Patterns, causes, and prevention of the mass mortality of juvenile blacklip pearl oyster Pinctada margaritifera (L.) cultured in Okinawa, subtropical Japan

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Blacklip pearl oyster, spat, mass mortality, subtropical Japan, infection

Abstract

The blacklip pearl oyster, *Pinctada margaritifera* (L.), provides many benefits for aquaculture in Okinawa and subtropical Japan. The spat have died off in massive numbers recently, possibly damaging the aquaculture industry. In the present study, the patterns, causes, and prevention of the mass mortality were investigated. Most investigations were made at 2 to 6 m depths in Kabira Bay, Okinawa, from September to December 2002.
Patterns: The spat were reared in perforated trays for 3 months starting in September 2002. Their cumulative deaths sharply increased starting at the end of October and finally reached and average death rate of 50.8 % while exceeding 90.5 % for half of the trays.
Causes: These deaths are attributable to species-specific factors of *P. margaritifera*, which may include pathogens rather than abiotic factors, predation, spat nutritional condition, or harmful phytoplankton, as indicated below. Only *P. margaritifera* spat showed rapid and massive death (mean cumulative mortality: 49.7 %), when reared together with the bivalves *Barbatia virescens* (Reeve) (17.2 %) and *Gafrarium tumidum* (Röding) (0.8 %), which suggests species-specific fatal factors. The abiotic factors (water temperature, salinity, turbidity, dissolved oxygen) showed no similar temporal fluctuation with the *P. margaritifera* cumulative mortality and/or were within safe ranges (20 to 28 °C, 32.5 to 34.7 PSU, 0.5 to 1.1 FTU, 7.0 to 9.1 mg / l). Predators killed only 2.2 % of spat on average, not accounting for the high cumulative mortality of spat. Malnutrition is unlikely to kill *P. margaritifera* spat quickly and massively, since most spat were found to endure starvation for > 70 days; and no serious malnutrition appears to occur, from sufficient chlorophyll *a* abundance (> 0.36 µg / l).

The abundances of possibly-harmful phytoplankton showed no similar temporal fluctuations with the spat mortality and were within safe ranges (< 5 cells / ml). The existence of a pathogen is suggested from infection experiments: the *P. margaritifera* spat possibly infected with pathogen in the sea were reared together with the spat that had been protected from any pathogen in the laboratory. This resulted in the mortalities of both spat groups. Although the sectioned tissue of the spat from the sea showed no identifiable pathogen during the mass-mortality season, it had deteriorated, and this suggests the symptoms of an infection. Prevention: Pathogens and/or other fatal factors appeared to be influential between November 19 – 26, 2002. 21 groups of *P. margaritifera* spat were reared in the sea and then transferred on different days into the laboratory. Only those transferred after November 19, 2002 showed mortality. Another 21 groups were transferred from the labs to the sea. Only those transferred before November 26, 2002 showed mortality. The other spat from the sea were evacuated into the laboratory between November 19 –26, 2002, and these spat survived. In conclusion, *P. margaritifera* spat begun to die massively from the end of October. This was probably a result of species-specific factors including pathogen, which can be prevented by evacuating spat into the laboratory for a short time during a dangerous period.

Introduction

The blacklip pearl oyster, *Pinctada margaritifera* (L.), produces black pearls, which provide many benefits (Shokita *et al.* 1991). The aquaculture techniques of *P. margaritifera* have been intensively studied since the beginning of 20th century. The spat of *P. margaritifera* can now be reared in the sea to produce black pearls. As a result, *P. margaritifera* is now cultured at many sites in the tropical and subtropical Indo - Pacific regions (Coeroli *et al.* 1984; Shokita *et al.* 1991).

There is, however, a problem. The spat of *P. margaritifera* are vulnerable to environmental stresses (Coeroli *et al.* 1984), and have died in massive numbers in the Solomon Islands, Tuticorin, India, and Marutea, French Polynesia. In the Solomon Islands, approximately 80 % of spat died during a 6 month period in some lantern nets due to predation by fish and invertebrates (Friedman and Southgate 1999). At Tuticorin, India, more than 80 % of spat died during a 45 day period in lantern nets for unknown causes (Alagarswami *et al.* 1989). At Marutea, French Polynesia, “heavy mortality” occurred due presumably to hypoxia (Sano 1998). The patterns and causes of the spat mortalities appear to differ between localities and thus should be investigated at each place.

In Okinawa, subtropical Japan, the spat of *P. margaritifera* appear to die off in massive numbers in some fish farms in recent years (Katsumata and Nakamori 2002, 2003). The spat were artificially produced in the laboratory, reared in trays in the fish farms, and showed mass mortality from September to November (local pearl producers, personal communication). These deaths appears to occur in only the fish farms, not laboratory tanks (Katsumata and Nakamori 2002). Hence, the mass mortality is

attributable in part, at least, to some environmental factors specific to the fish farms. Yet, the patterns, causes, and prevention of the mass mortality have not been fully investigated thus far.

In the present study, we first clarify the mortality patterns of *P. margaritifera* spat unique to a fish farm in Okinawa (Chapter 1). We then reveal the causes of the mortality (Chapter 2). Finally, we develop prevention methods.(Chapter 3).

1. Patterns

Aims

In this chapter, we reveal the patterns of the day-to-day and tray-to-tray variations in cumulative mortality of *P. margaritifera* spat.

Material and Methods

The study site is Kabira Bay (24° 27.3’ N, 124° 8.7’ E) at Ishigaki Island, Okinawa, subtropical Japan **(Fig. 1).** To this bay, pearl producers usually transfer *P. margaritifera* 1 month after an artificial hatch in July through September. They rear *P. margaritifera* at 2 to 6 m depths in lantern nets, trays, and panel nets that are suspended from headlines near the sea surface.

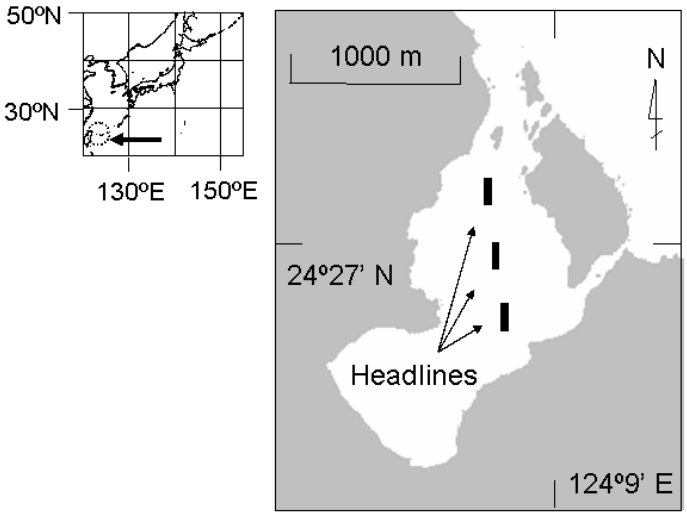

Figure 1. Maps of Kabira Bay.

In the observation of mortality pattern, *P. margaritifera* spat were artificially produced from wild parents. The spat were reared in the laboratory tanks, fed living *Chaetocerous* sp. (> 5,000 cells / ml). On 13th September 2002, 3456 spat with mean ± SD dorsoventral measurement (Sims 1993) being 14.5 ± 2.4 mm were transferred to the study site. These spat were kept in perforated trays with a lid (33 cm W × 21 cm L × 8 cm H; covered with a 2 mm × 2 mm mesh). Rearing conditions were varied among the trays. There were either 12, 24, 36, 72 or 144 spat placed in each tray. Trays were suspended at depth of either 2 or 6 m. The number of cells dividing spat was either one or four. Such variations between trays resulted in no significant difference in among the cumulative mortality rate (Kurihara *et al.* submitted). For each rearing condition, 3 trays were prepared (60 trays in total) and suspended from different headlines **(Fig. 1**). Under each headline, the trays were arranged in a random order at 1.4 m intervals in a horizontal direction. On a boat, these trays were checked weekly for the count of dead spat and were washed for the removal of fouling until 19th December 2002. The dead spat and predators (ranellid gastropods, portunid crabs, polyclads) were removed.

The cumulative mortality for each survey date was calculated for the spat in each tray according the following formulas: 100 × (cumulative number of individuals having died until the date) / (total number of individuals at the start of survey). The cumulative mortality was averaged across: 1) all 60 trays; 2)the 30 trays in the upper-half class in which cumulative mortality was the 1^{st} to 30^{th} on the last survey date; 3) and the 30 trays in the remaining, lower-half class. Such calculations for different classes can show a tray-to-tray variation in cumulative mortality.

Results

The averaged cumulative morality averaged across all trays sharply increased on October 31st, finally reaching 50.8 % (**Fig. 2**). Such a sharp rise was caused by rapid increases in cumulative mortality by only a few tray. On the last survey date, the mean mortality for the upper-half class reached as high as 90.5 %, whereas that for the lower-half class remained only 11.1 %.

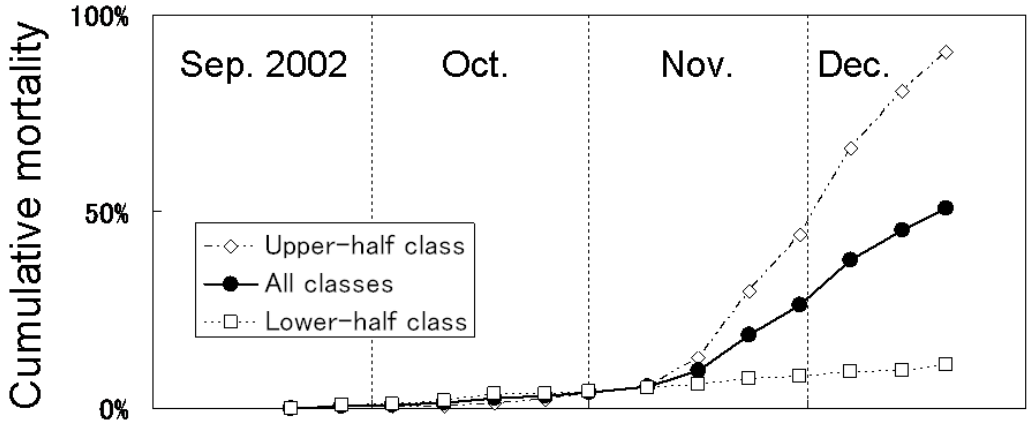

Figure 2. *Pinctada margaritifera*. Mean cumulative mortalities (%) for trays in the lower-half, upper-half, and all classes.

Discussion

Both the average and tray-to-tray variation of *P. margaritifera* spat mortality are much higher in the present study site than in previous study sites (Friedman and Southgate 1999; Southgate and Beer 2000). Further, the mortality rate in some trays increased nearly 90 % within 2 months, and such rapidness had never been reported previously (Alagarswami *et al.* 1989; Friedman and Southgate 1999; Southgate and Beer 1997, 2000). The next chapter explores why such massive and rapid mortality occurred in only a few trays.

2. Causes

Aims

In this chapter, we examine the causes of the mass mortality found in *P. margaritifera* spat. First, we examine whether only *P. margaritifera* spat died off in massive numbers among several bivalve species. If so, the *P. margaritifera* mortality may be due to species-specific factors, and not to more general factors such as harmful phytoplankton (Parry *et al.* 1989; Fukuyo *et al.* 2002). We then examine the factors that have been related with the mass mortalities of cultured bivalves (Ford 1992; Burreson

and Calvo 1996; Friedman and Southgate 1999; Fukuyo *et al*. 2002; Gosling 2003), namely: (a) abiotic factors (water temperature, salinity, turbidity and dissolved oxygen); (b) predation; (c) nutritional condition of spat; (d) harmful phytoplankton; and (e) pathogen.

Materials and Methods

In this chapter, we mainly investigated in Kabira Bay. First, we conducted three-species rearing experiments in which *P. margaritifera* were reared with filter-feeding bivalves, an ark shell *Barbatia virescens* (Reeve, 1844), and a Venus clam *Gafrafium tumidum* (Röding, 1798). These two species were chosen because preliminary experiments demonstrated that they were easily handled and because the species were abundant near the study site. The two species were collected between September 5-9, 2002. Each of *B. virescens* (mean ± SD shell length = 23.4 ± 6.6 mm; the number of measured individuals = 63) and *G. tumidum* (29.6 ± 4.3; 43) were reared in 18 trays consisting of one cell with no sediments. The rearing conditions were the combinations of 12, 24, or 36 individuals per tray at 2 or 6 m depths with three replications. The trays holding *B. virescens* and *G. tumidum* were suspended under the foregoing *P. margaritifera* trays (Chapter 1) in a way that the three trays with the same rearing condition were set along the same vertical rope (termed as "3-Sp set"). *B. virescens* and *G. tumidum* having died were counted during the observation of *P. margaritifera* mortality pattern (Chapter 1).

The water temperature, salinity, and turbidity were measured at 2 and 6 m depths near the center of each headline (**Fig. 1**) simultaneously with the observation of *P. margaritifera* mortality pattern (Chapter 1). They were determined by censors "Compact-CT" and "Compact-CL" (Allec Co. LTD. http://www.alec-electronics.co.jp/) with the accuracies of ± 0.02 °C, ± 0.02 PSU, and ± 2 FTU. Dissolved oxygen was measured at 3 m depth near the center of the central headline at 30-minute intervals from 10th October to 24th December 2002. These measurements used the "Compact-DOW" (Allec Co. LTD.) with the accuracy of ± 0.001 mg / l.

The mortality of *P. margaritifera* spat due to predation was evaluated for each tray. Dead *P. margaritifera* whose shells were attached or probably broken by predators (i.e., ranellid gastropods and portunid crabs) were counted and compounded with observations of *P. margaritifera* mortality pattern (Chapter 1). Cumulative predation rate for each date was calculated as: 100 × (cumulative number of individuals having died of predation until the date) / (total number of individuals at the start of survey).

To examine whether malnutrition of *P. margaritifera* spat caused the mass mortality, various investigations were conducted. First, the abundance of phytoplankton, the food of *P. margaritifera* spat, was estimated from chlorophyll *a* abundance (μg / l), measured at 2 and 6 m depths near the center of each headline (**Fig. 1**). This measurement concurrently compared the observation of the *P. margaritifera* mortality pattern (Chapter 1), using a Compact-CL with the accuracy of ±0.02 μg / l. Further, the nutritional condition of *P. margaritifera* was estimated from glycogen content, RNA/DNA ratio, and the relative weight of digestive organ. The higher these indices are the better the nutritional condition may be. Each index was measured at 3 to 7 day intervals from September 17 to December 3, 2002. On each day, the glycogen content (i.e., the ratio of glycogen weight to soft-tissue wet weight; ‰) and RNA/DNA ratio (the ratio of RNA weight to DNA weight) were measured following the methods described in Okumura *et al.* (2002) and references therein for 20 oysters. Of these, 10 oysters had been kept at 3 m depth in Kabira Bay, and the remaining 10 had been reared in tanks in the laboratory since September 12, 2002. The relative weight of the digestive organs (= wet weights of stomach and style sac / wet weight of all soft tissue) was measured for 10 one year old *P. margaritifera* that had been kept at 3 m depth in Kabira Bay since 12th September 2002. In addition to these investigations, starvation experiments were conducted to examine how long *P. margaritifera* spat can endure starvation. 10 *P. margaritifera* spat were kept from July 22 - November 19, 2002 in each of four aquariums (35.0 cm W × 21.5 cm L × 26.0 cm H) in the laboratory. The spat were fed living *Chaetocerous* sp. (> 5,000 cells / ml) in two aquariums, whereas others starved in the remaining two. Seawater wsa added to these aquariums and regulated at 25 °C or left natural. The mortalities of the spat were checked every day.

The abundance of possibly harmful phytoplankton (Parry *et al.* 1989; Fukuyo *et al.* 2002) was estimated by sampling 5 l sampled of seawater at 3 m depth near the central headline (**Fig. 1**) at the intervals of 3 to 7 days between September 17 - December 24, 2002. The seawater sampled was preserved with 5% formalin and filtered through 10-μm mesh. The sample remaining on the mesh was identified to the lowest possible taxonomic level. The phytoplankton was found to consist of as many as 42 taxonomic groups (mainly species). Of these, only possibly - harmful phytoplankton were analyzed, after grouped at either family level (Prorocentraceae, Gymnodiniaceae, Peridiniaceae, and Rhizosoleniaceae) or order level (when family-level identification was difficult; Gymnodiniales and Peridiniales).

Infection experiments were conducted to examine whether some infectious causing fatality factors could spread from spat to spat. They were carried out in 2001 (Run 1)

and 2002 (Run 2) Each year 10 to 20 possibly infected spat were taken from the sea just after *P. margaritifera* spat died off in massive numbers in Kabira Bay. They were transferred into either 4 or 7 aquariums in the laboratory with running seawater. We also transferred 10 to 60 spat that had been kept in the laboratory (thus, unlikely to be infected by pathogen) into each aquarium. Only when some of the spat from the sea were infected by pathogen would both groups from the sea and laboratory die. Thus, mortalities of both groups would be positively correlated. The spat were kept in aquariums for 30 to 40 days. Their mortality rates during such different periods were unified by calculating mortality per 35 days according to Akçakaya et al. (1999). In addition to the infection experiments, the soft tissue of spat was observed for symptoms of infection by pathogen. Spat were sampled from a fish farm in Urasoko Bay (24°27' N, 124°13' E) near Kabira Bay just before and after the massive kills on October 8 and 12, 1999. The spat were preserved in Davidson's Solution. The sections of its gills were stained with hematoxylin-eosin for the preparation of histological slides.

Results

In the three-species rearing experiments, the cumulative mortality of *P. margaritifera* showed species-specific fluctuation patterns (**Fig. 3**). Mortality rates sharply increased after October 31st, finally getting very high: the means within the lower-half class, across all trays, and within the upper-half class were 10.7 %, 49.7 %, and 88.7 %, respectively. In contrast, the cumulative mortality of *B. virescens* slowly increased after October 11 and finally remained low (4.1 %, 17.2 %, and 30.4 %). The cumulative mortality of *G. tumidum* remained very low throughout the experiments (0.0%, 0.8%, and 1.6%).

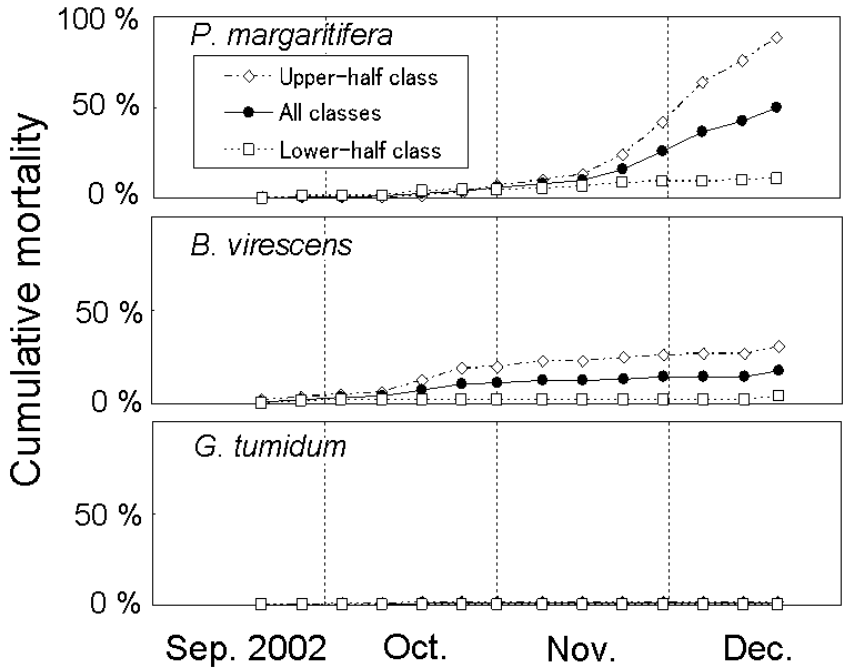

Figure 3. *Pinctada margaritifera, Barbatia virescens, and Gafrarium tumidum*. Mean cumulative mortalities (%) for trays in the lower-half, upper-half, and all classes.

Among abiotic environmental factors (**Fig. 4**), only the water temperature correlated with the sharp increase in *P. margaritifera* cumulative mortality rates after the end of October (**Fig. 2**). From this period, the mean water temperature decreased from 26.3 °C to 20.2 °C (max.: 28.1 °C on 4th October). After the same period, the averages of salinity (33.6 to 34.4 in range during the study period), turbidity (0.5 to 1.1 FTU), dissolved oxygen concentrations (7.0 to 9.1 mg / l) repeatedly increased and decreased.

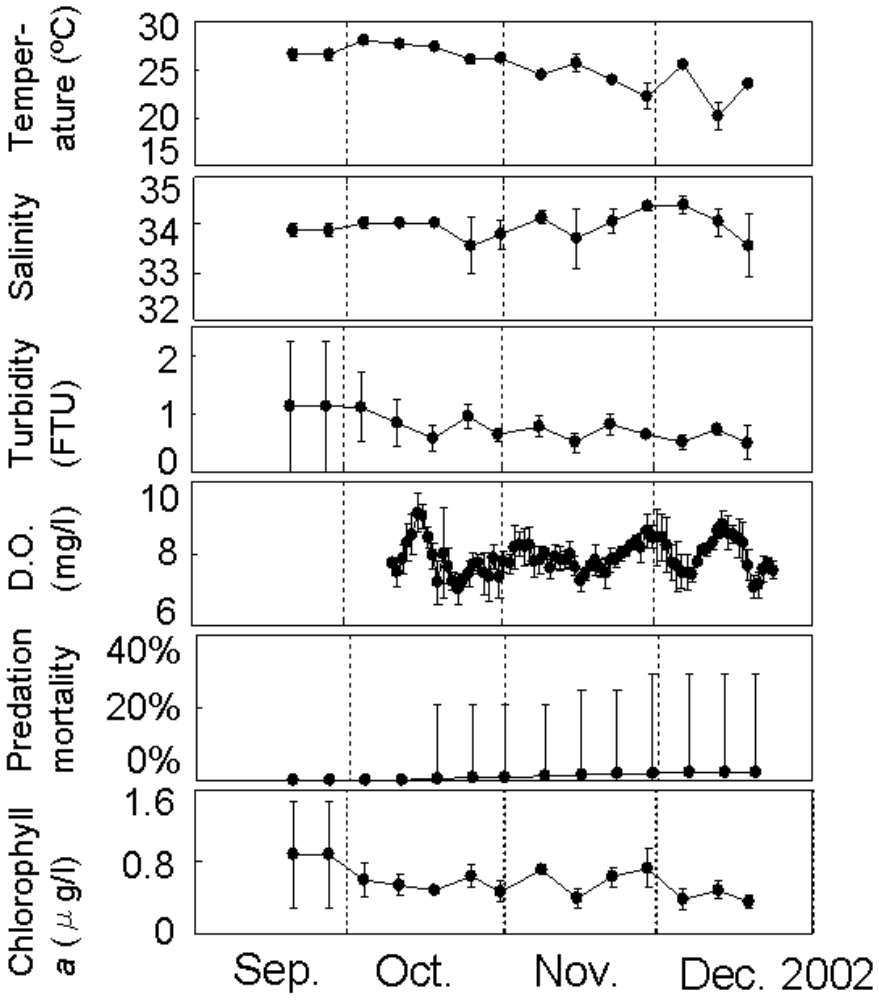

Figure 4. Mean water temperature (ºC), salinity, turbidity (FTU), dissolved oxygen (mg / l), cumulative predation rate (%), and chlorophyll *a* abundance (μg / l). Error bars denote either [maximum minus mean] of predation mortality rate or ± 1 SD of the other factors.

Cumulative predation rate (**Fig. 4**) did not correspond to the sharp increase in *P. margaritifera* mortality. The average of cumulative predation rate slowly increased from 11th October, namely, before the outbreak of *P. margaritifera* mass mortality. On the last day of the survey, it remained only 2.2 %, and the maximum was only 29.2 %.

The mean abundance of chlorophyll *a* repeatedly increased and decreased (range: 0.36 to 0.88 μg / l; **Fig. 4**), not corresponding to the sharp increase in *P. margaritifera* cumulative mortality after the end of October. On the other hand, the indices of *P. margaritifera* nutritional condition (**Fig. 5**) corresponded somewhat to the *P. margaritifera* mortality pattern. That is, glycogen content and RNA/DNA ratio of the *P. margaritifera* in Kabira Bay gradually decreased from 8th or 15th October to 3rd December. And, relative weight of digestive organ remained low between 24th September and 26th November. In the starvation experiments (**Fig. 6**), it took at least 70 days under each temperature condition for the mortality of starved *P. margaritifera* to exceed the mortality of fed *P. margaritifera* by > 20 %.

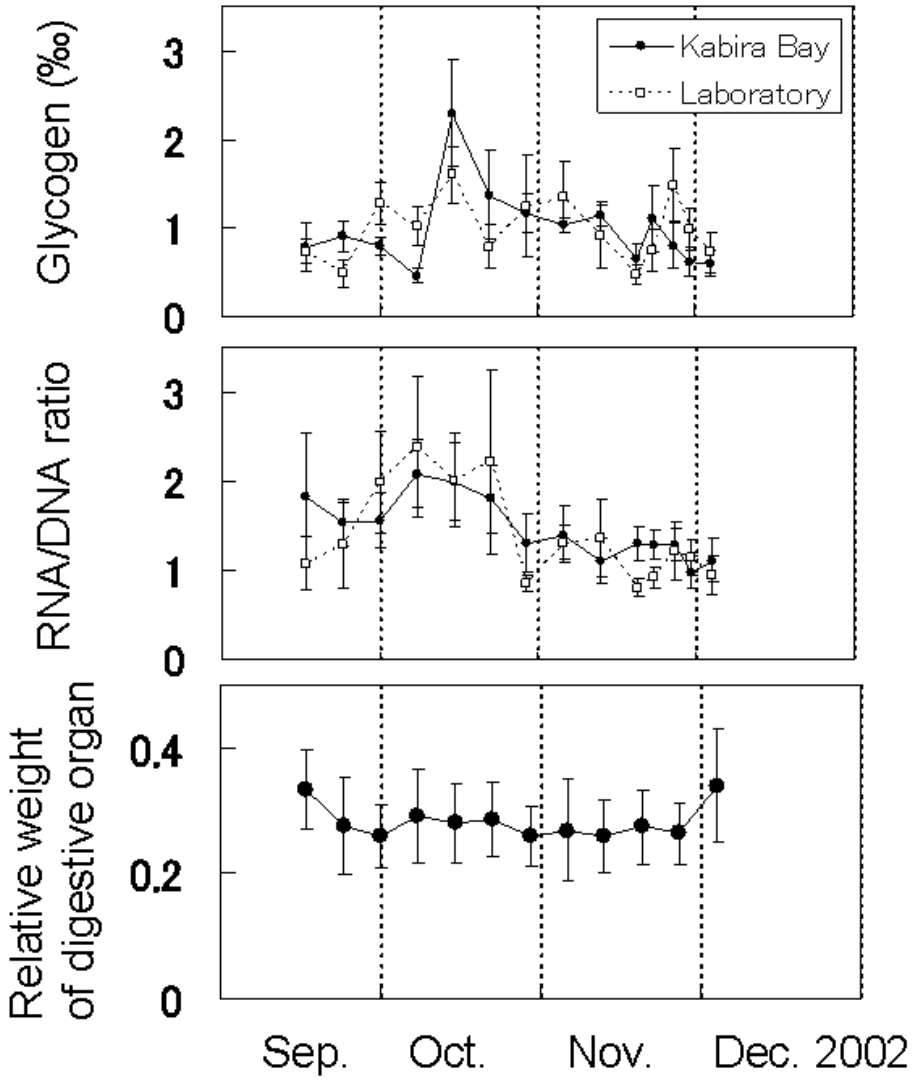

Figure 5. *Pinctada margaritifera*. Mean ± SD glycogen content (‰), RNA/DNA ratio, and relative weight of digestive organ.

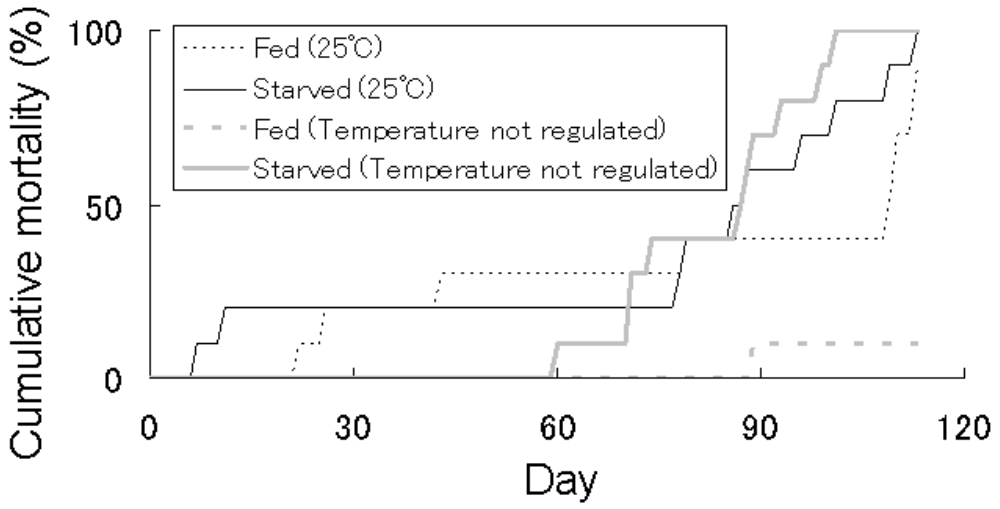

Figure 6. *Pinctada margaritifera*. Cumulative mortalities (%) in fed and starved spat.

The phytoplankton consisted of 42 taxonomic groups, each of which was scarce (mean abundance < 2.24 cells / ml; **Table 1**). These included 6 possibly-harmful groups: Prorocentraceae, Gymnodiniales, Gymnodiniaceae, Peridiniales, Peridiniaceae, and Rhizosoleniaceae. The abundances of these groups were low on each date (< 4.60 cells / ml; **Fig. 7**). They fluctuated up and down and did not correspond to the increase in *P. margaritifera* cumulative mortality rates after the end of October.

Table 1. Abundances of phytoplankton collected at 3 m depth (the number of samples: 27)

Possibly-harmful group	**Taxon**	**Mean ± SD abundance (cells / ml)**
PROROCENTRACEAE	*Prorocentrum mexicanum*	0.326 ± 0.811
GYMNODINIALES	GYMNODINIALES	0.716 ± 0.954
GYMNODINIACEAE	*Gymnodinium sanguineum*	0.037 ± 0.157
	Gymnodinium sp.	0.022 ± 0.115
PERIDINIALES	PERIDINIALES	0.307 ± 0.452
PERIDINIACEAE	*Protoperidinium bipes*	0.004 ± 0.019
	Protoperidinium oceanicum	0.004 ± 0.019
	Protoperidinium sp.	0.199 ± 0.395

RHIZOSOLENIACEAE	*Rhizosolenia alata (Proboscia alata)*	0.015 ± 0.046
	Rhizosolenia imbricata	0.230 ± 0.637
	Rhizosolenia stolterfothii (Guinardia striata)	0.122 ± 0.345
	Rhizosolenia sp.	0.015 ± 0.053
Others	Oscillatoriaceae	0.196 ± 0.313
	CRYPTOMONADALES	1.727 ± 2.843
	Distephanus speculum var.octonarius	0.004 ± 0.019
	Thalassiosira sp.	0.322 ± 0.879
	Thalassiosiraceae	0.393 ± 0.680
	Leptocylindrus danicus	0.141 ± 0.692
	Cerataulina dentata	0.004 ± 0.019
	Cerataulina pelagica	0.000 ± 0.000
	Bacteriastrum delicatulum	0.004 ± 0.019
	Chaetoceros curvisetum	0.115 ± 0.340
	Chaetoceros denticulatum	0.007 ± 0.038
	Chaetoceros pseudocurvisetum	2.000 ± 9.298
	Chaetoceros subtile	0.059 ± 0.308
	Chaetoceros sp.	2.104 ± 1.646
	Odontella sp.	0.007 ± 0.038
	Asterionella glacialis	0.411 ± 0.814
	Asterionella bleakeleyi var.notata	0.048 ± 0.231
	Asterionella sp.	0.022 ± 0.115
	Licmophora sp.	0.007 ± 0.027
	Striatella unipunctata	0.004 ± 0.019
	Thalassionema nitzschioides	0.156 ± 0.506
	Navicula sp.	1.678 ± 2.684
	Pleurosigma sp.	0.467 ± 0.912
	Bacillaria paxillifera (syn.Bacillaria paradoxa)	0.052 ± 0.233
	Cylindrotheca closterium	1.514 ± 3.031
	Nitzschia longissima	0.137 ± 0.248
	Nitzschia sp.	2.156 ± 3.768
	PENNALES	2.244 ± 1.913
	PRASINOPHYCEAE	0.104 ± 0.378
	Unidentified micro-flagellate	28.130 ± 18.325

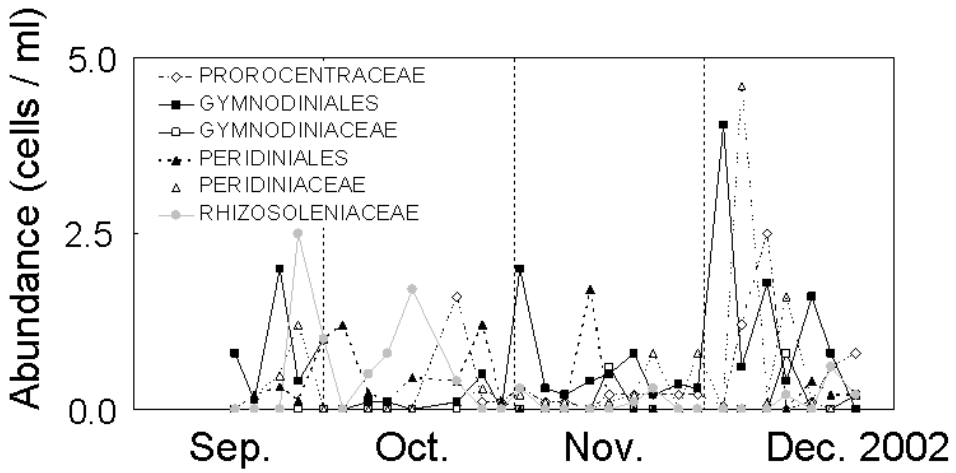

Figure 7. Abundances (cells / ml) of possibly - harmful groups of phytoplankton

In both runs of the infection experiment, the *P. margaritifera* collected from the sea and those having been kept in the laboratory showed positive correlations with the mortality rates (**Fig. 8**). A *P. margaritifera* had a normal tissue just before the breakout of mass-mortality, whereas after the breakout it possessed a deteriorated tissue consisting of shrunken cells without any recognizable pathogen (**Fig. 9**).

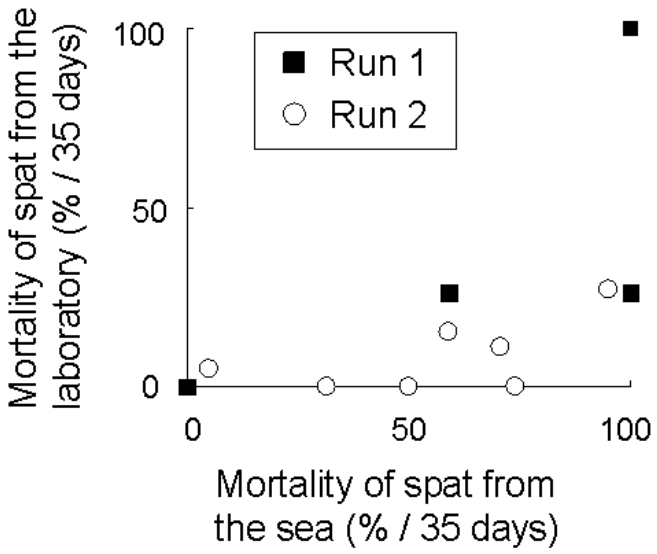

Figure 8. *Pinctada margaritifera*. Mortalities (% / 35 days) of spat groups from the sea and laboratory, presented for Runs 1 and 2 in the infection experiments.

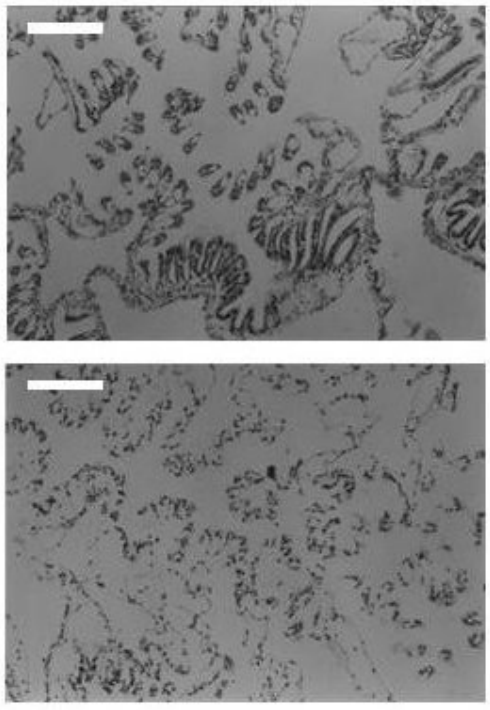

Figure 9. *Pinctada margaritifera*. Gills in spat before and after the outbreak of mass mortality (upper and lower panels, respectively). Scale bar: 1 mm.

Discussion

The mass mortality of *P. margaritifera* spat appears to be due mainly to its species-specific factors. This is suggested by the three-species rearing experiments in which the cumulative mortality of only *P. margaritifera* sharply increased after the end of October, finally getting very high in nearly half the trays. Although such species-specific factors might be intensified by some non-specific stresses (as suggested from the death of some *B. virescens* reared with *P. margaritifera*), the non-specific stresses themselves are somewhat unlikely to explain the sudden and massive death of only *P. margaritifera*.

Such species-specific fatal factors for *P. margaritifera* are unlikely to include abiotic environmental factors. Although the water temperature showed a similar pattern of temporal fluctuation with the cumulative mortality of *P. margaritifera*, its direct influence on the mass mortality is unlikely. This conclusion is drawn from the range of water temperature during the study period, 20 to 28 °C, in which *P. margaritifera* seldom died in laboratory tanks (see Chapter 3). In addition, the range was approximately within the optimum ranges regarding bioenergetic terms (23 to 28 °C; Yukihira *et al.* 2000) and oxygen consumption (15 to 33 °C; Sugiyama and Tomori 1988). The salinity showed a temporal fluctuation dissimilar to the mortality fluctuation. Further, it was within the range of 32.5 to 34.7, in which *P. margaritifera* is reported to survive in the present study site (25.0 to 34.5: Katsumata and Nakamori 2002). The turbidity fluctuation was dissimilar from the mortality fluctuation. The dissolved oxygen was also dissimilar and, in addition, appears to be sufficient for shellfishes to survive (> 7.0 mg /l).

None of predation, possibly-harmful phytoplankton, and malnutrition may appear to have directly killed many *P. margaritifera* spat either. Predators killed only 2.2 % of *P. margaritifera* on average, not accounting for the high cumulative mortality of *P. margaritifera* (mean: 50.8 %). The abundances of possibly-harmful phytoplankton temporally changed in dissimilar manners from the *P. margaritifera* mortality. In addition, they were far lower (< 5 cells / ml) than the abundance of a phytoplankton species previously reported to kill bivalves (> 50 to 20,000 cells / ml: Parry *et al.* 1989; Matsuyama *et al.* 1997, 1998; Nagai *et al.* 2000). Further, although harmful phytoplankton kills many bivalve species simultaneously in general (Parry *et al.* 1989;

Fukuyo *et al.* 2002), such phenomena were not found in the three-species rearing experiments. Malnutrition may not directly lead to the mass mortality of *P. margaritifera* spat either. This is because food abundance may be sufficient for the survival of *P. margaritifera*, as suggested by the chlorophyll *a* abundance exceeding 0.36 μg / l (Vacelet *et al.* 1996). Further, malnutrition may not quickly kill *P. margaritifera* spat, as suggested by the long-term survival of experimentally starved spat. Yet, it should be noted that the nutritional condition indices of the spat were low during the *P. margaritifera* mass-mortality period. The nutritional condition might perhaps be so low that the spat would be vulnerable to some fatal factors.

In comparison with the foregoing factors, pathogen are more likely to kill many *P. margaritifera* spat. This is indicated from the infection experiments in which the spat possibly infected with pathogen apparently caused the death of the other spat through infection. Pathogens can only properly explain the rapid and massive death of spat found a part of trays (Chapter 1). Pathogen may easily spread within only a part of trays that held infected spat because to the closed environment and the aggregation of spat with byssus (Southgate and Beer 2000). Although no pathogens were identified, the existence of pathogens could also explain the deteriorated tissue found in spat during the mass-mortality period. However, the results of the infection experiments should be carefully interpreted. A priori environmental factors might be much worse in one aquarium than in another aquarium. Thus in the former aquarium each spat might die more easily, and this would lead to apparent infection from spat to spat. Even if a dead spat has no pathogen, the dead body itself might do damage to the other spat in the same aquarium through, say, perishing the water, which may also lead to apparent infection.

3. Prevention

Aims

The sharp increase in cumulative mortality from the end of October (Chapter 1) might indicate that pathogens and/or other fatal factors are influential within only a short period. If so, one might be able to prevent the mass mortality by evacuating *P. margaritifera* spat from the sea to a safer place during this short period. In this chapter, we first examine whether pathogen and/or other fatal factors are influential only for a short period. We then examined whether evacuation during such a period is effective to avert the mass mortality. We evacuated the spat into the laboratory tanks with filtered

sea water because this has been suggested to be safe for the spat by local pearl producers through personal communications.

Material and Methods

We conducted the transference experiments to determine when *P. margaritifera* spat begin to be exposed to danger in the sea (the first experiment) and when the vulnerability ends (the second experiment). In the first experiment, 21 groups of spat (46 to 50 individuals / group) had been reared at 3 m depth at the central headline (**Fig. 1**) in Kabira Bay starting on September 12, 2002. Each group was evacuated into the laboratory aquariums on different days at nearly weekly intervals from September 17, 2002. These evacuated groups continued to be reared in the aquariums until December 24, 2002 or later, and their cumulative mortalities were determined. If it was too late to evacuate a group from risk factors occurring in Kabira Bay, the group would show very high mortality. Hence, by examining the evacuation dates from which spat groups begun to show high mortality, the beginning of dangerous period may be estimated. In the second experiment, the spat were transferred in the opposite direction of the first experiment. Twenty-one groups of spat (12 to 50 individuals / group) had been reared in the laboratory since its birth. Each group was transferred to 3 m depth in Kabira Bay on different days at nearly weekly intervals from September 17, 2002. These transferred groups were reared in Kabira Bay until February 13, 2003, and their cumulative mortalities were determined. If a group was transferred too early to Kabira Bay while fatal factors were still influential, such a group would show very high mortality. Hence, by examining the transference dates results the end of dangerous period may be estimated.

We conducted evacuation experiments, considering the results of the above-mentioned experiments that the dangerous period may be November 19-26, 2002 (see Results). In this period only, four trays holding 12 to 38 spat were evacuated in aquariums in the laboratory. Except for this period, the trays were suspended at 3 m depth in Kabira Bay from September 12, 2002 - February 13, 2003. The cumulative mortality was estimated for each tray. For each experiment, cumulative mortality was unified by calculating mortality per 100 days according to Akçakaya et al. (1999).

Results

Of the *P. margaritifera* groups evacuated from the sea to the laboratory, only those evacuated after November 19th showed high mortality frequently (22.4 to 95.6 % / 100 days; **Fig. 10**). Of the groups transferred in the opposite direction, only those transferred before 26th November died often (6.6 to 77.6 % / 100 days). Thus, November 19-26 was the period within which the *P. margaritifera* groups transferred in either direction died was. All of the additional four spat groups evacuated around this period survived.

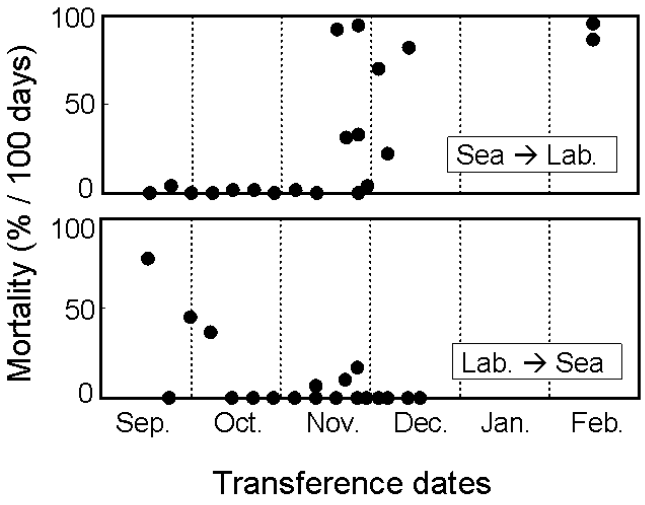

Figure 10. *Pinctada margaritifera*. Mortalities (% / 100 days) of spat groups transferred from the sea to the laboratory (upper panel) and from the laboratory to the sea (lower panel).

Discussion

The transference experiments and evacuation experiments suggest that fatal factors existed between November 19-26, approximately. During this period, fatal factors such as pathogens may be greatly influential in the sea, and/or their effects may be intensified by the other factors such as malnutrition of *P. margaritifera* spat (see Chapter 2). Such a dangerous period is likely to differ between years and between sites in Kabira Bay. That is, during yrs 2000 to 2002 the spat began to die off in massive

numbers at the end of October (Chapter 1; Katsumata and Nakamori 2002, 2003), whereas before then they had started to die earlier, such as the beginning of October (Kurihara, personal data; local pearl producers, personal communication). In trays within a 10 to 100 m distance from each other, the spat started to die with a difference of 30 days at maximum (Katsumata and Nakamori 2003; Kurihara, personal data). Hence, considering such annual and spatial variations in dangerous periods, one should evacuate spat for a long period that is inclusive of November 19-26. To determine the evacuation period, one should monitor spat mortality in a part of the trays every autumn at various sites in Kabira Bay. If some of the trays begin to show high mortality, one should evacuate the other trays into the laboratory; and if monitored trays stopped showing mortality, one may as well return the evacuated trays to the sea.

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