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Mass mortality of the Japanese pearl oyster *Pinctada fucata martensii* in relation to water temperature, chlorophyll *a* and phytoplankton composition

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ABSTRACT: Mass mortalities of the Japanese pearl oyster *Pinctada fucata martensii* have widely occurred in western Japan since 1994. The causes of these mass mortalities are at present not thoroughly understood. In this study, we investigated oyster survival in relation to some environmental factors such as water temperature, concentration of chlorophyll *a* and density or composition of phytoplankton. The examined mass mortality occurred from September to December 1998, and the color on the adductor muscle of the oysters was red-brown, suggesting an infectious disease. Oysters that became moribund during the experiment lost weight, while the weight of unaffected oysters increased. The cell density of *Nitzschia* spp., an inedible algae for the oyster, in Uchiumi Bay increased before and during the mass mortality event. From the results of our study, we hypothesize that *P. fucata* martensii was weakened by starvation because of the dominance of inedible food and then contracted an infectious disease that resulted in mortality.

KEY WORDS: Pearl oyster · Mortality · Water temperature · Phytoplankton

INTRODUCTION

Mass mortalities of bivalves occur every year in many places around the world due to various diseases (Pass et al. 1987, Friedman et al. 1991, Davis & Barber 1994, Chou et al. 1998). Diagnosed diseases are often secondary effects that follow deterioration of the normal environmental conditions (Pass et al. 1987, Chou et al. 1998), although the actual mass mortalities of bivalves are generally caused by infectious agents, such as bacteria (Pass et al. 1987, Friedman et al. 1991) and viruses (Chou et al. 1998). To understand the predisposing causes of the mortalities, it is important to determine the ambient conditions prevailing at the time and place of the mass mortality event (Fukushima 1970, Yamaguchi & Hasuo 1977). Extensive culture of the pearl oyster *Pinctada fucata martensii* occurs in Yusu Bay and Uchiumi Bay, Ehime Prefecture, Japan, and mass mortalities have occurred at these same sites every year since 1994, causing loss to the pearl industry. Such mass mortalities have also occurred in other bays in West Japan, and pathological investigations have been conducted to determine the cause (Suzuki et al. 1998, Kurokawa et al. 1999, Miyazaki et al. 1999). These studies have suggested that the mass mortalities were partly due to infectious disease.

In the present study, we investigated the relationships between some environmental factors such as water temperature, chlorophyll *a* concentration and phytoplankton composition and *Pinctada fucata mar-*

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tensii survival at several depths in 2 bays in order to further understand the cause of the observed mass mortalities.

MATERIALS AND METHODS

We carried out experiments in Yusu Bay and Uchiumi Bay, Shikoku west coastal area, Ehime Prefecture, Japan (Fig. 1). Yusu Bay is located within Uwajima Bay where the pearl oyster *Pinctada fucata martensii* and many other species of fish are extensively cultured. Uchiumi Bay is on the south side of the Yura Peninsula and open to Bungo Channel, where fish culture is rare. The water depths at the study sites in Yusu Bay and Uchiumi Bay are ca 50 and 60 m, respectively.

The experimental equipment and the densities of *Pinctada fucata martensii* placed in cages and bags

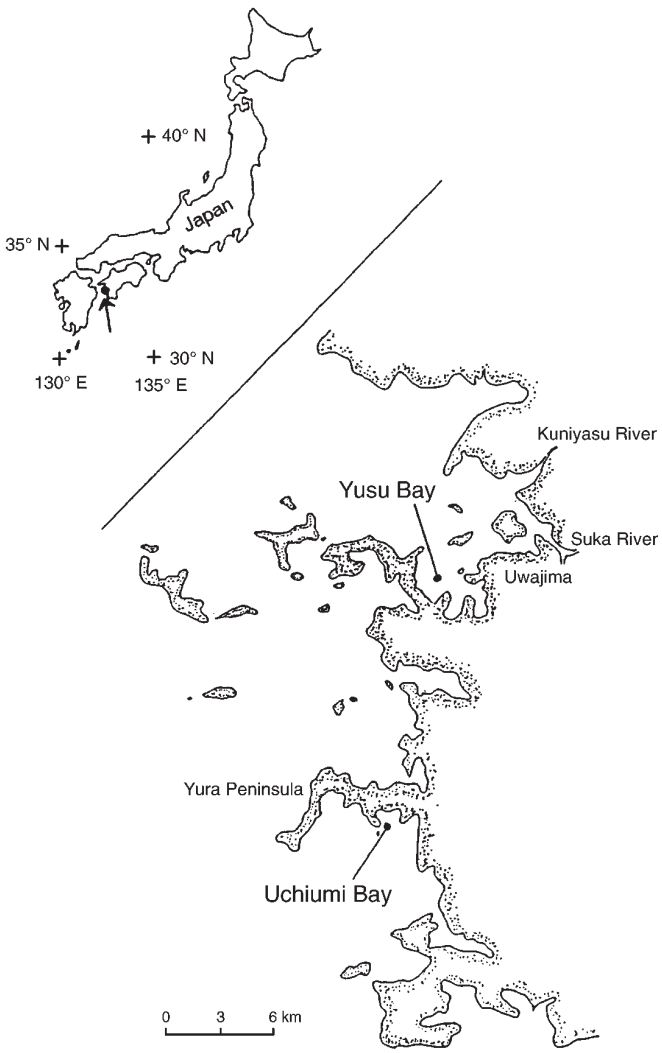

Fig. 1. Location of the study sites

were the same as these used in the actual pearl oyster culture method; the height of an oyster is ca 1 to 2 cm at the beginning of the culture and ca 6 to 7 cm after 1 yr. *P. fucata martensii* were collected on Japanese cedar leaves from 1 to 10 m depths at Uchiumi Bay from June to August, 1997 (Tomaru et al. 1999). They were cultured in blue nylon mesh (1 × 1 cm) cages (30 × 30 cm) at 2, 5 and 30 m in both Yusu Bay and Uchiumi Bay. At both sites, 4 oyster cages containing 100 oysters each were suspended at each depth from September 1997 to January 1998. The 100 oysters from the cages at each depth were randomly subsampled and weighed. After weighing, we returned the oysters to their cages. Fouling organisms were removed with a knife every month from all the oysters. From January to March 1998, 8 cages (50 oysters cage^{-1}) were monitored while 13 cages (ca 30 oysters cage^{-1}) were monitored from March to June 1998 in both bays.

In June 1998, we picked out 100 *Pinctada fucata martensii* at random from the oysters at each depth, and put them into 2 vertical bags (60 × 80 cm) at a density of 50 oysters bag^{-1}. From June 1998 to February 1999, all survivors for the sample month were weighed. The wet weight of each oyster was determined with an electronic balance once a month from September 1997 to February 1999. The number of dead oysters was also counted. Percentage survival (P_S) and cumulative mortality (CM) were calculated as follows:

$$P_S\,(\%) = P_{SB} \times [N_L/(N_L + N_D)]$$
$$CM\,(\%) = 100 - P_S$$

where P_{SB} is the percentage survival of the previous month, and N_L and N_D are, respectively, the number of living and dead oysters in the current month.

In Uchiumi Bay individual oysters could be identified and followed throughout the experiment. Oyster deaths were recorded and grouped into 6 groups: Group A, oysters dead in September; Group B, oysters dead in October; Group C, oysters dead in November; Group D, oysters dead in December; Group E, oysters dead in January; Group F, oysters which survived the study period (Tables 1 & 2). We calculated the average weight each month of oysters in each group (W_D) and those which survived throughout the study period (W_S), and the percentage of W_D to W_S was determined to give the relative weights of those which died.

At both stations water temperature was measured at each depth with a profiler Chlorotech (Alec Electronics Co., ACL-208-DK), and a 50 ml water sample from each depth was filtered through 0.2 µm pore Nuclepore filters to retain seston. The chlorophyll *a* content of the seston was determined by the fluorometric method (Rami & Porath 1980). Integrated water tem-

Table 1. *Pinctada fucata martensii.* Group classification of the oysters which were dead in September, October, November, and December 1998, and in January 1999 and those which survived throughout the study period in Uchiumi Bay

No. of identified oysters	Month of death	Group
1	Sep	A
2	Dec	D
3	Survived	F
4	Sep	A
5	Oct	B
6	Nov	C
7	Oct	B
8	Survived	F
9	Jan	E
10	Dec	D
11	Survived	F
12	Sep	A
13	Nov	C
14	Oct	B

peratures (*WT*) and chlorophyll *a* concentrations (*Chl*) were calculated based on this monthly data:

$$I = \sum_{(i = 1 \sim N)} (WT \text{ or } Chl)_i$$

where *I* and *N* indicate the integrated water temperature or chlorophyll *a* concentration and the number of month, respectively.

A 500 ml water sample taken at 2 m depth in Uchiumi Bay was fixed with acid Lugol's solution at a final concentration of 1% every month during the present experiment, and concentrated to 1000 times by natural sedimentation. Phytoplankton thus concentrated was enumerated with a haemocytometer under a microscope.

RESULTS

Water temperatures at 2, 5 and 30 m began rising about March, peaked in August to September, then declined throughout February in all bays (Fig. 2). Water temperature in Uchiumi Bay was 2 to 3°C higher than that of Yusu Bay during October to February (Fig. 2) at all depths. The minimum water temperatures were 14 and 16.4°C in March, and maxima were 27.9 and 26.5°C in August, in Uchiumi Bay and Yusu Bay, respectively. The maximum water temperature at 30 m occurred in September in Yusu Bay and October in Uchiumi Bay.

Chlorophyll *a* concentrations in Uchiumi Bay (Fig. 3) began rising in May, peaked in November 1998 (5.1 to 5.9 µg l^{-1}) at 2 and 5 m depths and in September 1998 at 30 m (3.7 µg l^{-1}) then declined through January 1999 (0.3 µg l^{-1}). Chlorophyll *a* concentrations in the surface layer were usually higher than the concentration at 30 m during the experiment. In Yusu Bay, the chlorophyll *a* concentrations at 2 and 5 m gradually increased from March 1998, decreased from June to July or August, and temporarily increased in September (12.9 to 15.0 µg l^{-1}), then decreased again (Fig. 3). At 30 m chlorophyll *a* concentration temporarily increased in September 1998 and remained relatively high during November 1998 to February 1999, despite being equal to or lower than that of the surface layer concentrations (Fig. 3).

Table 2. Number of *Pinctada fucata martensii* in groups A, B, C, D, E, and F

Depth (m)	A	B	C	D	E	F
2	21	7	4	10	0	33
5	29	12	3	10	1	34
30	17	14	24	9	0	34

Dominant phytoplankton species during the experiment in Uchiumi Bay (Fig. 4) were *Chaetoceros* spp., *Skeletonema* spp. and *Nitzschia* spp. The density of

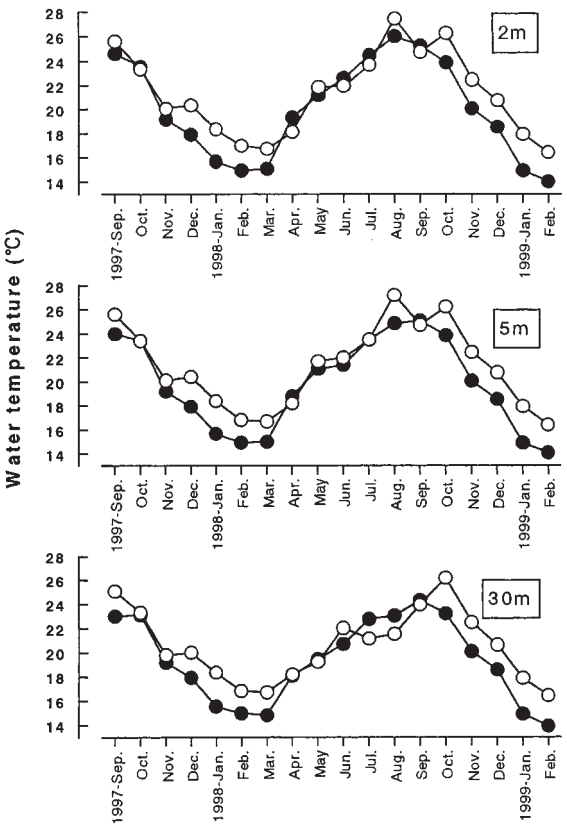

Fig. 2. Water temperature at 2, 5 and 30 m in Yusu Bay (●) and Uchiumi Bay (○) from September 1997 to February 1999

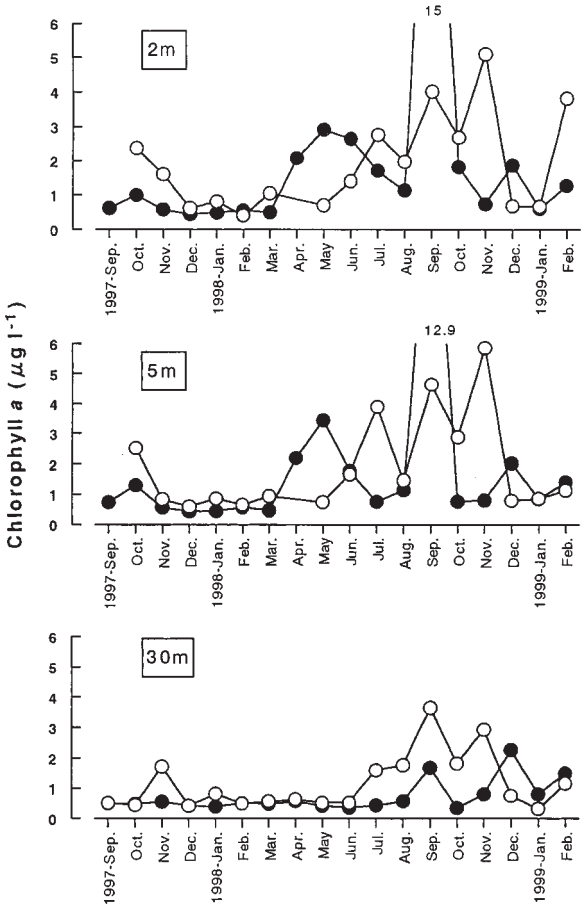

Fig. 3. Concentrations of chlorophyll *a* at 2, 5 and 30 m in Yusu Bay (●) from May 1998 and Uchiumi Bay (○) from September 1997 to February 1999

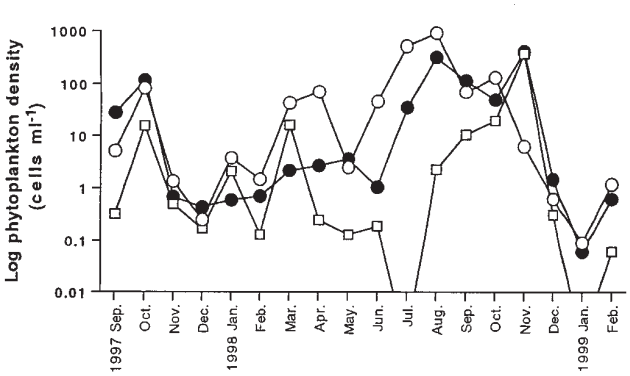

Fig. 4. Cell densities of the dominant phytoplankton, *Chaetoceros* spp. (○), *Skeletonema* sp. (□) and *Nitzschia* spp. (●), respectively, at 2 m in Uchiumi Bay from September 1997 to February 1999

Chaetoceros spp. temporarily increased between September and October 1997 (4 to 90 cells ml^{-1}), decreased through December 1997 before sporadically increasing again up to August 1998 (1000 cells ml^{-1}), and then decreased again through January 1999 (0.1 cells ml^{-1}) (Fig. 4). The seasonal density pattern of *Nitzschia* spp. was almost the same as that of *Chaetoceros* spp. throughout the experiment, as was that of *Skeletonema* spp. until March 1998, after which time this species declined earlier and increased later than the other 2 species. In July 1998 and January 1999 we could not detect cells of *Skeletonema* spp. (Fig. 4).

Oyster growth rates during the whole study period at 2 and 5 m, in both bays, were almost the same, ca 75 mg oyster^{-1} d^{-1}. The pattern of growth at both bays was similar from the beginning of the culture period until March 1998 (Fig. 5). In Yusu Bay, the oysters at 2 m grew particularly well from July 1998 to January 1999, and those at 5 m from June to October 1998. The growth rate of the oysters at 30 m in Uchiumi Bay was almost constant (51 mg oyster^{-1} d^{-1}) and somewhat higher than the growth rate of those at the same depth in Yusu Bay (35 mg oyster^{-1} d^{-1}). Unfortunately, we could not observe the oysters at 30 m in Yusu Bay after October 1998 because the net slipped away.

The percentage survival of the oysters at 2 and 5 m in Uchiumi Bay gradually declined from December 1997 to August 1998, then suddenly decreased steeply from September 1998 to January 1999 (Fig. 6). There was a similar but less pronounced pattern of decline at the same depths in Yusu Bay (Fig. 6). The adductor muscle of the fresh dead oysters at both sites usually had turned a red-brown color. After September 1998 the percentage survival at 2 and 5 m in Yusu Bay was higher than in Uchiumi Bay and, at the end of the experiment, was ca 50% in Yusu Bay but approximately 30% in Uchiumi Bay. At 30 m, in Uchiumi Bay, survival decreased steeply at the start of the experiment to 65% in February 1998. It then decreased only slightly from March to August before decreasing rapidly again after September 1998. Such a remarkable initial decrease in survival was not detected at 30 m in Yusu Bay; here a gradual decline occurred between December 1997 and October 1998.

The relationship between cumulative mortality and integrated water temperature from August 1998 to the end of the experiment was significant with logistic regression analysis ($p < 0.01$) (Fig. 7), whereas that between cumulative mortality and integrated chlorophyll *a* concentration was not significant ($p > 0.05$).

The relative weight of oysters in each group that died (see 'Materials and methods') when compared to survivors tended to decrease from June 1998 to the month before death (Fig. 8), indicating that the physiological state of the oysters whose weight was reduced became worse during the study period.

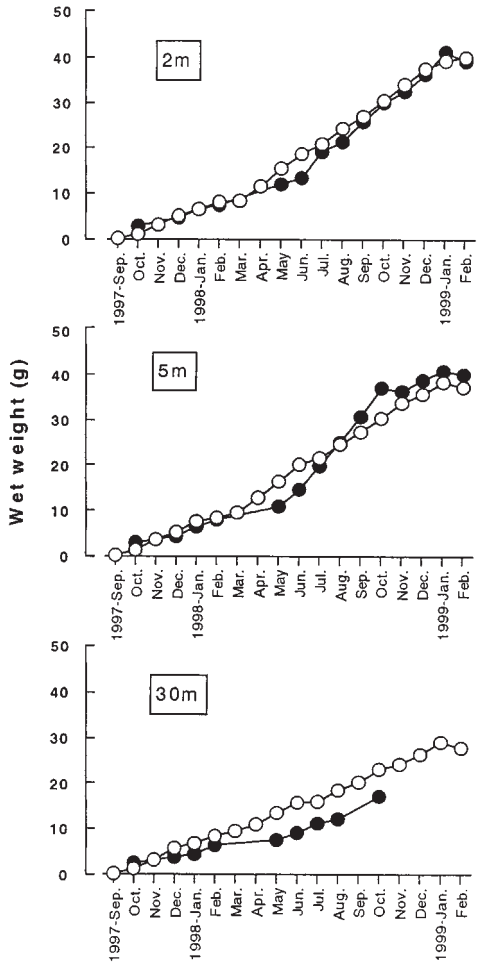

Fig. 5. *Pinctada fuccata martensii.* For surviving oysters: wet weight per oyster at 2, 5 and 30 m for Yusu Bay (●) and Uchiumi Bay (○) from September 1997 to February 1999

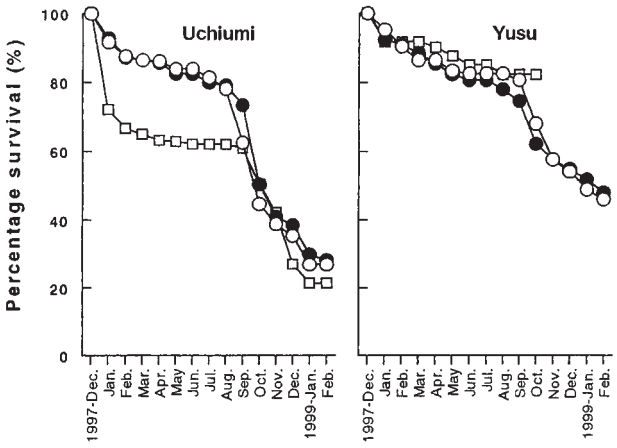

Fig. 6. *Pinctada fuccata martensii.* Percentage survival at 2, 5 and 30 m (○, ● and □, respectively) in Yusu Bay and Uchiumi Bay from December 1997 to February 1999

DISCUSSION

Bivalve activity and mortality are affected by various environmental factors, e.g. water temperature (Widdows 1973, Numaguchi & Tanaka 1986), toxic algal blooms (Shiokawa et al. 1966, Shumway et al. 1990, Numaguchi 1994, Fukuyo 1998), food availability (Toyoshima et al. 1958, Widdows 1978, Numaguchi 1995a,b), and synergistic effects from these factors (Yamaguchi & Hasuo 1977, MacDonald & Thompson 1985).

The physiological activity of bivalves is generally controlled by water temperature. The oxygen consumption of *Pinctada fucata martensii* increases consistently with water temperature from 13 to 28°C (Uemoto 1968). Above this temperature the survival rate decreases with increasing temperature when the oxygen consumption of the oyster suddenly increases (Uemoto 1968, Itoh 1976). *P. fucata martensii* becomes dormant below 13°C (Kobayashi & Tobata 1949). In the present experiment, since water temperatures were all within the range at which *P. fucata martensii* can grow, it is likely that water temperature did not directly affect mortality.

Nevertheless there was a significant relationship ($p < 0.01$) between cumulative mortality and integrated water temperature from August 1998 to the end of the experiment (Fig. 7). This suggests that there was some indirect relationship between the mass mortality of *Pinctada fucata martensii* after August 1998 and water temperature during the period.

Percentage survival of the oysters gradually decreased after August 1998, and after October 1998 survival in Yusu Bay was higher than in Uchiumi Bay (Fig. 6), suggesting that the environmental conditions

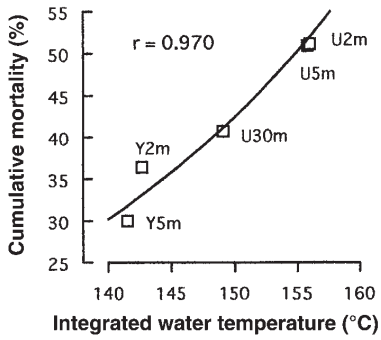

Fig. 7. Relationship between the integrated water temperature and cumulative mortality of *Pinctada fuccata martensii* from August 1998 to February 1999 at 2 and 5 m for Yusu Bay (Y) and 2, 5 and 30 m for Uchiumi Bay (U)

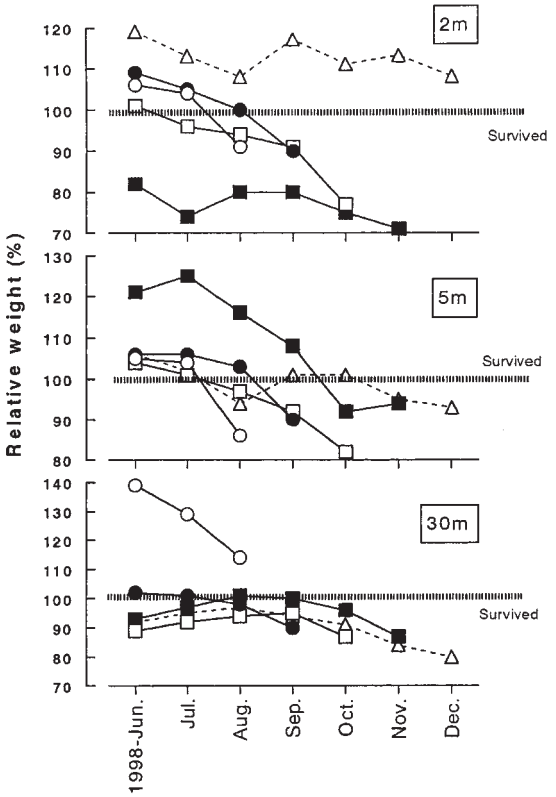

Fig. 8. *Pinctada fuccata martensii.* Percentage of the average weight of the oysters classified as Group A (○), Group B (●), Group C (□), Group D (■), Group E (△), and Group F (▥)

in Uchiumi Bay were less favorable than those in Yusu Bay. It has been shown (Kurokawa et al. 1999) that mass mortality of *Pinctada fucata martensii* is caused by a disease that changes the color of their adductor muscles to red-brown. Pathological studies of the affected oysters showed unusual conditions in their digestive glands and that the disease was caused by an infectious agent (Kurokawa et al. 1999). In the present experiment, the color of the adductor muscle of the oysters was also red-brown, which suggests that the mass mortality have was also caused by the same infectious disease. Therefore, we suggest that there might be some relationship between the infectious disease and the water temperature during August 1998 to February 1999. We are not, however, able to elaborate on the relationship between the disease and water temperature on the basis of the results of the present experiment.

Toxic algal blooms which usually consist of dinoflagellates or Raphidophyceae have caused mass mortality of bivalves (Shumway 1990, Shumway et al. 1990, Fukuyo 1998). For example, high concentrations of sulfide, produced by anoxic water as a result of to decomposition of *Gonyaulax polygramma*, bring about mass mortality of *Pinctada fucata martensii* (Koizumi et al. 1996). The algae *Chattonella antiqua* (Okaichi 1987), *Gymnodinium breve* (Shimizu 1987), and *Heterocapsa circularisquama* (Matsuyama et al. 1995, 1997), due to their toxicity, induce mortality of bivalves. We did not, however, have toxic algal blooms in either bay during the present experiment. Thus, it is unlikely that toxic algae were responsible for the mortality of *P. fucata martensii* in our study.

Shinomiya et al. (1997) suggested that the cause of the mass mortality associated with the red-brown colored adductor muscle in autumn (September to November) was starvation. Even when starved, *Pinctada fucata martensii* can survive using reserves such as glycogen for about 2 mo at water temperatures of 15 to 28°C, but the weight of the oyster does not increase during such a period (Numaguchi 1995a). Prolonged food deficiency for more than 2 mo causes high mortality of *P. fucata martensii* (Numaguchi 1995b). When starving, the whole body weight of *P. fucata martensii* decreases before they die (Numaguchi 1995a,b), which is similar to the results of the present experiment (Fig. 6) in which the average weight of the senescent oysters gradually decreased up to the month before they died (Fig. 8), and this suggests that the mass mortality in the present experiment was due to starvation.

Fukushima (1970) reported that phytoplankton composition was important for the survival of *Pinctada fucata martensii* and that mortalities occurred when algae of the genus *Nitzschia* (Bacillariophyceae), which is an inedible food for *P. fucata martensii*, dominated. In contrast, mass mortality did not occur when *Chaetoceros*, *Thalassionema*, *Bacteriastrum* and *Rhizosolenia*, all of which are edible food for the oyster, were dominant (Fukushima 1970). In the present study, there were high densities of *Nitzschia* spp. (>100 cells ml^{-1}), which accounted for ca 30 to 50% of the phytoplankton density in Uchiumi Bay during August to November 1998 (Fig. 4), and the mass mortality of *P. fucata martensii* occurred at that time. This is similar to the results of Fukushima (1970) and suggests that food limitation and starvation were important factors contributing to the mass mortality of *P. fucata martensii* in the present study, at least in Uchiumi Bay. During the mass mortality event, the abundance of edible phytoplankton *Chaetoceros* spp. available to the oyster was almost the same as that available to *Nitzschia* spp., but it might not have been enough to sustain the activity of *P. fucata martensii*.

Based on the above, we suggest that the mass mortality of *Pinctada fucata martensii* may be explained by the following hypothesis, at least in Uchiumi Bay. When *Nitzschia* spp. dominated in the culture area, the health of the oyster deteriorated due to food limitation and high water temperature (25 to 27°C). Subse-

quently, the digestive glands of *P. fucata martensii* were attacked by an infectious agent. The oyster was thus unable to assimilate food and the resulting mortality was due to nutritional deficiency. Nonetheless, we do not have data on phytoplankton composition and density in Yusu Bay, so this hypothesis needs to be treated with some caution.

Even during the high mortality period from August 1998 to January 1999, the surviving oysters grew well, at 75 and 150 mg whole body weight oyster^{-1} d^{-1} in Uchiumi and Yusu Bay, respectively (Fig. 5). These *Pinctada fucata martensii* growth rates were similar to those of oysters from other bays where there was no mass mortality and the chlorophyll *a* concentration was very similar (Seki 1972, Numaguchi 1994). This suggests that the oysters which survived are resistant to the disease, but it is unclear why, according to the results of the present experiment, survivors grew at a normal rate throughout the mortality period. Further experiments are necessary to fully understand the survival of these oysters.

The percentage survival of the oysters at 30 m in Uchiumi Bay decreased rapidly during the initial winter of 1997 to 1998, whereas that of oysters in Yusu Bay did not (Fig. 6). During that period, there was no difference in water temperature with depth in Uchiumi Bay (Fig. 2) and the concentration of chlorophyll *a* at 30 m was almost same as that at 30 m in Yusu Bay (Fig. 3). Hence, water temperature and food availability do not appear to be associated with this decline. We do not know if the adductor muscle of the survivors at 30 m depth in Uchiumi Bay has a red-brown color or not. Neither can we explain the steep initial decrease in the percentage survival at 30 m in Uchiumi Bay from the results of our study.

In the present study, we have proposed a hypothesis which needs further experimental analysis. We suggest that the mass mortality of *Pinctada fucata martensii* was primarily induced by unusual or suboptimal environmental conditions, such as food limitation, although infectious disease was the final cause of oyster death. Continuous monitoring of various environmental factors in the culture areas is necessary if we are to understand the relationship between an occurrence of bivalve disease and environmental conditions.

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