

# **TRANSFER EFFICIENCY FROM PRIMARY PRODUCERS TO *RUDITAPES PHILIPPINARUM* ON AN INTERTIDAL FLAT IN HIROSHIMA BAY, JAPAN**

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Fish yields of *Ruditapes philippinarum* have been decreased and the resources have not yet recovered. It needs to clarify food sources of *R. philippinarum*, and relationship between primary and secondary production of it. The purpose on this study is to reveal transfer efficiency from primary producers to *R. philippinarum* and food sources of *R. philippinarum*.

The field investigation was carried out to quantify biomass of *R. philippinarum* and primary producers on intertidal sand flat at Zigozen beach in Hiroshima Bay, Japan. In particular, photosynthetic rates of primary producers such as *Zostera marina*, *Ulva* sp. and microphytobenthos were determined in laboratory experiments. The carbon and nitrogen stable isotope ratios for *R. philippinarum* and 8 potential food sources (microphytobenthos, MPOM etc) growing in the tidal flat were also measured.

In summer 2015, the primary productions of *Z. marina*, *Ulva* sp. and microphytobenthos were estimated to be 70.4 kgC/day, 43.4 kgC/day and 2.2 kgC/day, respectively. Secondary production of *R. philippinarum* was 0.4 kgC/day. Contribution of microphytobenthos to *R. philippinarum* as food source was 56-76% on the basis of those carbon and nitrogen stable isotope ratios. Transfer efficiency from microphytobenthos to *R. philippinarum* was estimated to be 10-14%. It was suggested that microphytobenthos might sustain the high secondary production of *R. philippinarum*, though the primary production of microphytobenthos was about 1/10 compared to other algae.

Key words: *Intertidal flat, Transfer efficiency, Primary and secondary production, Ruditapes philippinarum, Stable isotope*

## **I. INTRODUCTION**

*Ruditapes philippinarum* is important fishery source in Japan. Fish yields of *R. philippinarum* have been decreased and the resources have not yet recovered in Japan. Especially, fish yields of *R.*

*philippinarum* as fishery source in Seto Inland Sea have decreased ca10,000 tons in 2010, in contrast ca 120,000 tons in 1970 [1].

There are numerous of ecology study in *R. philippinarum* such as food sources [2] and function of purification [3] and larval recruitment [4] were conducted. Furthermore, it was reported that the cause of reduction in production of *R. philippinarum* were decline of primary production [5], hypoxia impact [6], deterioration in habitat [7], increment in predation [8]. It's thought that a decrease of fish yields of *R. philippinarum* has been implicated as one of these important factors.

However, it needs for increment of fishery source to study and investigate, considering to understand ecology and environmental characteristic in habitat. Moreover, information on secondary production and their contribution to organic carbon pathways in various food webs is completely lacking. In this study, we estimated secondary production of *R. philippinarum* and provided quantitative estimates of the efficiency in channelling primary production to higher trophic levels. It needs for recovery of sources to clarify food sources of *R. philippinarum*, and relationship between primary and secondary production of it. The purpose on this study is to reveal transfer efficiency from primary producers to *R. philippinarum* and food sources of *R. philippinarum*.

## II . MATERIAL AND METHOD

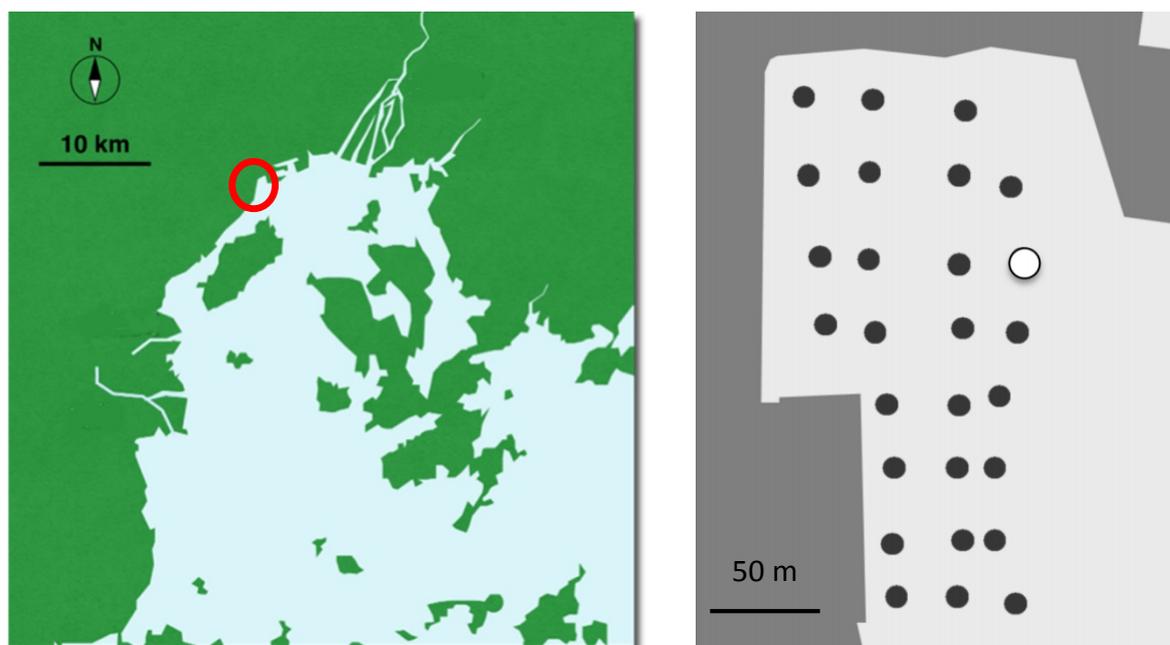
### *Investigation area*

Zigozen beach is located in the Hiroshima Bay, in west Japan (Fig. 1) and fore-shore type sandy tidal flat. Our field investigation was carried out on a sand tidal flat which is about 44,000 m<sup>2</sup>, there are biodiversity such as *Zostera marina*, *Zostera japonica*, *Ulva* sp., and *R. philippinarum*.

To clarify the primary and secondary production in the entire tidal flat, we conducted investigation about monthly fluctuation and distribution. Study of distribution was performed at 27 stations in the entire tidal flat on May 16, 2015 (Fig. 1). Biomass investigation of macroalgae and *R. philippinarum* were carried out and distribution area were measured using GPS.

### *Biomass and secondary production of R. philippinarum*

Quadrat (50×50×10 cm) samples of *R. philippinarum* were collected to examine the abundance using 2 mm mesh sieve at 4 stations during the period from July 2014 to October 2015. Monthly investigation was carried out in white circle station in Fig.1. Investigation of distribution of *R. philippinarum* was carried out in 27 black circle stations in Fig.1 including above station.



*Fig.1. Investigation station*

The shell length of *R. philippinarum* was measured using calipers. The Shell length-frequency data from *R. philippinarum* were analyzed. Cohort analysis was performed with the frequency distribution of shell length for secondary production.

*Estimation of primary production of Microphytobenthos*

Chl. *a* as microphytobenthos biomass in surface sediments which top 0-0.5 cm samples were collected by using an acrylic core tube (30 mm in diameter) and measured. Microphytobenthos were *in situ* surface sediments collect by exploiting their phototactic movement [9] during one day.

Primary production of microphytobenthos was measured by the  $^{13}\text{C}$  tracer method [10]. In the laboratory, microphytobenthos was suspended in filtered seawater and dispensed in polycarbonate bottle (500 mL).

After the addition of  $\text{NaH}^{13}\text{CO}_3$  (about 10% of dissolved inorganic carbon), the samples were incubated for about 4 hours in a tank. The incubations were conducted at *in situ* temperature with running surface seawater under the corresponding light intensity, which was regulated by density filters. The light intensity inside the tank was about  $200 \mu\text{mol}/\text{m}^2/\text{sec}$ .

All the samples were filtered through precombusted 47 mm Whatman GF/F filter ( $450^\circ\text{C}$  for 4 h), after incubation. The filters were treated with 1N HCl to remove inorganic carbon and washed by 3% NaCl. The filters were dried at  $60^\circ\text{C}$  and stored until isotope analysis.

Particle organic carbon concentration was analyzed by the elemental analyzer (Flash EA1112: Thermo Fisher Scientific), the  $^{13}\text{C}/^{12}\text{C}$  isotope ratios in the filter samples were analyzed by Stable isotope ratio mass spectrometer (DELTAplus Advantage: Thermo Fisher Scientific).

It was calculated photosynthetic activity ( $\mu\text{gC}/\mu\text{gChl.a}/\text{hour}$ ) using primary production in the bottle ( $\mu\text{gC}/\text{L}/\text{hour}$ ) and Chl.*a* concentration ( $\mu\text{g}/\text{L}$ ). Furthermore, primary production ( $\text{mgC}/\text{m}^2/\text{day}$ ) was calculated by Chl.*a* ( $\mu\text{gChl.a}/\text{m}^2$ ) in the sediment and sediment surface photic layer (Ca. 1.7mm) which estimated from particle size composition of the sediment [11].

*Estimation of primary production of Macroalgae*

Quadrat (30×30 cm) samples of macroalgae such as *Zostera* spp. and *Ulva* sp. were collected to examine the abundance at 3 each stations during the period from May 2015. In the laboratory, dry weight and carbon content of macroalgae were measured.

After filtering the seawater in the laboratory, the filtered seawater was sterilized using an autoclave (HIRAYAMA, HV-35LB). *Zostera marina* and *Ulva* sp. were cut a portion at a certain size to detect photosynthetic activity. The portion samples were incubated for about 2-4 hours incubator (NK system, LP-130P). The incubations were conducted at in situ temperature with running under light and dark condition. The light intensity was about 200  $\mu\text{mol}/\text{m}^2/\text{sec}$ . The dissolved oxygen concentration was measured before and after experiment (HACK, HQ40d). Primary production was calculated which photosynthesis quotient as 1.

#### *Estimation of food resource of R. philippinarum*

We collected *R. philippinarum* and potential 8 food sources such as microphytobenthos (MPB), surface sediments (SOM), particulate organic matter of river origin (RPOM) and that of marine water (MPOM), *Z. marina*, *Z. japonica*, *Ulva* sp. and attached matter on *Z. marina* for measuring carbon and nitrogen stable isotope ratios (DELTAplus Advantage: Thermo Fisher Scientific).

SIAR 4.0 [12] was used to estimate the contribution of each potential primary producer source to *R. philippinarum*.

### III. RESULT AND DISCUSSION

#### *Secondary production of R. philippinarum*

Figure 2 showed fluctuation of growth of *R. philippinarum* using cohort analysis. Two or three cohorts were detected during the sampling period. Density of *R. philippinarum* fluctuated 35-465 ind./ $\text{m}^2$  from August 2014 to October 2015. Figure 3 showed fluctuation of secondary production. It was estimated that secondary production was 4.3-37  $\text{mgC}/\text{m}^2/\text{day}$ . Secondary production was high value in early spring and summer.

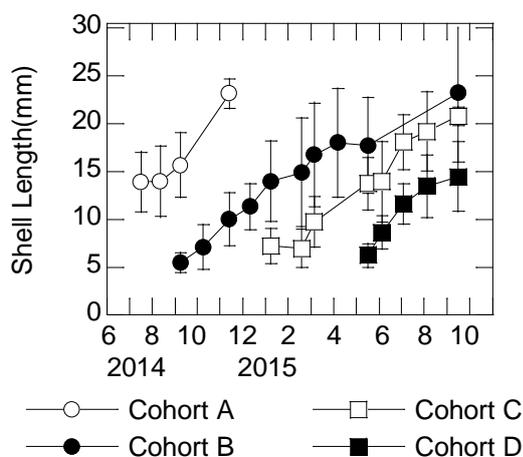


Fig.2. Fluctuation of growth of *R. philippinarum*

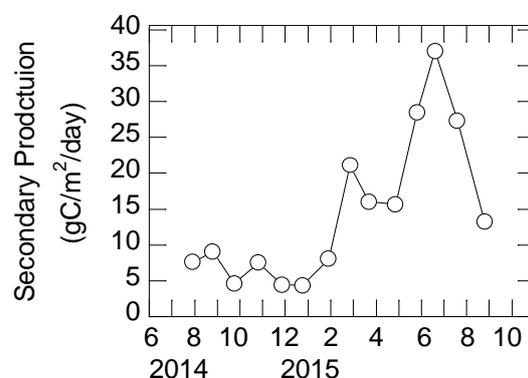


Fig.3. Fluctuation of secondary production

*Primary production of algae*

Figure 4 shows fluctuation of photosynthetic activity and primary production. Photosynthetic activity of *Ulva* sp. was 30.0-76.5 mgC/dry g/day, photosynthetic activity of *Z. marina* was 14.3-40.6 mgC/dry g/day, photosynthetic activity of microphytobenthos was 4.48-22.1 mgC/mgChl.a/day. Though units of photosynthetic activity of microphytobenthos is different from the other macroalgae, photosynthetic activity fluctuation of the other macroalgae was greater than microphytobenthos. Photosynthetic activity of *Ulva* sp. was tendency higher than that of *Z. marina*. Photosynthetic activity of *Z. marina* did not clearly fluctuate.

Primary production of *Ulva* sp. was 3.29-21.1 gC/m<sup>2</sup>/day, primary production of *Z. marina* was 2.20-28.0 gC/m<sup>2</sup>/day, primary production of microphytobenthos was 0.074-0.240 gC/m<sup>2</sup>/day. For macroalgae, primary production of *Ulva* sp. increased in spring, primary production of *Z. marina* tended to have increased in summer. Primary production of microphytobenthos showed a maximum value in early summer, but primary production of microphytobenthos was smaller than that of the other macroalgae. Photosynthetic activity and primary production of microphytobenthos showed similar

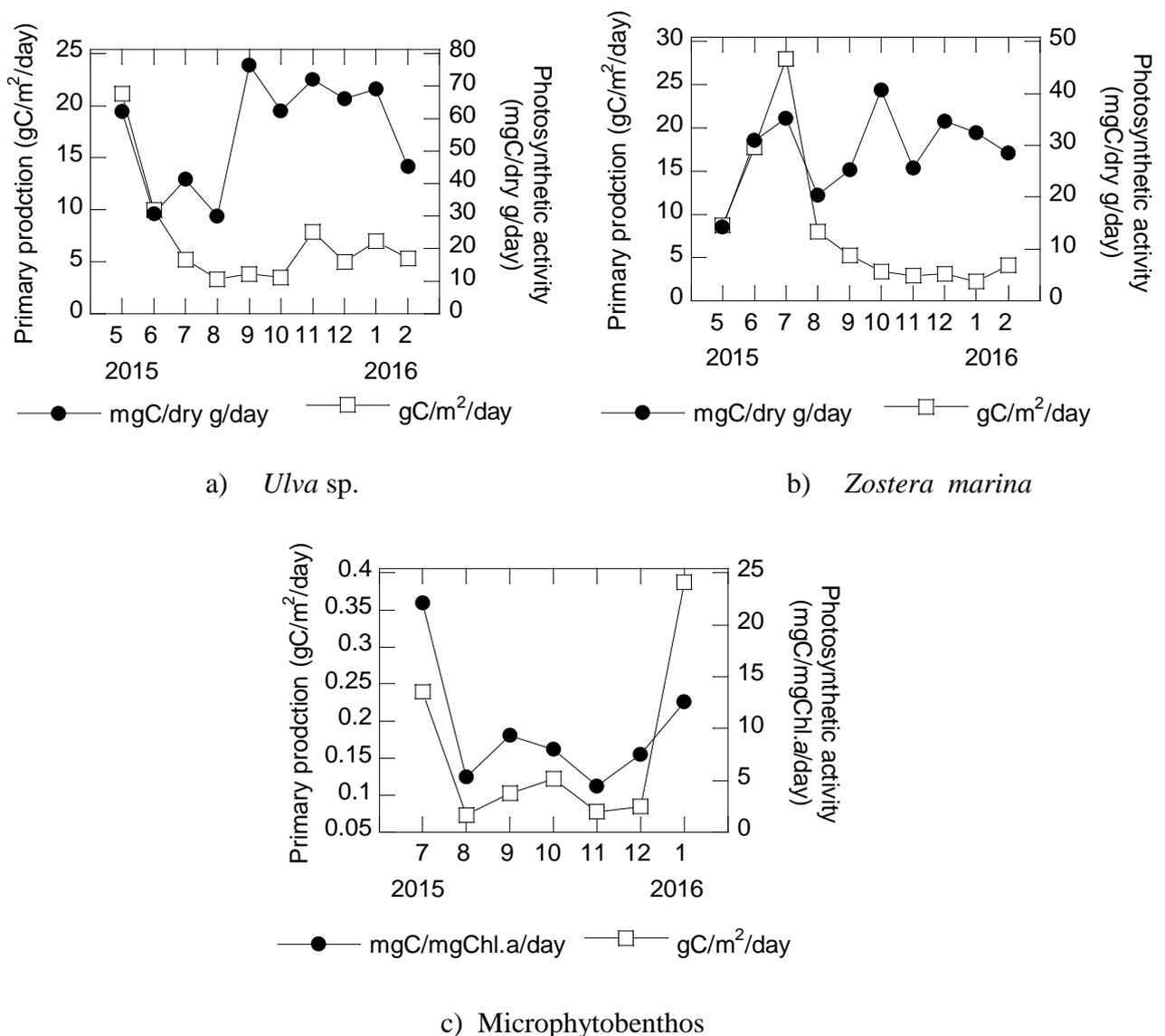


Fig. 4. Fluctuation of primary production and photosynthetic activity

fluctuation.

### Carbon and nitrogen stable isotope ratios

Figure 5 shows the carbon and nitrogen stable isotope ratios for *R. philippinarum* and 8 potential food sources. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *R. philippinarum* were -18.7‰ to -15.1‰ and 10.4‰ to 14.4‰, respectively.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of Macroalgae such as *Z. marina*, *Z. japonica* and *Ulva* sp. were not plotted around *R. philippinarum*, it was difficult to think that macroalgae were not food source of *R. philippinarum*. On the other hand, microphytobenthos, MPOM, RPOM, SOM and attached matter on *Z. marina* were plotted around *R. philippinarum*. Therefore, it was conducted to calculate that contribution rate as food source of *R. philippinarum* using those 5 potential food sources.

Figure 6 shows the contribution of 5 food resource for *R. philippinarum*. The plot in Fig 6 indicated range of 95% confidence interval. Contribution of microphytobenthos to *R. philippinarum* as food source was 56-76%, *R. philippinarum* mainly utilizes microphytobenthos as food source, contribution of MPOM (phytoplankton) and RPOM was less than 10%. Contribution of microphytobenthos to *R. philippinarum* was 35-64% in Hichirippu Lagoon bordering the Pacific Ocean in Hokkaido, Japan [13], it was high contribution of microphytobenthos to *R. philippinarum* as food source in this study.

Table 1. Primary and secondary production per tidal flat

Species	Area [ $\times 10^3 \text{ m}^2$ ]	Biomass [gDW/m <sup>2</sup> ]	Production	
			[gC/m <sup>2</sup> /day]	[kgC/day]
<i>R. philippinarum</i> □	44	2.0	0.010	0.40
<i>Zostera</i> spp.	8	465.1	9.5	76.2
<i>Ulva</i> sp.	13	95.0	2.9	37.4
Microphytobenthos	29	34.9*	0.14	2.2

\*[mgChl./m<sup>2</sup>]

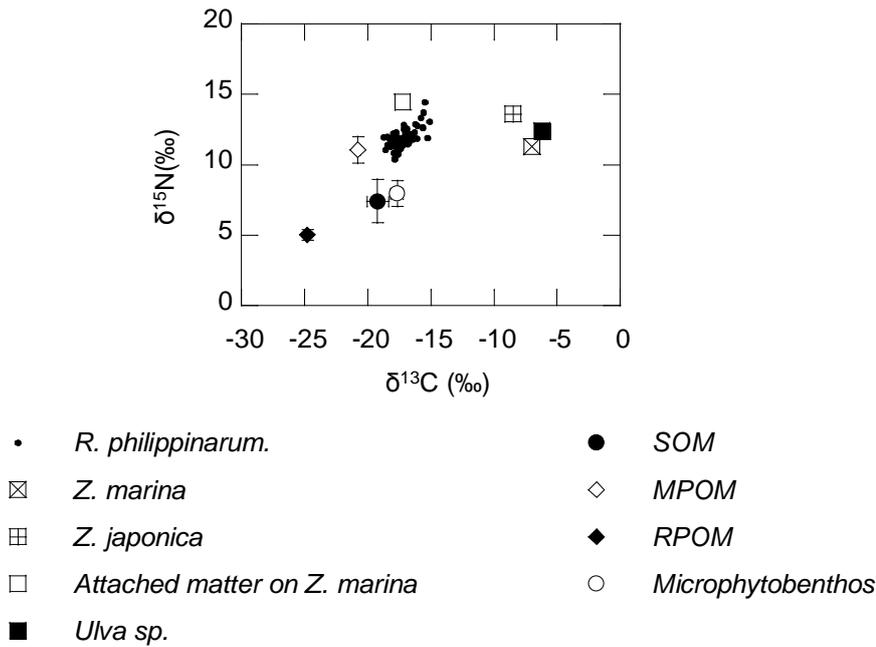


Fig.5. The carbon and nitrogen stable isotope ratios for *R. philippinarum* and 8 potential food sources

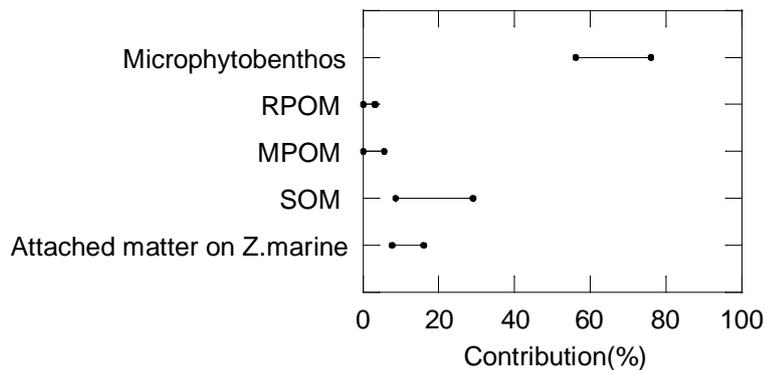


Fig.6. The contribution of 5 food source for *R. philippinarum*

#### Transfer efficiency

Table 1 shows primary and secondary production per tidal flat in summer 2015 from July to September. Area of *R. philippinarum*, *Zostera* spp., *Ulva* sp. and microphytobenthos were  $44 \times 10^3 \text{ m}^2$ ,  $8 \times 10^3 \text{ m}^2$ ,  $13 \times 10^3 \text{ m}^2$  and  $29 \times 10^3 \text{ m}^2$ , respectively. The primary productions of *Z. marina*, *Ulva* sp. and microphytobenthos were estimated to be 70.4 kgC/day, 43.4 kgC/day and 2.2 kgC/day, respectively, considering of tidal flat area. Secondary production of *R. philippinarum* was 0.40 kgC/day. Contribution of microphytobenthos to *R. philippinarum* as food source was 56-76% on the basis of those carbon and nitrogen stable isotope ratios (Fig 6). Therefore, transfer efficiency from microphytobenthos to *R. philippinarum* was estimated to be 10-14%. It might efficiently transferred from primary production to secondary production in summer, because secondary production in summer was 5-6 times higher than that in winter, and highest through year (Fig.3).

Montani *et al.* [14] reported transfer efficiency from microphytobenthos to *R. philippinarum* was estimated to be 16% on Shin and Kasuga River tidal flat located in Seto Inland Sea, transfer efficiency in this study was substantially the same value. It thought that transfer efficiency in Montani *et al.* [14] was overestimated, since contribution of food resource has not been considered.

It was suggested that microphytobenthos might sustain the high secondary production of *R. philippinarum*, though the primary production of microphytobenthos was about 1/10 compared to other algae.

#### IV. CONCLUSION

- The primary productions of *Z. marina*, *Ulva* sp. and microphytobenthos were estimated to be 70.4 kgC/day, 43.4 kgC/day and 2.2 kgC/day, respectively.
- Contribution of microphytobenthos to *R. philippinarum* as food source was 56-76%, *R. philippinarum* mainly utilizes microphytobenthos as food source.
- Transfer efficiency from microphytobenthos to *R. philippinarum* was estimated to be 10-14%, microphytobenthos might sustain the high secondary production of *R. philippinarum*.

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