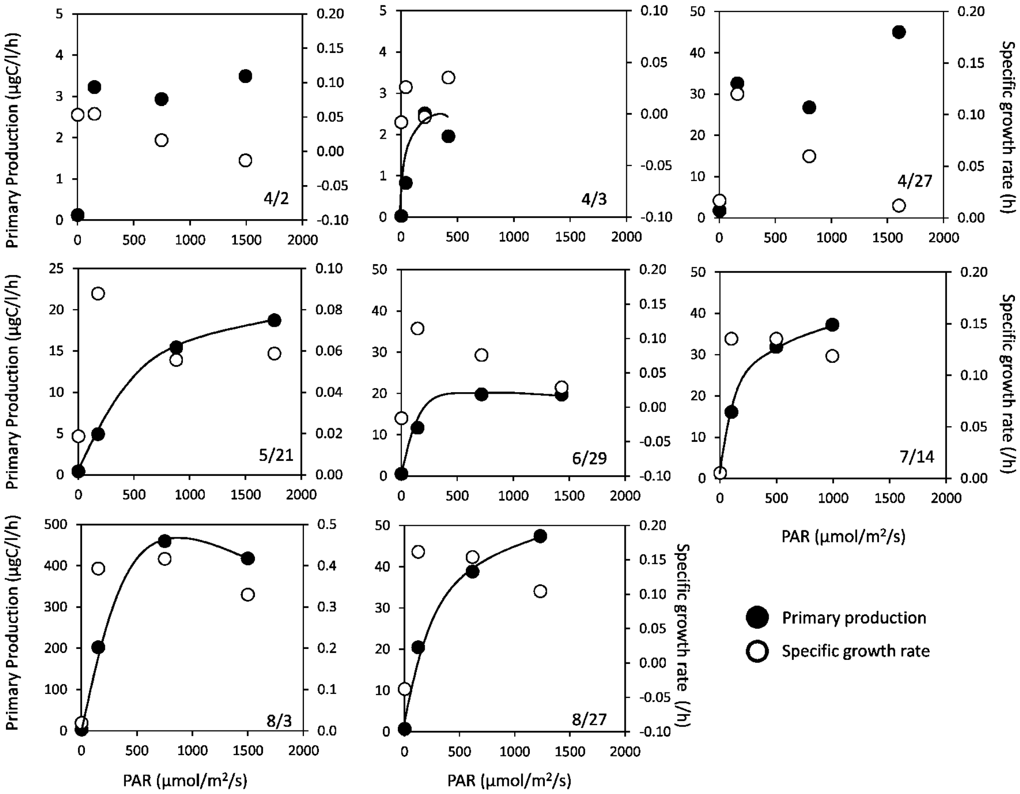

Fig. 6. Primary production (filled circles) and specific growth rate of Chl. *a* concentration (open circles) at various PAR observed in 2015. The solid line shows the approximate curve of the primary production rate obtained from PLATT *et al.* (1980).

14th July, 27th August, and 21th May, differences between the increase rates with L-10% and L-100% light were small, when solar radiation gradually decreased or was stable during the incubation (Fig. 6, 7), although solar radiation increased on other experimental days (Fig. 6, 7).

The initial DIN concentration was higher from July to September, although it differed widely (1.13–62.5 μM) depending on the observation day (Fig. 8). Similarly, PO_4 and $Si(OH)_4$ concentrations varied in the ranges 0.34–6.24 μM and 1.48–123 μM, respectively (Fig. 9, 10). Decreases in nutrient concentration during the incubation were common when Chl. *a* concentration increased substantially, such as in July to September. The largest decrease in nutrient concentration in the bottle was observed in 14th August 2006. The concentrations of DIN, PO_4, and $Si(OH)_4$ decreased from 29.5 to 11.9 μM, 6.24 to 4.29 μM, and 82.3 to 80.7 μM, respectively (Fig. 8, 9, 10). Similarly, in 3rd August 2015, they decreased from 18.4 to 0.70 μM, 4.47 to 2.40 μM, and 52.0 to 35.7 μM, respectively (Fig. 8, 9, 10).

3.3 Primary production rate

The primary production rate in L-100% measured in 2014 varied in the range of 24.0–43.0 μgC/l/h and was highest, 116 μgC/l/h, on 30 July (Fig. 11). In the experiment in 2015, the primary production rate was low, 3.50 and 1.96 μgC/l/h, on 2nd and 3rd April, respectively. It increased in the range of 18.7–47.5 μgC/l/h after 27th April (Fig.

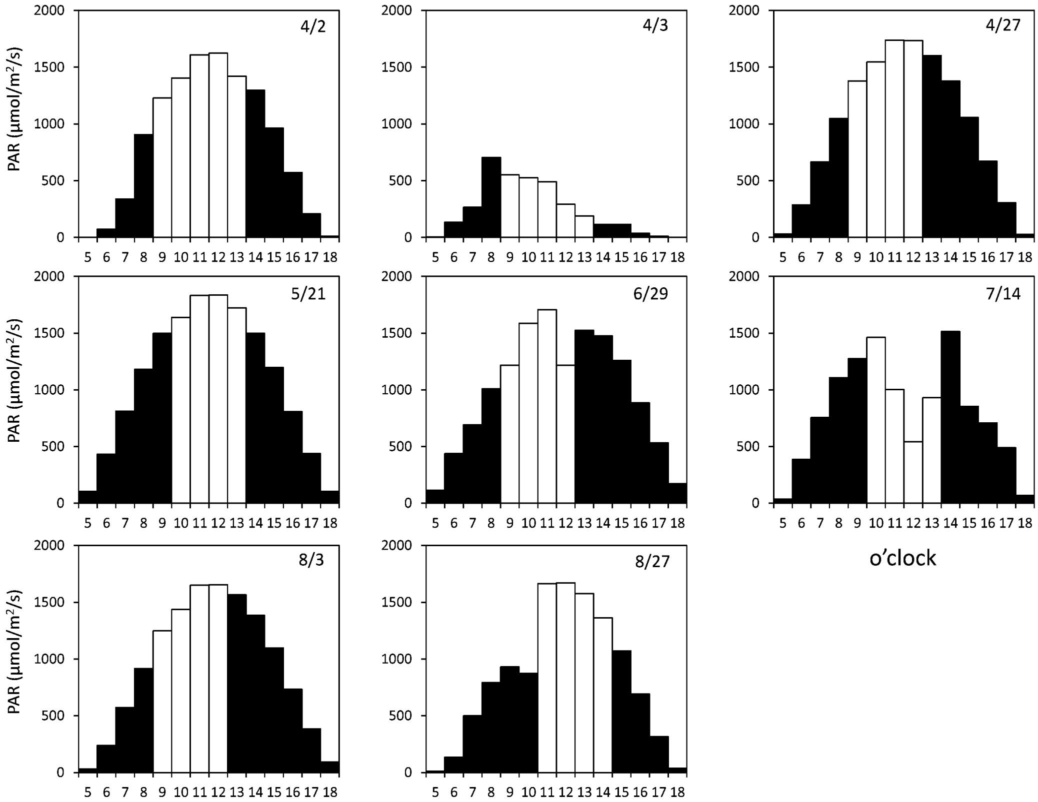

Fig. 7. Temporal variations in PAR by observation day. White shows the incubation period.

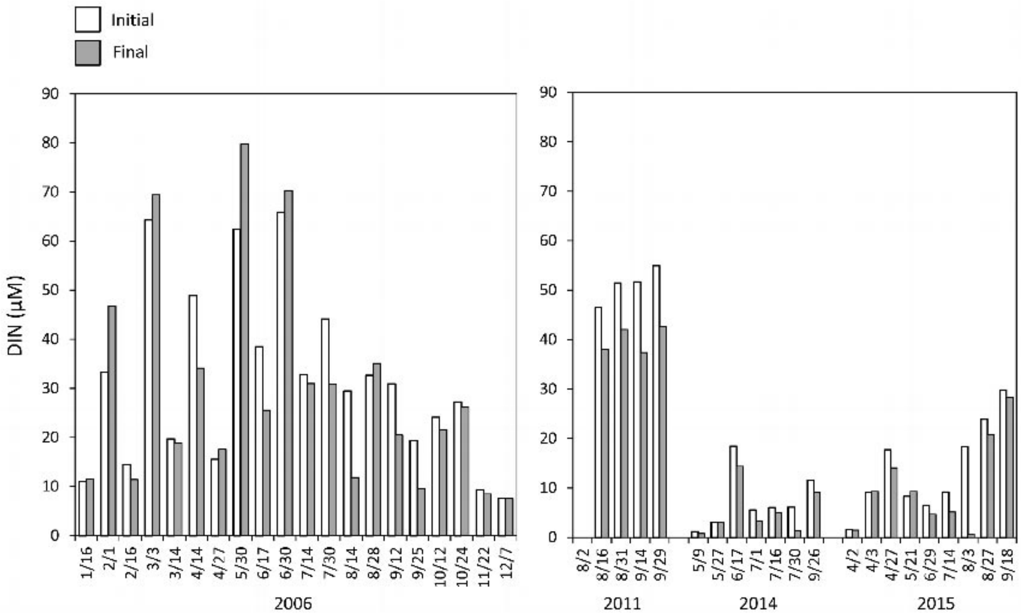

Fig. 8. Initial and final concentrations of DIN in the incubation bottles.

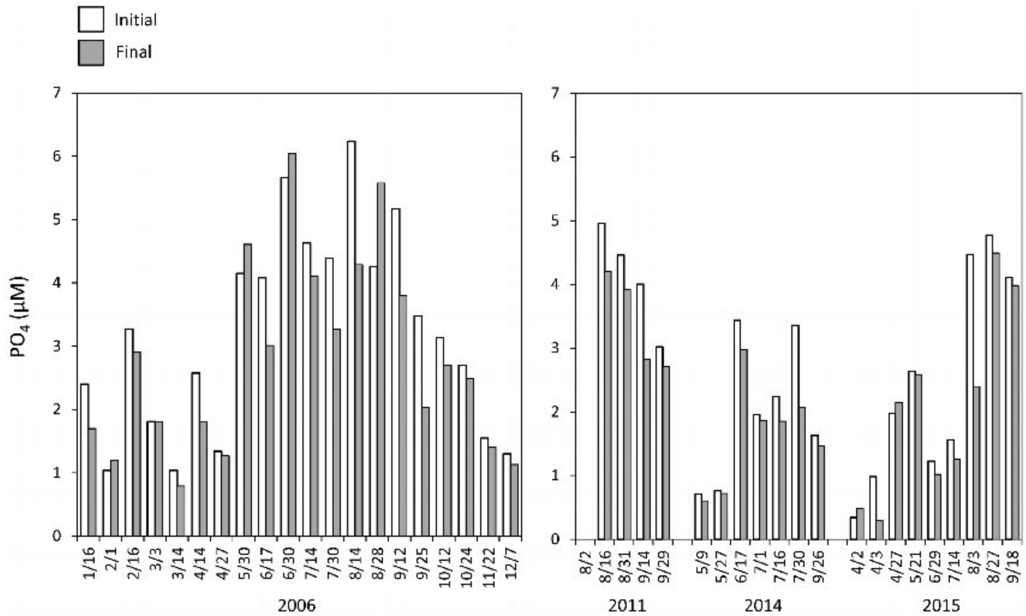

Fig. 9. Initial and final concentrations of PO_4 in the incubation bottles.

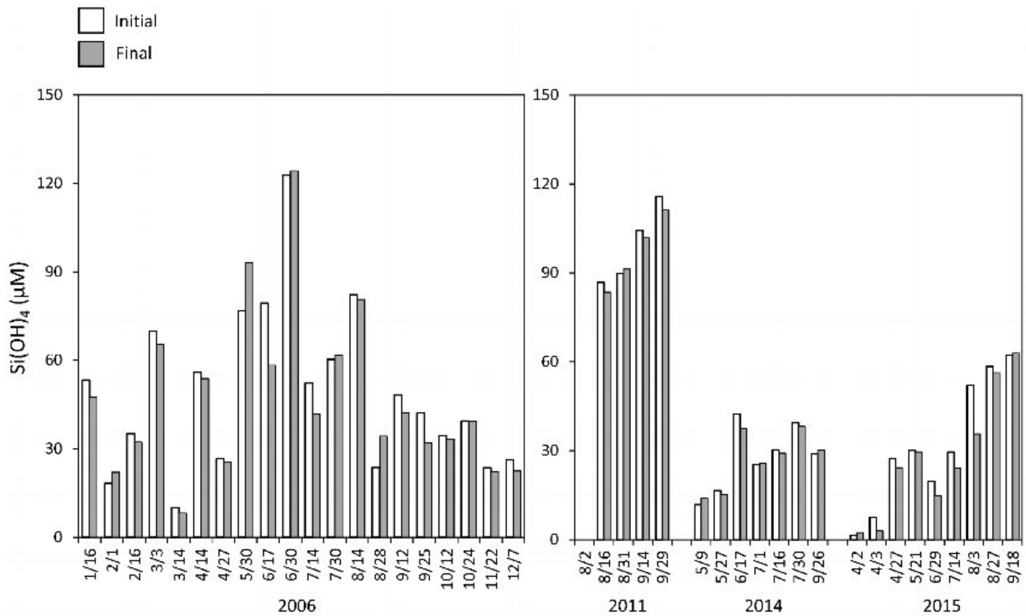

Fig. 10. Initial and final concentrations of Si $(OH)_4$ in the incubation bottles.

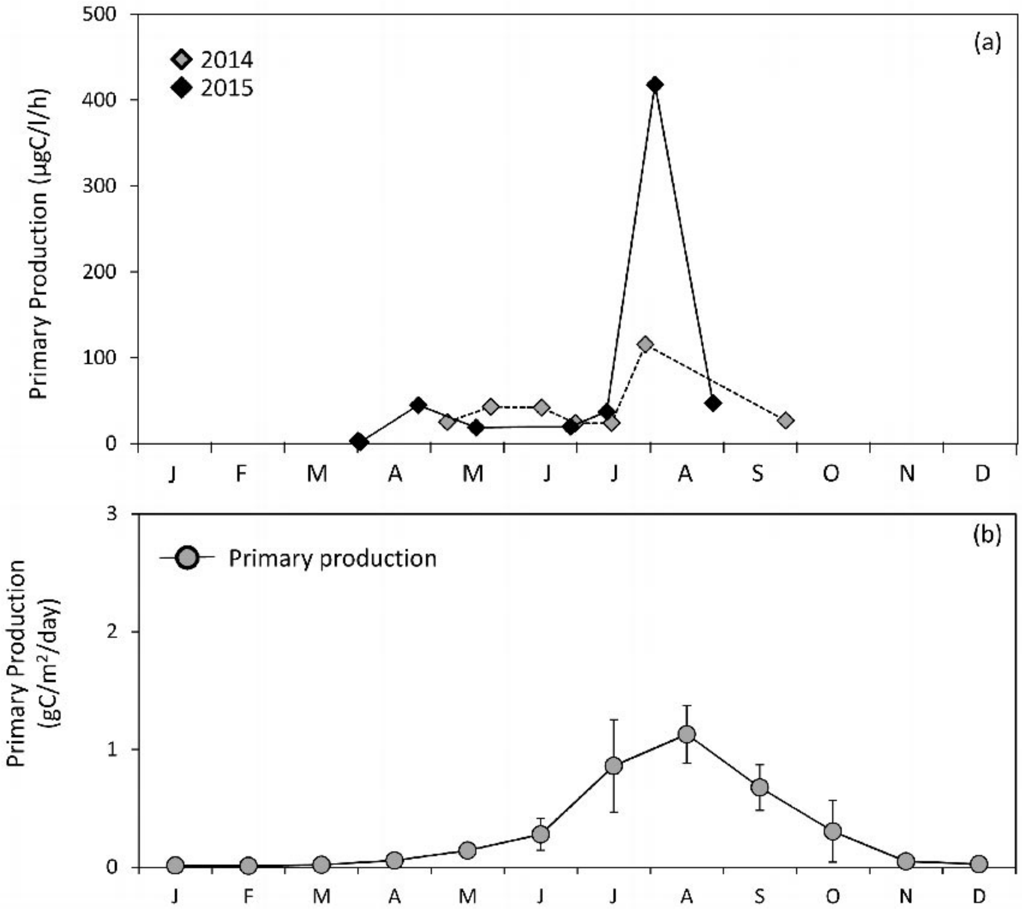

Fig. 11. Primary production measured in 2014/2015 (a), and estimated from AIC analysis (b).

11). An extremely high primary production rate, 418 μgC/l/h, was observed on 3rd August (Fig. 11).

Maximum primary production rates were observed with L-50% and/or L-100% (Fig. 6). Saturating light intensity for primary production (E_k) calculated in accordance with PLATT *et al.* (1980) was < 100 μmol/m^2/s on 3rd April and 150-700 μmol/m^2/s on other observation days, although the PI curve could not be determined on 2nd and 27th April.

4. Discussion

4.1 Effect of nutrients, water temperature, and light intensity on the increase of Chl. *a* and primary production rate

N/P and N/Si ratios in the tested seawater samples were from 1.58 to 35.5 and 0.10 to 1.95, respectively, which was smaller than the Redfield ratio (N/P = 16, N/Si ≒ 1; REDFIELD, 1963; BREZEZINSKI, 1985) (Fig. 12). In general, primary production in coastal seas is limited by low DIN concentration (HOWARTH, 1988). The N/P and N/Si ratios in the present study were relatively low; therefore, it seems the growth of phytoplankton was potentially limited by DIN concentration.

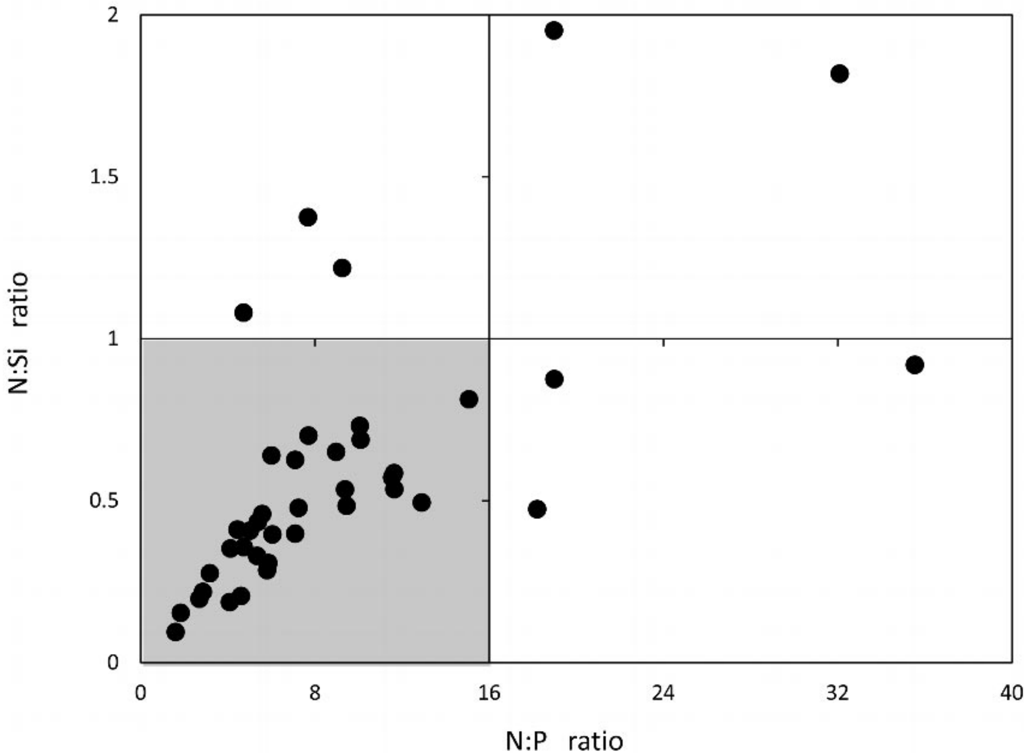

Fig. 12. Relationship to N/P and N/Si ratios calculated from the initial concentrations in the incubation bottle. Gray shaded areas show the decline of phytoplankton growth by DIN limitation.

On the other hand, it was reported that the half saturation constant of DIN concentration for phytoplankton growth in estuaries was 0.1–5.9 μM (EPPLEY *et al.*, 1969; PAASHE, 1973; CONWAY *et al.*, 1976; CONWAY and HARRISON , 1977). The salinity of the tested seawater samples was often lower than 30, which means that Shinkawa river fresh water was present in the water samples. The river water contained high nutrient concentrations (ICHIMI *et al.*, 2011), thus, the DIN concentration of the tested seawater was mostly higher, 1.13–65.8 μM, than the half saturation concentration. On the other hand, the remaining DIN was only 0.70 μM on 3rd August 2015, when the increase of Chl. *a* was very high (Fig. 6). Therefore, if phytoplankton growth is extremely high, the nutrient concentration for growth may be insufficient. Moreover, low DIN concentrations were often observed in spring (Fig. 8). YAMAGUCHI *et al.* (2015) reported recently that low DIN concentrations (< 3 μM) have been observed in spring in the Bisan Strait, which is located near the study area. Thus, primary productivity may be limited temporarily by low nutrient concentrations in this season. It will be necessary to verify this and examine potential reasons as part of future studies.

Light intensity is also an important factor affecting primary productivity. The increase rate of Chl. *a* with L–10% was apparently higher than that with L–100% (Fig. 6, 7) when solar radiation increased during sample incubation. In contrast, differences in the increase rate between L–10% and L–100% were small when solar radiation decreased or was stable during sample incubation (3rd April, 21th May, 14th July, and 27th

August). When phytoplankton was exposed to 200–1,500 μmol/m^2/s, the intracellular content of Chl. *a* decrease to half to one-third of the level with 20–100 μmol/m^2/s (STRAMASKI and MOREL, 1990; BENERAGAMA and GOTO, 2010; CHAUHAN and PATHAK, 2010; CARNEIRO *et al*., 2013). The decline in increase rate with L–100% was considered to occur because the intracellular Chl. *a* content of phytoplankton in the L–100% sample was reduced in high light conditions. Most of the incubation experiments were carried out when solar radiation was increasing gradually (Fig. 7). Therefore, it was considered that the increase rate in Chl. *a* declined at low water temperatures. A decrease in intracellular Chl. *a* content could also occur at high water temperatures. However, significant higher increase rates in Chl. *a* were observed. The positive increase rate was caused by high growth at high light intensity. Primary production rates with L–100% were clearly higher than those with L–10%; therefore, the low increase rate of Chl. *a* with L–100% does not represent low photosynthetic activity.

The primary production rate was higher in high light intensity (Fig. 6). It is well known that E_K, saturating light intensity for photosynthesis (TALLING, 1957), rises with higher water temperature. A similar trend was also observed in the present study (Fig. 6). E_K at high water temperature was 200–600 μmol/m^2/s (Tillman *et al.*, 2000; Shaw and Purdie, 2001; DOMINGUES *et al*., 2011; YAMAGUCHI *et al*., 2015). E_K observed in this study (150–700 μmol/m^2/s) was quite similar to previous reports. In general, the primary production rate in estuaries is usually limited by low light intensity, which is caused by high turbidity due to re-suspended sediment or suspended particles from rivers (CLOERN , 1987; COLE *et al*., 1992; IRIGOIEN and CASTEL , 1997). In this study area, however, about 60% of light at the sea surface reached the bottom even at high tide (HIGASHIZONO *et al*., in preparation). In addition, the primary production rates of L–50% and L–100% were similar; therefore, the primary production rate in the intertidal area was not limited by low light. The intertidal area is exposed in full sunlight, and photosynthesis is sometimes influenced by photo-inhibition, which occurs in high light conditions such as 200–800 μmol/m^2/s (STEEMAN-NIELSEN *et al*., 1962; BELAY and FOGG, 1978). However, the primary production rates obtained in this study site were not reduced even at over 1,000 μmol/m^2/s (Fig. 6). Thus, it was considered that light intensity was a minor factor to explain primary production in this area throughout the year.

Both the increased rate of Chl. *a* and primary production rate showed a good correlation with water temperature (Fig. 5). It was reported that the primary production rates in various estuaries were high when the water temperatures were high (COLIJN and de JONGE, 1984; KROMKAMP and PEENE, 1995; MORTAZAVI *et al*., 2000). High Chl. *a* concentrations were also observed in summer in this study area (HIGASHIZONO *et al*., in preparation) , thus, water temperature may be a primary factor for phytoplankton growth and primary production rate in the Shinkawa River estuary.

In order to examine in detail how primary production rates obtained in this study were affected by any environmental factors, was performed multiple regression analysis (an indicator of Akaike information criteria of [AIC; AKAIKE, 1973]) with a dependent variable. As a result, water temperature and PAR were selected as independent variables for primary production (Primary production = $6.64 \times 10^{-4} \times$ PAR + $1.99 \times 10^{-1} \times$ water temperature − 1.63, Adjusted R^2 = 0.833, AIC = 371). Judging from the results obtained in this study, primary production rate was considered to be limited in the order water temperature > light intensity > nutrients in the

Shinkawa River estuary.

In this study, however, primary production rates were not measured during autumn to winter when solar radiation was significantly reduced. Primary productivity in the season should be measured and discussed in the future.

4.2 Annual primary production rate and characteristics of the study area

The estimate from the model equation obtained from the AIC monthly primary production rate varied in the range 0.01 ± 0.00 to 1.13 ± 0.24 gC/m^2/day (Fig. 11). It was higher than 1 gC/m^2/day in August but lower than 0.10 gC/m^2/day during low temperature periods (Fig. 11). Estimated primary production rates showed a good correlation with water temperatures (Fig. 5). The annual primary production rate was calculated from monthly production rates as 125 ± 10.7 gC/m^2/year.

The primary production rate in this area varied in the range 0.83–47.5 μgC/l/h, except for > 100 μgC/l/h (Fig. 11). Monthly and annual primary production rates in this area varied in the range 0.01 ± 0.00 to 1.13 ± 0.24 gC/m^2/day, equating to 110 ± 11.8 gC/m^2/year (Fig. 11). The primary production rate in this study area was the same or somewhat higher compared with the primary production rate in the Seto Inland Sea, which varied in the range 0.41–32.1 μgC/l/h (Tada et al., 1998). Monthly and annual primary production rates in the Seto Inland Sea varied in the range 0.29–0.97 gC/m^2/day, equating to 285 gC/m^2/year (TADA *et al.*, 1998). Primary production rates in this study area were low in winter, about one-fifth, and higher in summer, 1.2-fold, compared with that in the Seto Inland Sea (TADA *et al.*, 1998). This difference was considered to be due to the difference in water temperature, with the water temperature in the Seto Inland Sea about 10℃ in winter and about 25℃ in summer, whereas, that in the present study area was lower than the 5℃ in winter and greater than 30℃ in summer (Fig. 2). Primary production rates in other brackish areas have been investigated. For example, previously reported rates have included 85 gC/m^2/year in Tagus River estuaries in Portugal (GAMEIRO *et al.*, 2011), 106 gC/m^2/year in the Douro River estuary (AZEVEDO *et al.*, 2006), 153 gC/m^2/year in the Scheldt River estuary in the Netherlands (GAZEAU *et al.*, 2005), 307 gC/m^2/year in the Delaware River estuary in the USA (PENNOCK and SHARP, 1986), and 467 gC/m^2/year in the North River estuary (BOYER *et al.*, 1993). The annual primary production rate (125 gC/m^2/year) in this study area was intermediate compared with these other estuaries. The results of the primary production rate was measured by the ^{13}C and ^{14}C methods is known to be similar (Hama et al., 1983). Therefore, it should be possible to compare the primary production rates measured using these two methods. However, the primary production rate in the Shinkawa River estuary included subtidal to offshore areas and was presumed to be larger. Primary production rate in this study was measured at a station on the tidal flats, but the annual average Chl. *a* concentration at a station located offshore (water depth 6 m, 3.6 μg/l; HIGASHIZONO *et al.*, in preparation) from this study station did not change significantly compared with that at Stn. B (5.0 μg/l; HIGASHIZONO *et al.*, in preparation). In addition, about 60% of the annual light total at the sea surface reached the bottom even at high tide at the present experimental station (HIGASHIZONO *et al.*, in preparation). Thus, although it was presumed that the primary production rate across the entire Shinkawa River estuary may be higher than in this study alone.

Assimilation number (Ass. No.) represents photosynthetic activity variation over the range 1.10–16.2 μgC/μgChl. *a*/h, except for 3rd August

2015 when the primary production rate was extremely high. The reported previously, Ass. No. in other Seto Inland Sea areas varied in the ranges 0.45–11.6 μgC/μgChl. *a*/h in Osaka Bay (YAMAGUCHI and IMAI, 1996), 0.63–12.3 μgC/μgChl. *a*/h in Dokai Bay (TADA *et al.*, 2001), 2–14 μgC/μgChl. *a*/h in Shitaba Bay, Uwa (YAMAGUCHI *et al.*, 2011), 1.0–11.0 μgC/μgChl. *a*/h in Suo-Nada (YAMAGUCHI *et al.*, 1984), and 2.5–15.0 μgC/μgChl. *a*/h in Hiroshima Bay (YAMAGUCHI *et al.*, 1994). In addition, Ass. No. varied in the ranges 1.3–10.3 μgC/μgChl. *a*/h in Chesapeake Bay (FLEMER, 1970), 1.0–8.4 μgC/μgChl. *a*/h in the Tagus River estuary in Portugal (GAMEIRO *et al.*, 2011), 4.53–20.5 μgC/μgChl. *a*/h in the Guadiana estuary in Spain (DOMINGUES *et al.*, 2011). Therefore, the photosynthetic activity in this study area was similar to that in other estuaries in Europe and America adjacent to large rivers, and to other Seto Inland Sea areas. Ass. No. is usually calculated using Chl. *a* concentration at the start of incubation. However, Ass. No. for the primary production rate was very high on 3rd August 2015, at 37.4 μgC/μgChl. *a*/h. On the other hand, Ass. No. calculated using Chl. *a* concentration at the end of incubation was 9.97 μgC/μgChl. *a*/h. This value was within the range of previous studies. This confirmed that phytoplankton communities in this study area had similar Ass. No. compared with in other areas. However, the growth rate, the increase in the rate of Chl. *a*, in phytoplankton communities that inhabit in this study area was significantly higher.

The primary production rate measured in 3rd August 2015 (418 μgC/l/h) was approximately 2-fold higher than in Chesapeake Bay (202 μgC/l/h; FLEMER, 1970) and Dokai Bay (219 μgC/l/h; TADA *et al.*, 2001) and higher than primary production rates recorded in marine waters previously. In addition, Chl. *a* concentration, for which the primary production rate measured > 200 μgC/l/h in Chesapeake Bay and Dokai Bay, was higher than about 30 μg/l (FLEMER, 1970) and 22.6 μg/l (TADA *et al.*, 2001). Chl. *a* concentration previously showed a positive correlation with primary production rate (HAMA *et al.*, 1997; TADA *et al.*, 1998); therefore, this result was reasonable. In contrast, in this study, the initial Chl. *a* concentration associated with this high primary production rate of 418 μgC/l/h, was 11.2 μg/l (Fig. 11). In addition, the initial Chl. *a* concentration in the incubation bottle in 14th August 2006, which had an estimated primary production rate of 197 μgC/l/h by equation (3), was 4.70 μg/l and not as high as the previous measurement. The sporadically extremely high primary production rate per unit volume at high water temperature (for example, 30th July 2014 and 3rd August 2015) was thought to be due to small diatoms, which have very high growth rates of about 10 d^{-1} in high temperature (30℃) and light intensity (700 μmol/m^2/s) culture conditions, are known to inhabit this study area (ICHIMI *et al.*, 2012). However, these very high primary production rates were not measures for all incidences of high temperature and light intensity. In the future, it will be necessary to consider the environmental conditions and timing of days with significantly higher primary production rates.

The annual primary production rate calculated from the monthly production rates was 125 gC/m^2/year. On the other hand, the annual primary production rate, 434 gC/m^2/year, by benthic microalgae was also measured using an oxygen method in this study area (MONTANI *et al.*, 2003). Although the annual primary production rate in this benthic habitat was higher than that in the water column (this study), the production rates in August to October were the same (Fig. 13). Major primary producers in Shinkawa River estuary could be microphytobenthos; however,

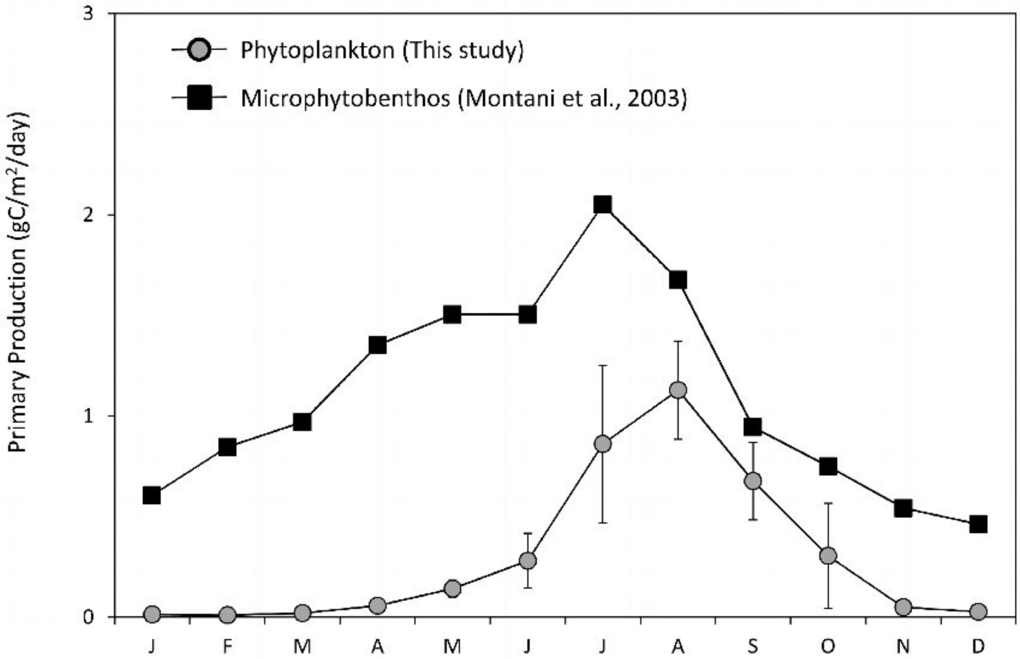

Fig. 13. Temporal variations in primary production of phytoplankton and microphytobenthos in the Shinkawa River estuary.

phytoplankton is also an important primary producer, especially in summer and autumn. Moreover, phytoplankton growing in the intertidal zone can be easily flushed out into the coastal sea during each ebb tide. It will be necessary to verify how this flow of phytoplankton is utilized by higher trophic organisms and what effect it has on coastal environments.

5. Summary

In this study, seasonal variations in the increase rate of Chl. *a* and the primary production rate were measured by *in situ* seawater incubation in a small estuary in the Seto Inland Sea, Japan. As a result, nutrients levels were sufficient throughout the year and were not a limiting factor for primary production. Moreover, about 60% of annual light exposure at the sea surface reached the bottom even at high tide at the study site, thus saturation light intensity for photosynthesis (100–700 μmol/m^2/s) reached to the bottom layer. Therefore, light intensity was also not a limiting factor for primary production. We concluded that water temperature was the major limiting factor for the primary production in this estuary. Primary production rate (mgC/m^2/d) in this study area was similar to other estuaries and coastal waters. The most significant feature related to productivity at this study site was the recording of extremely high production rates in seasons with high water temperature. It is necessary to verify how this high productivity contributes to secondary production and affects the coastal environment.

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