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Perkinsus olseni (Lester and Davis 1981) infection in the Manila clam (*Ruditapes philippinarum*) in Korea; species identification, impacts and spatio-temporal distribution

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Abstract: Asari or Manila clam *Ruditapes philippinarum* (=*Venerupis philippinarum*) is commonly distributed on the west and south coast of Korea, supporting the local shellfisheries industry. Studies examined Manila clams in Korean waters have reported Perkinsosis, a parasitic disease caused by protozoan belonging to the Genus *Perkinsus*. PCR analysis of the Perkinsosis agent indicated that *P. olseni* is only the Perkinsosis agent in Manila clam in Korean waters, while *P. houshuensis*, first discovered in Japan from Japanese Manila clam, detected from *R. variagata* in coastal Jeju Island. Several surveys carried out in Korean waters for the past decade revealed that infection intensity of *P. olseni* over $1x10^6$ cells/g tissue caused necrosis and lesion of the gills and the mantle tissues. Heavy infection with *P. olseni* also retarded reproductive process of the clams, including slow gonad maturation and decrease in the reproductive effort. Clams survived from mass mortality event occurred during post-spawning period on the west coast also showed high infection prevalence and intensity of *P. olseni*, suggested that *P. olseni* is closely linked to the mass mortality event. Despite the high infection intensity, some clam populations on the coastal Yellow Sea where the phytoplankton biomass was relatively higher, showed relatively higher condition factor, suggested that high level of food supply may compensate the energetic loss caused by high load of the parasite in Manila clam.

Key words: *Ruditapes philippinarum*, *Perkinsus*, parasitic disease, food condition factor

Endemic to the western Pacific, Manila clam or Asari *Ruditapes phillipinarum* is commonly distributed on the West and South coast of Korea, supporting the local shellfisheries industry. For the past two decades, the annual landings of Manila clams in Korea have declined due to decrease in the population size, although the main cause of this decrease remains unclear. Several studies have reported that recurring mass mortalities of Manila clam on the west coast seems one of the reasons attributable to the decline of the population size and the mass mortality events were focalized to certain areas, where Manila clams have been cultured intensively for the past several decades. Field surveys carried out during the mortality events suggested that the clam mass mortality is in part, associated with a protozoan parasite, *Perkinsus olseni*. The level of *P. olseni* infection among clams from the west coast often exceeded over million cells per gram tissue, considered to be high enough to evoke Perkinsosis, a parasite disease caused by the protozoan parasite belonging to the Genus *Perkinsus*.

Species of genus *Perkinsus* have been reported to cause mass mortalities in cultured and wild mollusks throughout the world. *P. marinus* has affected the oyster *Crassostrea virginica* populations of the USA, causing mass mortality and dramatic economic losses (Andrews, 1996). *P. qugwadii* was identified from Japanese scallop *Mizuhopecten yessoensis*, which was introduced from Japan to west Canada,

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and associated with mortality of the hosts (Bower *et al.*, 1998). *P. olseni* was blamed for mortalities of abalone species *Haliotis* spp. in Australia and New Zealand and clams *R. philippinarum* and *R. decussatus* in Europe (Goggin and Lester, 1995; Choi and Park, 2010). Moreover, *P. olseni* may be playing a major role in the decrease in Manila clam recourse in Japan because high infection levels in clam populations that have suffered decline in population size. *P. honshuensis* was first discovered in Japan from Manila clam (Dungan and Reece, 2006), although it was not found from Manila clam population in Korea. *Perkinsus* species, except for *P. qugwadi*, have propagation phase in host tissue and free living phase in seawater in their life cycle (Fig. 1). Trophozoites are the propagative stage living in host tissue. Death of the host results in anaerobic condition which stimulates the growth of trophozoites into prezoosporangia. When they are exposed to seawater, prezoosporangia become zoosporangia, which release the infective stage, zoospores.

Current Status of Perkinsosis in Korean Waters

In Korea, *Perkinsus* infection was first discovered and reported from Manila clam population in Gomso bay, off the west coast by Choi and Park (1997). PCR analysis (ITS, 2, NTS and 5.8rRNA sequences) of the Perkinsosis agent found from Manila clams revealed 99.9% genetic similarity between other *P. olseni* strains, indicating that *P. olseni* is the Perkinsosis agent in Manila clams in Korean waters (Park *et al.*, 2005). Park *et al.* (1999) investigated

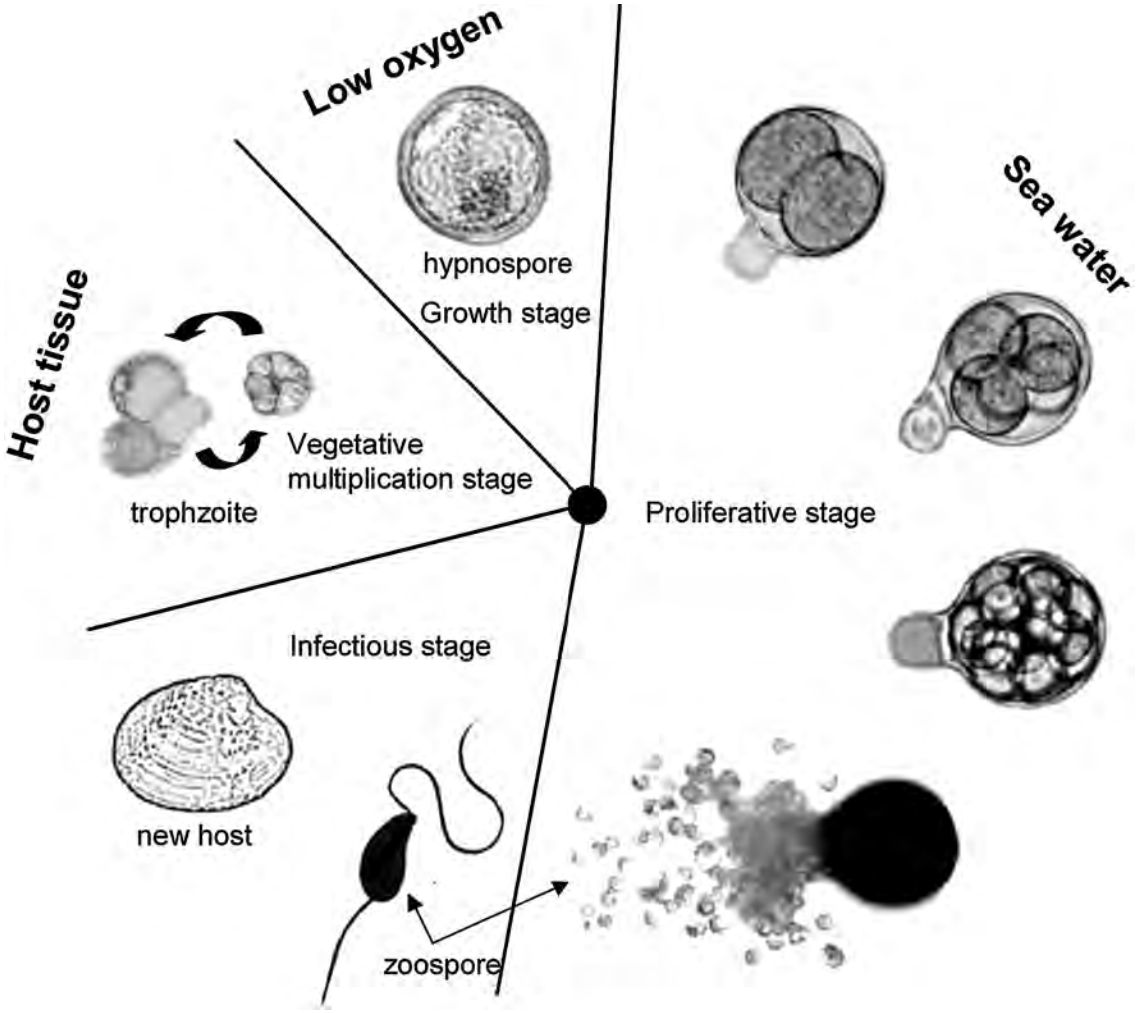

Fig. 1. Lifecycle of *Perkinsus olseni* infect Manila clam *Ruditapes philippinarum* (from Choi and Park, 2010).

P. olseni infection in Manila clam population in Gomso Bay, where recurrent mass mortalities of the clams occurred during post spawning season in fall. The authors reported 100% of prevalence and high infection intensities (up to 2,000,000 cells/g wet tissue) from the clams and suggested heavy infection with *P. olseni* the mass mortalities. Park and Choi (2001) first surveyed *P. olseni* infections in Manila clam populations on the west, south, east and in Jeju Island (Fig. 2). The authors surveyed the infection intensity and prevalence of *P. olseni* from 22 locations using Ray's fluid thioglycollate medium assay (RFTM). As the data revealed, the heavily infected clam populations were concentrated on sandy-mud tidal flats on the west coast, where the tidal flats have been used as a culture ground for the past decades. It was also noticeable that no *P. olseni* infection was observed from clam populations in sand beaches on the east coast and north Jeju Island. The survey suggested that environmental conditions, such as sediment types, salinity and nutrient in the water, determine *P. olseni* infection.

Several studies carried out in Korean waters surveying Perkinsosis for the past decade revealed that heavy infection with *P. olseni* cause necrosis and lesion of the gills and the mantle tissues (Park and Choi, 2010). Such a heavy infection in gill tissues would deteriorate filtration activity, resulting in decline in growth and condition of the host. Heavy infection with *P. olseni* also retarded reproductive process of Manila clams, including slow gonad maturation and decline in the reproductive effort. Park *et al.* (2006) first investigated impacts of *P. olseni* infection on egg production of adult Manila clams in Gosoe Bay, Korea using ELISA, which were developed by Park and Choi (2004). In their study, the amount of egg in an individual clam was negatively correlated with *P. olseni* infection, demonstrating that more heavily infected females produced less amount of egg during spawning season (Fig. 3). Histology also revealed that *P. olseni* trophozoites were commonly found in the connective tissues of female and male gonad (Park and Choi, 2001; Choi *et al.*, 2002), suggested that *P. olseni* interfered the host reproduction. Those data clearly demonstrated that higher level of *P. olseni* infection deteriorates reproduction of clams, resulting in decline in Manila clam populations on the tidal flats in Korea.

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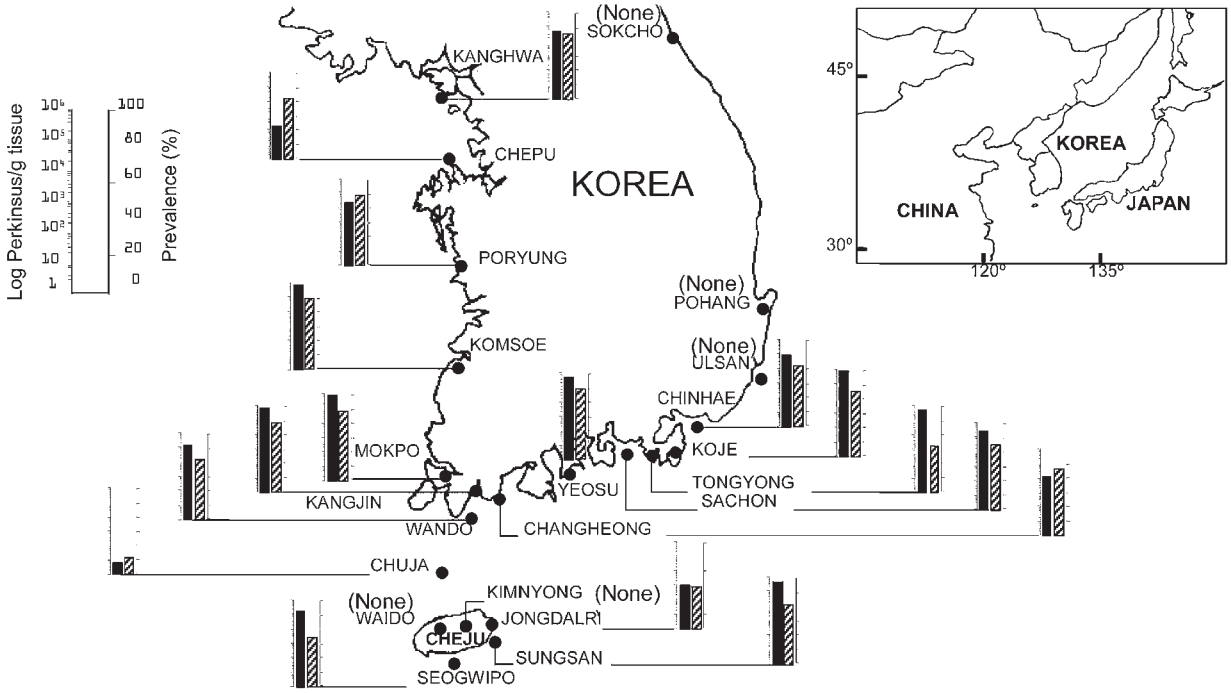

Fig. 2. Mean infection intensity (cells/g wet tissue) and prevalence of *Perkinsus olseni* in Manila clam population in Korea (from Park and Choi, 2001).

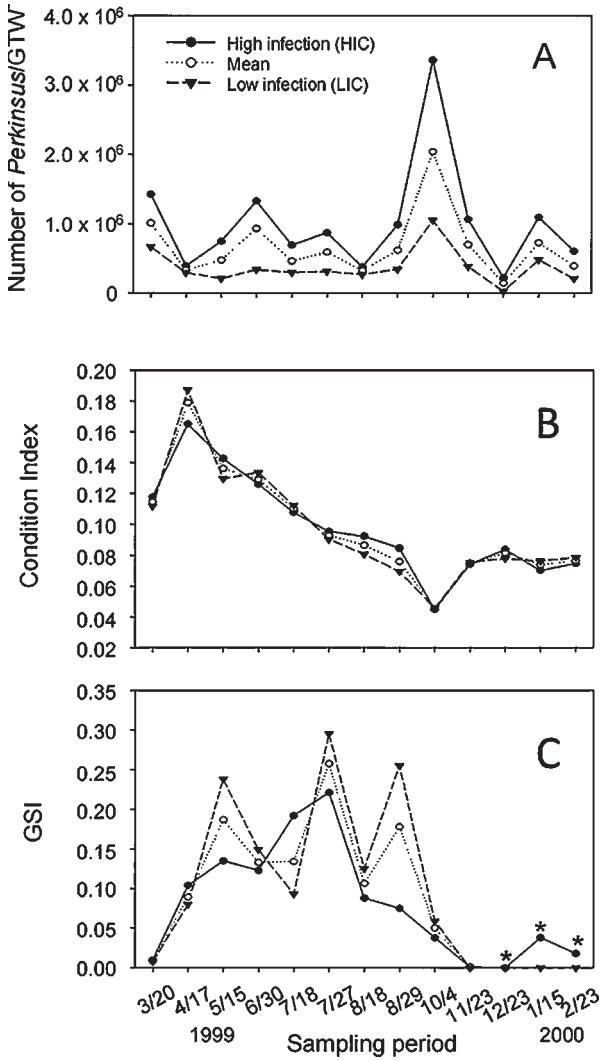

Fig. 3. Seasonal changes in *Perkinsus* infection, protein concentration, condition index and GSI in Gomso Bay, Korea. GTWT represents gram tissue wet weight of Manila clams (from Park *et al.*, 2006).

Institute (NFRDI) of Korea have reported mass mortalities of Manila clams occurred recurrently from clam culture grounds on the west coast of Korea for the past decade. According to NFRDI, the mass mortalities of Manila clam mostly occurred in Incheon Bay and Taean area in early spring and fall. In particular, the mass mortality of Manila clam occurred during fall was likely associated with high level of *P. olseni* infection and post-spawning period in some major clam culture grounds. According to Park and Choi (2001) and Park *et al.* (2006), most of adult clams at major clam culture grounds in Taean and Gomso Bay exhibit high level of *P. olseni* infection and the parasite numbers exceed 10^6 cells/g wet tissue. Waki and Yoshinaga (2013) demonstrated that a cultured strain of *P. olseni* became lethal to Manila clams when geometric mean infection intensities reached ca. 10^6 cells/g wet tissue in juvenile (ca. 5 mm in shell length) and adult Manila clams (ca. 20 mm in shell length). Interestingly, the infection intensity of *Perkinsus* was ca. 10^6 cells/g wet tissue in wild Manila clam populations with the highest infection levels in Korea (Park and Choi, 2001), suggested that *P. olseni* appeared to have had a significant effect on the survival of wild Manila clams in Korea.

Despite the high infection intensity, some clam populations on the coastal Yellow Sea, where the phytoplankton biomass was relatively higher, showed relatively higher condition factor. Accordingly, it is believed that that high level of food supply in these areas may compensate the energetic loss caused by high load of *P. olseni* in Manila clam, resulting in high condition factor. Previous studies have reported that condition of Manila clams is determined by several biotic and abiotic factors, including water temperature, salinity, and level of available food in the environment. As the data showed, Manila clam condition is determined not only by the parasite load including *P. olseni*, but also by environment factors, especially by level food supply.

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