



Studies on mass summer mortality of cultured zhikong scallops (*Chlamys farreri* Jones et Preston) in China

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Abstract

Mass mortalities of cultured zhikong scallops (*Chlamys farreri*) have occurred each summer in most culture areas of northern China since 1996. Among the hypothesized causes are high culture density, infectious disease and genetic inbreeding. To investigate these potential agents, *C. farreri* were deployed at three densities (low, medium and high) at three sites (Jiaonan, Penglai and Yantai) in the summer of 2000. Scallops were sampled for survival, growth and histopathology before, during and after a mortality episode. Most of the mortality occurred in July and August, during and toward the later part of the spawning season, when water temperature reached 23–26 °C. Final cumulative mortalities reached 85% to 90% at all three sites. Scallops in the medium and high densities had higher initial death rates than did those at the low density. High densities also inhibited growth. Ciliates from the genus *Trichodina*, larvae of various organisms and anomalous secretions were observed in sections of the gill cavity, with highest prevalence during and at the end of the mortality period. Prokaryotic inclusion bodies were found in the soft tissues, but their prevalence was low and apparently without correlation with mortalities. Genetic analysis with random amplified polymorphic DNA markers showed slightly lower heterozygosity in the cultured stocks (0.301) than in the wild stocks (0.331). It is possible that the mortalities are caused by a combination of several factors such as stress associated with reproduction, high temperature, overcrowding and poor circulation in the growout cages, opportunistic invaders or pathogens, and possibly inbreeding.

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1. Introduction

The zhikong scallop, *Chlamys farreri* Jones et Preston, is one of the most important cultured molluscs in China. In 1996, China produced 1 million

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metric tons of scallops by aquaculture, about 75–80% of which were from the zhikong scallop (Guo et al., 1999). The coast of Shandong province (Fig. 1) is the main culture area with about 80% of the total production. The *C. farreri* culture was first developed between 1973 and 1983, using hatchery-produced seed, in the Shandong province. After 1983, culture operations greatly expanded in northern Shandong, and cultured populations gave rise to naturalized populations. Consequently, “wild” seed collected from the hatchery-derived populations became abundant and were used for scallop culture. In the decade between 1986 and 1996, total production increased 42 fold (Zhang and Yang, 1999a; Guo et al., 1999). Zhikong scallops are cultured in lantern nets on suspended longlines. To augment production, farmers have been growing scallops at increasing densities within lantern nets and adding more longlines to the culture areas. Then in the mid-1990s, summer mortalities began occurring with increasing frequency.

Heavy mortality of cultured zhikong scallops was first observed in 1994 at Rizhao (Shandong province, Fig. 1), the southern most site for zhikong scallop culture (Guo et al., 1999). From 1996 on, the continuing heavy mortalities have been widespread, affecting most zhikong scallop farms along both southern and northern coasts of Shandong. Zhikong scallops are grown from wild seed collected in autumn for nursery

growout, then placed in lantern nets for final growth. Deaths occur as the scallops are entering their second year, from late July into August depending on the site, when the water temperature reaches 25 °C.

Production has declined sharply as a consequence of the mortalities. For example, over 60% of cultured zhikong scallops died during the summer of 1997 in Shandong province, resulting in a loss of about US\$180 million (Zhang and Yang, 1999b). The cause of mortalities has not been identified, but among the hypothesized causes are excessive stocking densities, pathogens, and inbreeding (linked to the original hatchery seed). In an effort to methodically document the mortality and test these hypotheses, we deployed scallops at three densities in commercial lantern nets at three culture sites in Shandong province in 2000. Samples were collected for mortality, growth, histopathological and genetic analysis before, during, and after the mortality.

2. Materials and methods

2.1. Deployment and sampling at three sites in the Shandong province

Three sites, Jiaonan, Yantai and Penglai, which are located at major scallop culturing regions in Shandong

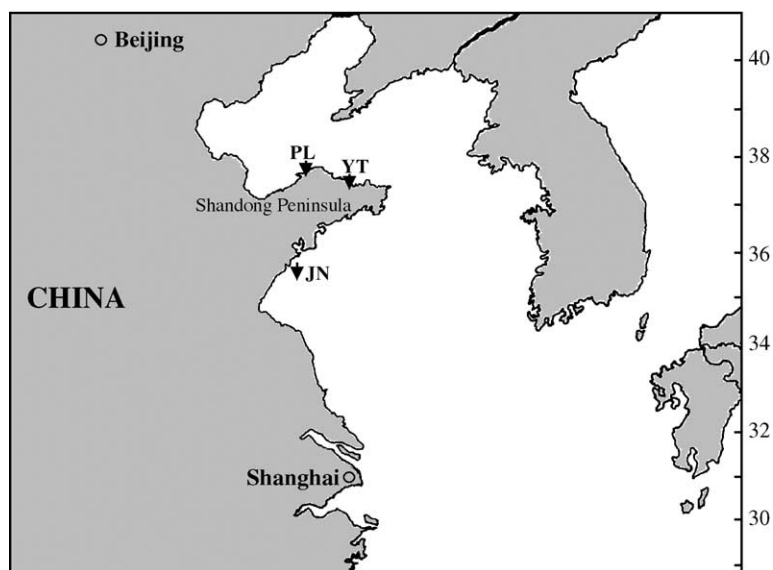


Fig. 1. A map of North China showing three experimental sites: Jiaonan (JN), Penglai (PL) and Yantai (YT).

province, were chosen for this study. Jiaonan is located on the south side of the Shandong peninsula, and Yantai and Penglai are on the north side of the peninsula (Fig. 1). Scallop seed that had been collected in the summer of 1999 were deployed at the three sites on May 29, June 1 and June 2, 2000, respectively. Seed for Penglai were collected from a nearby site. Seed for Jiaonan and Yantai were from two different collections at the same collection area in Yantai. At deployment, 30 scallops from each site were randomly selected, weighed and measured. Seed for the three sites differed significantly ($p < 0.05$) in initial size, ranging from 8.15 to 15.79 g in whole wet body weight, and 39.4 to 46.5 mm in shell height (Table 1).

The juvenile scallops were placed in 2-m long lantern nets with 10 layers. Each layer was a growout compartment that was 35 cm in diameter and 20 cm in height. The nets were hung on suspended longlines supported by rubber buoys so that the top compartment was 2–3 m below the surface. All experimental nets were placed on longlines that were used for commercial culture. Since scallop size varied among sites, stocking densities were based on biomass (average whole wet weight per compartment) and the known commercial stocking density at each site. At each site, the medium density was the typical density for a given size recommended by experts and local farmers. The specific numbers for low (L), medium (M) and high (H) densities were 20, 40, and 60 scallops per layer at Jiaonan; 30, 60, and 90 per layer at Penglai; and 25, 50, and 75 per layer at Yantai (Table 1). Nine layers of each net were stocked: the upper three contained replicates of one density, the middle three replicates of the second density, and the bottom three, replicates of the third. The three densities (L, M and H) were arranged so that the vertical distribution of each density was the same. The set of

three density–position combinations (LMH, HLM, MHL) was itself replicated twice (Fig. 2). Thus, each site contained 6 lantern nets, with a total of 54 compartments divided equally among three densities with 18 compartments per density.

After the initial deployment (T0), each site was sampled three times: before (T1), during (T2) and after (T3) summer mortalities. At each sampling, we counted all live and dead scallops in each compartment. The shells of dead scallops were not removed to maintain the original shell volume in the compartment and to imitate commercial operations. Fifty scallops per site from each density, distributed approximately equally among compartments, were randomly collected for size measurements, histology and genetic analysis. Whole wet weights were obtained, and shell height and length measured, before the scallops were dissected and fixed.

2.2. Determination of mortality and growth

Dead scallops included all gapers (newly deceased with soft tissues present), boxes (shells without soft tissues), and disarticulated shells (which were divided by 2). The percentage mortality (M) for each of the two intervals of observation (T1 to T2 and T2 to T3) and cumulative percent mortality (CM) at T3 for every layer were calculated as follows:

$$M_1(\%) = D_1 / (D_1 + L_1) \times 100$$

$$M_2(\%) = D_2 / (D_2 + L_2) \times 100$$

$$CM(\%) = M_1 + (1 - M_1/100) \times M_2$$

where D and L are the number of dead and live scallops, and the subscripts 1 and 2 refer to T2 and T3, respectively (Tomaru et al., 2001).

Table 1

Number, size and total biomass of scallops at deployment at low (L), medium (M) and high (H) densities and three experimental sites

Location	Jiaonan			Penglai			Yantai		
Date	May 29, 2000			June 2, 2000			June 1, 2000		
Density	L	M	H	L	M	H	L	M	H
Number	20	40	60	30	60	90	25	50	75
Biomass (g)	315.8	631.6	947.4	244.5	489.0	733.5	278.8	557.5	836.3
Shell height (mm)	46.5 ± 0.8			39.4 ± 0.7			43.6 ± 0.8		
Wet weight (g)	15.8 ± 0.8			8.2 ± 0.4			11.2 ± 0.7		

Shell height and whole wet weight (mean ± standard error) were determined for 30 scallops at each site. Biomass was estimated by multiplying the mean whole wet weight by the number of scallops in each compartment.

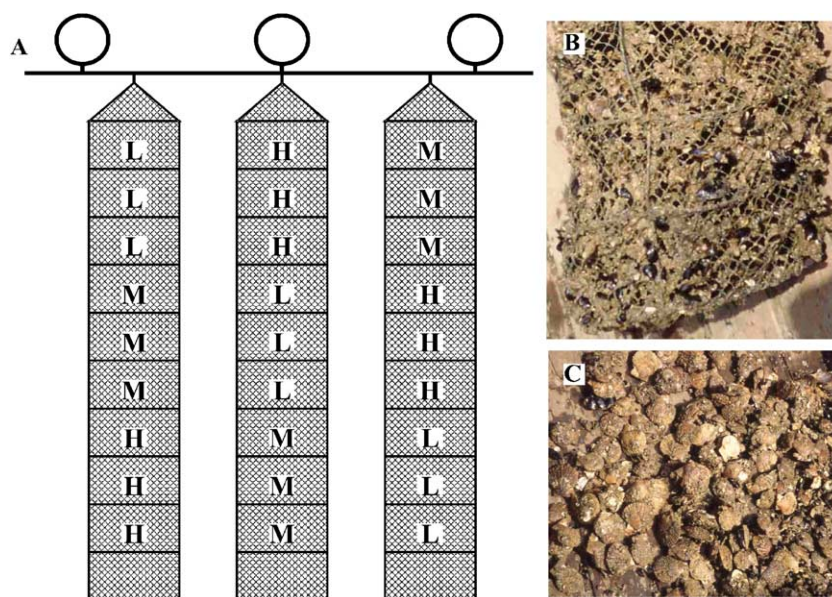


Fig. 2. Lantern nets on suspended longlines used for scallop deployment. A: A diagram showing the vertical distribution of compartments at low (L), medium (M) and high (H) densities; B: heavily fouled lantern net; and C, fouled scallops.

To standardize growth estimates among sites, where starting sizes differed, instantaneous growth rates of weight (RW) and height (RH) during mortality were calculated (Bricelj et al., 1992) for each interval, including T0 to T1, as follows:

$$RW = ((\ln W_2 - \ln W_1)/t) \times 100$$

$$RH = ((\ln H_2 - \ln H_1)/t) \times 100$$

where W_1 , W_2 and H_1 , H_2 are initial and final whole wet weight and shell height, and t is the interval (in days) between two samplings.

2.3. Histopathology

For histological examination, a section about 6–7 mm thick extending from the hinge to the mantle edge, and including digestive gland, gut, adductor muscle, kidney, gonad, gill, and mantle, was placed in a plastic cassette in Davidson's fixative (seawater: 95%:EtOH: 40% v/v: formaldehyde solution: glycerol: acetic acid = 3:3:2:1:1) for 24–48 h, then transferred into 70% EtOH for storage. Tissues from selected samples were subsequently embedded and processed into tissue slides using standard procedures, and stained using a modification of Masson's tri-

chrome stain (Humason, 1979). A total of 180 individuals (20 per density \times 3 densities \times 3 sites) collected before, during, and after mortality were examined with light microscopy.

2.4. Genetic analysis

To study genetic variability of the cultured scallops, samples from Jiaonan and Penglai ($n=30$ for each population) were analyzed along with two wild populations using RAPD (randomly amplification polymorphic DNA) markers. Cultured scallops from Yantai were not included as they were from the same area as the Jiaonan scallops. The wild scallops were collected from areas without a history of aquaculture: one group from Dalian ($n=30$) and one from Korea ($n=20$).

Total genomic DNA was isolated from the adductor muscle using published techniques (Grewe et al., 1993). The DNA quality was verified by running the samples on a 0.8% agarose gel. Seven oligonucleotide primers (Sangon Inc., Canada) were selected based on their ability to amplify polymorphic bands. PCR amplification was carried out using a PE9600 thermal cycler in a 25 μ l reaction volume containing 50 ng DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM

dNTPs, 0.5 μ M 10-mer primer, and 1 U of *Taq* DNA polymerase (TaKaRa Inc. Japan). Cycling was set as following: initial denaturing, 3 m at 94 °C; 40 cycles of 60 s at 94 °C, 120 s at 36 °C, and 120 s at 72 °C; and a final extension, 10 m at 72 °C. Amplification products were run on 1.4% agarose gels in 1X TAE buffer, at 6 V/cm for about 4 h, then stained in 0.5 μ g/mL ethidium bromide solution for about 15 m. Gels were imaged by the Gel Documentation System 7600.

The amplified bands were scored on the basis of their presence or absence. Because RAPDs are dominant markers, allele frequencies were calculated assuming Hardy–Weinberg equilibrium, heterozygosity (H) was estimated using the formula $H = 1 - \sum P_i^2$, where P_i is the frequency of the i th allele (Nei and Roychoudhury, 1974).

3. Results

3.1. Mortality

The first sampling was conducted in mid-June, about two weeks after deployment. No mortality was observed at any of the three sites. Water temperature at this time was about 18 to 20 °C at Jiaonan (Fig. 3). Temperature at the two northern sites was not available for this period, but is generally 1–4 °C lower than that at Jiaonan. But by mid July, when water temperature was 24.5 °C, mortality had reached 50% to 66% at Jiaonan (Table 2). At the end of July

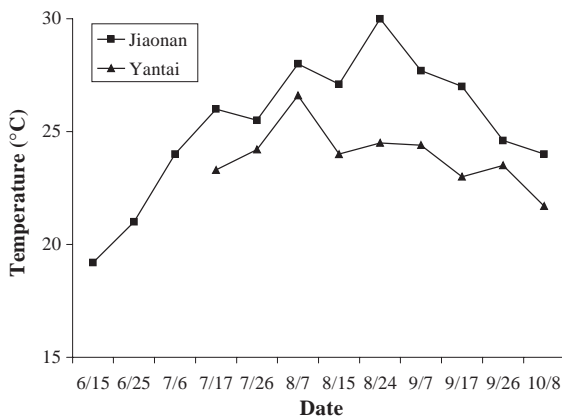


Fig. 3. Water temperature during the period of this study at Jiaonan and Yantai.

and beginning of August, mortalities were ongoing at Penglai and Yantai, with totals averaging between 26% and 49%. Water temperature at Yantai peaked at 26.6 °C on August 7th, when cumulative mortality reached about 40% (or half of the final total). Cumulative mortality reached 90% at Jiaonan by August 6, and comparable levels were recorded at Penglai and Yantai by the end of August. Although our sampling regime was not frequent enough to allow precise determination of the onset and duration of the highest mortality rate, onsite personnel estimated that over 60% of the scallops died within 2–3 weeks when water temperature reached about 25 °C. Temperature records from Jiaonan also indicated that most of the mortality occurred around 23–25 °C, before the peak of summer temperature, 26–29 °C, was reached (Fig. 3).

Although cumulative mortality at T3 was not associated with density at any site, density was a factor at T2 ($p < 0.05$) (Table 2). At this time, scallops in the low density compartment had suffered relatively fewer deaths than those at the middle and higher densities, which were similar. No relationship was observed between mortality and the vertical position (top, middle, and bottom) of compartments used (Table 2).

There was a slight decrease over time in the ratio of females to males at Jiaonan and Penglai, and a slight increase at Yantai. However, the changes were not statistically significant (Table 3) suggesting that the mortalities were not sex-specific. Interestingly, the Penglai population had a significantly higher female-to-male ratio (1.44) than that in Jiaonan (1.11) and Yantai (1.04) at deployment.

During the sampling at T2 and T3, it was noted that the lantern nets were heavily fouled, both on the inside and the outside, by mussels, tube-building worms and algae (Fig. 2B). These organisms were often abundant on the scallops themselves (Fig. 2C).

3.2. Growth

Instantaneous growth rates for shell height and whole wet weight of scallops were above zero throughout the study period. There were statistically significant differences among sampling intervals at most sites, although the pattern of change was similar among sites (Table 4). Shell growth rate was highest

Table 2

Cumulative mortality (mean ± standard error) of *C. farreri* at two sampling dates (T2 and T3), three densities (low, medium and high) and three vertical positions (top, middle and bottom)

Location	Jiaonan		Penglai		Yantai	
Sampling Date	T2 7/18	T3 8/6	T2 7/26	T3 8/29	T2 8/9	T3 8/28
<i>Density</i>						
Low	50.5(5.0)	92.9(2.3)	26.3(3.4)	90.5(2.0)	38.2(3.9)	85.4(2.6)
Medium	70.6(2.4)	89.1(1.8)	43.9(2.9)	88.1(1.2)	43.3(2.7)	80.4(2.1)
High	66.3(3.3)	88.9(1.4)	43.4(1.9)	87.7(1.2)	48.9(1.7)	88.8(1.8)
<i>p</i> -value*	0.001	0.246	0.000	0.365	0.040	0.030
<i>Position</i>						
Top	64.1(5.3)	91.8(1.9)	40.0(3.3)	88.2(1.7)	43.9(3.3)	81.9(2.4)
Middle	63.5(3.7)	89.7(1.8)	38.4(3.3)	88.2(1.4)	41.3(3.2)	86.0(2.3)
Bottom	59.8(3.6)	89.2(2.0)	35.2(3.9)	89.8(1.5)	45.2(2.7)	86.6(2.1)
<i>p</i> -value*	0.742	0.595	0.630	0.696	0.659	0.298

p-values are from one-way ANOVAs with density as the treatment.

No mortality was noted at any location at T1 (6/11 at Jiaonan; 6/16 at Penglai, and 6/14 at Yantai).

between T0 and T1 at all sites, then steadily decreased. In contrast, growth rate in total wet weight was the highest during the interval T1 to T2 and lowest, on average, during the interval T2 to T3. The large weight increase during the second interval at the same time that shell growth was decreasing suggests that a period of rapid soft tissue growth occurred just before and during the early stages of mortality.

Density had little effect on growth during the early part of the study or when the scallops were small. A negative effect of density on whole body weight was first observed at T1 at Jiaonan, where the stocking density was greatest at deployment, and when the scallops reached about 20 g (Fig. 4). Effects of density were not significant at T3, probably because biomass

had been reduced by heavy mortalities. At Yantai, negative effects of density on body weight became evident at T2 when the scallops reached about 25 g. At Penglai, the scallops started small and reached only 20 g in whole weight at T3, and density had no significant effect on body size.

Difference in body size between the two sexes was observed only at Penglai during the pre-mortality (T0) sampling. At T0 in Penglai, female scallops weighed 10.2 g ($n=88$, standard error=0.3); the males weighed 9.0 g ($n=61$, standard error=0.3). The difference was significant ($p=0.011$). No size difference between the two sexes was detected at any other sampling dates and at the other two sites.

Table 3

Sex-ratio (female:male) of *C. farreri* at three sites before (T1), during (T2) and after (T3) summer mortality

Sites	Date	Female	Male	Sex-ratio	<i>p</i> -value
Jiaonan	T1	79	71	1.11	0.875
	T2	73	71	1.03	
	T3	60	61	0.98	
Penglai	T1	88	61	1.44	0.813
	T2	87	65	1.34	
	T3	69	56	1.23	
Yantai	T1	74	71	1.04	0.458
	T2	69	80	0.86	
	T3	76	66	1.15	

Table 4

Instantaneous growth rates for shell height and whole wet weight at the three experimental sites for sampling intervals during the study period

Location	T0–T1	T1–T2	T2–T3	<i>p</i> -value
<i>Shell height</i>				
Jiaonan	0.911	0.210	0.357	0.000
Penglai	0.515	0.392	0.150	0.035
Yantai	0.398	0.322	0.161	0.156
<i>Whole weight</i>				
Jiaonan	0.410	0.563	0.142	0.007
Penglai	0.401	0.932	0.537	0.000
Yantai	0.553	0.960	0.168	0.001

**p*-values are from one-way ANOVAs with interval as the treatment.

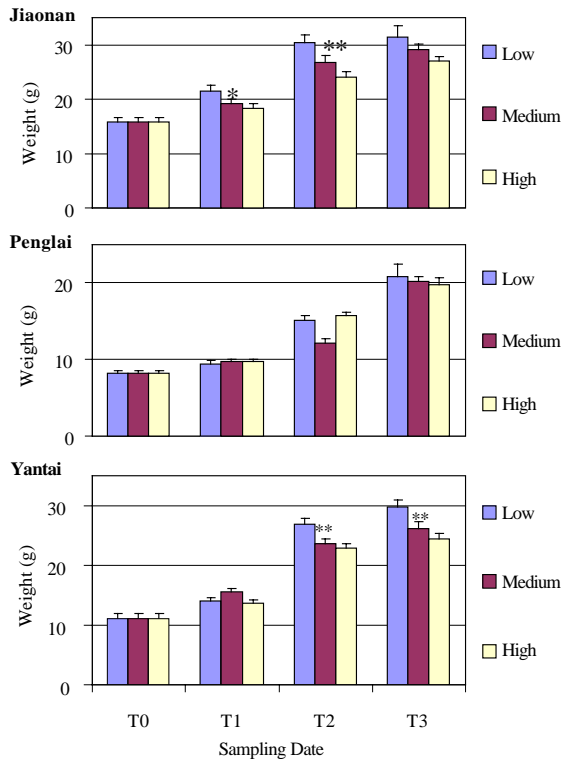


Fig. 4. Average whole wet weight (+standard error of the mean) of *C. farreri* deployed at three densities (low, medium and high) and three sites (Jiaonan, Penglai and Yantai). Sample size for each bar equals to 95–154 scallops. Significant effects of density were designated by * ($p < 0.05$) and ** ($p < 0.01$).

3.3. Histopathology

No abnormal conditions were observed in gonads, adductor muscle or heart, and food was present in the gut of most individuals. The connective tissue of the mantle of several individuals appeared degenerated, but the prevalence of this condition was below 10% and not associated with sample time or site. Similarly, the kidneys of a few scallops contained disintegrated cells and anomalous granules were observed inside degenerated cells of two scallops. Prokaryotic inclusion bodies resembling rickettsiales- or chlamydiales-like organisms were present in the digestive tubule epithelia of 16.4% of the scallops (Fig. 5A). They were more prevalent at T2 in Jiaonan and Penglai, and highest in Yantai at T1 (Table 5). Heavy infections (over 20 inclusion bodies per tissue section) were found in only two scallops, which were collected at

Yantai at T1. Most of the other individuals had fewer than 5 inclusion bodies per section. Furthermore, they were not found in other locations such as mantle, gill, or connective tissue.

The gill cavities of numerous scallops contained relatively large invading organisms, including ciliates and what appeared to be larvae of bivalve species. They were more prevalent at T2 and T3 than at T1 (Table 5). Ciliates identified as being in the genus *Trichodina* were observed in the mantle cavity of up to 67% (Penglai, T3) of the scallops examined, with up to 116 per section (Table 5). They were found at all three sites, but were most prevalent at Penglai and Yantai where they increased during the mortality episode. Depending on the angle of the section, these ciliates appeared dome-like (~30 μm in height) or circular (~50 μm in diameter) and displayed the characteristic basal denticle ring (Fig. 5B). Hemocyte infiltration and deformation of the gill epithelium were common adjacent to these ciliates. *Trichodina* sp. were also found between the adductor muscle and gills, and adjacent to the mantle. No *Trichodina* sp. was observed within the tissues of any organ.

Up to 40% (Penglai, T2) of the scallops had relatively large organisms present in their mantle cavities, which resembled bivalve larvae (Fig. 5C). The overall mean prevalence was 14%, with higher prevalence at Penglai and Yantai than at Jiaonan (Table 5). In sections, these organisms ranged from 150 μm to 1 mm in largest dimension. They were large enough to severely displace adjacent organs such as gills. They were most prevalent during the mortality. No other organisms or pathological conditions were observed consistently or with high frequency.

About 12% of the individuals examined exhibited secretions along the gill epithelium (Fig. 5D–F). These varied in appearance, but were typically composed of thin sheet-like layers (Fig. 5D) that often contained cells and debris (Fig. 5E). Although some of the cells appeared to be hemocytes, others were not identifiable (Fig. 5F).

The gonads of most scallops were mature and many appeared to be in the process of spawning. But only 2 individuals at Penglai (T2) and 4 at Yantai (3 at T2 and 1 at T3) showed a completely spawned-out condition. Noteworthy was the abundance of eggs in the mantle cavities of most females. While this condition was undoubtedly partly due to the dislod-

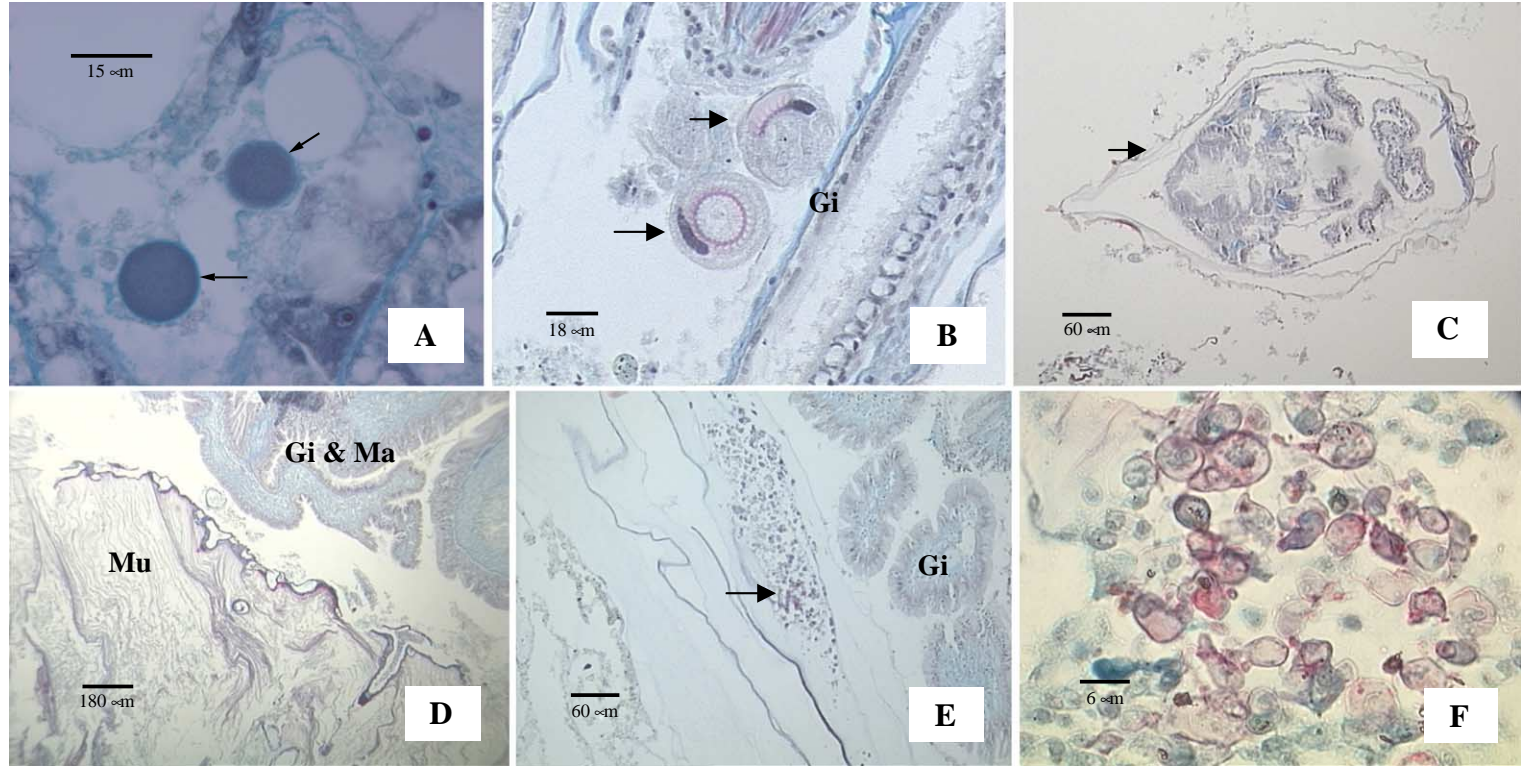


Fig. 5. Histological sections of *C. farreri* sampled in this study: A, prokaryotic inclusion bodies (arrows) in digestive tissue; B, *Trichodina* spp. marked by arrows with thin-epithelia gills around; C, larvae of unknown organisms found in body cavity (not tissue) with shell vestige (arrow) around viscera; D, mucus like secretion; E, secretion layers enclosing cells (arrow); F, enlargement of region marked by arrow in E showing cluster of unidentified cells. Abbreviations are: Mu = mucus, Gi = gill, Ma = mantle.

Table 5

Prevalence of *Trichodina* sp. ciliates, larvae of an unknown organism, prokaryotic organisms and secretions observed in tissues and mantle cavities of *C. farreri* at each sampling time and location

Location	Sample	<i>Trichodina</i> sp.	Larvae	Prokaryotes	Secretion
Jiaonan	T1	0.0	13.4	6.7	6.7
	T2	6.7	0.0	28.6	13.3
	T3	0.0	0.0	7.1	13.3
Penglai	T1	6.7	6.7	16.7	6.7
	T2	30.0	40.0	32.1	16.7
	T3	66.7	26.7	16.7	6.7
Yantai	T1	0.0	6.7	35.7	6.7
	T2	20.0	3.3	3.8	3.3
	T3	33.3	26.7	0	33.3

ging of eggs from the gonad into the mantle cavity during the dissection procedure, eggs were often wedged in between gill filaments and some appeared degenerate compared to those still in the gonad, suggesting they had been present before dissection.

3.4. Genetic analysis

From the seven RAPD primers (Table 6), 37 clear and stable bands were scored and among them, 30 bands were polymorphic (at the 95% level), corresponding to an overall polymorphism of 81.1% (Table 6). Among the four populations analyzed, heterozygosity was slightly lower in the cultured than wild populations, ranging from 0.286 to 0.316 in cultured populations and from 0.325 to 0.336 in the wild populations (Table 7). When combined, the two cultured populations had an average heterozygosity of

Table 6

Sequences of seven RAPD primers used, number of bands amplified and number of polymorphic loci in two cultured and two wild populations of *C. farreri*

Primers	Sequence (5'–3')	Total bands scored (No)	Polymorphic bands (No)	Polymorphism (%)
S30	GTGATCGCAG	4	3	75.0
S37	GACCGCTTGT	4	3	75.0
S103	AGACGTCCAC	7	5	71.4
S143	CCAGATGCAC	9	8	88.9
S234	AGATCCCGCC	4	4	100.0
S462	TCGGCACGCA	5	4	80.0
S476	CCAAGCTGCC	4	3	75.0
Total		37	30	81.1

Table 7

Heterozygosity and percent polymorphic loci at 30 RAPD loci in two cultured and two wild populations of *C. farreri*

Population	Description	<i>n</i>	Heterozygosity	Polymorphic loci (%)
Dalian	Wild, Dalian, Liaoning	30	0.336	78.4
Korea	Wild, from Korea	20	0.325	78.4
Jiaonan	Cultured, Jiaonan, Shandong	30	0.316	73.0
Penglai	Cultured, Penglai, Shandong	30	0.286	75.0

0.301, which is 9% lower than that of the wild populations (0.331), but the difference is not statistically significant ($p=0.103$). The percentage of polymorphic loci was lower in the cultured than the wild populations (Table 7).

4. Discussion

To our knowledge, this study represents the first attempt to document the timing and extent of zhikong scallop mortality in a systematic manner at several sites along the Chinese coast and to test specific hypotheses as to its cause(s) (Zhang and Yang, 1999a, b; Guo et al., 1999; Wang and Xiang, 1999). Other studies have collected scallops only during the period of mortality, have been limited to a single site, or have examined for potential pathogens only (He et al., 2003; Wu et al., in press). In our study, mortalities reached about 90% at all three experimental sites and, as reported earlier (Guo et al., 1999), occurred in July and August during the warmest period of the year starting before and not at the peak of temperature (Yang et al., 1999). Mortalities began in early July at Jiaonan and mid-July at Penglai and Yantai, and when the water temperature was about 23 or 4–6 °C below the peak summer temperature. This period corresponds to the late part of the spawning season of zhikong scallops, and histological examination showed that most individuals were reproductively mature. Zhang et al. (1956), who documented spawning of zhikong scallops from 1953 to 1956, found that the peak-spawning season is from mid-May to mid-July for zhikong scallops in Shandong. It is possible that stress related to spawning is a major contributing factor to the mortalities observed.

The zhikong scallop mortalities are reminiscent of “unexplained” mortalities of cultured oysters such as the “Summer Mortality” of the Pacific oyster, *Crassostrea gigas*, reported from Japan, the west coast of the United States, and France (Mori, 1979; Koganezawa, 1975; Perdue et al., 1981; Cheney et al., 2000; Lacoste et al., 2001) and blue mussels, *Mytilus edulis*, on both coasts of North America (Myrand and Gaudreault, 1995 and references therein). All occur when water temperature is highest; among two-year-old or older stocks at a time when the gonad is at peak development; and are prevalent in cultured stocks growing in nutrient-enriched waters. Mori (1979) and Perdue et al. (1981) concluded that the mortalities were caused by physiological stress associated with reproduction, high metabolism, and warm, eutrophic water. Later, Friedman et al. (1998, 1991), and Elston et al. (1987) showed that a bacterium (*Nocardia crassostreae*) was also a contributing factor. The current concept is that Summer Mortality has multiple factors that stress the oysters (Cheney et al., 2000; IFREMER, 2003). Juvenile Oyster Disease (JOD) of *Crassostrea virginica* in the northeastern United States occurs under similar conditions, although affected oysters display symptomatic, anomalous, shell growth and deposits. A bacterial etiology is suspected for JOD, but has yet to be confirmed (Boettcher et al., 2000, Ford and Borrero, 2001).

Histopathological examination of scallops collected before, during, and at the end of mortality showed no parasites that could be implicated as the cause. We deliberately sampled just before the mortality began, as well as during the mortality, and did not limit our collection to moribund scallops (although scallops near death were obviously included) in order to avoid the problem of designating as causative agents what are actually secondary invaders of dead and dying organisms. Prokaryotic organisms have been associated with mass mortality of scallops (Gulka et al., 1983; LeGall et al., 1988), and Wu et al. (in press) reported finding heavy infections of what they identified as rickettsia, in zhikong scallops during summer mortality episodes and strongly suggested an association between these microbes and the mortality. Two of our sites, Penglai and Yantai, were near the site sampled by Wu et al. (in press). Although we found prokaryotic organisms in the scallops we examined at all sites, they were present at a prevalence and inten-

sity too low to be associated with the mortality and were not linked with pathological alterations of the tissue. Similarly, He et al. (2003) concluded that rickettsia-like organisms were not the cause of mortality in the zhikong scallop. Although it is possible that we missed some light prokaryotic infections, detectable only by transmission electron microscopy (Hines and Diggles, 2002), it is also likely that any such infection capable of causing or exacerbating mortality also would have caused pathological lesions visible by light microscopy (Bower and Meyer, 1991; Hines and Diggles, 2002). Whereas the transmission electron micrographs presented by Wu and Pan (2000) for oysters and Wu et al. (in press) for scallops clearly depict prokaryotes, the eosinophilic structures represented as dense concentrations of prokaryotic inclusion bodies in light micrographs do not resemble typical chlamydiales or rickettsiales inclusions, but may be misidentified secretory cells.

We found no evidence at all of *Perkinsus*-like protists, which have also been associated with the mortality (Liang et al., 2002). Amoebae and small ciliates were found at low prevalence in the gill cavity, but the most obvious single organism observed in the histological sections was a *Trichodina* sp. ciliate, present in and on the gill filaments of numerous scallops where it elicited localized hemocytic infiltration into, and disruption of, the epithelial layer. Trichodinids are common in the gill cavities of bivalves (Lauckner, 1983). In most instances, they are probably innocuous inhabitants (Bower et al., 1994), although they can cause localized tissue damage (Boussid et al., 1999) and have been implicated in mortalities of the cockle *Cardium edule* in northern Europe (Lauckner, 1983). Interestingly, their prevalence in *C. edule* increased during the summer and with cockle density. In a survey of oysters, *C. gigas*, in the Bay of Brest, France, high prevalence of trichodinids was reported in the summer and at a site with high bacterial load (Boussid et al., 1999). *Trichodina* sp. are filter-feeding ciliates and they probably consume bacteria present in the gill cavities of bivalves (Lauckner, 1983). Lauckner (1983) hypothesized that healthy bivalves can control infestations by maintaining a good water flow through their gill cavities, which expels ciliates and prevents large populations from developing. Under conditions of weak water flow, however, concentrations of trichodinids could build to damaging

proportions. In the case of the zhikong scallops, the presence of a large number of trichodinids during the mortality episode is probably more a measure of a degraded environment and stressed scallops (high density of scallops, high bacterial loads in the water, poor circulation through the growout compartments, and weak flushing through the scallops' mantle cavities) than it is an indication of a direct causative agent. The presence of other invaders of the mantle cavity, which appeared to be larval stages of bivalves, during the mortality, is consistent with the same scenario. Further, the presence of secretions along the gill is evidence of irritation, either by the invading organisms or by some other factor.

Our failure to find evidence of a histologically detectable pathogen in the scallops does not necessarily negate the possibility that either bacteria or viruses (Wang et al., 2002) are associated with the mortality. Bacterial aggregates in molluscan tissues can be detected histologically in some cases such as infections by *N. crassostreae*, which has been associated with Summer Mortality in *C. gigas*, but not when bacteria cause disease by attacking in the mantle cavity, as is the case of Brown Ring Disease of Manila clams, *Ruditapes philippinarum*, and is suspected in the case of Juvenile Oyster Disease of *C. virginica* (Paillard and Maes, 1994; Boettcher et al., 2000). Viral infections, too, often cause damage, particularly cellular or nuclear hypertrophy, which can be observed at the light microscope level (Comps, 1988; Bower et al., 1994, Elston, 1986). Nevertheless, we did not find any consistent lesions that would suggest either bacterial or viral pathogens in the tissues of the scallops we examined, although we did not focus exclusively on moribund animals.

The presence of food in the guts of examined scallops indicated that absence of food was not a cause for the mortalities. Actually, zhikong scallops in our study experienced a period of rapid soft tissue growth just before and at the beginning of mortality. High density clearly had some negative effects on scallops. Scallops at the high and medium densities began dying earlier than those at the low density even though cumulative mortalities were similarly high (~90%) for all groups at the end of the observation period. High density also inhibited growth toward the end of our studying period. It is not known whether the association of higher density with slower growth

was due to food limitation or physical disturbance from adjacent scallops and fouling organisms, or both.

It has been observed that the scallop mortality appears to be species specific, occurring only in the zhikong scallops and not in bay scallops (*Argopecten irradians*) on the same or adjacent longlines. Whether this is because the zhikong scallop is more susceptible to the agent(s) of mortality or because of different growout cycles (Guo et al., 1999) for the various species has yet to be tested experimentally. Zhikong scallops are grown from wild seed collected in summer (late June to early July) or fall (late August to early September). The seed (about 10 mm in size) are placed in lantern nets and grown in nursery areas until the following March when they are transferred to growout lantern nets where they remain until harvested in December. They experience the mortalities as they are entering their second year. In contrast, bay scallops are grown from hatchery seed produced in late winter or spring. From the hatchery, they are transferred to nursery areas until later in the summer when they are placed in lantern nets for final growout. Because of their high growth rate, they, too, can be harvested in December. Thus, the bay scallops are placed in the growout system in clean lantern nets and at lower biomass, albeit the same numerical density, as the zhikong scallop (Guo et al., 1999) at the same time as the latter, which have been resident in the same lantern nets for several months and have reached a mean shell height of >40 mm, are already experiencing mortalities in heavily fouled nets. Under this growout cycle, the bay scallops would never encounter the same combination of high biomass, fouling, and high temperature experienced by the zhikong scallop.

Because zhikong scallop seed used for culture were collected from populations originally derived from hatchery production, there have been widely-held suspicions that the cultured "stock" may be genetically degenerated or suffer from inbreeding depression (Guo et al., 1999). Our results confirm that heterozygosity in cultured populations is slightly lower than that from wild populations, a result that is supported by other studies (Li et al., 2002; Cheng et al., 2001). Reduced heterozygosity has been observed in hatchery stocks in many shellfish species (Song et al., 1999; Hirschfeld et al., 1999; Xu et al., 2001; Gaffney et al., 1996; Yu and Guo, 2005). Positive correlations

between heterozygosity and growth have been reported in oysters, scallops, mussels and clams (Fujio, 1982; Bierne et al., 1998; Gaffney et al., 1990; Koehn et al., 1988; Koehn and Gaffney, 1984; Koehn and Shumway, 1982; Pogson and Zouros, 1994). Severe inbreeding may affect general fitness including larval and adult survival. However, it is not clear to what extent the reduction in heterozygosity has contributed to the mortalities observed in zhikong scallops. Hybrid zhikong scallops made using populations from China, Japan and Korea have shown increased immune activities than their parental populations, but there was no difference among the three parental populations (Li et al., 2002), suggesting that heterosis, rather than heterozygosity, is the contributing factor. It is unlikely that the 9% reduction in heterozygosity that we recorded can cause massive mortality alone, although it is possible that reduced vigor may make the scallop particularly susceptible to stress and/or opportunistic pathogens. A comparative study on mortalities in cultured and wild populations is needed to determine the effects of inbreeding on survival.

This experimental study tested three of the hypotheses put forward to explain the recurring summer mortalities of cultured zhikong scallops in China. Culture density at the compartment level affected the onset of mortalities, but the final mortality was the same for all densities. There was no indication of a pathogen in tissue sections examined by light microscopy, and no obvious pathology other than that associated with apparent mechanical pressure and irritation of gill tissue by ciliates and *Trichodina* sp. ciliates. We conclude that the organisms that we found at relatively high prevalence during the mortality are opportunistic invaders of the mantle cavity, although ones that could cause tissue damage. Recent studies suggest that the mortality is caused by viral agents (Wang et al., 2002; He et al., 2003), which we could not confirm with light microscopy. Genetic variability was lower in the cultured than the wild populations, but it is unknown if a 9% reduction in heterozygosity can be the primary cause for the heavy mortalities observed. It is possible that the scallop mortalities are caused by a combination of several factors similar to those associated with “Summer Mortalities” of oysters and mussels. These may include high temperature, stress associated with spawning, poor circulation in

growout compartments due to overcrowding and fouling, opportunistic pathogens, and possibly reduced vigor due to inbreeding.

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