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Review on the Protozoan Parasite *Perkinsus olseni* (Lester and Davis 1981) Infection in Asian Waters

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Abstract—Perkinsosis is a shellfish disease caused by protozoan parasite belonging to the genus *Perkinsus*. Perkinsosis has been reported in some commercially important shellfishes including oysters, clam, abalone and scallop. Heavy infection with *Perkinsus* often results in tissue inflammation and mass mortalities. *Perkinsus* trophozoites are commonly occurring in gills, digestive glands, mantle and gonadal connective tissues. *Perkinsus* is also believed to be responsible for the decline in clam landings for the past decades in Korea. *Perkinsus* infection was also reported in China, Japan and Thailand, from Japanese short-necked clam and the undulated clam. Microscopic features of different life stages and DNA sequences from the non-transcribed spacer and internal transcribed spacer indicated that *Perkinsus* sp. discovered in Asian waters are *P. olseni*. Field survey results suggested that reduced growth and reproduction as well as mass mortalities observed in some major clam beds in Korea was in part, associated with high level of *Perkinsus* infection.

Keywords: *Perkinsus olseni*, *Ruditapes philippinarum*, shellfish disease, protozoan parasite, pathogen

1. INTRODUCTION

In the year 2007, global aquaculture production rose to 50,329,007 MT and shellfish production accounted for 26.0% (13,071,573 MT) of the world aquaculture production (FAO, 2009). It is remarkable that over 80% of the world shellfish production in 2007 originated from Asia, mostly from China, Japan and Korea. Oysters, clams and cockles, scallops and mussels were the main shellfish species that covered over 90% of the world shellfish aquaculture production. Currently these species are cultured in high density using intensive culture systems, which requires relatively less area for the culture. However, culture of these species in high density and limited space often cause outbreaks of epidemic diseases. Shellfish disease outbreaks have been recognized as a significant constraint to aquaculture production and trade, affecting both the economic development and socio-economic revenue. To date, several shellfish diseases have been reported from some commercially important marine bivalves.

Table 1. *Perkinsus* species reported in the world.

Species	Host species	Location	Author
P. marinus	*Crassostrea gigas*	Gulf of Mexico Atlantic coast of USA	Mackin et al. (1950)
P. olseni	*Haliotis rubra*	Australia	Lester and Davis (1981)
P. atlanticus	*Ruditapes philippinarum*, *R. decussatus*	Portugal, Spain, France, Italy, possibly in Korea	Azevedo (1989)
P. qugwadi	*Patinopecten yessoensis*	Pacific coast of Canada	Blackbourn et al. (1998)
P. andrewsi	*Macoma baltica*	Atlantic coast of USA	Coss et al. (2001)
P. chesapaeki	*Mya arenaria*	Atlantic coast of USA	McLaughlin et al. (2000)
P. mediterraneus	*Ostrea edulis*	Mediterranean Sea	Casas et al. (2005)
P. honshuensis	*Venerupis philippinarum* (=*R. philippinarum*)	Japan	Dungan and Reece (2006)
P. beihaiensis	*C. hongkongensis*, *C. ariakensis*	Southern China	Moss et al. (2008)

FAO has listed several significant shellfish diseases including Bonamiosis, Marteiliosis, Haplosporidiosis, Marteilioidosis and Perkinsosis (see Bondad-Reantaso et al., 2001). In Asian waters, several studies have reported marine bivalve diseases associated with *Perkinsus olseni*, a protozoan pathogen responsible for the mass mortalities of the venerid clams of the genus *Ruditapes* (i.e., *Tapes* or *Venerupis*) inhabiting along the Mediterranean and Atlantic coasts of Europe (Da Ros and Cansonier, 1985; Chagot et al., 1987; Sagrista et al., 1995; Canestri-Trotti et al., 2000). In this paper, we review life cycle, host organisms, pathologic features and impacts of *Perkinsus*.

2. HOST ORGANISMS AND LIFE CYCLE OF *PERKINSUS*

Since the first report of *P. marinus* (=*Dermocystidium marinum*) in the Gulf of Mexico (Mackin et al., 1950), several species of *Perkinsus* have been identified from various marine mollusks including oysters, scallops, clams and abalones in the world. Table 1 summarizes types of *Perkinsus* and their host organisms reported so far. Azevedo (1989) first reported on the occurrence of *Perkinsus* infection in clam *R. decussatus* in Portugal and he named this new *Perkinsus* parasite as *P. atlanticus*. Murrell et al. (2002) compared the internal transcribed spacer (ITS) and non-transcribed spacer (NTS) regions of DNA of *P. olseni* collected in Australian water with other *Perkinsus* species. Genetic similarity of *P. olseni* and *P. atlanticus* observed in the ITS and NTS sequences was very high, indicating that *P. olseni* and *P. atlanticus* is conspecific. Accordingly, *P. atlanticus* become synonym of *P. olseni* due to the taxonomic priority. *Perkinsus*-like organism was also found in undulated surf clam *Paphia undulata* in Gulf of Thailand (Leethochavalit et al., 2004).

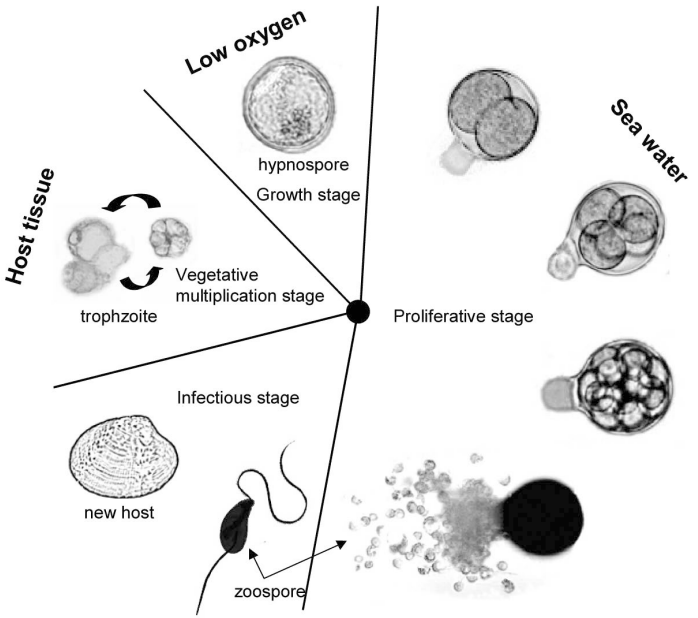

Fig. 1. Life cycle of *P. olseni* parasitizing in the Manila clam *R. philippinarum* (Modified from Auzoux-Bordenave et al. 1995, Choi et al., 2005).

Analysis of ITS 1, 2 and NTS with 5.8S ribosomal RNA of *Perkinsus* sp. isolated from the undulated surf clam strongly suggested that *Perkinsus* sp. discovered in Gulf of Thailand is *P. olseni* (Leethochavalit et al., 2003). Park et al. (2005) also compared ITS 1, 2, NTS and 5.8S rRNA sequences of *Perkinsus* sp. isolated from *R. philippinarum* in Korean waters with those of *P. olseni* reported elsewhere. The sequence analysis revealed that there is 99.9% genetic similarity between *Perkinsus* sp. in Korean waters and *P. olseni* reported in Australia. Accordingly, they concluded that *Perkinsus* sp. isolated from clams in Korea is *P. olseni* (Park et al., 2005). *P. olseni* was also discovered in the Venus clam, *Protothaca jedoensis* distributed on the south coast of Korea (Park et al., 2006b). Recently, Dungan and Reece (2006) reported a new *Perkinsus* species in Japanese little-neck clam *Venerupis philippinarum* (=*R. philippinarum*) in Gokasho Bay, Mie Prefecture and they named it as *P. honshuensis* (Dungan and Reece, 2006). New *Perkinsus* species was also isolated and identified from oysters in the southern Chinese coast. In 2008, Moss et al reported *P. beihaiensis*, a new *Perkinsus* species found in oyster *Crassostrea hongkongensis* and *C. ariakensis* in the southern Chinese waters.

Perkinsus olseni has a unique life cycle (Fig. 1) consisting of trophozoite, hypnospore and zoospore (Auzoux-Bordenave et al., 1995; Perkins, 1996; Choi et al., 2005). In clam tissues, *Perkinsus* trophozoite occurs as a single cell (=tomont) of

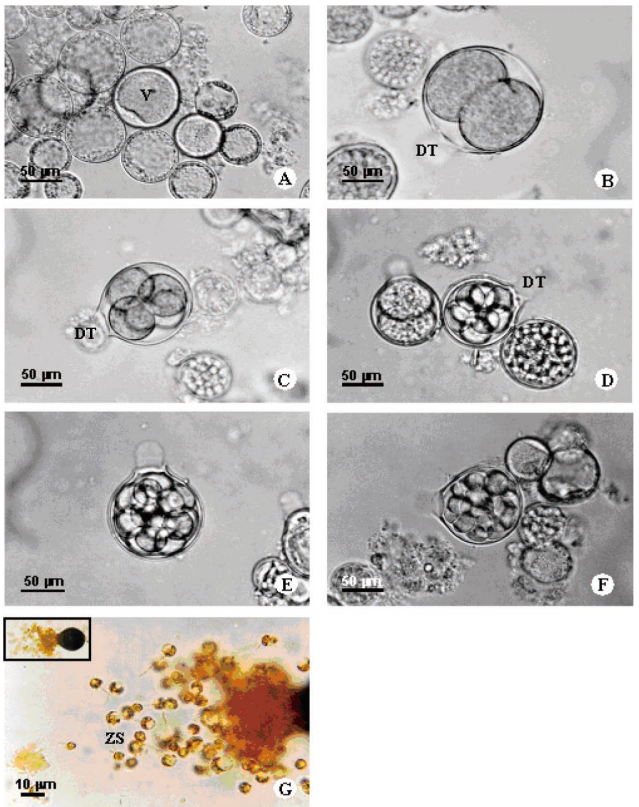

Fig. 2. *In vitro* sporulation of *Perkinsus* sp. in GF/C filtered seawater. A: Beginning of eccentric vacuole subdivision, B: 2 cell-stage, C: 4 cell-stage, D: 8 cell-stage, E: 16 cell-stage, F: 32 cell-stage, G: Discharge of motile zoospores. Scale-bar = 25 μm. Vacuole (V); Discharging tube (DT); Zoospore (ZS) (Park et al., 2005)

multi-nucleated form. Once the trophozoites are placed in an anaerobic condition such as in fluid thioglycollate medium (FTM) or in necrotic tissues, they develop a dormant form of hypnospores (=prezoosporangia), which are characterized as enlarged cell size and thick cell wall stained as dark blue or brown with iodine. When the hypnospores are placed in aerated seawater, they undergo zoosporulation (Fig. 2). Two to three days after initial incubation at room temperature, a pore which later forms a discharge tube, is observed in the cell wall of hypnospore as early as in 2-celled stage (Fig. 2). Successive bipartition of the nuclei results in 4, 8, 16 to 64 cells of bi-flagellated zoospores. After two to three days of incubation, the zoospores are released from mature hypnospores via the discharge tube (Fig. 2G). *In vitro* induction of the zoosproluation was also confirmed in *P. honshuensis* and *P. beihaiensis* (Dungan and Reece, 2006; Moss et al., 2008).

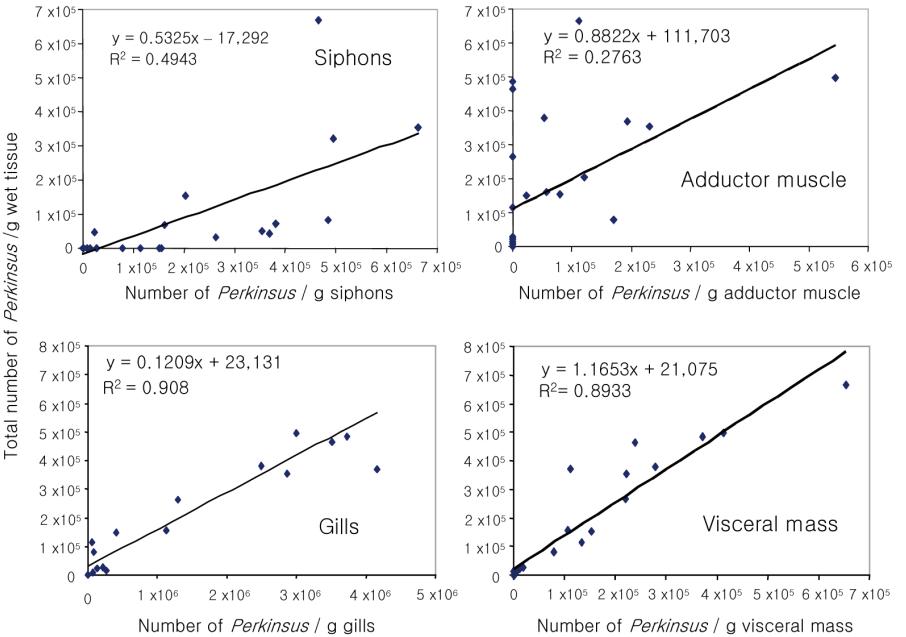

Fig. 3. Correlation between the total number of *Perkinsus* per gram tissue wet weight and the number of *Perkinsus* per gram in siphons, adductor muscle, gills and visceral mass (Choi et al., 2002).

3. DIAGNOSTICS OF *PERKINSUS* INFECTION

Numerous methods have been applied in the diagnosis of *Perkinsus* infection including histology and electron microscopy (Mackin, 1951; Perkins and Menzel, 1966; Azevedo, et al., 1990; Navas et al., 1992; Montes et al., 1996; Bower et al., 1998), the fluid thioglycollate medium (FTM) technique (Ray, 1953, 1966; Choi et al., 1989; Bushek et al., 1994; Rodriguez and Navas, 1995; Fisher and Oliver, 1996; Almeida et al., 1999; Ford et al., 1999), immunology (Choi et al., 1991; Dungan et al., 1993; Romestand et al., 2001) and PCR assays (Marsh et al., 1995; Penna et al., 2001; Park et al., 2002; Dungan and Reece, 2006; Moss et al., 2008). Since first use of FTM in *P. marinus* diagnostic by Ray (1953), Ray's FTM assay (it is often called as RFTM) is currently the most widely used in *Perkinsus* study. RFTM technique has been successfully applied in the study of *Perkinsus* spp. infection in clams in Europe and Asia (Auzoux-Bordenave et al., 1995; Rodriguez and Navas, 1995; Choi and Park, 1997; Cigarria et al., 1997; Almeida et al., 1999; Park and Choi, 2001; Park et al., 2006a). For quantitative assessment of *Perkinsus* infection, total number of *Perkinsus* cells in a clam (i.e., total body burden) was counted by dissolving a whole clam with 2 M NaOH after incubation in FTM (Choi et al., 1989; Almeida et al., 1999; Park et al., 1999; Park and Choi, 2001). The NaOH digestion assay is affordable and sensitive enough to detect only a few cells in an individual clam (Park and Choi,

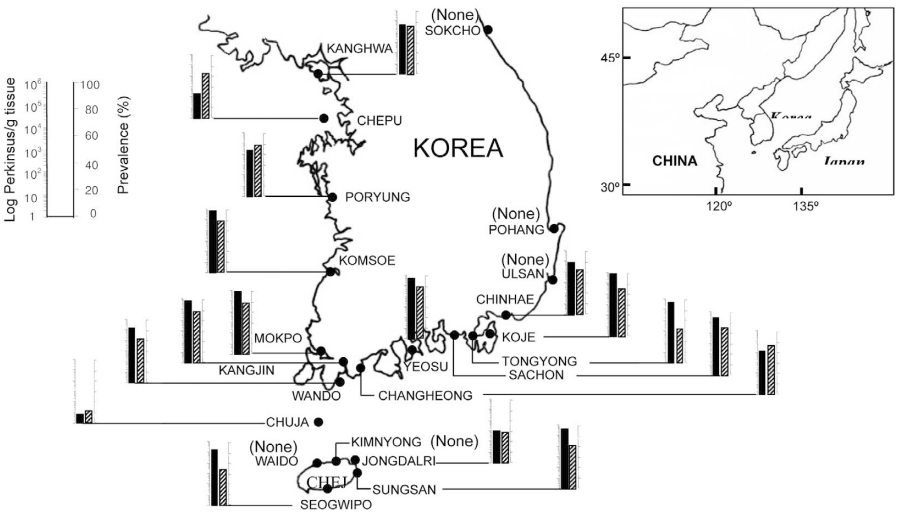

Fig. 4. Mean infection intensity (*Perkinsus* cells/g wet tissue) and prevalence (% of infected clams). "None" represents no infection observed (Park and Choi, 2001).

2001). Rodriguez and Navas (1995) also suggested that the total body burden analysis with FTM followed by 2 M NaOH digestion method is a method of choice for accurate diagnosis of *Perkinsus* infection. Choi et al. (2002) found that there is a strong positive correlation between the total number of *Perkinsus* cells in clams and the number of *Perkinsus* cells in gill tissues of the clams collected from Isahaya Bay, Japan (Fig. 3). They recommended the gill assay for the routine diagnostic of *Perkinsus* infections in clams, instead of whole clam assay.

4. *P. OLSENI* INFECTION STATUS IN ASIAN WATER

Since the first report on the occurrence of *Perkinsus* by Choi and Park (1997) in Korean waters, several studies have surveyed *Perkinsus* infection in different clam populations. Park et al. (1999) investigated prevalence (i.e., percentage of infected clams) and infection intensity (i.e., number of *Perkinsus* cells per unit weight) of *Perkinsus* in Manila clams in Gomsoe Bay on the west coast in late summer when a mass mortality of the clams occurred. It was noticeable that almost all of the clams examined were infected with *P. olseni* (i.e, prevalence = 100%) and the infection intensity ranged 11,000–2,000,000 cells/g wet tissue. Based upon the survey, they suggested that mass mortalities of clams observed in Gomsoe Bay in late summer were closely associated with the extremely high level of *Perkinsus* in clams. Using RFTM and histology, Park and Choi (2001) also surveyed the prevalence and infection intensity of clam populations in 22 sites located on the west, south and east coasts. The survey revealed that most clams from commercial clam beds on the west and south coasts, where clams are cultured on sandy-mud tidal flats with high density

were heavily infected with *P. olseni* with the intensity ranging 0–870,000 cells/g wet tissue. In contrast, clams inhabited in sand beaches on the east and Jeju Island were free from the infection (Fig. 4). The survey suggested that water temperature, salinity, density of clam and types of substrate are the key environmental factors that govern the infection intensity and prevalence (Park and Choi, 2001). *Perkinsus* infection in *R. philippinarum* was also reported from clam populations distributed on the northern coast of the Yellow Sea along the Liaodung Peninsula, China. Using RFTM, Liang et al. (2001) surveyed the prevalence and infection intensity in clam populations in Dalian on the northern Yellow Sea. As high as 4,391,732 *Perkinsus* cells/individual or 2,271,883 cells/g wet tissue were observed in the northern China.

Perkinsus-like pathogen was also reported from clam populations in Hiroshima, Kumamoto and Nagasaki areas in Japan (Hamaguchi et al., 1998; Maeno et al., 1999; Choi et al., 2002). Hamaguchi et al. (1998) first diagnosed *Perkinsus* infection in clams in Kumamoto and Hiroshima using FTM, histology and PCR. In histology, trophozoite of *Perkinsus* could be found all types of the tissue and they could observe white spots (i.e., nodules), sign of *Perkinsus*-associated tissue inflammation on the surface of the body of some heavily infected clams. FTM assay also indicated that 64–94% of clam analyzed in their study were infected with *Perkinsus*. DNA sequences of *Perkinsus* isolated from clam populations in Kumamoto and Hiroshima also suggested that the pathogen found in the clam populations was *P. atlanticus* (=*P. olseni*). Choi et al. (2002) examined prevalence and infection intensity of *Perkinsus* in a clam population in Isahaya Bay in Ariake Sound using FTM. The survey showed that clams collected in October 2001 from Isahaya Bay are rather moderately infected with *P. olseni* with the prevalence of 57% and infection intensity of 226,000 cells/g wet tissue or 352,000 cells/individual. Similarly, Park et al. (2008) reported *P. olseni* infection intensity in the Japanese short-neck clam in Kumamoto tidal flats as 0–464,000 cells/g wet tissue with prevalence of 20–97%.

5. IMPACTS OF *PERKINSUS* INFECTION

High level of *Perkinsus* infection often results in slow growth, tissue necrosis and mass mortalities in clam and oyster populations (Mackin, 1962; Park et al., 1999; Park and Choi, 2001). In particular, *P. atlanticus* (=*P. olseni*) has been blamed for mass mortalities of the venerid clams of the genus *Ruditapes* (i.e., *Tapes* or *Venerupis*) inhabiting Mediterranean and Atlantic coasts of Europe (Da Ros and Cansonier, 1985; Chagot et al., 1987; Sagrista; et al., 1995; Canestri-Trotti et al., 2000). In Manila clam, *Perkinsus* trophozoites are mostly aggregated in gills, digestive diverticulars and mantle while they are less common in the foot, adductor muscle and siphons (Fig. 5). *Perkinsus* cells are also found in connective tissues of female and male gonadal tissues (Park and Choi, 2001; Choi et al., 2002). As shown in Fig. 5, heavily infected clams often exhibit white nodules on their mantle, foot and gills due to inflammatory reaction of the clams (Figs. 5B, D). The heavily infected clams also exhibited numerous clusters of trophozoites on their gill plica and connective tissue of the digestive tubules with severe hemocytic infiltration (Figs. 5E, F). Such a heavy infection in gill tissues would deteriorate the filtering

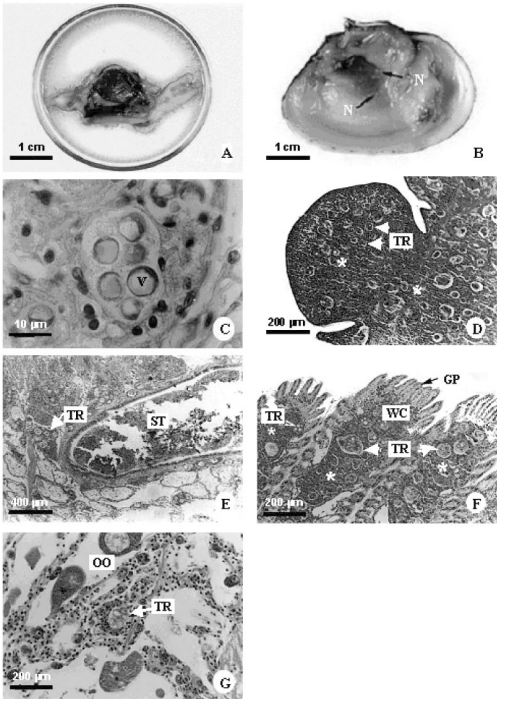

Fig. 5. Internal and external features of *Perkinsus* infection in the Manila clam. A; Lugol's iodine stained *Perkinsus* hypnospores after FTM incubation. B; Nodules on the clam body caused by inflammatory reaction against the parasite. C; Trophozoites of *Perkinsus* in clam tissues. D; A nodule showing the infiltration of host's hemocytes around *Perkinsus* cells. E; Trophozoites parasitizing around the stomach. F; Gills infiltrated with trophozoites and clam hemocytes. G; Trophozoites in the connective tissues of the female gonad. Nodule (N); Eccentric vacuole (V); Trophozoite (TR); Stomach (ST); Gill plica (GP); Water chamber (WC); Oocyte (OO); Asterisk-infiltrated clam hemocytes (Park and Choi, 2001).

activity and its efficiency and, in turn, retards growth of the host animal. Infestation of *Perkinsus* in digestive tubules would cause digestive tubule atrophy and exert deleterious effects on the food digestion, as reported by Lee et al. (2001). *Perkinsus* was also observed among the connective tissues of female and male gonads (Fig. 5G), indicating that *Perkinsus* parasitism also interferes reproductive activity of clams in some way.

Deleterious effects of *Perkinsus* infection on the host animal reproduction have been reported for the past decades. According to Choi et al. (1989), high level of *P. marinus* infection in the American oyster *C. virginica* continuously depletes the net energy of oysters that supposedly used in the growth and reproduction. As a consequence, the heavily infected oyster exhibits reduced growth and reproductive effort (Choi et al., 1993). Choi et al. (1994) also reported a negative correlation

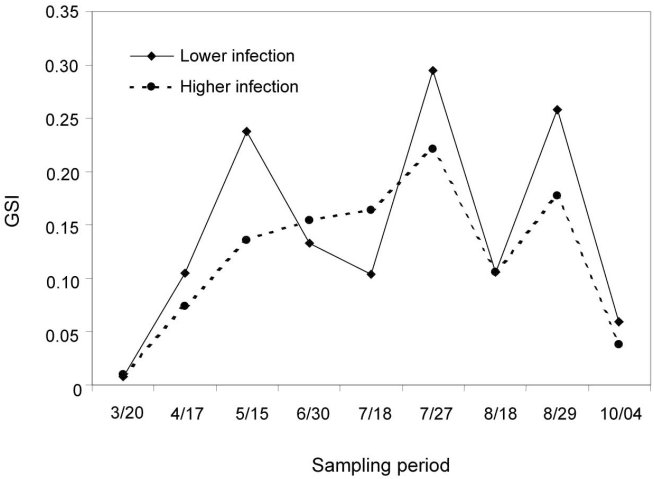

Fig. 6. Seasonal variation in the gonad-somatic index (GSI, mg egg/mg dry-tissue) of *Ruditapes philippinarum* in Gomsoe Bay, Korea in 1999. Higher infection; clams with the infection intensity higher than that of the monthly mean, and lower infection, clams with the infection intensity lower than that of the monthly mean (Park et al., 2006).

between infection intensity of *P. marinus* and instantaneous rate of reproduction in the American oysters; the heavier the infection, the longer it took the oyster to become ready for spawning. Dittman et al. (2001) observed *P. marinus* infection-induced decrease in relative gonad size and reproductive effort in the female oysters in Delaware Bay.

Park et al. (2006a) investigated impacts of *Perkinsus* infection on reproductive effort (i.e., the quantity of egg) of *R. philippinarum* in Gomsoe Bay, Korea using immunoassay and FTM technique. For quantification of the egg mass, they developed polyclonal antibody against the clam egg protein and the reproductive effort was estimated using enzyme-linked immunosorbent assay (ELISA, Park and Choi, 2004). Figure 6 shows the monthly changes in reproductive effort and *P. olseni* infection intensity in Gomso Bay. To investigate the impact, reproductive effort of clams (i.e., gonad somatic index, GSI) measured from March to October in 1999 was grouped into 1), those of clams with infection intensity higher than the monthly mean intensity (i.e., higher infection in Fig. 6) and 2), the other with their infection intensity lower than the monthly mean (i.e, lower infection in Fig. 6). In an annual reproductive cycle of clam in Gomso Bay, three distinct GSI peaks could be identified in the lower infection clams, indicating that less heavily infected clams spawned at least three times during the spawning period (mid May, late July and late August). In each spawning peak, the lower infection clams produced eggs as much s 25 to 30% of their body weight. In contrast, quantity of eggs produced from the clams in heavy infection group was much smaller, approximately one-half of the clams in light infection

during the first spawning peak in May. The heavily infected clams also exhibited lower GSI in late July and August relative to clams in light infection group. The data clearly demonstrated that high level of *Perkinsus* infection interfere reproductive activity of clam resulting in reduced egg production and retarded gonad maturation.

In conclusion, *Perkinsus* has been reported in Asian waters, including Korea, China, Japan and Thailand. Heavy infection with *Perkinsus* in the Manila clams resulted in various pathologic symptoms such as tissue inflammation and hemocyte infiltration around the infected areas. High level of *Perkinsus* infection also reduced reproductive effort as well as retarding gonad maturation of host animals including clams and oysters.

REFERENCES

Almeida, M., F. Berthe, A. Thebault and M.T. Dinis. 1999. Whole clam culture as a quantitative diagnostic procedure of *Perkinsus atlanticus* (Apicomplexa, Perkinsea) in the clam *Ruditapes decussatus*. *Aquaculture* **177**: 325–332.

Auzoux-Bordenave, S., A.M. Vigario, F. Ruano, I. Domart-Coulon and D. Doumenc. 1995. *In vitro* sporulation of the clam pathogen *Perkinsus atlanticus* (Apicomplexa, Perkinsea) under various environmental conditions. *Journal of Shellfish Research* **14**: 469–475.

Azevedo, C. 1989. Fine structure of *Perkinsus atlanticus* n. sp. (Apicomplexa, Perkinsea), a parasite of the clam *Ruditapes decussatus* from Portugal. *Journal of Parasitology* **75**: 627–635.

Azevedo, C. L. Corral and R. Cachola. 1990. Fine structure of zoosporulation in *Perkinsus atlanticus* (Apicomplexa: Perkinsea). *Parasitology* **100**: 351–358.

Blackbourn, J., S.M. Bower and G.R. Meyer. 1998. *Perkinsus qugwadi* sp. nov. (incertae sedis), a pathogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Canadian Journal of Zoology* **76**: 942–953.

Bondad-Reantaso, MG., S.E. McGladdery, I. East and R.P. Subasinghe. 2001. *Asia diagnostic guide to aquatic animal diseases*. FAO Fishereis Technical Paper 402/2. FAO, Rome, 240 pp.

Bower, S.M., J. Blackbourn and G.R. Meyer. 1998. Distribution, prevalence, and pathogenicity of the protozoan *Perkinsus qugwadi* in Japanese scallops, *Patinopecten yessoensis*, cultured in British Columbia, Canada. *Canadian Journal of Zoology* **76**: 954–959.

Bushek, D., S.E Ford and S.K Allen. 1994. Evaluation of methods using Ray's fluid thioglycollate medium diagnosis of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*. *Annual Review of Fish Diseases* **4**: 201–217.

Canestri-Trotti, G., E.M. Baccarani, F. Paesanti and E. Turolla. 2000. Monitoring of infection by protozoa of the genera *Nematopsis*, *Perkinsus*, and *Porospora* in the smooth venus clam, *Callista chione*, from the northwestern Adriatic Sea (Italy). *Diseases of Aquatic Organisms* **42**: 157–161.

Casas, S.M., A. Grau, K.S. Reece, K. Apakupakul, C. Azevedo and A. Villalba. 2005. *Perkinsus mediterraneus* n. sp., a protistan parasite of the European flat oyster *Ostrea edulis* from the Balearic Islands, Mediterranean Sea. *Diseases of Aquatic Organisms* **58**: 231–244.

Chagot, D., M. Comps, V. Boulo, F. Ruano and H. Grizel. 1987. Histological study of a cellular reaction in *Ruditapes decussatus* infected by a protozoan. *Aquaculture* **67**: 260–261.

Choi, K.-S. and K.-I. Park 1997. Report on occurrence of *Perkinsus* sp. in the Manila clam, *Ruditapes philippinarum*, in Korea. *Journal of the Korean Aquaculture Society* **10**: 227–237.

Choi, K.-S., E.A. Wilson, D.H. Lewis, E.N. Powell and S.M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. *Journal of Shellfish Research* **8**: 125–131.

Choi, K.-S., D.H. Lewis, E.N. Powell, P.F. Frelier and S.M. Ray. 1991. A polyclonal antibody developed from *Perkinsus marinus* hypnospores fails to cross react with other life stages of *P. marinus* in oyster (*Crassostrea virginica*) tissue. *Journal of Shellfish Research* **10**: 411–415.

Choi, K.-S., D.H. Lewis, E.N. Powell and S.M. Ray. 1993. Quantitative measurment of reproductive output in the American oyster, *Crassostrea virginica* (Gmelin), using an enzyme-linked immunosrobent

assay (ELISA). *Aquaculture and Fisheries Management* **24**: 375–398.

Choi, K-S., E.N. Powell, D.H. Lewis and S.M. Ray. 1994. Instantaneous reproductive effort in female American oysters, *Crassostrea virginica*, measured by a new immunoprecipitation assay. *Biological Bulletin* **186**: 41–61.

Choi, K.-S., K.-L. Park, K.-W. Lee and K. Matsuoka. 2002. Infection intensity, prevalence and histopathology of *Perkinsus* sp. in the Manila clam, *Ruditapes philippinarum*, in Isahaya Bay, Japan. *Journal of Shellfish Research* **21**: 119–125.

Choi, K.-S., K.-I. Park, M. Cho and P. Soudant. 2005. Diagnosis, pathology, and taxonomy of *Perkinsus* sp. isolated from the Manila clam *Ruditapes philippinarum* in Korea. *Journal of the Korean Aquaculture Society* **18**: 207–214.

Cigarria, J., C. Rodrigues and J.M. Fernandez. 1997. Impact of *Perkinsus* sp. on the Manila clam, *Ruditapes philippinarum*, beds. *Diseases of Aquatic Organisms* **29**: 117–120.

Coss, C.A., J.A. Robledo, G.M. Ruiz and G.R. Vasta. 2001. Description of *Perkinsus* andrewsi n. sp. isolated from the Baltic clam (*Macoma balthica*) by characterization of the ribosomal RNA locus, and development of a species-specific PCR-based diagnostic assay. *Journal of Eukaryotic Microbiology* **48**: 52–61.

Da Ros, L. and W. J. Canzonier 1985. *Perkinsus*, a protistan threat to bivalve culture in the Mediterranean basin. *Bulletin of the European Association of Fish Pathology* **5**: 23–27.

Dittman, D.E., S.E. Ford and D.K. Padilla. 2001. Effects of *Perkinsus marinus* on reproduction and condition of the eastern oyster, *Crassostrea virginica*, depend on timing. *Journal of Shellfish Research* **20**: 1025–1034.

Dungan, C.F. and K.S. Reece. 2006. *In vitro* propagation of two *Perkinsus* spp. Parasites from Japanese Manila clams *Venerupis philippinarum* and description of *Perkinsus honshuensis* n. sp. *Journal of Eukaryotic Microbiology* **53**: 316–326.

Dungan, C.F. and B.S. Roberson 1993. Binding specificities of mono- and polyclonal antibodies to the protozoan oyster pathogen *Perkinsus marinus*. *Diseases of Aquatic Organisms* **15**: 9–22.

FAO. 2000. *FAOSTAT fisheries data*. http://apps.fao.org/page/collections?subset=fisheries.

Fisher, W.S. and L.M. Oliver. 1996. A whole-oyster procedure for diagnosis of *Perkinsus marinus* disease using Ray's fluid thioglycollate culture medium. *J. Shellfish Res.* **15**: 109–117.

Ford, S.E., A. Schotthoefer and C. Spruck. 1999. *In vivo* dynamics of the microparasite *Perkinsus marinus* during progression and regression of infections in eastern oysters. *Journal of Parasitology* **85**: 273–282.

Hamaguchi, M. N. Suzuki, H. Usuki and H. Ishioka. 1998. *Perkinsus* protozoan infection in the short-necked clam, *Tapes* (=*Ruditapes*) *philippinarum*, in Japan. *Fish Pathology* **33**: 473–480.

Lee, M.-K., B.-Y. Cho, S.-J. Lee, J.-Y. Kang, H.-D. Jeong, S.-H. Huh and M.-D. Huh. 2001. Histopathological lesions of Manila clam, *Tapes philippinarum*, from Hadong and Namhae coastal areas of Korea. *Aquaculture* **201**: 199–209.

Leethochavalit, S., E.S. Upatham, K.-S. Choi, P. Sawangwong, K. Chalermwat and M. Kruatrachue. 2003. Ribosomal RNA characterization of non-transcribed spacer and two internal transcribed spacers with 5.8S ribosomal RNA of *Perkinsus* sp. found in undulated surf clams (*Paphia undulata*) from Thailand. *Journal of Shellfish Research* **22**: 431–434.

Leethochavalit, S., K. Chalermwat, E.S. Upatham, K.-S. Choi, P. Sawangwong and M. Kruatrachue. 2004. The occurrence of *Perkinsus* sp. in undulated surf clams *Paphia undulata* from the Gulf of Thailand. *Diseases of Aquatic Organisms* **60**: 165–171.

Lester, R.J.G. and G.H.G. Davis. 1981. A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone, *Haliotis ruber*. *Journal of Invertebrate Pathology* **37**: 181–187.

Liang, Y.-B., X.-C. Zhang, L.-J. Wang, B. Yang, Y. Zhang and C.-L. Cai. 2001. Prevalence of *Perkinsus* sp. in the Manila clam, *Ruditapes philippinarum*, along the northern coast of the Yellow Sea in China. *Oceanologica et Limnologica Sinica* **32**: 502–511 (in Chinese with English abstract).

Mackin, J.G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. *Bulletin of Marine Science* **1**: 72–87.

Mackin, J.G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publication of Institute of Marine Science, University of Texas* **7**: 132–229.

Mackin, J.G., H.M. Owen and A. Collier. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp., in *Crassostrea virginca* (Gmelin). *Science* **111**: 328–329.

Maeno, Y., T. Yoshinaga and K. Nakajima. 1999. Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) from Manila clam, *Tapes philippinarum*, in Japan. *Fish Pathology* **34**: 127–131.

Marsh, A.G., J.D. Gauthier and G.R. Vastar. 1995. A semiquantitative PCR assay for assessing *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*. *Journal of Parasitology* **81**: 577–583.

McLaughlin, S.M., B.D. Tall, A. Shaheen, E. Elsayed and M. Faisal. 2000. Zoosporulation of a new *Perkinsus* species isolated from the gills of the softshell clam *Mya arenaria*. *Parasite* **7**: 115–122.

Moss, J.A., J. Xiao, C.F. Dungan and K.S. Reece. 2008. Description of *Perkinsus beihaiensis* n. sp., a new *Perkinsus* sp. parasite in oysters of southern China. *Journal of Eukaryotic Microbiology* **55**: 117–130.

Montes, J., M. Durfort and J. Garcia-Valero. 1996. When the venerid clam, *Tapes decussatus*, is parasitized by the protozoan *Perkinsus* sp. it synthesizes a defensive polypeptide that is closely related to p225. *Diseases of Aquatic Organisms* **26**:149–157.

Murrell, A., S.N. Kleeman, S.C. Barker and R.G.L. Lester. 2002. Synonymy of *Perkinsus olseni* Lester & Davis, 1981 and *Perkinsus atlanticus* Azevedo, 1989 and an update on the phylogenetic position of the genus *Perkinsus*. *Bulletin of the European Association of Fish Pathology* **22**: 258–265.

Navas, J.I., M.C. Castillo, P. Vera and M. Ruiz-Rico. 1992. Principal parasites observed in the clams, *Ruditapes decusastus* (L), *Ruditapes philippinarum* (Adams et Reeve), *Venerupis pullastra* (Montagu), and *Venerupis aureus* (Gmelin), from Huelva coast (S.W. Spain). *Aquaculture* **107**: 193–199.

Park, K.-I. and K.-S. Choi. 2001. Spatial distribution of the protozoan parasite, *Perkinsus* sp., found in the manila clam, *Ruditapes philippinarum* in Korea. *Aquaculture* **203**: 9–22.

Park, K.-I. and K.-S. Choi. 2004. Application of enzyme-linked immunosorbent assay (ELISA) for studying of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca: Bivalvia): I. quantifying eggs. *Aquaculture* **241**: 667–687.

Park, K.-I., K.-S. Choi and J.-W. Choi. 1999. Epizootiology of *Perkinsus* sp. found in the Manila clam, *Ruditapes philippinarum*, in Komsoe Bay, Korea. *Journal of the Korean Fisheries Society* **32**: 303–309 (in Korean with English abstract).

Park, K.-I., Y.-M. Park J.-H. Lee and K.-S. Choi. 2002. Development of PCR assay for detection of the protozoan parasite *Perkinsus*. *Korean Journal of Environmental Biology* **20**: 109–117 (In Korean with English abstract).

Park, K.-I., J.-K. Park, J. Lee and K.-S. Choi. 2005. Use of molecular markers for species identification of Korean *Perkinsus* sp. isolated from Manila clam *Ruditapes philippinarum*. *Diseases of Aquatic Organisms* **66**: 255–263.

Park, K.-I., A. Figueras and K.-S. Choi. 2006a. Application of enzyme-linked immunosorbent assay (ELISA) for the study of reproduction in the Manila clam *Ruditapes philippinarum*: (Mollusca: Bivalvia): II. impacts of *Perkinsus olseni* on clam reproduction. *Aquaculture* **251**: 182–191.

Park, K.-I., T.T.T. Ngo, S.-D. Choi, M. Cho and K.-S. Choi. 2006b. Occurrence of *Perkinsus olseni* in the venus clam *Protothaca jedoensis* in Korean waters. *Journal of Invertebrate Pathology* **93**: 81–87.

Park, K.-I., H. Tsutsumi, J.-S. Hong and K.-S. Choi. 2008. Pathology survey of the short-neck clam *Ruditapes philippinarum* occurring on sandy tidal flats along the coast of Ariake Bay, Kyushu, Japan. *Journal of Invertebrate Pathology* **99**: 212–219.

Penna, M.-S., M. Khan and R.A. French. 2001. Development of multiplex PCR for the detection of *Haplosporidium nelsoni*, *Haplosporidium costale* and *Perkinsus marinus* in the eastern oyster (*Crassostrea virginica* Gmelin, 1791). *Molecular and Cellular Probe* **15**: 385–390.

Perkins, F. O. 1996. The structure of *Perkinsus marinus* (Mackin, Owen, and Collier, 1950) Levine, 1978, with comments on the taxonomy and phylogeny of *Perkinsus* sp. *Journal of Shellfish Research* **15**: 67–87.

Perkins, F.O. and R.W. Menzel. 1966. Morphological and cultural studies of a motile stage in the life cycle of *Dermocystidium marinum*. *Proceedings of the National Shellfisheries Association* **56**: 23–30.

Ray, S.M. 1953. Studies on the occurrence of *Dermocystidium marinum* in young oysters. *Proceedings of the National Shellfisheries Association* **44**: 80–92.

Ray, S.M. 1966. A review of the culture method for detecting *Dermocystidium marinum* with suggested modifications. *Proceedings of the National Shellfisheries Association* **54**: 55–69.

Rodriguez, F. and J.I. Navas. 1995. A comparison of gill and hemolymph assays for the thioglycollate diagnosis of *Perkinsus atlanticus* (Apicomplexa, Perkinsea) in clams, *Ruditapes decussatus* (L.) and *Ruditapes philippinarum* (Adams et Reeve). *Aquaculture* **132**: 145–152.

Romestand, B., J. Torreilles and P. Roch. 2001. Production of monoclonal antibodies against the protozoa, *Perkinsus marinus*: estimation of parasite multiplication *in vitro*. *Aquatic Living Resources* **14**: 352–357.

Sagrista, E., M. Durfort and C. Azevedo. 1995. *Perkinsus* sp. (Phylum Apicomplexa) in Mediterranean clam *Ruditapes semidecussatus*: ultrastructural observations of the cellular response of the host. *Aquaculture* **132**: 153–160.

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