The role of crabs (*Macrophthalmus japonicus*) burrows on organic carbon cycle in estuarine tidal flat, Japan

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**ABSTRACT**

The objective of this study is to elucidate the burrow structure and to clarify the role of burrows in material cycle in the tidal flat. In our work, we focused on the dominant species in muddy tidal flat, crab *Macrophthalmus japonicus*.

Burrow structure of *Macrophthalmus japonicus* was investigated on a Katsuura river tidal flat in Tokushima prefecture, Japan, using in situ resin casting. Sampling was conducted in August 2006, and a total of 48 burrow casts were obtained. Burrows consisted mainly of J-shaped structures (98%) while the rest belonged to U-shaped structures (2%). The maximum measured burrow volume was 120 cm³ and wall surface area was 224 cm², while maximum burrow length and depth were 23.2 cm and 16.5 cm, respectively. Burrow volume and surface area were strongly correlated with carapace width of *M. japonicus*.

Investigation of the individual number of *M. japonicus* in 13 quadrats (50 × 50 × 20 cm) was conducted using 2 mm sieve. The number of *M. japonicus* was 15–31 ind./m². Using cohort analysis we estimated that surface area of burrows was 0.07–0.15 m²/m².

CO₂ emission rate was measured at the surface sediment during the period from June to December 2008. Results varied from 13.8 ± 2.2 to 49.4 ± 3.2 mg CO₂/m²/h, and organic carbon decomposition was 3.8 ± 0.6–13.5 ± 0.9 mg C/m²/h. This leads the increase of organic carbon decomposition by 1.1 times, because of the expansion of the tidal flat surface area by burrowing activity. Organic carbon decomposition in burrow walls therefore contributed to organic matter decomposition in the tidal flat. These results indicated that *in situ* activities of *Macrophthalmus japonicus* significantly influence the material cycle and it is important to consider the existence of burrow in order to understand the fluxes of materials and to evaluate the purification function of the tidal flat.

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1. Introduction

Intertidal areas of estuaries are productive and dynamic systems with extreme and variable environmental conditions (Heip et al., 1995). They are functionally connected to adjoining coastal waters via advective transfers of water and dissolved, particulate material, and ecological food chain of macrobenthos. These interactions are fundamental to understand the roles that estuaries play in the functioning of coastal waters. Especially, organic matter is a major component of estuarine sediments, one of the most important food sources and organic matter decomposition occurs mainly in sediments (Nedwell et al., 1999). Moreover, rates and pathways of carbon flux have been measured infrequently in tropical coastal sediments (Taylor and Allanson, 1995; Middelburg et al., 1996; Alongi et al., 1999).

Macrobenthos are common in tidal flat and play an important role in purification functions. Organic matter decomposition in sediments may be mediated by the activity of macrobenthos (Herman et al., 1999). They can affect sediments chemistry by bioturbation such as burrow activity, tube-building, ingestion, defecation, respiration and mucus secretion (e.g. Kostka et al., 2002). Therefore, the influence of bioturbation on material cycle in tidal flat caused by their living activities cannot be ignored. The purification functions of Nereidae and Bivalvia, which are the macrobenthos have been intensively studied (e.g. Jordan and...
It is well known that some crabs reduce carbon and nutrient exports from mangrove forests by actively burying litter within their burrows (Robertson, 1986; Twilley et al., 1997), in addition, it was reported that mangrove crabs are ecosystem engineers (Kristensen, 2008). However, Ocypodids have been hardly detail studied regarding the material cycle in tidal flat, although the species is abundant.

We focused on the crab *Macrophthalmus japonicus*, which is distributed widely and dominantly in Japan (Kitaura et al., 2002). The crab, relatively large (up to 35 mm carapace width) and one of the most abundant macrobenthos, is also a burrowing species that inhabit muddy tidal flats. In previous studies, it has been observed that reproduction (Henmi and Kaneto, 1992; Henmi, 1992b), life-history (Henmi, 1989, 1992c), wandering (Henmi, 1992a) of *M. japonicus*. Although these biologies have been revealed, crab bioturbation is expected to affect material cycle in tidal flat. We speculated that the burrowing activities play an important role in the purification function in the tidal flat. Furthermore, indirect effects of bioturbation such as burrowing activity should be considered to evaluate purification in tidal flat, as well as direct effects of production and consumption of macrobenthos.

The objectives of this study are to elucidate the burrow structure and to clarify the role of burrows in material cycle in the tidal flat.

2. Material and methods

Katsuura River (34°02′N, 134°34′E) is located in the eastern part of Shikoku Island, in west Japan (Fig. 1). Our field investigation was carried out on a muddy tidal flat which is about 0.5 ha of whole 9 ha, from the river mouth to about 2 km upstream. The muddy tidal flat lies the reed, *Phragmites australis*, grows thickly around it. The sediment of the muddy tidal flat consists of mud containing about 50% silty clay.

Quadrat (50×50×20 cm) samples of *Macrophthalmus japonicus* were collected to examine the abundance using 2 mm mesh sieve at 13 stations during the period from June to December 2008. The carapace width of *M. japonicus* was measured using calipers.
The carapace width–frequency data from *M. japonicus* were analyzed using PROGEAN VER.4.0J (Tsutsumi and Tanaka, 1994). Cohort analysis was performed with the frequency distribution of carapace width for secondary production. Sediment samples down to 4 cm were collected by using an acrylic core tube (15 mm in diameter) which was measured top 5 mm surface sediment Chl. a at 6 stations. Chl. a was measured as Whitney and Darley (1979), and primary production of *Nitzschia* sp. was estimated by Montani et al. (2003).

We investigated burrows of *Macrophthalmus japonicus* to elucidate its burrow structure. Burrow casts were obtained in August 2006, using polyester resin (Eporak G-226P and G-226PS, Nihon Shokubai) as Kinoshita (2002). Liquid resin was poured in frames that covered several burrows. About 24 h later, resin casts were pulled out and recovered. In the laboratory, burrows' depth and length were measured directly resin casts using measure. Surface area was calculated by Scion Image (Scion Corp.) covered aluminum foil with resin casts. Volume was detected water volume spilled out from container into resin casts.

Two ways of experiments were performed to quantify respiration rate of *Macrophthalmus japonicus* in ebb and flood tide. We demonstrated that oxygen consumption of *M. japonicus* in the air (O2UP TESTER, TAITEC). This equipment measured only oxygen consumption, with sodium hydroxide absorbed CO2 which *M. japonicus* respired. Two experiments were conducted on same individuals and under the condition, with sodium hydroxide and without it. Hence, it was measured that only oxygen consumption with sodium hydroxide, and oxygen consumption plus carbon dioxide without sodium hydroxide in order to estimate carbon dioxide from two experiments. The experiment was conducted under the 20°C (spring), 30°C (summer), 20°C (fall), 10°C (winter) in air temperature. After 24 h starvation period in the laboratory, crabs were acclimated to the equipment for 2 h.

We demonstrated that oxygen consumption of *Macrophthalmus japonicus* in the water. After 24 h starvation period in the laboratory, crabs were acclimated to the flasks (370 mL) in the seawater for 2 h to reduce stress, and afterwards a water sample was exchanged to

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**Fig. 3.** Relationships of carapace width with burrow measurements.

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**Fig. 4.** Results of cohort analysis.
saturated sterilization filtration seawater to determine the initial concentration of oxygen (DO21-P, TOA-DDK). Later, flasks were sealed for 1 h, after measuring final concentrations. One flask without a crab for each treatment was added as a control. The experiment was conducted under 30, 20, 10 °C in water temperature.

We conducted measurements of CO2 emission rate from the surface sediment in the field during the ebb tide to examine the organic matter decomposition rates. In situ CO2 emission rate from the surface sediment was measured using CO2 sensor (Telaire 7001, Onset) placed in an acrylic chamber (9 cm, height: 20 cm) from June to December 2008. We carefully detected that 5 min measuring Onset) placed in an acrylic chamber (9 cm, height: 20 cm) from June to December 2008. We carefully detected that 5 min measuring CO2 emission rate after the value was stable condition. Because June to December 2008. We carefully detected that 5 min measuring CO2 emission rate after the value was stable condition. Because

3. Result

3.1. Burrow structure

A total of 48 burrow casts were obtained. Structurally, the burrows consisted of J shape type and U shape type. 98% of the burrows were J type and 2% of the burrows were U type (Fig. 2). The relationship between carapace width and various burrow parameters is shown in Fig. 3. Burrow surface area ranged from 8.4 to 224.1 cm² and burrow volume ranged from 1.5 to 119 cm³, burrow depth ranged from 3.3 to 16.5, burrow length ranged from 5.2 to 23.2 cm, ranging from 5.05 to 30.50 mm of carapace width. Formulas to estimate burrow parameters from carapace width (CW, mm) as follows: (1) surface area (cm²) = 0.505CW², R² = 0.88; (2) volume (cm³) = 0.024CW², R² = 0.90; (3) depth (cm) = 1.063CW⁰.⁶₂, R² = 0.44; (4) length (cm) = 2.274CW⁰.⁶¹, R² = 0.41. In the case of carapace was 25 mm, for instance, it was estimated that surface area was 136.7 cm² and volume was 54.4 cm³.

A similar result was obtained that average burrow depth was 10–15 cm and the burrow casts almost consisted of J shape type using plaster (Utashiro, 1966). In addition, it was revealed that there was significant relationship between carapace width and various burrow parameters.

3.2. Primary production and secondary production

Chl. a as microphytobenthos biomass in surface sediments (0–0.5 cm) fluctuated 3.0–12.6 mg Chl. a/m² and primary production was estimated 1.6–9.1 mg C/m²/h in this study. Montani et al. (2003) and Colijn and de Jonge (1984) reported that primary production was 33–215 (average: 89.4) and 1–120 (average: 37.0) mg C/m²/h, respectively, which are higher than in our study.

Fig. 4 indicates results of cohort analysis. Three cohorts were detected during the sampling period. The smallest cohort (cohort 3), which recruited in August 2008 at a mean size of about 4.4 mm, grew to about 6.1 mm by December. The middle cohort (cohort 2) grew to 5.6 mm in 6 months. No apparent growth of cohort 1 was observed. Density fluctuated 15–31 ind./m², and cohort 2 was high in population. It was estimated that secondary production was 0–1.43 mg C/m²/h.

3.3. Respiration rate

3.3.1. In the air

Fig. 5 indicates oxygen consumption of Macrophthalmus japonicus in the air. Oxygen consumption of M. japonicus increased as the temperature rose. Oxygen consumption in 30 °C and 10 °C was 0.36 and 0.11 mL O₂/h (Table 1), respectively, and oxygen consumption of M. japonicus at 30 °C was 3.3 times higher than that at 10 °C. This is because M. japonicus became more active with the temperature rise in the daytime. A significant correlation was recognized between oxygen consumption and carapace width of M. japonicus in all season. Formulas for relationships of carapace width (CW, mm) with oxygen consumption (OC, mL O₂/h) of M. japonicus in the air as follows: (spring, 20 °C) OC = 0.0042CW¹.¹⁷, R² = 0.24; (summer, 30 °C) OC = 0.022CW¹.⁶⁰, R² = 0.75; (fall, 20 °C) OC = 0.018CW⁰.⁷⁷, R² = 0.15; (winter, 10 °C) OC = 0.0060CW¹.⁶⁴, R² = 0.31.

3.3.2. In the water

Fig. 6 indicates oxygen consumption of Macrophthalmus japonicus in the water. Oxygen consumption of M. japonicus increased as the temperature rose as in the air. Oxygen consumption in 30 °C and

![Fig. 5. Oxygen consumption of M. japonicus in the air.](image)

![Fig. 6. Oxygen consumption of M. japonicus in the water.](image)

### Table 1

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Oxygen consumption (mL O₂/h)</th>
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<tbody>
<tr>
<td></td>
<td>In the air</td>
</tr>
<tr>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td>20</td>
<td>0.18</td>
</tr>
<tr>
<td>30</td>
<td>0.36</td>
</tr>
<tr>
<td>Ono (1965)</td>
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### Table 2

<table>
<thead>
<tr>
<th>Season</th>
<th>Temperature (°C)</th>
<th>Respiration quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>20</td>
<td>0.788</td>
</tr>
<tr>
<td>Summer</td>
<td>30</td>
<td>0.741</td>
</tr>
<tr>
<td>Fall</td>
<td>20</td>
<td>0.781</td>
</tr>
<tr>
<td>Winter</td>
<td>10</td>
<td>0.776</td>
</tr>
</tbody>
</table>
10 °C is 0.42 and 0.09 mL O₂/h (Table 1), respectively, and oxygen consumption of M. japonicus at 30 °C was 4.7 times higher than that at 10 °C. The values were similar to that of in the water and air; oxygen consumption in 30 °C was similar with Ono (1965) (Table 1). It was reported that oxygen consumption of crab was different in the water and air (Teal, 1959; O’Mahoney and Full, 1984), but there was no difference in oxygen consumption of M. japonicus in water and air in this study. Formulas for relationships of carapace width (CW, mm) with oxygen consumption (OC, mL O₂/h) of M. japonicus in the water as follows: (spring and fall, 20 °C) OC = 0.026CW^{0.75}, R² = 0.65; (summer, 30 °C) OC = 0.028CW^{0.95}, R² = 0.69; (winter, 10 °C) OC = 0.0082CW^{0.84}, R² = 0.52.

3.3.3. Respiration quotient

Respiration of Macrophthalmus japonicus was also detected in the air, and respiration quotient was estimated from oxygen consumption and CO₂ emission each seasons. Table 2 indicates relationship air, and respiration quotient was estimated from oxygen consumption of M. japonicus (December–February). Respiration rate in the population of summer (June–August), fall (September–November), winter (December–February). Fig. 7 indicates CO₂ emission rates in organic carbon cycle. Organic carbon cycle consists of organic carbon decomposition by bacteria in sediment, the fixation of the organic carbon of secondary production of macrobenthos and primary production of microphytobenthos. In addition, organic carbon decomposition in burrow wall. We defined organic carbon cycle as follows.

\[ Y = Sc + Bc + Mc - Mp - Pp \]

Y: organic carbon cycle (g C/m²/month), Sc: organic carbon decomposition rates in sediment (g C/m²/month), Bc: organic carbon decomposition rates in burrow (g C/m²/month), Mc: respiration rate (g C/m²/month), Mp: secondary production (g C/m²/month), Pp: primary production (g C/m²/month).

Since a significant difference was not recognized statistically oxygen consumption between organic carbon of sediment of surface (0 cm) and burrow wall (~10 cm) (unpublished data), we assumed that was similar to organic carbon decomposition rates in sediment surface and burrow wall. Moreover, the fixation of the organic carbon was assumed to be 0, when the secondary production of Macrophthalmus japonicus indicated a negative value.

Here, it was thought that the organic carbon was not decomposed in ebb tide, because there was little dissolved oxygen in water almost remained in the burrow (Koike and Mukai, 1983; Wolfrath, 1992, unpublished data), and actually area in the burrow wall up to approximately 5 cm in depth was exposed to atmosphere. Therefore, we estimated burrow depth from carapace width of Macrophthalmus japonicus and calculated organic carbon decomposition in depth 5 cm in ebb tide. As a result, the surface area to depth 5 cm for the surface area to all depth was 82–98% of the surface area in the burrow. On the contrary, the dissolved oxygen in burrow was recovered when a tidal flat surface was covered with water, it was assumed that organic carbon decomposition by the whole burrow was performed.

Table 3 indicates organic carbon cycle. Organic carbon cycle in July 2008 indicated, for example, organic carbon decomposition rate in sediment was 7.10 g C/m²/month and organic carbon decomposition rate in burrow was 0.68 g C/m²/month. In addition,
respiration rate was 2.69 g C/m²/month. The amount of release of organic carbon was 10.47 g C/m²/month. On the other hand, secondary production was 1.03 g C/m²/month and primary production was 5.21 g C/m²/month. It means that organic carbon accumulation was 6.24 g C/m²/month. In fact, organic carbon was totally released by 4.22 g C/m²/month in this month.

A significant statistical correlation was recognized between sediments temperature and organic carbon decomposition rate in sediment ($P < 0.01$, $r = 0.90$, $n = 7$) and in burrows ($P < 0.01$, $r = 0.93$, $n = 7$). It was not recognized that a significant statistical correlation between sediments temperature and primary production, secondary production, respiration rate, respectively. There were also not a significant statistical correlation among parameters.

The burrowing activity of Macrophthalmus japonicus increased the surface area of tidal flat and also organic carbon decomposition. This leads the increase of organic carbon decompositon by 1.1 times, because of the expansion of the tidal flat surface area by burrowing activity. Organic carbon decomposition in burrow walls, therefore, burrowing activities increased surface area which contributed to organic carbon decomposition. As for the organic carbon release, contribution by organic carbon decomposition of surface sediment was highest. In deed, respiration rate of $M. japonicus$ was higher than organic carbon decomposition in burrow. However, the effects of removed sediment from the surface sediment after burrowing activity were not included in this calculation such as oxidation of the removed sediment itself and respnseusation at the flood tide. Total amount of organic carbon cycle of $M. japonicus$ will increase if these effects would be elucidated. Moreover, it needed to show the date though at least one year, considering temporal and spatial variability in the future.

Earlier studies indicated that macrobenthos contribute to the organic carbon cycle (Dye, 1981; Kikuchi, 1987). However, these researches were conducted only with the organic carbon decomposition of surface sediment and respiration rate of the macrobenthos. In this paper, we considered the organic carbon decomposition of burrow wall and production of the macrobenthos as parameters and analyzed their contribution. In this study area, it was indicated that organic carbon cycle increased about 1.1 times when the existence of Macrophthalmus japonicus was considered. When production and respiration rate were considered in the effect of the bioturbation of burrowing activity such as polychaetes (Kikuchi, 1987) and Helice tridens (Kurihara et al., 1989) as well as $M. japonicus$, organic carbon cycle on the tidal flat showed increase more than it had been considered. These results indicated that in situ activities of $M. japonicus$ significantly influence the material cycle and it is important to consider the existence of burrow in order to understand the fluxes of materials and to evaluate the purification function of the tidal flat.

5. Conclusion

As for the burrow, many of its structure was J-shaped type, and it was recognized statistically significant correlation among carapace width, surface area and volume. It was estimated that tidal flat surface area increased 1.1–1.2 times by burrowing activity which corresponds to 7–15% of this muddy tidal flat area.

Two ways of experiments were performed to quantify respiration rate of Macrophthalmus japonicus in water and air. There was no difference in oxygen consumption of $M. japonicus$ in water and air. It was also recognized statistically significant correlation between carapace width and oxygen consumption of $M. japonicus$ in water and air, respectively. Thus, we could estimate burrow parameters and respiration rate from carapace width.

The organic carbon cycle in the tidal flat increased 1.1 times by considering the existence of Macrophthalmus japonicus burrow. These results indicated that in situ activities of $M. japonicus$ significantly influence on the organic carbon cycle and it is important to consider indirect effects of bioturbation as burrowing activity and direct effects such as production, consumption of macrobenthos to evaluate the purification function of the tidal flat.

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