

Note

Pathology Survey of the Manila Clam *Ruditapes philippinarum* from Hwangdo Tidal Flat in Cheonsu Bay on the West Coast of Korea

Hyun-Sung Yang^{1,2}, Young-Ghan Cho¹, Jong-Seop Shin¹, Heung-Sik Park³, and Kwang-Sik Choi^{1*}

¹Department of Marine Life Science and Marine Science Institute (BK21 FOUR),
Jeju National University, Jeju 63243, Korea

²Jeju Marine Research Center, Korea Institute of Ocean Science & Technology, Jeju 63349, Korea

³Research Project Development Department, Korea Institute of Ocean Science & Technology,
Busan 49111, Korea

Abstract : Manila clam *Ruditapes philippinarum* is present at high rates of density in tidal flats in Cheonsu Bay on the west coast of Korea, where clams often exhibit mass mortalities in late summer. We monitored the pathologic condition of clams at Hwangdo tidal flat (HD) to understand the parasitic impacts on clam fitness. Manila clams were fully ripe in July and spawned during August and September, as the histology indicated. The histology revealed that clams in HD tidal flats were heavily infected by the protozoa parasite *Perkinsus olseni*, as the monthly prevalence ranged from 53% (September) to 93% (August). In addition, Manila clams were co-infected by the metazoan parasite *Cercaria tapetis* and *Parvatrema duboisi* with the prevalence of 0-33% and 0-14%, respectively. Massive hemocyte infiltration and subsequent inflammation were commonly observed from the gills of *P. olseni* infected clams. Clusters of *P. olseni* trophozoites and heavy hemocyte infiltration were also observed from the female gonad, suggesting that *P. olseni* interferes with host gonad maturation. The larval trematode occupied almost the entire host gonad, resulting in gonad castration. In addition, Metacercaria of *P. duboisi* were observed from the subsurface of the mantle. Ray's fluid thioglycollate medium assay (RFTM) indicated that clams collected in August and September contained approximately 4.0×10^6 *P. olseni* cells/g gills. Condition Index (CI) declined gradually from spring to early summer, and the decline in CI was interpreted as a consequence of the heavy parasitism, as the parasites drain the host's net energy to be used in somatic growth and gamete production.

Key words : parasitism, *Ruditapes philippinarum*, *Perkinsus olseni*, *Cercaria tapetis*, *Parvatrema duboisi*, condition index

1. Introduction

In the coastal Yellow Sea, the suspension-feeding Manila clam *Ruditapes philippinarum* occurs at a high density from low intertidal to the shallow subtidal, linking benthic primary production to the upper trophic level. On the west coast of Korea, the well-developed sandy-mud tidal flats are often licensed to the local shellfish unions to explore or culture the clams, where the clam density often reaches over 1,000 individuals/m² (Park et al. 2013). In particular,

Cheonsu Bay and the adjacent coast contain numerous tidal flats, and these areas are utilized as a clam culture ground. South Korea has produced 30,000 to 50,000 tons of Manila clams annually for the past decades, and approximately one-third of the annual landings are known to originate from the tidal flats in the Taean coast and Cheonsu Bay. However, Manila clam landings in Korea have declined for the past twenty years, and recent studies reported various pathogenic organisms associated with Manila clams. Previous studies reported that Manila clams in Korean waters are infected by the protozoan *Perkinsus olseni*, and the heavy infection retards clam growth and reproduction (Park and

*Corresponding author. E-mail: skchoi@jejunu.ac.kr

Choi 2001; Park et al. 2006; Lee et al. 2020a, 2021).

During summer and early fall in 2004, Manila clams in HD tidal flat in upper Cheonsu Bay exhibited mass mortality. Field survey and laboratory analysis revealed that clams collected from the tidal flats in the late fall were heavily infected by *P. olsenii* and other metazoan parasites (Lee et al. 2021). The poor fitness, such as low condition factor and low carbohydrate levels observed from the clams collected in October 2004, was likely linked to the unusually high level of the parasite infection previously reported from Asia and Europe (Park and Choi 2001; Villalba et al. 2004; Park et al. 2005; Nam et al. 2018; Waki et al. 2018).

We monitored the health condition and pathology of Manila clams distributed in HD tidal flat from April to September 2005, a year after the clam mass mortality occurred, to better understand the impacts of the parasites. This study reports monthly variation in the condition index and histopathology of Manila clams from HD tidal flat.

2. Materials and Methods

Located in the upper Cheonsu Bay (Fig. 1), the study site has a well-developed tidal flat (1.65×5.15 km) with numerous tidal channels (Choi et al. 2011). Near the low tide line, Manila clam occurs at a high density reaching 660 clams/m² (Park et al. 2013). To monitor the health

condition of clams, we collected 60 adults clams with shell length (i.e., the longest axis of the shell, SL) 30–40 mm from April to September 2005. After measuring SL, the body tissue was removed and weighed to mg using an electronic balance. Condition Index (CI) was determined as a ratio of the wet tissue weight to the dry shell weight (Uddin et al. 2012; Lee et al. 2020b).

For histology, a 3mm-thick longitudinal section was made in the middle of the body and preserved in Davidson's fixative. The cross-section included the gills, mantle, foot, and digestive system. After 24 hrs of fixation, the clam tissue went through a series of ascending ethanol to dehydrate then embedded in paraffin. The paraffin blocks were sliced to 5–6 μ m, deparaffinized, stained with hematoxylin, counterstained with eosin Y, and examined under a light microscope. Manila clam reproductive phase was categorized into 1) resting, 2) early developing, 3) late developing, 4) ripe, 5) spawning, and 6) spent (Park et al. 2006; Uddin et al. 2012). The percentage of the infected clams was estimated and expressed as infection prevalence.

The infection intensity of *P. olsenii* in Manila clam was determined using Ray's fluid thioglycollate medium assay (RFTM, Ray 1966). For the assay, a piece of gill tissue was excised from clam and incubated in FTM media according to Park and Choi (2001) and Park et al. (2005, 2006). The FTM incubated clam tissues were dissolved in

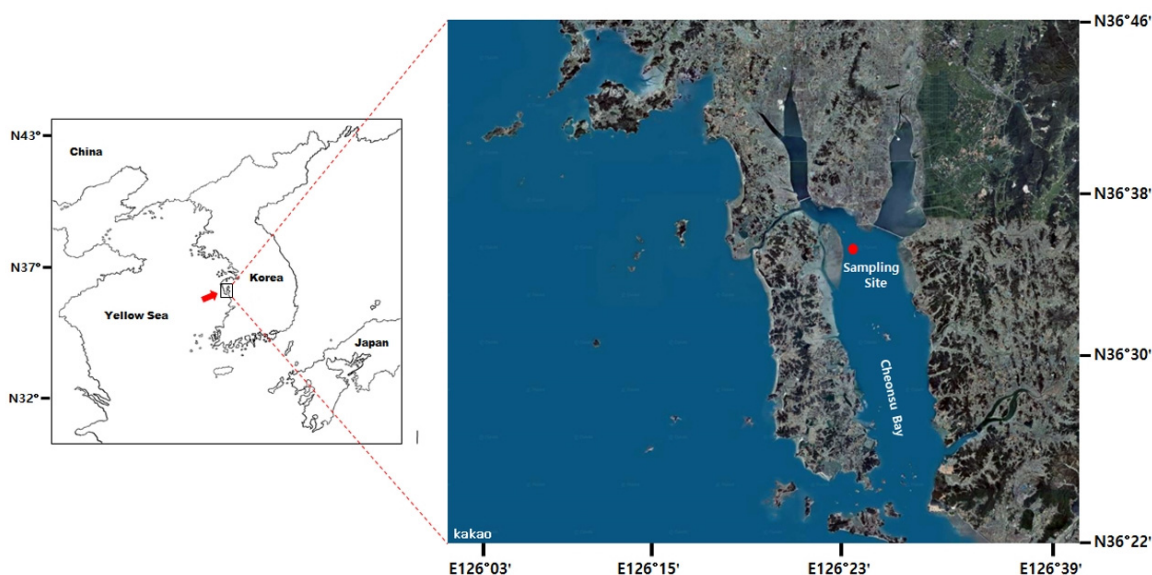


Fig. 1. Hwangdo tidal flat in Cheonsu Bay on the west coast of Korea

2M NaOH, washed several times in phosphate-buffered saline solution (0.15M NaCl, pH = 7.6), and counted *P. olsenii* cell using a hemocyte cell counter (Choi et al. 1989; Yang et al. 2010, 2012). Finally, the infection intensity was recorded as *P. olsenii* cells/g gills.

Monthly means of CI and *P. olsenii* infection intensity were compared using one-way analysis of variance and Duncan's range test. SAS statistical package was used to run the statistical test. The significant statistical level was set at $P < 0.05$.

3. Results and Discussions

Table 1 summarizes the number and size of clams collected and analyzed in this study. A total of 360 clams with SL ranging from 34.8 to 38.2 mm were collected from April to September 2005. Fig. 1 plots the monthly mean CI of Manila clams in HD tidal flat during the study. CI declined significantly from April (0.87) to June (0.74, $P < 0.05$). The mean CI determined in September (0.502) was lower significantly than the values determined in other months ($P < 0.05$).

Histology revealed that one-half of the clams in April were in the late developing stage, and 16% were in the ripe stage (Fig. 2). In July, approximately one-half of the clams analyzed were in the ripe stage, and no clams showed signs of spawning. In August, 37.5% of the clams collected were engaged in spawning. Histology also indicated that the spawning continued to September, as 62.5% of the clams were partially spawned (i.e., spawning), and approximately 10% reached the spent stage.

In histology, *P. olsenii* was found dominantly in the gills (Fig. 4). As shown in Fig. 4a and b, numerous clam hemocytes surrounded the *P. olsenii* trophozoites, a typical sign of inflammation, resulting in swollen gill tissues. The trophozoite stage was also observed in the female gonad

(Fig. 4c) with a sign of massive hemocyte infiltration. Clusters of *P. olsenii* trophozoites appeared in the connective tissue of the digestive tubules (Fig. 4d), suggesting that *P. olsenii* interferes with the gonad maturation and host food absorption process. The larval trematode of *Cercaria tapetis* (Park et al. 2008) was observed commonly in the visceral mass and the branchial connective tissues (Fig. 4e). The sporocysts containing developing germinal balls were also common in the visceral mass (Fig. 4f). The metacercaria stage of the trematode *Parvatrema duboisi* (Cho et al. 2020) occurred at a high density in the mantle epithelial tissue (Fig. 4g). Like *P. olsenii* infection, hemocyte infiltrations were observed along the host tissues occupied by the larval trematodes.

Fig. 5 plots monthly variation in the infection prevalence of *P. olsenii*, *C. tapetis*, and *P. duboisi*. The infection preva-

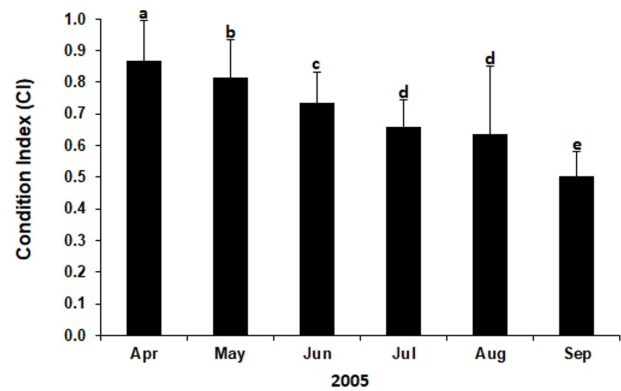


Fig. 2. Monthly mean Condition Index (CI) of Manila clams collected from Hwangdo tidal flat from April to September 2005. The different letters on the bars indicate the significant differences among different months (ANOVA, $P < 0.05$)

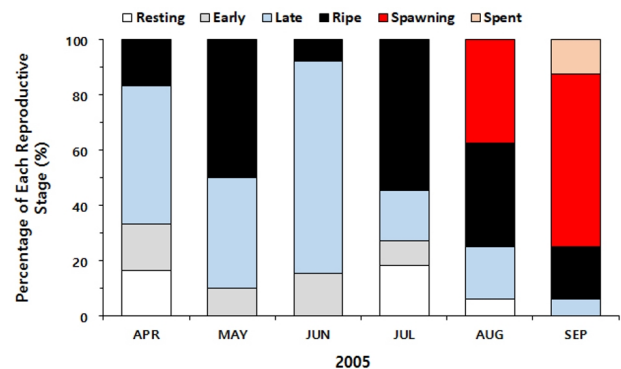


Fig. 3. Reproductive stages of Manila clams collected from April to September 2005 in Hwangdo tidal flat in Cheonsu Bay on the west coast of Korea

Table 1. Summary of the sampling effort

Year	Month	N	SL (mm)±SD
2005	APR	60	35.0±2.7
	MAY	60	36.7±2.7
	JUN	60	37.5±2.5
	JUL	60	34.8±2.1
	AUG	60	38.2±3.0
	SEP	60	37.3±3.2

lence of *P. olseni* increased markedly from April (58.6%) to May (86.7%) then declined in June and July. The highest *P. olseni* prevalence was recorded in August at 93.1%. The larval *C. tapetis* infection prevalence ranged from 3.45 (August) to 33.3% (June), and *P. duboisi* showed its range from 0 (September) to 13.8% (April and August).

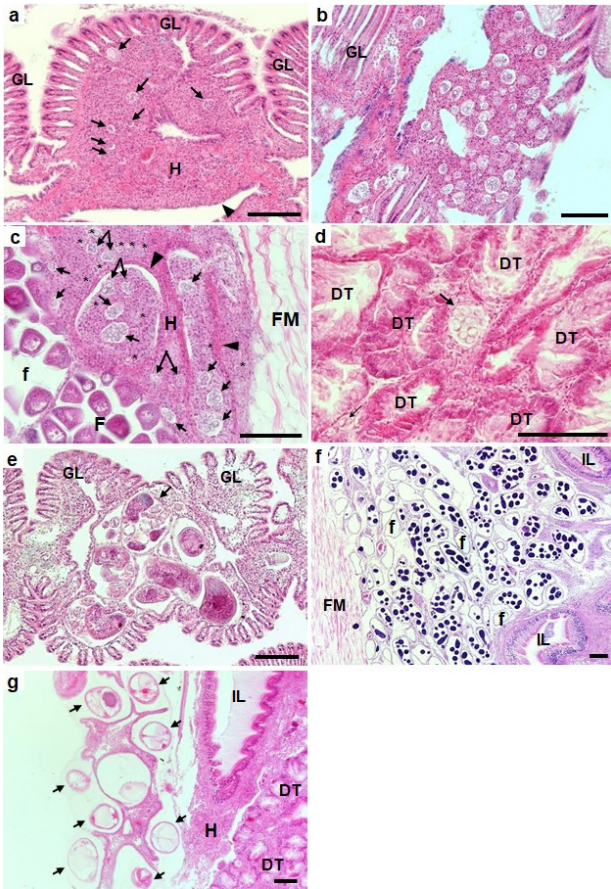


Fig. 4. Photomicrographs showing *Perkinsus olseni* (A–D) and trematodes *Cercaria tapetis* and *Parvatrema duboisi*. (E–G) appeared in the different host tissues (scale bar = 100 μ m). A, clam hemocytes surrounding *P. olseni* trophozoites (arrows) and heavy infiltration of hemocytes (H and the arrowhead) in the branchial connective tissue of gill lamella (GL). B, numerous *P. olseni* clusters and hemocyte infiltration in the gills. C, *P. olseni* trophozoites (* and arrows) in the female follicular connective tissues. D, *P. olseni* (arrows) in the connective tissues of the digestive tubules (DT), E, sporocysts of mature *Cercaria tapetis* in the branchial connective tissue of the gill lamella, F, heavy infestation of *C. tapetis* sporocyst in the gonad, IL, intestinal lumen, G, metacercaria of *Parvatrema duboisi* in the mantle tissue (arrow)

The infection intensity of *P. olseni* in Manila clams varied from 1.5×10^6 to 4.1×10^6 cells/g gills (Fig. 6). When most clams were actively spawning, the intensity increased dramatically from July (2.0×10^6 cells/g gills) to August (4.0×10^6 cells/g gills). One-way ANOVA and Duncan's test indicated that the mean infection intensities determined in September and August were significantly higher than the intensities recorded in May and June ($P < 0.05$).

Lee et al. (2021) first reported parasitic organisms associated with Manila clams in HD tidal flat, where mass mortalities of the clams occurred recurrently for many years. The pathology survey carried out in October 2004 in HD tidal flat, shortly after the mass mortality of Manila clams, revealed that *P. olseni* heavily infected clams with the prevalence of 100% and the mean infection intensity

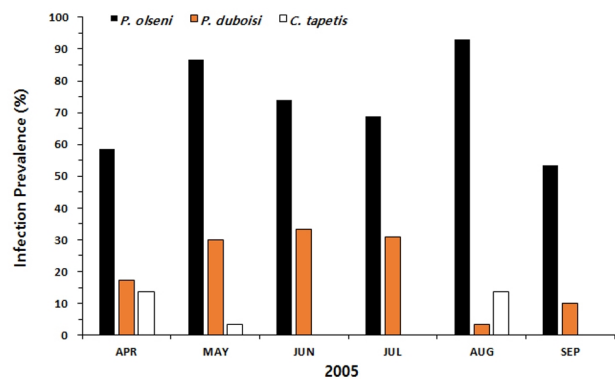


Fig. 5. Monthly variation in the infection prevalence (i.e., percentage of the infected clam) of *Perkinsus olseni*, *C. tapetis*, and *Parvatrema duboisi* in Manila clams in HD tidal flat

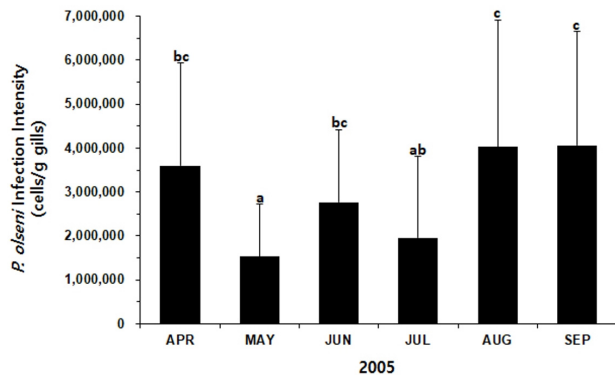


Fig. 6. *P. olseni* infection intensity (cells/g gills) determined using RFTM assay. The different letters on the bars indicate the significant differences among different months (ANOVA, $P < 0.05$)

of 1.74×10^6 cells/g gills. Compared to Lee et al. (2021), the infection intensity measured in August (4.02×10^6 cells/g gills) and September 2005 (4.07×10^6 cells/g gills) is much higher. Park et al. (2006) also monitored *P. olseni* infection in Manila clams in Gomso Bay, approximately 115km to the south of the current study site. According to Park et al. (2006), *P. olseni* infection intensity was the highest in October (2.03×10^6 cells/g gills), when most clams completed spawning. In addition, the female clams heavily infected by *P. olseni* in Gomso Bay showed retarded gonad maturation and produced fewer eggs during spawning, suggesting a sublethal effect of a high level of *P. olseni* parasitism on the host health.

CI of Manila clams in HD tidal flat declined continuously from April to July when the clams were ready to spawn. However, contrary to the present study, Uddin et al. (2012) observed a marked increase in CI in Manila clams distributed in Begmiri tidal flat in Incheon Bay from April to July. In Begmiri tidal flat, CI of Manila clam reached the annual highest in July when the clams were ripe and ready to spawn. A high CI prior to spawning in Manila clam was also observed in Seonjaedo Island tidal flat in Incheon Bay, where Manila clam exhibited the highest CI in May as the clams were fully ripe and ready to spawn (Uddin et al. 2010). The gradual decrease in CI observed in this study can be, in part, explained by the high level of parasitism. As demonstrated by Choi et al. (1989, 1994), the main impact of *Perkinsus* parasitism on the host is a continuous net energy drain. As a parasite, *Perkinsus* spp. supply their metabolic energy used in respiration, cell growth, and reproduction entirely from the host. Consequently, host clams or oysters heavily infected by *Perkinsus* spp. may reserve insufficient net energy for their growth and/or reproduction, resulting in poor fitness (i.e., condition factor or condition index) and slow gonad maturation (Choi et al. 1989, 1994; Lee et al. 2020a).

Acknowledgments

We are grateful to the Shellfish Aquaculture and Research Laboratory of Jeju National University staff for their help in sampling and histology preparation. This study was supported by the research grant of Jeju National University in 2021. These authors (Yang HS, Cho YG) contributed equally.

References

- Cho YG, Kang HS, Le CT, Kwon MK, Jang MS, Choi KS (2020) Molecular characterization of *Urosporidium tapetis* sp. nov., a haplosporidian hyperparasite infecting metacercaria of *Parvatrema duboisi* (Dollfus 1923), a trematode parasite of Manila clam *Ruditapes philippinarum* on the west coast of Korea. *J Invertebr Pathol* **175**(2020):107454
- Choi JK, Oh HJ, Koo BJ, Lee S, Ryu JH (2011) Macrobenthos habitat mapping in a tidal flat using remotely sensed data and a GIS-based probabilistic model. *Mar Pollut Bull* **62**:564–572
- Choi KS, Powell EN, Lewis DH, Ray SM (1994) Instantaneous reproductive effort in female American oysters, *Crassostrea virginica*, measured by a new immunoprecipitation assay. *Biol Bull* **186**:41–61
- Choi KS, Wilson EA, Lewis DH, Powell EN, Ray SM (1989) The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. *J Shellfish Res* **8**:125–131
- Lee HM, Cho YG, Jeung HD, Jang MS, Hwang JY, Choi KS (2020a) Are juvenile Manila clam *Ruditapes philippinarum* free from *Perkinsus olseni* infection in Korean waters?. *Ocean Sci J* **55**:573–579
- Lee HM, Kim HJ, Park KI, Choi KS (2020b) Enhanced growth, gonad maturation, and low-level parasite infection in juvenile Manila clam *Ruditapes philippinarum* cultured in subtidal cages on the south coast of Korea. *Aquaculture* **526**:735410. doi:10.1016/j.aquaculture.2020.735410
- Lee HM, Park KI, Yang HS, Choi KS (2021) Negative impacts of *Perkinsus olseni* infection in Manila clam *Ruditapes philippinarum* observed from tidal flats in Anmyeondo Island on the west coast of Korea during the post-spawning period. *Ocean Sci J* **56**:307–316
- Nam KW, Jeung HD, Song JH, Park KH, Choi KS, Park KI (2018) High parasite burden increases the surfacing and mortality of the Manila clam (*Ruditapes philippinarum*) in sandy intertidal mudflats on the west coast of Korea during hot summer. *Parasite Vector* **11**:42. doi:10.1186/s13071-018-2620-3
- Park KI, Choi KS (2001) Spatial distribution of the protozoan parasite, *Perkinsus* sp., found in the Manila clam, *Ruditapes philippinarum* in Korea. *Aquaculture* **203**:9–22
- Park KI, Figueras A, Choi KS (2006) 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 KI, Park JK, Lee J, Choi KS (2005) Use of molecular markers for species identification of Korean *Perkinsus* sp. isolated from Manila clams *Ruditapes philippinarum*. *Dis Aquat Org* **66**:255–263
- Park KI, Tsutsumi H, Hong JS, Choi KS (2008) Pathology survey of the short-neck clam *Ruditapes philippinarum* on sandy tidal flats along the coast of Ariake Bay, Kyushu, Japan. *J Invertebr Pathol* **99**:212–219
- Park KJ, Song JH, Choi YS, An KH (2013) Changes in density and culture conditions of the Manila clam *Ruditapes philippinarum* on the west coast of Korea. *Korean J Malacol* **29**:207–216
- Ray SM (1966) A review of the culture method for detecting *Dermocystidium marinum* with suggested modifications and precautions. *Proc Natl Shellfish Ass* **54**:55–69
- Uddin MJ, Yang HS, Choi KS, Kim HJ, Hong JS, Cho M (2010) Seasonal changes in *Perkinsus olseni* infection and gametogenesis in Manila clam, *Ruditapes philippinarum*, from Seonjedo Island in Incheon, off the west coast of Korea. *J World Aquacult Soc* **41**:93–101
- Uddin MJ, Yang HS, Park KJ, Kang CK, Kang HS, Choi KS (2012) Annual reproductive cycle and reproductive efforts of the Manila clam *Ruditapes philippinarum* in Incheon Bay off the west coast of Korea using a histology-ELISA combined assay. *Aquaculture* **364–365**:5–32
- Villalba A, Reece KS, Ordas MC, Casas SM, Figueras A (2004) Perkinsosis in molluscs: a review. *Aquat Living Resour* **17**:411–432
- Waki T, Takahashi M, Eki T, Hiasa M, Umeda K, Karakawa N, Yoshinaga T (2018) Impact of *Perkinsus olseni* infection on a wild population of Manila clam *Ruditapes philippinarum* in Ariake Bay, Japan. *J Invertebr Pathol* **15**:134–144
- Yang HS, Park KI, Donaghy L, Adhya M, Choi KS (2012) Temporal variation of *Perkinsus olseni* infection intensity in the Manila clam *Ruditapes philippinarum* in Gomso Bay, off the west coast of Korea. *J Shellfish Res* **31**:685–690
- Yang HS, Park KJ, Choi KS (2010) Pathologic survey on the Manila Clam *Ruditapes philippinarum* (Adams and Reeve 1850) from Haeju off the Western Coastal Yellow Sea. *Ocean Sci J* **45**:93–100

Author's Information

Hyun-Sung Yang

Full-time Research Scientist, Korea Institute of Ocean Science & Technology

Young-Ghan Cho

Post-doctoral Research Fellow, Jeju National University

Jong-Seop Shin

Graduate Research Assistant, Jeju National University

Heung-Sik Park

Principal Research Scientist, Korea Institute of Ocean Science & Technology

Kwang-Sik Choi

Professor, Jeju National University

Received Nov. 8, 2021

Revised Nov. 30, 2021

Accepted Dec. 1, 2021