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Short Communication

Digestive tube contents of Blood cockle (*Anadara granosa*) in a tropical mangrove estuary in Malaysia

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Abstract: This study was carried out to clarify the feeding biology of the blood cockle (*Anadara granosa*). We collected blood cockles from 8 stations in the Matang mangrove estuary of Malaysia in July and August 2010. The digestive tube contents of the specimens were stained with Congo red and observed under a light microscope. The results showed blood cockles take in particles containing cellulose as well as phytoplankton such as diatoms. As blood cockles in estuaries are known to exhibit cellulolytic enzyme activity in their digestive gland, the present results indicate blood cockles in estuaries feed on litter supplied from mangrove forests and terrestrial plants.

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Introduction

Blood cockles, *Anadara granosa*, inhabit the coastal areas of the Middle East, South Asia, Southeast Asia, East Asia, and Northern Australia (Faulkner, 2010). They mainly live in the silt clay muddy bottoms of estuaries or the inner parts of bays from the tidal zone to shallow areas (Narashimham et al., 1984; Nakao et al., 1989). The blood cockle is an important species for bivalve aquaculture industry due to being popular food in Southeast Asia, including Thailand and Malaysia (Watanabe, 2009; Yurimoto et al., 2014).

The Matang mangrove is approximately 40,000 ha and located on the west coast of Peninsular Malaysia. Moreover, there is a huge muddy tidal flat suitable for blood cockle culture that contains wide sowing aquaculture grounds (Pathansali and Song, 1958; Fontalvo-Herazo et al., 2011). Blood cockles occupy a large proportion of the biomass of the ecosystem of the Matang mangrove (Man et al., 2012). In addition to clarifying their ecological position, understanding the feeding behaviors of the blood cockle is necessary for stable production in sowing aquaculture in mangrove estuary ecosystems. Many benthic invertebrate organisms including clams such as *Ruditapes philippinarum* and *Corbicula japonica* in brackish waters contain cellulolytic enzymes (Sakamoto and Toyohara, 2009; Niiyama and Toyohara, 2011; Sakami and Higano, 2012). Moreover, the presence of cellulolytic enzymes was recently revealed in the digestive gland of the blood cockle, suggesting they can feed and digest organic matter including cellulose supplied from terrestrial plants including those from mangrove forests (Niiyama et al., 2012).

Therefore, this study aimed to clarify the feeding biology of the blood cockle in the Matang mangrove estuary (Peninsular Malaysia). Therefore, we collected the blood cockles from 8 stations in the Matang mangrove estuary and analyzed their digestive tube contents for terrestrial plant particles using Congo red staining, which adsorbs to cellulose (Samiey and Dargahi, 2010). In addition, the composition of digestive tube contents of the blood

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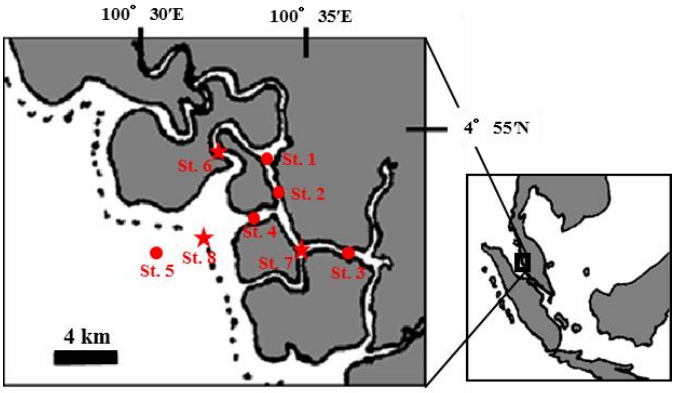

Figure 1. Sampling locations of the blood cockle, *A. granosa*, in the Matang mangrove estuary, Malaysia. ●: sampled in July 2010, ★: sampled in August 2010.

cockle was analyzed and compared among stations.

Materials and Methods

A total of 79 individual blood cockles with shell lengths ranging from 14-35 mm were collected from a total of 8 stations (St.) in Matang mangrove estuary in Peninsular Malaysia (Fig. 1). Sampling at St. 1-5 and 6-8 was carried out in July and August 2010, respectively. The digestive tube contents of specimens were analyzed. In brief, each specimen was fixed in formalin and dissected, and the digestive tube contents were aspirated from the lip with a micropipette. The contents were subsequently stained with Congo red solution to visualize cellulose and observed under a light microscope. In detail, the digestive tube contents were immersed with 1% (w/v) Congo red solution for at least 1 hour, and the extra solution after staining was washed with an alkaline alcohol solution [1 mL 1% (w/v) sodium hydroxide + 100 mL 50% (v/v) alcohol] and ion-exchanged water. The contents in the microtube were washed repeatedly with bleaching in alkaline alcohol solution followed by purified water. The contents were then flash centrifuged at 3,000 rpm to exchange the solution. Finally, the sample was diluted with a predetermined amount (500 µl) of purified water, dropped to a glass slide, and covered with a cover glass before observation under a light microscope. In addition, particles > 10 µm stained positive (i.e., land plant-derived particles) and negative (i.e., phytoplankton, etc.) with Congo red

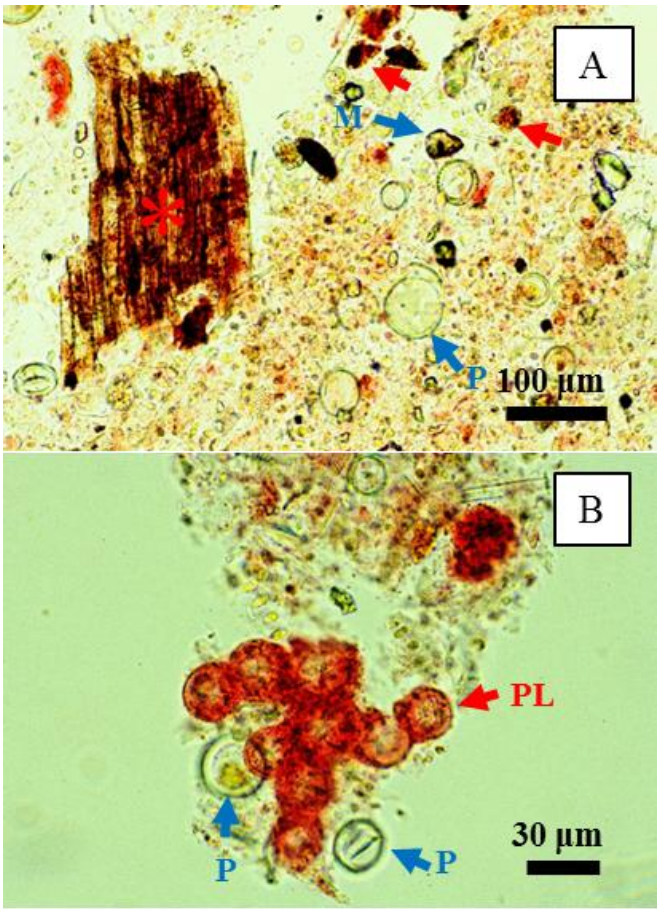

Figure 2. Photomicrographs of the digestive tube contents of blood cockle, *A. granosa*, stained with Congo red solution. Positive staining: Asterisk and red arrows show terrestrial plant fragments, and red arrow PL shows phytoplankton cell-like particle. Negative staining: blue arrows M and P show mineral particle and phytoplankton such as diatom, respectively.

were counted.

Results and Discussion

The numbers of collected specimens at each station are shown in Table 1; 5-14 individuals were collected from each station. However, only 3-7 individuals from each station were successfully analyzed. Thus, the success rate at each station ranged from 21-80%. In particular, the success rate was higher at stations from the inner part of the mangrove area than the estuaries in both July (70-80% at St. 1-3) and August (36% at St. 6). These results indicate that food consumption is higher in the inner part of the mangrove area than estuaries.

Photomicrographs of the gut contents of blood cockles are shown in Figure 2. The particles positively and negatively stained with Congo red are

Table 1. Number of the blood cockle specimens collected at each sampling station and success rate of the digestive tube contents sampling.

	July 2010					August 2010		
	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8
Number of specimens	10	5	7	10	10	11	14	12
Number of successful samples	7	4	5	4	3	4	3	3
Success rate (%)	70	80	71	40	30	36	21	25

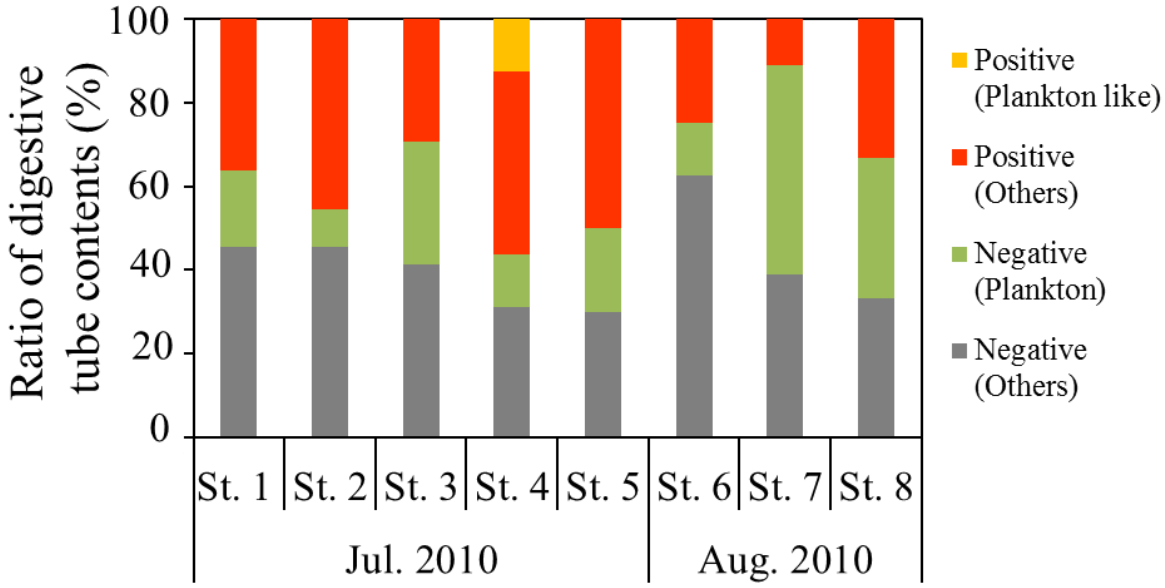

Figure 3. Composition of the digestive tube contents (>10 μm in diameter) of blood cockles, *A. granosa*, at each sampling station. The numbers of total counted particles and observed samples at each station are as follows: St. 1: n = 391 (7), St. 2: n = 204 (4), St. 3: n = 405 (5), St. 4: n = 319 (4), St. 5: n = 116 (3), St. 6: n = 112 (4), St. 7: n = 229 (3) and St. 8: n = 31 (3). Positive (Plankton like) indicates positively stained particles of phytoplankton-like cells (Fig. 2B, red arrow PL), Positive (Others) indicates positively stained terrestrial plant-derived particles (Fig. 2A, asterisk and red arrows), Negative (Plankton) indicates unstained particles identified as phytoplankton such as diatom (Fig. 2A, B, blue arrows P), and Negative (Others) indicates an unstained mineral particle (Fig. 2A, blue arrow M) or unidentified.

shown in Figure 2A; particles containing cellulose components from mangrove forests or terrestrial plants stained strongly with red (Fig. 2A, asterisk and red arrows). In addition, the silicate cell walls of diatoms (Fig. 2A, B, blue arrows P) (Hecky et al., 1973) and mineral particles (Fig. 2A, blue arrow M) as well as many other unidentified particles were not stained. Phytoplankton-like particles were stained strongly (Fig. 2B, red arrow PL) could be green algae cells, which contain cellulose in their cell walls (Sugiyama et al., 1991). The composition of the digestive tube contents (particles > 10 μm) from blood cockles collected from each sampling station is shown in Figure 3. The ratios of components differed somewhat among sites. Mineral and terrestrial plant particles and phytoplankton, which is a common food source for bivalves (Nakamura and Shinotsuka, 2007), were observed. Regarding staining, positively and negatively stained particles accounted for 11-57% and 43-89% of all particles at each sampling station, respectively. Among the positively stained particles, phytoplankton-like particles accounted for approximately 13% of all particles in samples from St. 4. Diatoms, and minerals and unidentified particles accounted for 9-50% and 30-63% of the negatively stained particles, respectively. In addition, the composition of digestive tube contents differed between survey months. There was a greater ratio of positively stained particles in July (St. 1-5) than August (St. 6-8). On the other hand, the ratio of negatively stained particles (e.g., diatoms) was higher in August than

July. In addition, the ratio of negatively stained particles was higher in mangrove areas (St. 1, 2, 3, 6 and 7) than estuaries (St. 4, 5, and 8), which had a higher ratio of positive to negative particles. These results indicate that the digestive tube contents of blood cockles vary with respect to environmental differences and changes in their habitat.

As mentioned above, the presence of cellulolytic enzyme activity has been confirmed in the mid-gut glands of blood cockles living in estuaries (Niiyama et al., 2012). Accordingly, the present results indicate that blood cockles in the Matang mangrove estuary take in terrestrial plant particles containing cellulose as well as phytoplankton. Thus, blood cockles digest particles containing cellulose in their digestive tube with cellulolytic enzymes.

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