Survey on the Ovarian Parasite, *Marteilioides chungmuensis* in the Cultured Pacific Oyster, *Crassostrea gigas* in Korea

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ABSTRACT

In Korea, oyster culture began in the early 1900's. Commercial oyster culture using the hanging system started in the 1960's, on the southern coast. After the 1960's, production of cultured oysters has increased every year to the late 1980's with the highest production recorded at 288,078 metric tons in 1987. This upward trend stagnated in the 1990's and production dropped to 174,117 metric tons in 2001. Since 1990, the oyster culture industry in Korea has faced difficulties because of insufficient seed collection and mass mortality of cultured oysters. National Fisheries Research and Development Institute (NFRDI) presumed that increased contaminants from the land, and self contamination in the growing area together with a genetic weakening of the broodstock caused the decline. The ovarian parasite of the oyster has been inplicated as a cause of poor seed collection and mass mortality. In Korea, the ovarian parasite was reported in 1970 for the first time, and was assigned to the genus *Acanthamoeba* (see Chun, 1979). Based on the morphological characters, however, Comps *et al.* (1986) assigned it to a new genus and species and named it *"Marteilioides chungmuensis"*. The infection rate of *M. chungmuensis* in Korea has increased every year and the parasite now occurs not only in the spawning season (August) but throughout the year. The related authorities such as Ministry of Maritime Affairs & Fisheries (MOMAF) and provincial governments have performed "Farming Area Cleaning Project" in an attempt to improve coastal conditions. Also NFRDI recommended that the oyster industry culture triploid oysters free from parasitic infection.

INTRODUCTION

Korean production of Pacific oysters, *Crassostrea gigas*, has increased since 1960s when a hanging culture method was introduced, making the country competitive with the world's leading countries in oyster production by early 1990s. Thereafter, the production entered into a stagnant status, positioning the country 3rd in global production following China and Japan. Oyster production contributed 26.5% of total Korean marine aquaculture production in 2001 and 5.2% of marine exports.

The Korean government started to invest in oyster farming following the Fisherman Income Expansion Plan and a Nation Economy Development Plan in the early 1960s. Subsequently, the Oyster Hanging Culture Fisheries Cooperative of Korea was organized in 1965. Under the Shellfish Sanitation Agreement for Export between Korea and the USA, November 1972, some farming areas could be designated as blue belts by the FDA, USA. This

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opportunity spurred farmers to practice oyster culture in these areas, resulting in increased exports to Japan of fresh oysters and to the United States of frozen or canned ones. The expanded farming areas of Tongyoung, Sacheon, Masan, Jinhae, Osong, Namhae, and Yeosu produce annually 30,000M/T of farmed oysters, 60% of which are exported, earning over US$ 110 millions annually. Oyster processing and marketing industries were developed, which in combination with oyster farming have played a key role in the economy of the southern coasts of the Korean peninsula.

Since 1992, a constraint on the production of the cultured oyster has appeared mainly by local failure to collect healthy seeds. Considerable amounts of ready-to-attach larvae and attached spats were imported from United States and Japan. Efforts to determine the causes of the failure have included biological, biochemical, and environmental approaches in the oyster seed grounds. Health assessments of larvae and broodstock and some environmental factors affecting larval growth have also been explored in the areas where seed collection problems were prevalent. During this investigation, an ovarian parasite of *Crassostrea gigas* was found and was probably related to the failure of seed collection in the areas. Here, the recent status of ovarian parasite occurrence and its pathological impact on the bloodstocks and larvae of *C. gigas* is reviewed.

OVARIAN PARASITE INFECTION RATE

This study was carried out in the southern coasts of Korea (Fig. 1). Two locations were chosen for this experiment. The first was an Osu in Hansan-Koje Bay with a total area of 56 km^2. This bay is a semi-enclosed system and the cultivated oysters were suspended in the inner side of the bay. The other is Josan in Jaran Bay, which is open to oceanic environments. Both of the sites have long been used as farming grounds for the Pacific oyster with very high densities compared to other locations. Sixty oysters were randomly sampled from the 30 ropes once a month from 1993 to 1998.

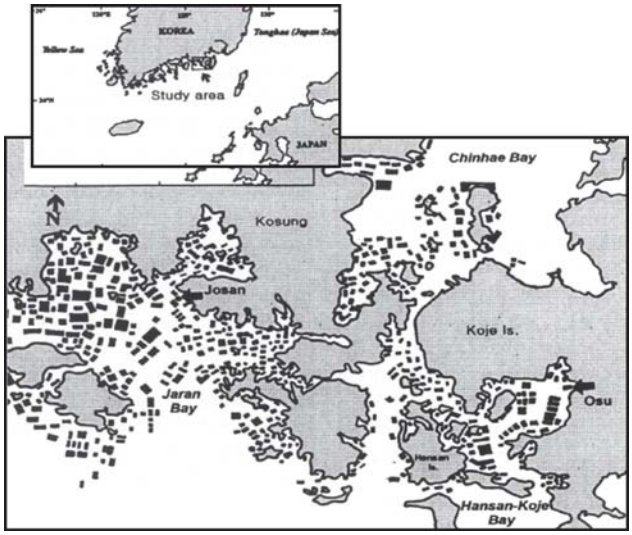

Figure 1. Location of the study area. Black rectangles indicate longline culturing grounds and arrows represent the sampling stations.

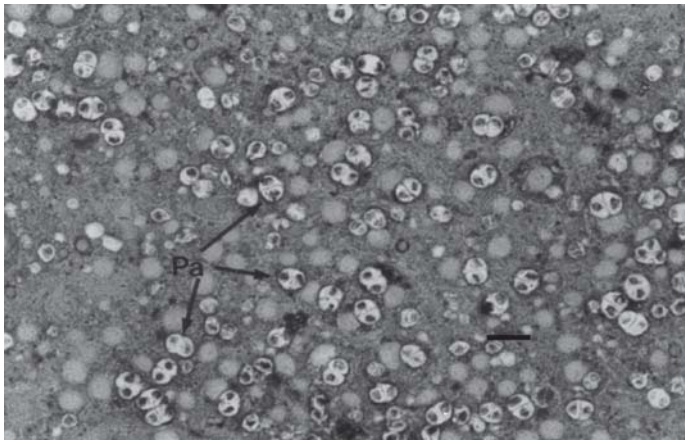

Figure 2. Photomicrograph of the infected ovary; Pa, parasite. Eosin-methylene blue (scale bar 50 m).

Sampled broodstock were washed with clean seawater and shucked by hand. The shucked oysters were fixed in Bouin's fixative for 24 h. The visceral mass was washed in flowing tap water for 24 h, and then dehydrated in serially diluted ethyl alcohol. The tissues were embedded in paraffin, sectioned at 3 to 5 μm and stained with Harris' hematoxylin-eosin on slides. The stained sections were observed with a light microscope. Because early stages of infection are difficult to detect by light microscope, additional tissue smears were made from ovarian tissues and stained with eosin-methylene blue (Fig. 2).

Ovarian parasites were prevalent from June to September, during the spawning season, with the highest infection rate occurring in September in Osu and August in Josan in every year except for 1998 in Josan (Fig. 3). The infection rate in Josan was lower than that at Osu.

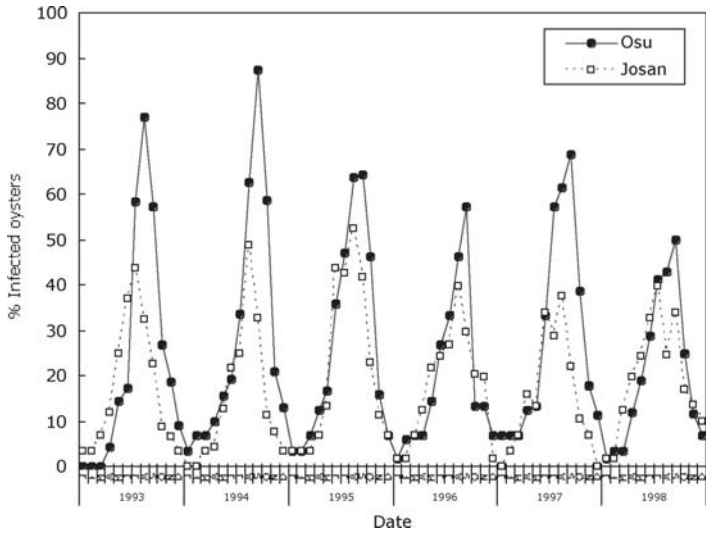

Figure 3. Infection rates of the ovarian parasite, *M. chungmuensis* of the Pacific oyster, *Crassostrea gigas.*

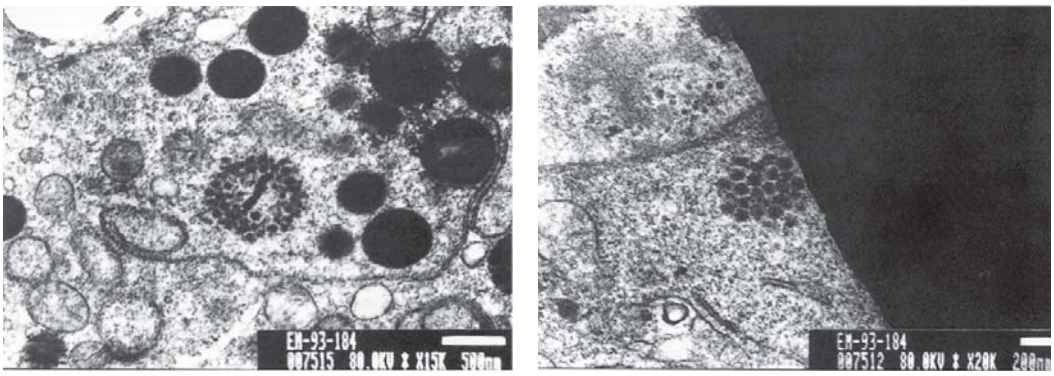

Figure 4. Virus-like particle appeared in the cytoplasm of an oocyte (upper scale bar 500 μm, lower 200 μm) (Park *et al*., 1999).

Park and Chun (1989) investigated ovarian parasite infection rates of Pacific oysters collected from Hwado in Hansan-Koje Bay, Ochon in West Sea and Sinchang in East Sea, from January, 1986 to December, 1987. Oysters were from hanging culture in Hwado, bottom culture in Ocheon and from the wild in Shinchang. The average infection rates per year were 5.3-6.7%, 4.2-2.8% and 0%, respectively. Infection occurred from June, when oysters start spawning, to November, two months after oysters finish spawning. In the present study, the ovarian parasite infection rates of cultivated oysters in Geoje Bay, Gyeongnam, were over 60% higher in August, the main spawning season in adult oysters.

Park and Chun (1989) reported that infected oysters had a prominent hemocytic infiltration around ovarian follicles. Using electron microscopy, we have found virus-like particles, 70-80 nm across, in the cytoplasm of infected egg cells (Fig. 4). The presence of viral particles in the cytoplasm of infected egg cells was not previously reported. Further study is required to characterize the virus and assess its pathological effects.

DEVELOPMENT OF LARVAE PRODUCED FROM PACIFIC OYSTER INFECTED BY OVARIAN PARASITE

The ovarian parasite *Marteilioides chungmuensis* is found in only the Pacific oyster, Crassostrea gigas, and it inhibits normal fertilization of eggs and normal development of larvae. The prevalence of *M. chungmuensis* had been increasing rapidly in cultured oysters on the southern coast of Korea since 1990, and has severely impaired the oyster culture industry due to reduced levels of seed collection.

To determine the effects of *M. chungmuensis* on the development and growth of oyster larvae produced from parasite infected oysters (Fig. 5), uninfected and infected oyster eggs were collected from the southern coast in Korea using the strip method (Park *et al*., 1999). The embryological characteristics of eggs and developing larvae were investigated. *C. gigas* imported from Hiroshima, Japan were used as uninfected controls.

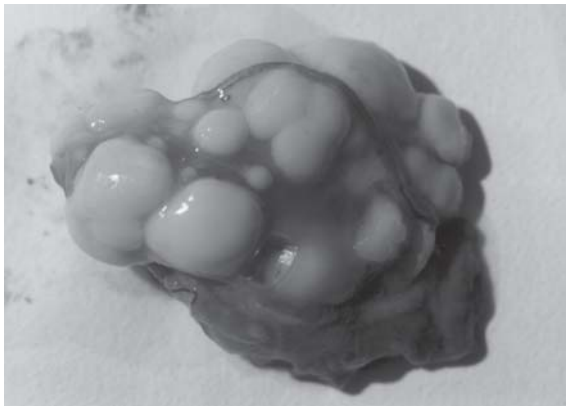

Figure 5. The external view of the oyster infected with parasite, *M. chungmuensis.*

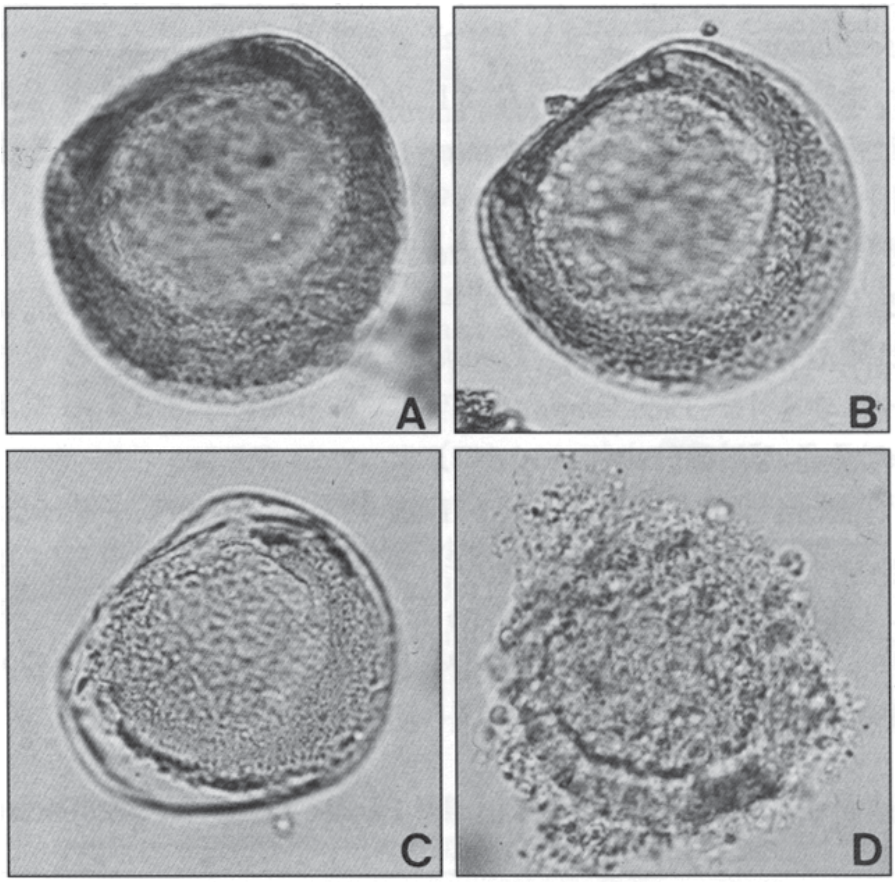

Figure 6. Necrotizing process of the Pacific oyster larva; A ' D (¥ 400) (Park *et al*., 1999).

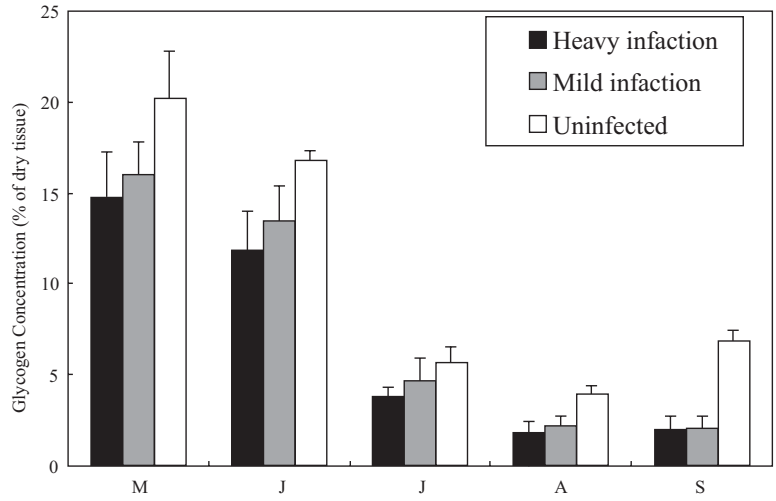

Figure 7. Glycogen concentration (± SD) in infected and uninfected oysters (Park *et al*., 2003).

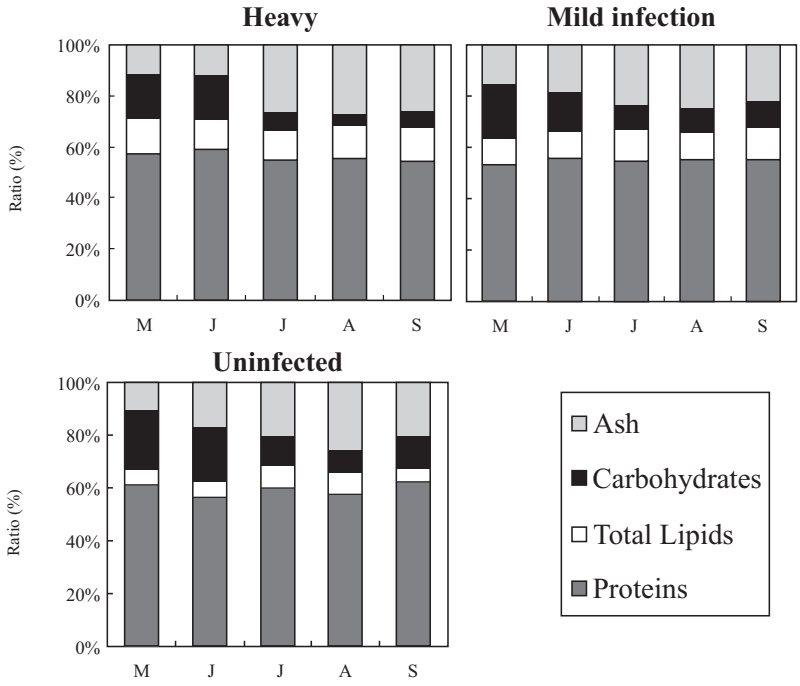

Figure 8. Biochemical composition of infected and uninfected oysters (Park *et al*., 2003).

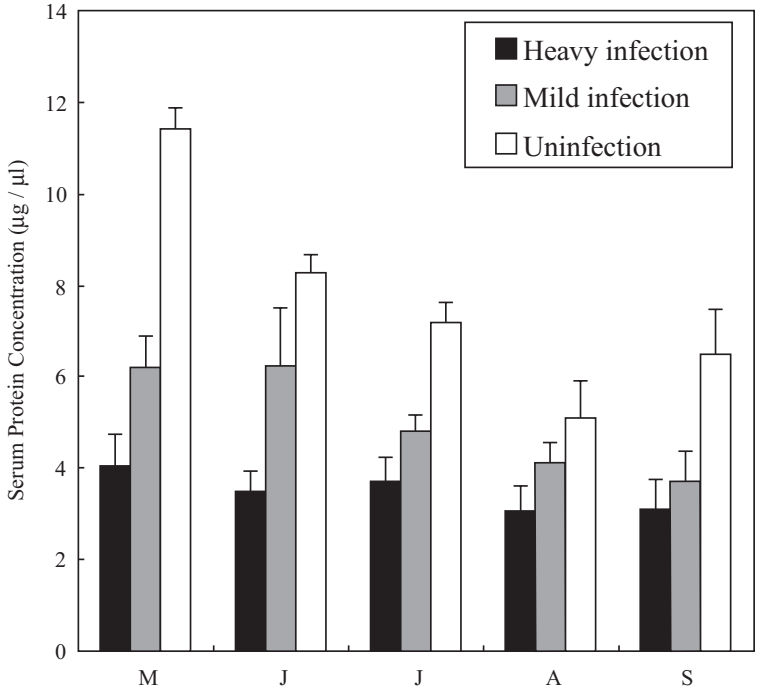

Figure 9. Serum protein concentration (± SD) in infected and uninfected oysters (Park *et al*., 2003).

To investigate development of parasitised and non-parasitised eggs derived from infected oyster gonad, the two kinds of eggs were fertilized with normal active sperm. The experiment was carried out three times at a water temperature of 25°C. The larvae were fed with *Pavlova lutheri, Isochrysis galbana, I. aff. galbana,* and *Chaetoceros calcitrans* daily. Both infected and uninfected eggs from an infected oyster had a low fertilisation rate and developed much slower than than the control uninfected eggs from C. gigas imported from Hiroshima. Only 13.8% survived to D stage and had an average length of 93.5 ± 1.87 μm compared to 58.2% and 76.4 ± 2.02 μm in the Hiroshima controls. All larvae from the infected oysters died before the umbo stage (Fig. 6) whereas in the controls the D-shaped larvae reached full grown lengths of 340.8 μm 20 days after fertilization (Table 1).

Table 1. Egg and larval development in ovaries infected by parasites, *M. chungmuensis* (Unit: %).

	Infected eggs	Uninfected Eggs	Control
Fertilization rate	0	88.7	89.4
Survival rates at D-shaped larvae	-	13.8	58.2
(Average length ± SD)	-	(73.1 ± 1.87)	(76.4 ± 2.02)
	0	36.7	
Survival rates at umbo-stage (10day-old)		(93.5 ± 3.18)	(165.2 ± 3.29)
(Average length ± SD)			

Table 2. Lipid content of oyster egg and larva.

	Lipid content (ng/cell)		
	Mature egg	Early D-shaped larva	7 day-old larva
Egg from *M. chungmuensis* infected oyster	6.3	4.2	9.9
(Average length)	(47.8 μ)	(73.1 μ)	(84.6 μ)
Control	9.9	8.9	31.4
(Average length)	(43.2 μ)	(76.4 μ)	(118.5 μ)

Seki (1933) reported that egg cells in abnormal egg masses with ovarian parasite could be fertilized, though Ogasawara et al. (1962) concluded that abnormal egg cells could not reach fertilization because of the necrosis of nucleus and cytoplasm.

The lipid contents of uninfected eggs and D-shaped larvae produced from infected oysters were analyzed to evaluate their health. The lipid content of the uninfected matured egg was 6.3 ng per egg, and in the early D-shaped larva and late D-shaped larva at 7 days after fertilization was 4.2 ng and 9.9 ng per larva respectively. In the uninfected control oysters, lipid content in the matured eggs and early D-shaped larva and in umbo-stage larva at 7 day after fertilization was 9.9 ng per egg, and 8.9 ng and 31.4 ng per larva, respectively (Table 2). Uninfected eggs from infected gonads were not released completely and remained in the gonad with fully ripe one throughout the spawning season. Further study is needed to identify the mechanism which prevents the normal development of uninfected eggs from parasitised oysters.

HEALTH CONDITION ACCORDING TO INFECTION STATUS

The oyster samples were divided into infected and uninfected groups. Health was evaluated using broodstock collected during the spawning season (May to September) because the presence of infection can be determined by the naked eye during the spawning season. Degree of infection was divided into two stages: over 50% of the gonad affected (heavy infection, ‘H’), and less than 50% (mild infection, ‘M’). Oyster h was evaluated by assessing the glycogen content of the oyster meat without the ovary. Glycogen content was analyzed by the method of Whyte and Englar (1982), the meat components were examined by the method of AOAC (1990), and serum protein was analyzed by the method of Lowry *et al*. (1951).

Glycogen content

The glycogen content of parasite infected oysters was lower than that of uninfected oysters. The glycogen content of normal broodstock increased after the spawning season with the degeneration of the ovary, but glycogen content in the parasite infected oyster did not vary after the spawning season (Fig. 7).

Body components

The lipid content in normal broodstock increased in March and reached its highest in July. However, lipid content in the infected groups (H; infection rate is above 50% and M group; infection rate is below 50%) was higher than that in uninfected oysters. Protein content in uninfected, H group and M group was 56.44-61.99, 55.05-58.87 and 53.15-55.79%, respectively (Fig. 8).

Serum protein

Serum protein in infected oysters was lower than that in uninfected oysters, with ranges of 5.12-11.41, 3.08-4.04, and 3.74-6.24 μg/μl for uninfected, H group and M group, respectively. Serum protein in the normal broodstock increased after spawning but decreased continuously in the infected broodstock (Fig. 9).

The parasite apparently infects only the ovary. It induces necrosis of oyster eggs, and death of oysters. In those eggs that survive, it inhibits growth (Matsuzato *et al*., 1977, Matsuzto and Masumura, 1988, Park and Chun, 1989). Park *et al*. (1999) examined in vitro, development of parasitised and unparasitised eggs, and found that the parasitised eggs were not able to be fertilized normally. Unparasitised eggs isolated from infected oysters were able to be fertilized but did not show normal development. Of these, more than 80 % showed abnormal development and all died in the early umbo stage. To date, there is no clear evidence that the parasite *M. chungmuensis* induces mortality of the mature oyster.

Glycogen is an essential component for gonad maturation and growth (Encomio and Chu, 2000). It accumulates in the digestive gland, gonad and mantle (Berthelin *et al*., 2000) with stores in the gonad and mantle used for gonad maturation. The low content of glycogen in infected broodstock means that the parasite may directly affect the reproduction of broodstock. Serum protein in normal broodstock increased after spawning but decreased steadily in the parasitised broodstock, and the reducted glycogen content could conceivably induce broodstock death. Protein is not used for gametogenetic effort but is an essential component of oyster tissues (Berthelin *et al*., 2000) and this growth is retarded in infected oysters.

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