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.
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).

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Host species</th>
<th>Location</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. marinus</em></td>
<td><em>Crassostrea gigas</em></td>
<td>Gulf of Mexico, Atlantic coast of USA</td>
<td>Mackin et al. (1950)</td>
</tr>
<tr>
<td><em>P. olseni</em></td>
<td><em>Haliotis rubra</em></td>
<td>Australia</td>
<td>Lester and Davis (1981)</td>
</tr>
<tr>
<td><em>P. atlanticus</em></td>
<td><em>Ruditapes philippinarum</em></td>
<td>Portugal, Spain, France, Italy, possibly in Korea</td>
<td>Azevedo (1989)</td>
</tr>
<tr>
<td><em>P. qugwadi</em></td>
<td><em>Patinopecten yessoensis</em></td>
<td>Pacific coast of Canada</td>
<td>Blackbourn et al. (1998)</td>
</tr>
<tr>
<td><em>P. andrewsi</em></td>
<td><em>Macoma baltica</em></td>
<td>Atlantic coast of USA</td>
<td>Coss et al. (2001)</td>
</tr>
<tr>
<td><em>P. chesapaeki</em></td>
<td><em>Mya arenaria</em></td>
<td>Atlantic Sea</td>
<td>McLaughlin et al. (2005)</td>
</tr>
<tr>
<td><em>P. mediterraneus</em></td>
<td><em>Ostrea edulis</em></td>
<td>Mediterranean Sea</td>
<td>Casas et al. (2000)</td>
</tr>
<tr>
<td><em>P. honshuensis</em></td>
<td><em>Venerupis philippinarum</em> (=<em>R. philippinarum</em>)</td>
<td>Japan, Southern China</td>
<td>Dungan and Reece (2006)</td>
</tr>
<tr>
<td><em>P. beihaiensis</em></td>
<td><em>C. hongkongensis</em>, <em>C. ariakensis</em></td>
<td>Southern China</td>
<td>Moss et al. (2008)</td>
</tr>
</tbody>
</table>
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
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 zoosporulation was also confirmed in *P. honshuensis* and *P. beihaiensis* (Dungan and Reece, 2006; Moss et al., 2008).
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., 2006a). 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,
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
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...
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 Gomso 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 as 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 retard gonad maturation of host animals including clams and oysters.

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