

Histopathology of the Japanese Scallop, *Mizuhopecten yessoensis*, Cultured in the Experimental Marine Farm in Minonosok Bay (Russian Far East)

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ABSTRACT

Health state of the Japanese scallop, *Mizuhopecten yessoensis*, cultured in the Experimental Marine Farm in Minonosok Bay (Peter the Great Bay, Sea of Japan) was investigated. The wide spectrum of histopathological changes has been identified in the internal organs and tissues of scallops: prokaryotic infection with prevalence 100%, destruction of digestive epithelium and other changes in digestive system, infiltration of organs by hemocytes and granulocytome formation. The most affected by prokaryotic infection organs are labial palps, lips, esophagus, intestine and gills. Several of the observed alterations seem to be related to prokaryotic infection.

Keywords: Histopathology, Scallop, Prokaryotic infection.

INTRODUCTION

The scallops are an important recreational and commercial species, in this connection the scallop fisheries from natural populations, as well as the organization of aquaculture farms (hatcheries) for stock enhancement and cultivation are established in many countries such as England, USA, Canada, New Zealand, Japan, China, Korea, and Russia. In

Asia-Pacific region, the Japanese scallop *Mizuhopecten yessoensis* is of considerable commercial value. In China, scallops (bay scallop *Argopecten irradians* and Japanese scallop *M. yessoensis*) took the third position among the cultured shellfish, the production reached 1,040,000 t in 2005 (Fang, 2006). In Zhang Zidao Island (Liaoning province, China) – the largest aquaculture area for Japanese scallop – annual production reached 40,000 t, in 2005 the total yields was about 13,130 t (Zhang *et al.*, 2006a). In Japan, current total harvest of scallops from stock enhancement in Hokkaido is similar to 300,000 t per annum (Uki, 2006). Korean production of *M. yessoensis* gradually increased beginning in 1990. For example, in Gangwon province, the volume of scallops produced using aquaculture increased 298 times from 6.2 t to 1,852 t from 1991 to 1996 (six years) (Choi, 2002).

The Japanese scallop is the most important commercial mollusc in the Russian Far East. From the beginning of 1971, the Experimental Marine Farm raising the Japanese scallop functions in Minonosok Bay (Posyet Bay, East Sea). Nevertheless, the parasites and infective diseases of scallops from this area have been studied insufficiently. The present paper was a part of five-year program on investigation of the state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth (The state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth. Vladivostok: Dalnauka,

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2001). The specific target of the present work was to perform histopathological study of different organs of Japanese scallop cultured in Minonosok Bay.

MATERIAL AND METHODS

The Japanese scallop *Mizuhopecten* (= *Patinopecten*) *yessoensis* (Jay) of different age (1, 2, 4 and 5 years old; the generation of three years old molluscs was absent owing to mass mortality in 1996) cultured in Experimental Marine Farm in Minonosok Bay (Fig. 1) were collected on July 22, 1999. One and two years old scallops were removed from the shell and preserved whole. Large scallops were dissected and the samples of labial palps, lips, esophagus, intestine, digestive gland, gills, kidney, gonad, adductor muscle of 25 animals of each age class were preserved for analysis. The whole animals or the samples of different organs were fixed with Bouin's fluid and embedded in paraffin wax using a routine protocol. Histological sections were stained with haematoxylin & eosin and analyzed for the presence of pathological changes.

RESULTS

In the epithelium of gills, labial palps, lips,

esophagus, and other organs, basophilically stained parasitophorous vacuoles (inclusions) containing prokaryotes were detected (Fig. 2 A). Basophilic inclusions were localized along the base of epithelia of listed organs or in the epithelia, squeezing the neighboring cells and deforming the epithelial stratum in whole. The size of basophilic inclusions changed from 30 to 180 μm (the longest dimension), it was much larger than the height of epithelia, particularly in gills (Fig. 2 B). In that case spherical and oval inclusions protrude from the surface of respiratory epithelium. Under releasing of microorganisms, the integrity of epithelium was broken nevertheless the reaction of surrounding tissues was negligible. The similar basophilic inclusions of smaller size were observed in digestive tubules with localization in the cytoplasm of digestive cells.

Three degrees of the intensity of epithelia infection have been distinguished: slight (one inclusion in the field of examination under magnification $\times 160$); moderate (up to 3 inclusions in the field of examination); severe (more 3 inclusions in the field of examination) (Fig. 2 C, D). On the presence and intensity of infection by microorganisms, the organs and tissues of 4-years old scallops should be arranged

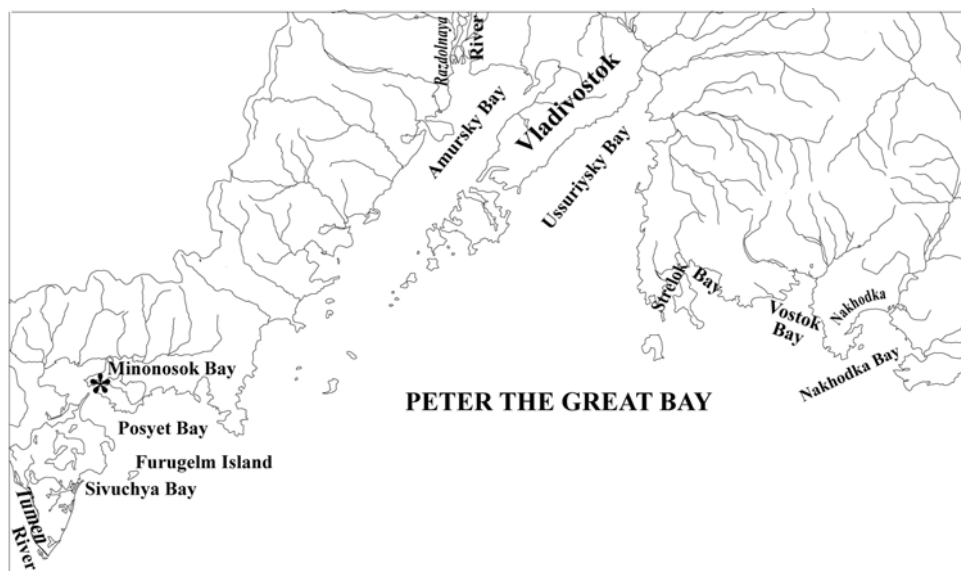


Fig. 1. Schematic map of location of Minonosok Bay (Peter the Great Bay, East Sea)

in descending order: epithelium of labial palps and lips \geq epithelium of esophagus \geq epithelium of intestine \geq epithelium of gills $>$ epithelium of digestive tubules $>$ connective tissue. Histological study revealed that the prevalence of infections was 100%. Investigation of the scallops of different ages has shown that molluscs of all the age groups were infected. Intensity of infection in one and two years old scallops was high too. Apart from the intracellular

inclusions containing prokaryotes, sparse protozoa were observed on the surface of gills.

Special attention was paid to 4-years old scallops because of the individuals of the same age from natural population in southwestern part of Peter the Great Bay were studied as well (unpublished data). The state of the digestive gland of 4-years old molluscs corresponds to the normal largely, but individual variations in the frequency of digestive

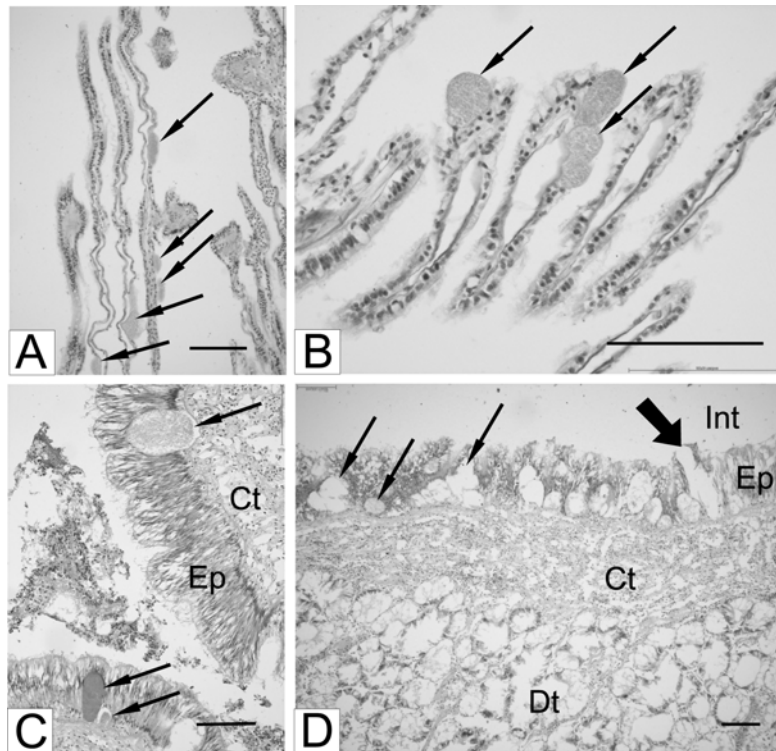


Fig. 2. Morphological changes in the organs of Japanese scallop *Mizuhopecten yessoensis* from Experimental Marine Farm in Minonosok Bay conditioned by prokaryotic infection. **A:** Severe degree of infection in the gills. At least 5 basophilic inclusions are distinguishable in the field of examination (arrows). Longitudinal section of gills; **B:** Basophilic inclusions (arrows) are localized in the respiratory epithelium, the size of inclusions is much larger than the height of epithelium. Transverse section of gills; **C:** Severe degree of infection of labial pulps. Parasitophorous vacuoles are shown by arrows. Intensity of basophilic staining of vacuoles are differed; **D:** Severe degree of infection of intestine, which pass through digestive diverticulum. Thin arrows show numerous vacuoles in the epithelium. Broad arrow shows on the vacuoles, the content of which dispersed into the lumen of intestine. Abbreviations: Int – intestine, Ct – connective tissue, Dt – digestive tubules, Ep – epithelium. Bar indicate 100 μ m.

tubules of different types were observed. In several individuals almost all digestive tubules were of I (holding) and IV (reconstituting) types (for description of tubule types in *Mizuhopecten yessoensis*, see Syasina *et al.*, 1997) with very low height of digestive cells and dilated lumen (Fig. 3 A). In other individuals the digestive tubules of II (absorptive) and III (fragmenting) types were also occurred that corresponds to the normal state at this time of year. The epithelial constitution was normal, no alterations of basophilic cells was detected. The characteristic of morphology of digestive cells was their vacuolization (occurrence 100%), the number of pigmented inclusions (tertiary lysosomes) was regular. Desquamation of digestive cells observed in 40% of molluscs result in the formation of erosive area with

bare spots of basement membrane (Fig. 3 B). Connective tissue between digestive tubules in all animals (100%) was infiltrated by hemocytes. The dilatations between digestive tubules in the digestive gland of cultured molluscs were found sometimes. The significant degenerative and destructive changes such as tubule atrophy and necrosis were registered only in 10% scallops.

On the moment of investigation (22 July 1999) gonads of 4-years old individuals were unfilled; the signs of gametogenesis were absent. Syncytium-like structures were present inside several acini.

In the kidney of scallops no remarkable changes were found. Renal concretions were of usual size and not numerous, local infiltration by hemocytes was occurred. Hemocytic infiltration of internal organs

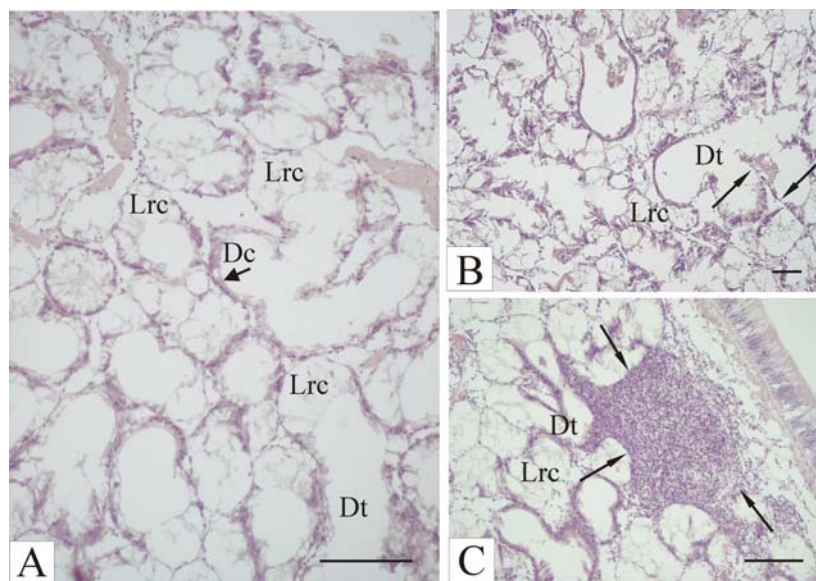


Fig. 3. Morphology of the digestive gland of Japanese scallop *Mizuhopecten yessoensis* from Experimental Marine Farm in Minonosok Bay. **A:** The state of the digestive gland in July that corresponds to the normal largely. The changes are: almost all digestive tubules are on the same stage of the digestive cycle. The epithelium is very low, the lumen is extended. The height of the digestive cells is highly reduced (arrow), lipid reserving cells with large empty vacuoles are hypertrophied; **B:** The state of the digestive gland is worse than on Fig. A, because the large part of epithelial cells of the tubules and ducts is desquamated. Arrows point to the erosive area of epithelium with bare spots of basement membrane; **C:** Inflammation and formation of granulocytome consisting of numerous hemocytes (arrows). Abbreviations: Dt – digestive tubules, Dc – digestive cells, Lrc – lipid reserving cells. Bar indicate 100 μ m.

Table 1. Rickettsia- and chlamydia-like infections in molluscs of the family Pectinidae

Mollusc species	Area	Microorganism ¹	References
<i>Argopecten irradians</i>	Canada (Prince Edward Island)	ChLOs	Morrison and Shum, 1982
	Canada (Prince Edward Island)	RLOs	Morrison and Shum, 1983
	Atlantic U.S.A. (NE)	RLOs	Leibovitz <i>et al.</i> , 1984
	Atlantic North America (NE)	ChLOs	Leibovitz, 1989
	China (Laizhou Bay)	RLOs	Li and Wu, 2004
<i>Chlamys varia</i>	Atlantic U.S.A. (NE)	RLOs	Le Gall <i>et al.</i> , 1991
<i>Chlamys opercularis</i>	Atlantic U.S.A. (NE)	RLOs	Le Gall <i>et al.</i> , 1991
<i>Chlamys deliculata</i>	New Zealand	n.c.	Hine and Diggles, 2002
<i>Patinopecten (Mizuhopecten) yessoensis</i>	Japan, Aomori prefecture	RLOs	Elston, 1986
	Russia, Peter the Great Bay	n.c.	Present study
<i>Pecten maximus</i>	France (Brittany), Sweden, Scottish	RLOs	Le Gall <i>et al.</i> , 1988, 1991; Le Gall and Mialhe, 1992
<i>Pecten novaezelandiae</i>	New Zealand	RLOs	Hine and Diggles, 2002
<i>Placopecten magellanicus</i>	Atlantic U.S.A. (Rhode Island)	RLOs	Gulka <i>et al.</i> , 1983; Gulka and Chang, 1984a

Note 1. Microorganisms were classified as rickettsiae (rickettsia-like) (RLOs) or chlamydiae (chlamydia-like) (ChLOs), n.c. - not classified.

testimonial about inflammation was characteristic for scallops from Minonosok Bay. The most advanced stage of this process - the formation of granulocytomes - was found in several individuals (Fig. 3 C). The nuclei in numerous hemocytes were lobular or fabiform, hemocytes which look like binuclear were observed too.

In whole the significant abnormality was found in scallops from Experimental Marine Farm in Minonosok Bay: prokaryotic infection with prevalence 100%, destruction of digestive epithelium and other changes in digestive system, infiltration of organs by hemocytes and granulocytome formation. The most affected by prokaryotic infection organs are labial palps, lips, esophagus, intestine and gills. The observed pathological alterations argue in favour of ill-being of cultured scallops.

DISCUSSION

The process of cultivation of different scallop species is similar in common, growth of molluscs occur under the high density, that facilitate the arising and spreading different infective diseases. Mass mortality

occurs among cultured scallops in result of damage by pathogenic organisms such as viruses, haplosporidia, rickettsia-like and chlamydia-like (Gulka and Chang, 1984a; Chu *et al.*, 1996; Arzul *et al.*, 2001; Hine and Diggles, 2002; Wang *et al.*, 2006). In Asia-Pacific region, the bay scallop *Argopecten irradians* is cultivated in China beginning 1982, the mortality was noticed in 1988 and spread to several facilities of Shandong and Liaoning provinces (Chu *et al.*, 1996). Significant mortality of cultured scallop *A. irradians* took place in 1999 (Laizhou Bay) (Li and Wu, 2004). An intracellular rickettsia-like organism was proposed to be responsible for scallop mortalities after an epidemic investigation.

Intermittent mortalities of up to 39% per annum have been reported among wild scallops *Pecten novaezelandiae* and *Chlamys deliculata* in New Zealand (Hine and Diggles, 2002). In *P. novaezelandiae*, two types of highly prevalent (95-100%) basophilic inclusion in the branchial epithelium contained rickettsia-like organisms. The possible role of these organisms in pathogenesis and as the cause of mass mortalities in wild scallop stocks was investigated (Hine and Diggles, 2002). Occurrence

of rickettsia- and chlamydia-like infections in molluscs of the family Pectinidae is summarized in Table 1.

In the present paper was shown that 100% of 4-years old scallops cultivated in Minonosok Bay highly infected by prokaryotic organisms. Mass mortality of scallops took place in some years, so in 1999 the generation of three years old molluscs was absent because spat collected in summer of 1996, have died in winter (personal communication of farm's workers). The presence of basophilic intracellular inclusions is characteristic feature of rickettsial or chlamydial infection in molluscs and fish. The first rickettsial infection in molluscs was reported from epithelial cells of digestive gland tubules (Comps *et al.*, 1977), and later on in other organs of the cultured Pacific oyster *Crassostrea gigas* from the south Atlantic coast of France (Renault and Cochenec, 1994). The colonies of microorganisms in epithelial gill cells of *C. gigas* were enclosed in a parasitophorous vacuole having a unit-type membrane. Colonies were composed of large irregular pleomorphic prokaryote cells from 0.4 to 4.0 μm in length and 0.2 to 2.0 μm in diameter.

At the present time intracellular rickettsia-like organisms (RLOs) and chlamydia-like organisms (ChLOs) have been found in more than 25 species of marine bivalve molluscs (Fryer and Lannan, 1994). Localization of RLOs and ChLOs detected in the epithelia of digestive gland (Harshbarger *et al.*, 1977; Buchanan, 1978; Comps and Deltreil, 1979; Gulka *et al.*, 1983; Elston and Peacock, 1984; Cajaraville and Angulo, 1991), gills (Elston, 1986; Le Gall *et al.*, 1988; Fries *et al.*, 1991; Renault and Cochenec, 1994; Hine and Diggles, 2002), kidney (Morrison and Shum, 1983).

The Japanese scallop *Mizuhopecten yessoensis* is listed among the bivalve molluscs for which infection by rickettsia/chlamydia-like organisms has been described (Elston, 1986). The similarity of lesion signs, the same localization of the infective agents in the organisms of scallops examined in the present study and described previously (Elston, 1986) allow us to propose that scallops from Experimental Marine Farm in Minonosok Bay infected by rickettsia- or

chlamydia-like organisms, but special methods are needed for precise diagnostic of these microorganisms. Parasites and infective diseases of Japanese scallop from southwestern part of Peter the Great Bay and Posyet Bay have been studied insufficiently. The list of registered pathogens includes 12 species, but only a few of them are really hazardous for this mollusc (Kurochkin *et al.*, 1986). Among them, 4 species are organisms boring the shell (sponge *Cliona* sp. and 3 species of polychaetes *Polydora*) from the remainder 8 species, which may be applied to the true parasites, 2 species (fungus *Sirolopidium zoophthorum* and apicomplexan parasite *Perkinsus* sp.) were occurred only once, 1 species (trematode metacercaria, *Podocotyle*) are occurred very seldom, the other 5 parasites (infusoria, copepode *Herrmannella longicaudata* and gastropods) could not induced any appreciable pathology in scallops under normal conditions (Kurochkin *et al.*, 1986).

Rickettsia- and chlamydia-like organisms belong to obligate parasites, infections appear to cause little damage except under conditions of intensive culture or in certain natural stress conditions (Elston, 1986), where increased infection intensities could impair various physiological processes. Labial palps and lips, esophagus, intestine and gills were the most infected organs in *Mizuhopecten yessoensis* cultured in Minonosok Bay, but no defense reaction of molluscs organism was observed in this organs. The intensity of digestive gland infection was much lower, nevertheless, hemocytic infiltration observed in all scallops. Probably we should not exclude the influence of other pathogenic conditions and factors. Several types of bivalve damage and response to prokaryotic infection are known today. In mussel *Mytilus galloprovincialis* (Basque coast) infected by chlamydia-like organisms, the spheric colonies were usually surrounded by lipofuscin-like yellow granules that could indicate a localized metabolic damage within infected digestive cells (Cajaraville and Angulo, 1991). Cellular defense response with participation of granular amebocytes was observed in mussel *Mytilus edulis* from Rhode Island (Atlantic U.S.A.) infected with rickettsia-like organisms (Gulka and Chang,

1984b). In *Argopecten irradians* – one of the most important commercial species of Pectinidae family in China, both granulocytes and hyalinocytes showed a phagocytic response to the RLOs (Zhang *et al.*, 2006b). The phagocytic ability of granulocytes was significantly higher than that of hyalinocyte.

A lot of histopathological changes were found in scallops cultured in Minonosok Bay. The observed pathological changes argue in favor of ill-being of cultured scallops. One of the reason observed pathological alteration is prokaryotic infections. The scallop yearlings farmed in Minonosok Bay were settled in Sivuchya Bay in June 1996 and at other localities in southwestern Peter the Great Bay too from 1972 through 1984, and in 1995, 1996, 1998 (Vyshkvartsev *et al.*, 2005). According to Vyshkvartsev *et al.* (2005) about 2,636,000 one-year-old scallops were transplanted from Minonosok Bay to near shore locations in southwestern part of Peter the Great Bay. It seems the introduction of scallop yearlings from Experimental Marine Farm has been facilitating the distribution of prokaryotic infections to natural scallop habitat. The effects of transfers and introductions of scallop and other bivalve molluscs are always more or less unpredictable (Mortensen, 2000).

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