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The after-effects of hypoxia exposure on the clam *Ruditapes philippinarum* in Omaehama beach, Japan

Yasunori Kozuki [a,*], Ryoichi Yamanaka [a], Maya Matsushige [b], Azusa Saitoh [b], Sosuke Otani [c], Tatsunori Ishida [a]

[a] Ecosystem Design, Institute Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
[b] Department of Ecosystem Engineering, College of Earth and Life Environmental Engineering, Graduate School of Advanced Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
[c] Research Center for Environmental Quality Management, Kyoto University, 1-2 Yumihama, Otsu, Shiga 520-0811, Japan

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A B S T R A C T

A number of reports describe the impact of hypoxic conditions on the manila clam *Ruditapes philippinarum*, but few deal with the after-effects of hypoxia on this clam species. Exposure experiments were carried out on the manila clam under conditions of low dissolved oxygen (DO, 0.5 mg/L). In the first exposure experiment (i.e., 3 days of hypoxia), a substantial change in mortality rate, glycogen content, and clearance rate was not observed in the period following the hypoxia. However, in the second exposure experiment (i.e., an additional 3 days of hypoxia following recovery from the first exposure), the mortality rate was significantly increased relative to that of the first exposure experiment, and glycogen content underwent a long-term decline. At the end of the experimental period, subsequent to the 2 exposures to hypoxia, the clearance rate had decreased until it was 77% of that of the normal manila clams unexposed to hypoxia. Moreover, the clearance rate was unable to recover following the second exposure. Thus, when the manila clams were repeatedly exposed to hypoxic conditions, the surviving individuals showed a residual disability, as reflected in the increased mortality and unrecovered clearance capacity.

Afterward, the after-effects of hypoxia exposure on the clearance function of the manila clams in Omaehama beach, Japan, were assessed using the experimental results and the observed data. An examination of the effects of the residual disability showed a more extensive decrease in filtering capacity when a formula accounting for residual disability was used rather than a standard one. The clearance volume using our new formula was 75% of that of a standard formula. The formula equations that have been used up until now have only evaluated whether the manila clams die. However, the surviving individuals have a residual disability, and our formula indicates this effect would cause a further decline in the purification function of the beach.

1. Introduction

The environmental impact of hypoxia in eutrophied coastal waters has been studied worldwide, especially in enclosed bays located near big cities (Sagasti et al., 2001). In these coastal areas, a hypoxic layer forms near the sea bottom in the summertime and results in the mass mortality of the macrobenthos (Kodama and Horiguchi, 2011). In Japan, this phenomenon has been observed in tidal flats every year and has become a major issue in coastal environmental conservation, since almost all of the manila clams (*Ruditapes philippinarum*), a fishery resource in Japan, die because of the hypoxic water (Kakino, 1986; Toba, 2004). Hence, the tolerance of the clam for anoxic conditions has been investigated by a number of researchers (de Zwaan and Mathieu, 1992; Sobral and Widdows, 1997). Moreover, the clearance function of the clam has often been treated as a scale for biological activity because this function plays an important role in material circulation in coastal areas (Jordan and Valiela, 1982; Nakamura et al., 1988).

According to previous research, the clam is able to tolerate hypoxic and anoxic conditions for a few days. The mechanism of the

* Corresponding author.
E-mail address: kozuki@eco.tokushima-u.ac.jp (Y. Kozuki).

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tolerance has been discussed by Hochachka (1980), and anaerobic metabolism has been evaluated in particular. Based on the findings of some studies, the clam is able to survive under hypoxic conditions by closing its shell and using glycogen that was stored prior to the occurrence of the hypoxic bottom water. For example, Higano (2005) found that manila clams can survive for about 3 days under hypoxic conditions (DO < 0.5 mg/L). Moreover, Uzaki et al. (2003) has suggested that the glycogen content of the clam is a determinant of its ability to tolerate hypoxia. Lee et al. (2011) and Okumura et al. (2005) have also suggested that the glycogen content can be used as an indicator of the nutritional state of bivalves.

In a previous study, we conducted field observations over the course of 3 years in a natural tidal flat named "Omaehama" located in the northern part of inner Osaka Bay, Japan. The water quality and the abundance of the natural clam were investigated every month, and hypoxia was observed to occur every summer. Of interest, we found that some of the clams that survived the hypoxic environment had a low survival rate and often died within a couple of months of the hypoxic event, a phenomenon that has not been previously reported for the manila clam (Kozuki et al., 2009, 2011). Based on this finding, we hypothesized that the clams that survive the hypoxia suffer from permanent damage. The objectives of the present study were to clarify in a laboratory setting the potential after-effects of the hypoxia on the clams and to evaluate the clearance rate and the glycogen content of clams that survived the hypoxic event. Moreover, the environmental impact on the purification capacity of the beach was estimated.

2. Material and methods

2.1. Laboratory experiments

One hundred manila clams were exposed to hypoxic water maintained at a constant dissolved oxygen (DO) concentration of 0.5 mg/L for 3 days with nitrogen and oxygen aeration. Afterward, the clams were moved to another tank filled with artificial seawater with sufficient oxygen (DO > 6.0 mg/L) until the clearance rate and glycogen content had recovered to the same values as the initial ones prior to the exposure to hypoxia. The clearance rate and glycogen content of the clams were measured and compared until both parameters recovered. Following recovery to a normal condition, which was the initial clearance rate before the exposure to hypoxia, the manila clams were again exposed to hypoxia (DO < 0.5 mg/L) for 3 days, and the effects of this second exposure to hypoxia were observed. The clearance rate and the glycogen content of the control manila clams in normoxic seawater were also measured. The artificial seawater in the tank was changed once a day to prevent the reduction in water quality that can influence the manila clams. The generation of hydrogen sulfide was not observed under the hypoxic conditions. The temperature and salinity of the artificial seawater were kept at 25 °C and 28 psu during the experiments, respectively. In addition, individuals that had never been exposed to hypoxia due to indoor farming were used in the experiments. The shell lengths of individuals used in the experiments were 28 ± 1.7 mm. The manila clams were kept in a water tank that generates tides automatically. This tank has tidal mud and produces conditions close to those of the environment. The manila clams were not fed during the exposure to hypoxia. *Skeletonema costatum* was fed to the manila clams during the recovery period in the normoxic water.

2.2. Measurement of the clearance rate

Skeletonema costatum, one of the plankton that causes red tide (Li et al., 2009), was added to the artificial seawater at the same concentration as that required to produce a red tide bloom, so that the clearance rate of the manila clams could be measured. One clam was placed in each beaker. The amount of plankton remaining in the beaker was measured at 10-min intervals for 1 h with a fluorometer (TURNER DESIGNS, Aquafluor TM) after the manila clams had extended their siphons.

We chose 10 surviving manila clams from the above hypoxic-exposure experiment and measured the clearance rate of each clam every few days. Clams that did not extend their siphons for 2 h were not used for the experiments. In addition, manila clams had to be sacrificed for measuring the glycogen. Moreover, the number of surviving manila clams decreased because manila clams died during the experiments. Hence, the number of experimental manila clams was not uniform. Coughlan's (1969) formula was used to determine the clearance rate:

$$m = M \times \frac{\log_{10}C_0 - \log_{10}C_t}{t \times n \times \log_{10}e} \quad (1)$$

where m: the clearance rate of a clam (L/h), M: the water volume available for clearance (L), n: the number of individuals, C_0: the starting value of the suspended solids (mg/L), and C_t: the value of the suspended solids after t hours (mg/L).

2.3. Glycogen content measurements

The glycogen content of several surviving manila clams in the hypoxic-exposure experiment was measured every few days. The glycogen content was measured by using the anthrone-sulfuric acid method (Yoshikawa, 1952), as follows:

(1) The meat of the clam was freeze-dried for over 24 h; (2) The freeze-dried meat was placed in a test tube, and a 30% KOH solution was added. The test tube was placed in a boiling water bath for 1 h; (3) The test tube was left in a coolant for 20 min, and ethanol was added to remove the supernatant liquid. The sediment was dissolved with the appropriate amount of distilled water; (4) The samples were placed in anthrone-sulfuric acid reagent, and the absorbance was measured at 620 nm.

The manila clams that did not react to the external stimulus of being poked were considered dead and were freeze-dried within 1 h before the meat began to decompose. The glycogen content was measured later.

2.4. Calculation of the effect of frequent hypoxia on the purification of the beach

The manila clams exposed to frequent hypoxia may have a residual disability in their clearance function. To access the broader effects of this possibility, we investigated the impact of this impaired function on the purification of the beach. In this study, we defined the purification of the beach as referring to the clearance volume. Omaehama beach, which is in an enclosed coastal area in the interior of Osaka bay (Fig. 1), can be used as a case study. This beach is one of the few natural beaches remaining in Osaka Bay. The sea near the beach becomes hypoxic every year, and the macrobenthos are greatly affected (Kozuki et al., 2009, 2011). In this study, manila clams occupying this site were observed in terms of the number of individuals and weight. The shallow bottom the manila clams frequently inhabit turns hypoxic, and at a DL (datum line) of 0 m, the water was hypoxic (DO, 0.24 mg/L) on the 4th day of September 2009. We set up this day as an observational date and as one for using our lab-derived experimental results for determining the effect of hypoxia. The effect of the residual disability of the manila clams at the beach was calculated for one month. Table 1 shows the scenarios for the effects of hypoxia on the mortality rate and residual disability. Scenario A assumes that normoxia was