

Mass mortality event of the blood cockle, *Anadara granosa*, in aquaculture ground along Selangor coast, Peninsular Malaysia

Tatsuya Yurimoto · Faizul Mohd Kassim ·
Reiko Fuseya · Alias Man

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Abstract A mass mortality event of blood cockles, *Anadara granosa*, occurred in aquaculture ground in Selangor in mid-February 2012. To identify factors contributing to this event, we estimated the changes in survival rate and population density of the blood cockles using data from field surveys carried out in late-January and mid-March, 2012. In addition, blood cockle samples were used for histological observation of various tissues with a light microscope. Furthermore, we examined the environmental factors that seemed to be involved in this mass mortality event using environmental data obtained in two surveys and weather data for the Selangor region from late-January to mid-March 2012. As a result of the field surveys and the histological observation, the mortality was estimated to be 30 % over, and the blood cockles were shown to be in the spawning season at this time of year. In addition, numerous individuals collected in mid-March had no food in the digestive duct, which suggested low food availability. Precipitation data for the Selangor region showed rainfall of 30 mm/day over four days in mid-February, which suggested an impact of freshwater flooding from rivers on the aquaculture ground. Normally, the blood cockles are tolerant to low-salinity environments. However, the fact that the flooding and associated environmental changes occurred, when the population was debilitated by reproductive activity, may have led to the mass mortality event in association with low food availability.

Keywords Blood cockle · *Anadara granosa* · Mass mortality · Malaysia

Introduction

The blood cockle, *Anadara granosa*, is a member of the Arcidae family and has red bodily fluid that contains hemoglobin, like blood. This species is widely distributed, from the Middle East to East Asia (Faulkner 2010).

T. Yurimoto (✉)

Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan
e-mail: yurimoto@outlook.com

F. Mohd Kassim

Penang Office of Japan International Research Center for Agricultural Sciences, 11960 Batu Maung, Penang, Malaysia

R. Fuseya

National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Ibaraki 314-0408, Japan

A. Man

Fisheries Research Institute, Department of Fisheries, 11960 Batu Maung, Penang, Malaysia



Its habitat is soft muddy bottoms of tidal flats formed in estuaries or the interior of bays (Narashimham et al. 1984; Nakao et al. 1989). It is also a major aquaculture species, with Malaysia and Thailand being among the Southeast Asian countries that have been actively developing its use in this field (Watanabe 2009). In particular, in the Selangor region of Malaysia, blood cockle aquaculture is flourishing because a large number of juvenile cockles naturally develop in the tidal flats of this region, and many of them are collected for aquaculture. These juveniles have been supplied as seeds for aquaculture grounds in not only the Selangor region but also surrounding areas.

The harvest of blood cockles in the Selangor region remained at less than 10,000 tons in the early 2000s, but increased after 2008, with a record harvest of about 40,000 tons in 2010. Factors thought to have contributed to this increase include the arrival of new aqua farmers and expansion of the area used for aquaculture. However, the harvest has declined to about 25,000 tons since 2011, with its level remaining unstable (Alias Man, unpublished data). Factors that may have had an impact on this have been identified, such as overcrowding cockles, sediment deterioration, flooding, and sediment disturbance in aquaculture grounds, but the main factor remains unidentified. Against this background, we heard from fishermen that a mass mortality event of blood cockles occurred at our monitoring site for aquaculture research in Selangor in mid-February, 2012. To achieve stable production in bivalve aquaculture, it is important to measure and control factors that negatively influence the growth of these organisms. Therefore, in order to clarify the causes of this mass mortality event, we examined the survival rate and population density of the cockles, alongside an environmental survey using data from field surveys that were conducted before and after the mass mortality event; in addition, the blood cockles were observed histologically after the two sampling periods, and the obtained results were discussed along with data on the weather at around this time.

Materials and methods

Sample collection

On January 30 and March 15, 2012, field surveys were carried out in a blood cockle aquaculture ground along the coast of Selangor (Fig. 1). To collect blood cockles, we performed quantitative sampling used a long hand dredge (basket size: 60 cm wide, 15 cm high, 30 cm deep) with a 1.5 cm mesh. The towing distance of this dredge was estimated from the hauling time and the navigation speed of the fishing boat to determine the collection area, and we calculated the population density of blood cockles in the aquaculture ground. In addition, the collected blood cockles were measured in terms of the shell length of each individual.

Histological observation

The collected blood cockles were brought back to a laboratory, and the interior of each organism was excised to produce tissue blocks of the various organs (gill, mantle, digestive gland, and gonads) after measurement of the shell length. These blocks were immersion fixed in 10 % formalin seawater solution. Fixed tissue was then embedded in paraffin to make a paraffin block after alcohol dehydration and lemosol replacement. The paraffin block was cut into thin sections 5–10 μm thick with a microtome, and the tissue was observed under a light microscope after hematoxylin & eosin staining. The various tissues were observed and the presence or absence of lesions, parasites, and food in the digestive duct were estimated.

Environment

The environmental features of the blood cockle aquaculture ground were examined by field sampling. Using a throw-in-type water-quality sensor (AAQ-RINKO, JFE Advantech Co., Ltd., Japan), water-quality parameters, such as temperature, salinity, dissolved oxygen, and chlorophyll-*a* concentration, were measured at a layer 20 cm from the bottom at the survey site. In addition, surface mud was collected with an Ekman-Barge bottom sampler at the survey site, and redox potential and pH of the surface sediments (0–1 cm) were measured. In addition, to clarify the relationship between weather and the mass mortality event, we obtained

Fig. 1 Sampling location (*asterisk*) of blood cockles, *Anadara granosa*, at an aquaculture ground in the Selangor region, Malaysia, at which a mass mortality event occurred

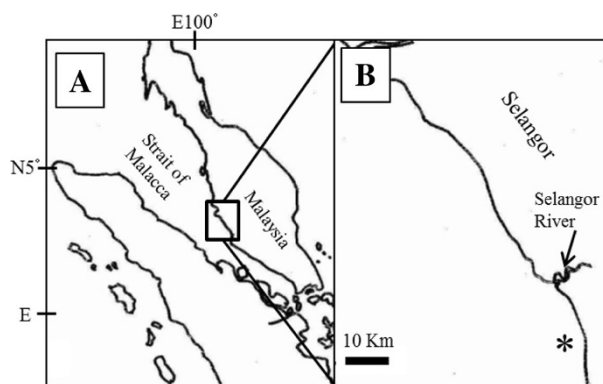


Table 1 Details of the sampling of blood cockles, *Anadara granosa*, collected in an aquaculture ground along the Selangor coast in late-January and mid-March, 2012

Date	Hauling area of dredge net (m ²)	Total number of individuals	Status of individuals		Mortality rate (%)	Density of alive individuals (m ²)
			Alive	Dead		
30th Jan., 2012	54	422	420	2	<0.5	7.8
15th Mar., 2012	27	191	129	62	32.5	4.8

rainfall data in the Selangor region from January to March, 2012, from the Water Resources Management and Hydrology Division, Department of Irrigation and Drainage, Malaysia.

Results

Mortality and population density

The population density (number of individuals per sampling area) of the blood cockles was calculated for each set of sampling data for late-January and mid-March. The density decreased from 7.8 to 4.8/m² over these two sampling dates (Table 1). In addition, the dead individual, it was showed no closed shells reaction upon stimulation or eluted molluscos part remained the shells, and the percentage among the collected blood cockle samples was less than 0.5 % in late-January, but rose to 32.5 % in mid-March (Table 1).

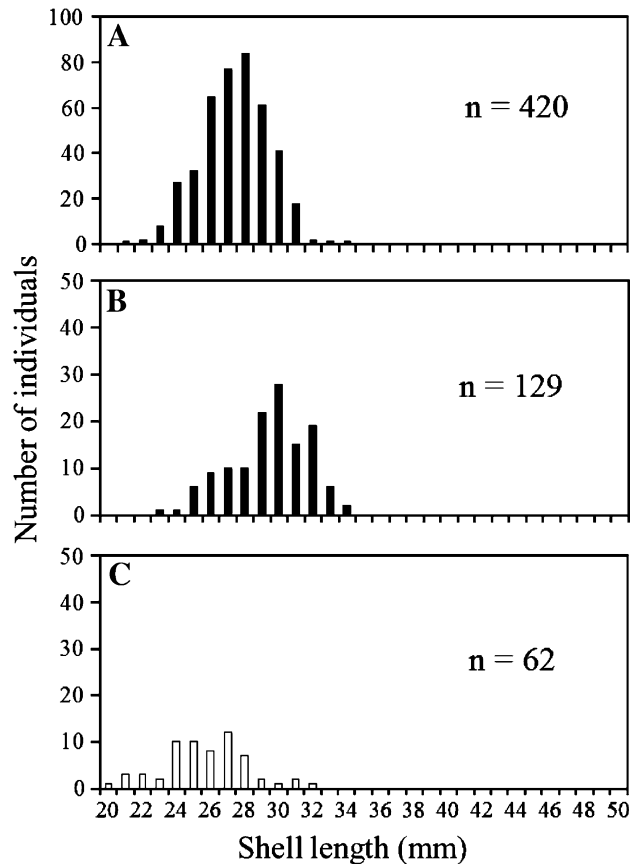
Shell length

The shell length histogram of the collected blood cockles was compared between late-January and mid-March (Fig. 2). The two sets of data exhibit single peaks: at 28 mm in late-January and 30 mm in mid-March. In addition, we also plotted the distribution of shell length of dead individuals collected in the survey in mid-March. A single peak was again shown, the same as for the other results. However, this peak was at 27 mm.

Parasitism

Parasite infection status was evaluated for the gonads ($n = 30$) and digestive gland ($n = 24$) in late-January, and for the gonads ($n = 30$), digestive gland ($n = 24$), mantle ($n = 18$), and gill ($n = 18$) in mid-March. However, parasites were not observed in these tissues.

Fig. 2 Shell length distribution of blood cockles, *Anadara granosa*, collected at an aquaculture ground along Selangor coast in late-January and mid-March, 2012. **a** Shell length distribution in late-January. **b** Shell length distribution in mid-March. **c** Shell length distribution of dead individuals in mid-March



Gonads

In accordance with the classification of gonad development in previous reports on blood cockles (Yurimoto et al. 2008, 2014), individuals of both sexes in the mature stage/spawning stage (stages III–IV) dominated, while some females had already reached the spent stage (stage V) in late-January (Fig. 3). Although individuals of both sexes in stage III or IV were still observed in March, they were less dominant and the number in stage V had increased.

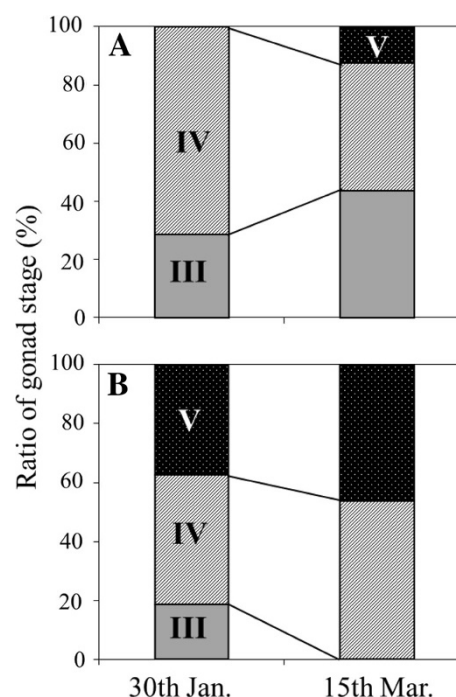
Digestive gland

To clarify the status of food availability for the blood cockles, we observed food content in the digestive duct and the tissue condition of the digestive tube. Epithelial cell condition in the digestive tube was classified into three categories: Class I represents good condition of epithelial cells, with large cell length and high cell density (Fig. 4I). Class II represents an intermediate condition of the epithelial cells, with reduced cell length and spaces being observed in the gland (Fig. 4II). Class III represents a worse condition of the epithelial cells, with the conditions described for class II being observed over a wide area of the digestive gland and the gland being thinner (Fig. 4III). The obtained results are shown in Fig. 5. In late-January, individuals categorized into class I accounted for more than 40 % of the total, but this proportion decreased to about 20 % in March.

Food availability

The digestive duct in the digestive gland was observed to estimate food intake. For the tissue sections of individuals who had been feeding, the digestive duct filled with fine particles, including diatoms, could be seen (Fig. 6a). On the other hand, such fine particles were not observed for individuals who had not been feeding sufficiently (Fig. 6b). The difference in this characteristic between late-January and mid-March is summarized

Fig. 3 Ratio of maturation stages of blood cockles, *Anadara granosa*, collected at an aquaculture ground along Selangor coast in late-January and mid-March, 2012. *III* Mature stage. *IV* Spawning stage. *V* Spent stage. **a** Male. **b** Female



in Fig. 7. Food intake was observed in more than 60 % of individuals in late-January, but this rate decreased to about 20 % in mid-March.

Environment

The results of environmental measurement of water temperature, salinity, dissolved oxygen, and chlorophyll-*a* concentration in the bottom layer and redox potential and pH in surface sediments in late-January and mid-March are summarized in Table 2. No parameter showed a significant difference between the two periods. The level of chlorophyll-*a* was about 5 µg/L in both months, the pH in surface sediments was 7.4–7.5, and the redox potential showed an oxidation state of over 60 mV in late-January and mid-March.

Weather

Daily precipitation in the Selangor estuary in January–March 2012 is shown in Fig. 8. The monthly amounts in January and March were lower than that in February; a rate of over 30 mm/day occurred on only one day in each of these two months. In contrast, such a rate was observed on four near-consecutive days during mid-February. In particular, on the 15th of February, the level exceeded 50 mm/day.

Discussion

There are many reports about mass mortality events in bivalves, such as pen shell, ark shell, Pacific oyster, and pearl oyster (Imai et al. 1969; Tomaru et al. 2001; Nakamura 2005; Maeno et al. 2006). Normally, an emergency survey or research on such events is conducted a thorough investigation to determine the cause because they can lead to major economic losses in the bivalve aquaculture industry. However, identification of the main causative factors is extremely difficult because these events involve a complicated interplay between the physiological condition of bivalves and environmental factors, so the cause of death can rarely be explained by a single factor. For example, even when bivalves are exposed to poor environmental conditions, such as hydrogen sulfide or hypoxia, they exhibit some resistance and do not die straight away (Nagasoe et al. 2011; Suzuki et al. 2012). For this reason, when a mass mortality event occurs, if the environment of fishing grounds is investigated after a mass mortality event occurs, it is difficult to identify the causative factor

Fig. 4 Microphotographs of the digestive gland of blood cockles, *Anadara granosa*, collected at an aquaculture ground along Selangor coast in late-January and mid-March, 2012. **I** Good condition of epithelial cells. **II** Partial flattening of epithelial cells (*arrows*). **III** Substantial flattening of epithelial cells (*arrows*). Scale bar represents 100 μ m

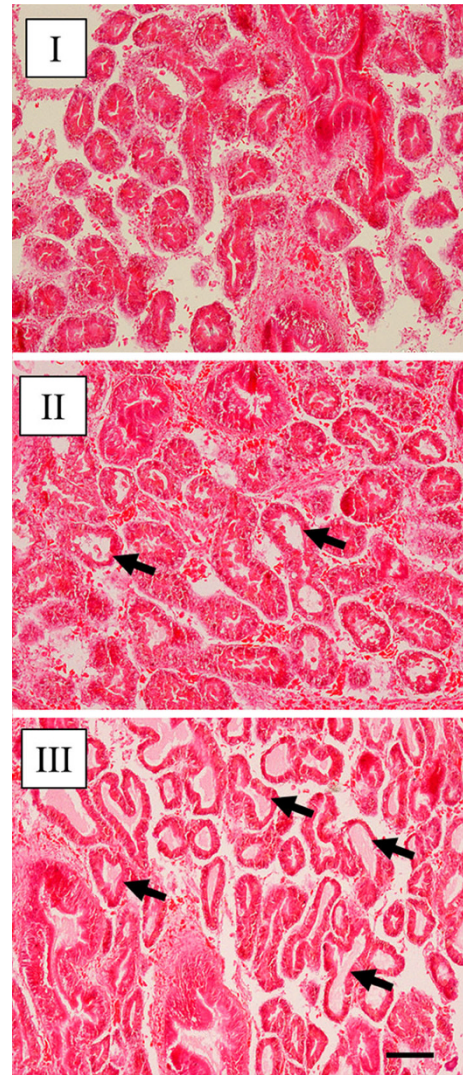
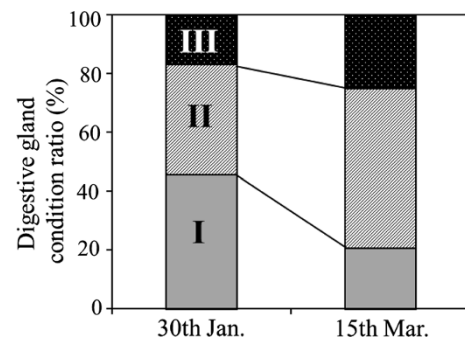


Fig. 5 Ratios of the digestive gland condition of blood cockles, *Anadara granosa*, collected at an aquaculture ground along Selangor coast in late-January and mid-March, 2012. *I* Good condition of epithelial cells. *II* Partial flattening of epithelial cells. *III* Substantial flattening of epithelial cells



because the environment has already changed. In addition, if the physiological state of the affected organisms is surveyed after a mass mortality event, the main causative factor is not clear in terms of whether the mortality is due to external environmental factors or internal physiological ones.

In this study, we collected biological and environmental data to clarify the cause of a mass mortality event that occurred in a blood cockle aquaculture ground in the Selangor region of Malaysia in mid-February, 2012. The shell length results also supported the occurrence of the mass mortality event in mid-February, because

Fig. 6 Microphotographs of the digestive duct of blood cockles, *Anadara granosa*, collected at aquaculture grounds along Selangor coast in late-January and mid-March, 2012. **a** The duct is filled with food. **b** The duct is empty (asterisk). Scale bar represents 100 μm

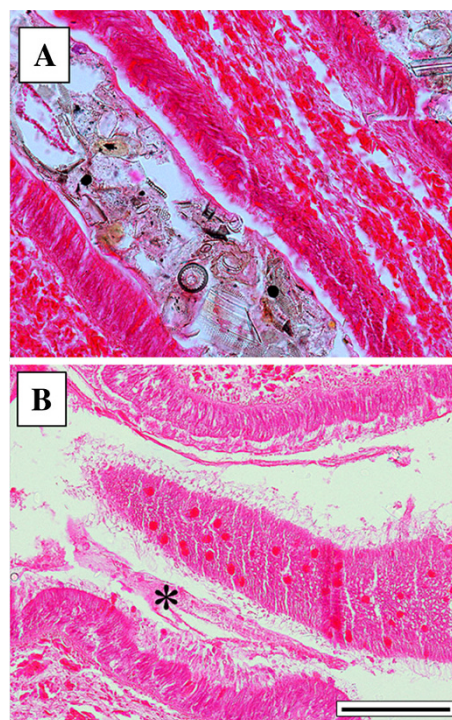


Fig. 7 Ratios of presence of food in the digestive duct of blood cockles, *Anadara granosa*, collected at an aquaculture ground along Selangor coast in late-January and mid-March, 2012. *P* Presence. *N* Non presence

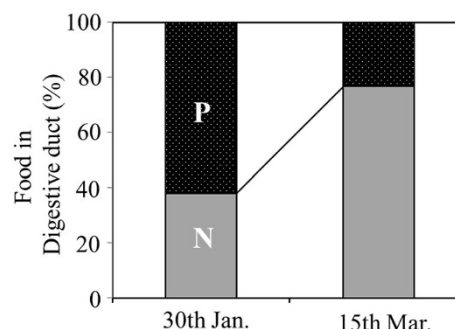


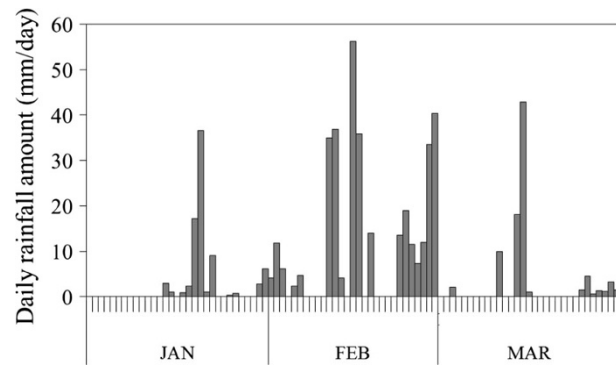
Table 2 Environmental data collected in an aquaculture ground on the sampling dates in late-January and mid-March, 2012

Date	Depth (m)	Water quality				Sediment quality	
		Temperature (°C)	Salinity (PSU)	DO (mg/L)	Chl- <i>a</i> (μg/L)	ORP (mV)	pH
30th Jan., 2012	2.5	29.7	29.9	5.1	5.2	118 ± 59	7.5 ± 0.2
15th Mar., 2012	2.7	29.1	29.5	5.0	4.6	66 ± 32	7.4 ± 0.2

DO dissolved oxygen, *Chl-a* chlorophyll-*a*, *ORP* oxidation–reduction potential, *pH* potential hydrogen, mean ± SD in Sediment quality

both shell length histograms of the living individuals in late-January and the dead individuals in mid-March were approximately the same. Thus, this result suggests that the dead individuals spawned while they were alive. Additionally, it became clear that many individuals had fed poorly in mid-March and that the blood cockles were in the spawning season when this mass mortality event occurred. Among the survived individuals in mid-March, we observed a phenomenon that epithelial cells in the digestive tube were flattened in severe cases; it is suggesting the blood cockles had a no feeding period caused both physiological and

Fig. 8 Data on daily rainfall in the Selangor region from January to March, 2012. Data are from the Water Resources Management and Hydrology Division, Department of Irrigation and Drainage, Malaysia



environmental factors (Ellis et al. 1998), and there was a high probability that nutrient absorption was difficult by the histological condition, so even in such individuals who had fed well recently. On the other hand, certain parasites, such as *Nematopsis* sp., trematode sporocysts, and Turbellaria, were reported to have been isolated from blood cockles along the coast of the Malacca Strait by Uddin et al. (2011). However, in the present study, no parasitic infections were found in the observed tissues in the gonads, digestive gland, mantle, and gill. Therefore, we considered it unlikely for parasitism to be related to the mass mortality event. In addition, we considered improper functioning of those tissues to be unlikely to be related to this event. Furthermore, abnormal water quality, such as the occurrence of a high water temperature, low salinity, and hypoxic water, was not observed in the aquaculture ground during the field surveys. The chlorophyll-*a* content was also appropriate for bivalves, which indicates that phytoplankton was present during the studied periods.

There was also no observed decrease in pH level due to the decomposition of organic matter, as shown by the pH measurement of surface mud, and the redox potential in the mud was also in the oxidation state, so we considered that the aquaculture ground was an appropriate environment for blood cockles. However, to characterize the environment of fisheries, since water quality is more likely to change over a short period than sediment, it is necessary to consider the changes in water quality on a day-to-day basis. In this study, from the daily rainfall data, it became clear that intensive precipitation of over 30 mm/day occurred on four days in mid-February.

As for other weather-related factors, using a weather report for Subang Jaya, which is located close to Selangor (WeatherSpark 2013), as a reference, we confirmed that there were no extreme weather events, such as high temperature, drought, or strong winds, in late-January to mid-March. This suggested that sediment disturbance caused by strong winds, high water temperature or reduction of the water level due to drought, or a long period of high temperature was unlikely to be causative factors; however, a decrease in salinity caused by flooding from nearby rivers due to heavy rainfall did occur in mid-February, when this mass mortality event occurred. It seems highly likely that such rainfall, even though we could not determine the salinity levels by a regular survey, would have had a large environmental impact via a large amount of fresh water flowing into the aquaculture ground from nearby rivers because of its location near an estuary.

Normally, blood cockles exhibit resistance to low salinity. For example, from a salt-resistance experiment on blood cockles by Davenport and Wong (1986), the cockles showed a clear shell-closing reaction upon exposure to salinity of less than 19 PSU; about 50 % of them survived at salinity of 16 PSU, while the mortality rate was 100 % upon exposure to salinity of lower than 10 PSU for a week. Therefore, mass mortality of blood cockles is extremely likely upon exposure to a low-salinity environment of lower than 10 PSU for a week or more, which affects the filtering activity that cockles can exhibit at a salinity level of 16–19 PSU.

In this study, the salinity changes at the aquaculture ground during the mass mortality event are unknown because we did not measure this variable. However, it is highly likely that a reduction in salinity due to flooding occurred because there was precipitation of over 30 mm/day on four days in mid-February, as shown by the weather data. However, to explain this mass mortality event of blood cockles, multiple factors should be considered. Normally, in the spawning season, bivalves have lower environmental resistance, which is known to be potentially associated with death due to even weak environmental stress (Cho and Jeong 2005). Thus, even though the salinity decrease in the aquaculture ground alone may not have been sufficiently severe



to kill the blood cockles, we should consider the possibility that the spawning season made these organisms particularly susceptible. In addition, from observation of the digestive gland tissue, many of the blood cockles collected in mid-March had no food in the digestive duct, and the epithelial cells in the tubes were also flattened. Thus, it is suggested that the state of the digestive gland made it difficult to absorb nutrients. As such, it is suggested that the blood cockles cultured in the Selangor region were debilitated by reproductive activity, and died due to a feeding disorder that was also influenced by low salinity due to the flooding in mid-February.

Conclusions

A mass mortality event of blood cockles occurred in an aquaculture ground in Selangor in mid-February, 2012. The mortality rate was estimated to be 30 % over upon comparison of the data from the two surveys in late-January and mid-March, and blood cockles were shown to be in the spawning season at this time of year. Many individuals collected in mid-March were also in a state of poor feeding. In addition, precipitation data showed four days on which there was rainfall of over 30 mm/day in mid-February, so the blood cockle aquaculture ground close to an estuary was considered to have been adversely impacted by flooding from nearby rivers. These results suggest that some individuals had been debilitated by reproductive activity, which led to their death upon low food availability due to low salinity and related environmental changes caused by flooding.

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Conflict of interest The authors declare that they have no competing interests.

Author's contributions T Yurimoto designed the study, collected and analyzed data and drafted the manuscript. F Mohd Kassim assisted the field sampling and collected environmental data. R Fuseya advised the study and discussed data. A Man organized the field survey and the study. All authors read and approved the final manuscript.

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