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Monitoring the Protozoan Parasite *Perkinsus olseni* Infection in Manila Clam *Ruditapes philippinarum* in Korean Waters During Post-Spawning Period

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ABSTRACT

The Manila clam (*Ruditapes philippinarum*), commonly found on mudflats and sandy beaches along the coastal Yellow Sea, is frequently infected by the protozoan parasite *Perkinsus olseni*. The intensity and prevalence of *P. olseni* infection in Manila clams vary spatially and seasonally, often peaking during the post-spawning period. In this study, we investigated the infection intensity and prevalence of *P. olseni* in Manila clams collected in October 2013, when most clams had completed spawning. Using Ray's fluid thioglycollate medium assay (RFTM), we found that, except for clams from Pohang and Imwon on the east coast, *P. olseni* infection was widespread among clams from tidal flats and sandy beaches on the west coast, with prevalence rates ranging from 87% to 100%. The infection intensity varied widely, from no infection (Pohang and Imwon) to over a million cells per gram of gill tissue at most sampling sites. Clams from sites on the west coast, such as Seonjae, Hwangdo, and Gomso, exhibited infection levels of 1.0 to 4.3 × 10^6 cells/g gill, while those from the south coast, including Wanddo, Yeosu, Tongyeong, and Masan, showed levels of 1.1 to 4.8 × 10^6 cells/g gill. Histological analysis revealed a similar range of infection intensity, from 0 (no infection in Pohang and Imwon) to 3.6 (Hwangdo). The high infection intensities and prevalence observed in the clams during this study may be partly attributed to elevated water temperatures and physiological stress during the post-spawning period, a pattern previously noted in clams from tidal flats along the west coast.

Keywords: *Perkinsus olseni*, *Ruditapes philippinarum*, Jeju Island, Infection intensity, RFTM assay, Histology

INTRODUCTION

Manila clams (*Ruditapes philippinarum*), widely distributed across tidal flats and sandy beaches in the East Pacific region, including China, Korea, and Japan, are a crucial species in the soft-bottom coastal ecosystem. As filter feeders, Manila clams contribute significantly to ecosystem functioning by removing large amounts of suspended organic and planktonic particles. They also enhance primary productivity by recycling nutrients through their feeding activities (Saurel *et al.*, 2014; Lavoie *et al.*, 2016; Hou *et al.*, 2023). The coastal Yellow Sea of China produces approximately 90% of the world's Manila clam supply, highlighting the species' economic importance (Martini *et al.*, 2025). In Korea, Manila clams thrive at high densities on sandy mud tidal flats along the west and south coasts, where they are either harvested commercially or cultured, supporting local shellfish industries (Park *et al.*, 2013; Ahn *et al.*, 2016). However, previous studies indicate that the growth and reproduction of Manila clams in Korean waters are affected by various parasitic organisms, such as the protozoan parasite *Perkinsus olseni* and

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larval trematodes (Park and Choi, 2001; Ngo and Choi, 2004; Park *et al.*, 2006; Subramaniam *et al.*, 2024; Le *et al.*, 2024).

Among the pathogenic protozoan parasites infecting Manila clams, *P. olseni* is a major cause of mass mortalities in European waters and the East Pacific region (Villalba *et al.*, 2004; Nam *et al.*, 2018; Lee *et al.*, 2021). Pretto *et al.* (2014) reported that a high prevalence of *P. olseni* infections contributed to 90–100% mortality of Manila clams in the lagoons of the Veneto Region, Italy, in 2011. In Korea, Park and Choi (2001) were the first to nationa-wide survey *P. olseni* infection in Manila clams, documenting heavy infections in tidal flats on the west and south coasts, with intensities exceeding one million cells per gram of tissue. Similarly, Lee *et al.* (2021) reported a mass mortality event on tidal flats along Korea's west coast in 2004 with high levels of *P. olseni* infection. The intensity of *P. olseni* infections in Manila clams is closely linked to environmental factors such as seawater temperature, salinity, and the physiological condition of the clams. Seasonally, infections are more intense in summer and late fall, when seawater temperatures remain high (Park *et al.*, 2006; Yang *et al.*, 2012). Subramaniam *et al.* (2024) noted that infection levels are particularly elevated during the post-spawning period in late summer to early fall on the west coast, when most clams are in a spent stage.

In this study, we investigated *P. olseni* infections in Manila clams during the post-spawning period in October 2013 to assess their spatial distribution in Korean waters. We report the intensity and prevalence of *P. olseni* infections in Manila clams collected from 11 sites along the west, south, and east coasts of Korea, using Ray's fluid thioglycollate medium assay (RFTM) and histological analyses.

MATERIALS AND METHODS

For this survey, we selected 11 representative sites, including Seonjae, Padori, Hwangdo, and Gomso tidal flats on the west coast; Wando, Yeosu, Tongyeong, and Masan tidal flats on the south coast; Seongsan muddy sand beach on Jeju Island; and Pohang and

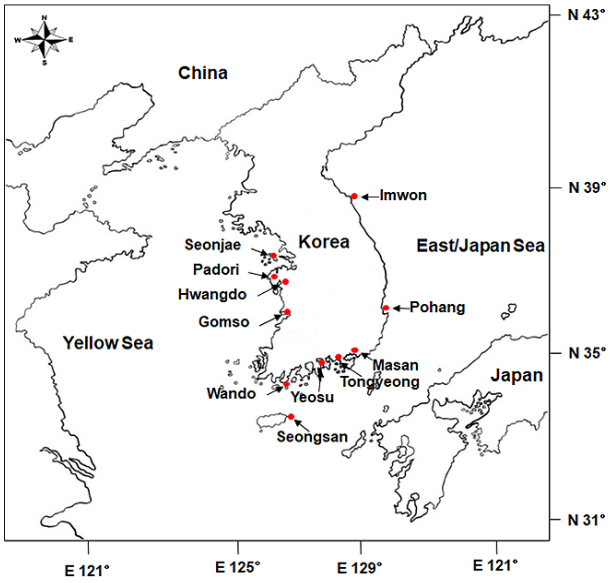

Fig. 1. Location of the sampling sites.

Imwon on the east coast (Fig. 1). In the second week of October 2013, we visited these sites and collected 30 to 40 adult clams with shell lengths (SL) ranging from 30 to 40 mm (Table 1).

The longest axis of each shell was recorded as the shell length (SL). The soft tissue was then removed, blotted to remove excess surface water, and weighed to the nearest milligram. The dry shell weight (DSW) was also measured to the nearest milligram using an electronic balance. The condition index (CI) was calculated based on the wet tissue weight (WTW) and dry shell weight (DSW) using the formula:

CI = WTW / DSW.

The prevalence and intensity of *Perkinsus olseni* infections were determined following the methodologies of Choi *et al.* (1989), Park *et al.* (2006), and Subramaniam *et al.* (2024). Briefly, a portion of the gills from each clam was excised, weighed to the nearest milligram, and placed into a 15 ml plastic tube containing sterilized fluid thioglycollate medium (FTM; Ray, 1966) supplemented with antibiotics. The gill tissues incubated in FTM were kept in the dark at room temperature for one week, dissolved using 2 M NaOH, and the *P. olseni* cells were counted using a

Table 1. The mean and standard error of shell length (mm) wet tissue weight (WTW), and dry tissue weight (DTW) of Manila clams used in this study. N, number of clams collected

Sites	N	SL (mm)	WTW (g)	DTW (g)
Seonjae	30	42.2 ± 0.38	2.502 ± 0.086	0.477 ± 0.019
Padori	30	39.0 ± 0.48	2.181 ± 0.079	0.435 ± 0.018
Hwangdo	30	34.3 ± 0.40	1.122 ± 0.053	0.213 ± 0.011
Gomso	30	41.6 ± 0.53	2.565 ± 0.118	0.497 ± 0.025
Wando	30	37.2 ± 0.32	1.960 ± 0.065	0.384 ± 0.014
Yeosu	30	30.5 ± 0.43	0.969 ± 0.024	0.193 ± 0.005
Tongyeong	30	39.9 ± 0.64	2.711 ± 0.127	0.573 ± 0.030
Masan	30	40.4 ± 0.63	2.335 ± 0.100	0.463 ± 0.019
Pohang	30	39.7 ± 0.42	2.155 ± 0.072	0.404 ± 0.015
Imwon	30	42.5 ± 0.37	2.702 ± 0.091	0.475 ± 0.019
Seongsan	30	39.6 ± 0.36	2.074 ± 0.062	0.400 ± 0.014

hemocyte counter (Choi *et al.*, 1989). Infection intensity was expressed as the number of *P. olseni* cells per gram of gill tissue.

For histological analysis, a 2–3 mm-thick longitudinal section was taken from the middle of each clam's body (previously used for the RFTM assay) and fixed in Davidson's solution. The wet weight of the remaining tissue was measured, freeze-dried, and the dry weight was determined. The dry tissue weight of each clam was estimated by multiplying the water content percentage of the remaining tissue by the wet weight of the whole tissue. A dry weight-based condition index was also calculated using the formula:

$$CI = (\text{dry tissue weight} / \text{dry shell weight}) \times 1{,}000$$

Histological slides were examined to grade *P. olseni* infection intensity on a scale from 0 (no infection) to 4 (systemic infection), following Ngo and Choi (2004) and Kim *et al.* (2024). Additionally, the reproductive condition of Manila clams was categorized into one of six stages: 1) resting, 2) early developing, 3) late developing, 4) ripe, 5) partially spawned, and 6) spent, as described by Uddin *et al.* (2012) and Subramaniam *et al.* (2024).

RESULTS

In October 2013, a total of 330 Manila clams were collected from 11 sites across the west, south, and east coasts (Table 1). The shell length (SL) of the clams ranged from 30.5 mm (Yeosu) to 42.5 mm (Imwon), indicating that the sampled clams were likely over three years old.

Histological analysis revealed spatial variation in reproductive conditions (Fig. 2). On the Gomso tidal flat, 40% of the clams were in the partially spawned stage, while clams from more northern sites, including Seonjae, Padori, and Hwangdo, had completed spawning and were mostly in the resting stage. In contrast, clams from Tongyeong (43.3%) and

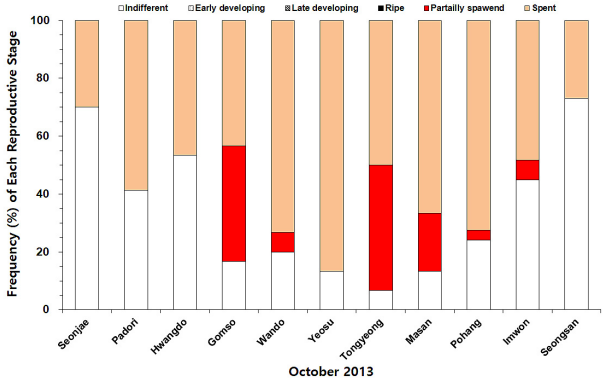

Fig. 2. Proportion of the reproductive stage of Manila clam determined by histology.

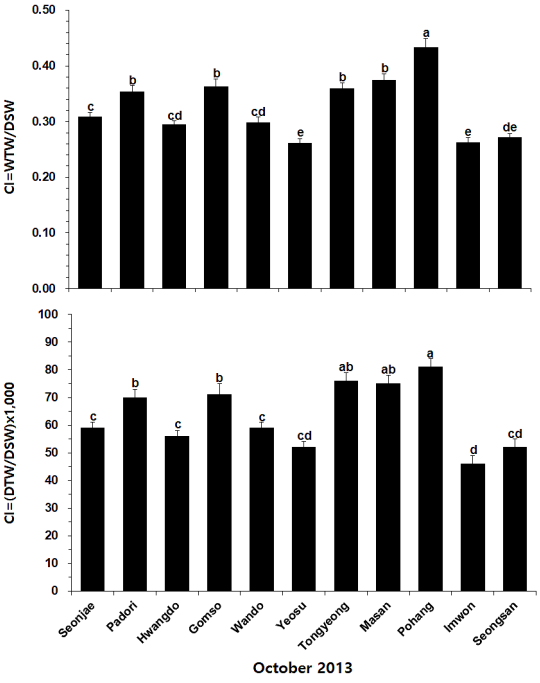

Fig. 3 Condition index (CI) of Manila clam collected during the survey carried out in October 2013. The letters on the bars indicate statistically significant differences between groups ($p < 0.05$, ANOVA and post-hog range test).

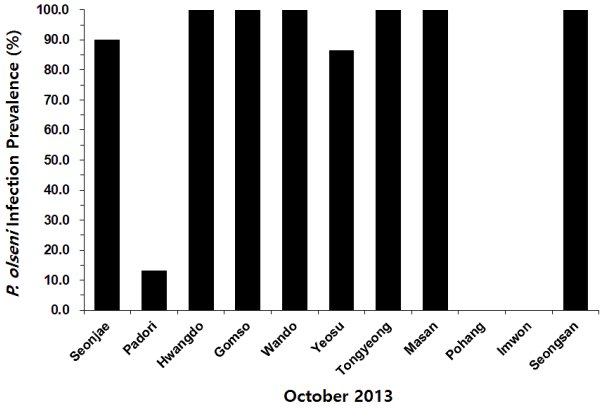

Fig. 4. Prevalence of *P. olseni* infection in Manila clams surveyed in October 2013.

Masan (20.0%) on the south coast were actively spawning.

The condition index (CI) of clams, determined using two different methods, is shown in Fig. 3. The mean CI based on the ratio of wet tissue weight to dry shell weight ranged from 0.261 (Yeosu) to 0.433 (Pohang). The mean CI based on the ratio of dry tissue weight to dry shell weight ranged from 46

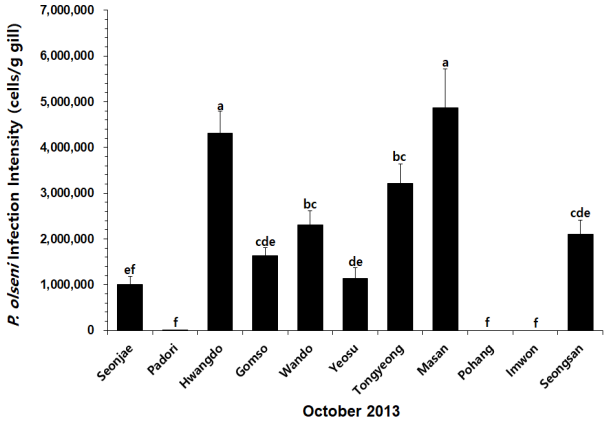

Fig. 5. *Perkinsus olseni* infection intensity in Manila clams collected determined using RFTM in October 2013. The letters on the bars indicate statistically significant differences between groups ($p < 0.05$, ANOVA and post-hog range test).

(Imwon) to 81 (Pohang). Clams from Pohang exhibited the highest CI, which was significantly different from those in other sites (ANOVA, $P < 0.05$).

The infection prevalence of *P. olseni*, assessed using RFTM, ranged from 0% (no infection) in Pohang and Imwon to 100% in Hwangdo, Gomso, Wando, Tongyeong, Masan, and Seongsan (Fig. 4). The infection intensity determined through RFTM also varied spatially, from no infection to over a million cells per gram of gill tissue (Fig. 5). Clams from Pohang and Imwon on the east coast were free of *P. olseni* infection. On the west coast, the lowest infection intensity was observed in Padori, where prevalence was 13.3%, and intensity was 0.007×10^6 cells/g gill. In contrast, significantly higher infection intensities were recorded at Seonjae (1.004×10^6 cells/g gill), Hwangdo (4.317×10^6 cells/g gill), and Gomso (1.627×10^6 cells/g gill). Similarly, high infection intensities were observed on the south coast, ranging from 1.138×10^6 cells/g gill (Yeosu) to 4.873×10^6 cells/g gill (Masan). ANOVA results indicated that infection intensities in clams from Hwangdo and Masan were significantly higher than those from other sites ($P < 0.05$).

Histological analysis revealed that the mean infection score also varied spatially, ranging from 0 to 3.6 (Fig. 6). The highest score (3.60) was recorded in Hwangdo, where RFTM assays also showed the

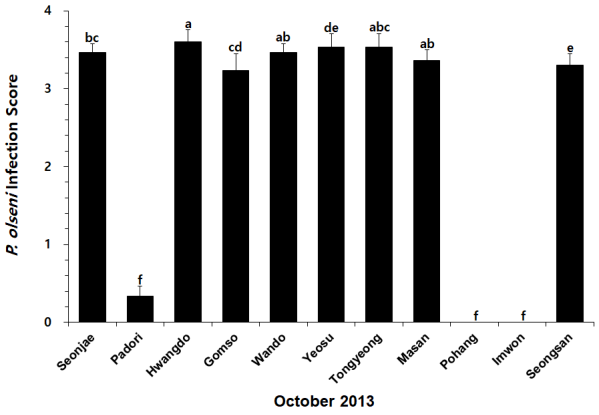

Fig. 6. *Perkinsus olseni* infection score as the intensity determined using histology. The letters on the bars indicate statistically significant differences between groups ($p < 0.05$, ANOVA and post-hoc range test).

highest infection intensity. The infection score at Hwangdo was significantly higher than scores from other sites (ANOVA, $P < 0.05$). Histological results confirmed that *P. olseni* was not detected in clams from Pohang and Imwon (Fig. 6).

DISCUSSION

The World Organization of Animal Health (WOAH, formerly known as the Office International des Epizooties, OIE) lists several parasitic protozoans including *Bonamia ostrea*, *B. exitiosa*, *Marteilia refringens*, *P. marinus*, and *P. olseni* as pathogens responsible for the "notifiable aquatic animal disease". These pathogens are a significant global concern, as they potentially affect the health of commercially crucial mollusks, including oysters, mussels, clams, and abalones (Conchas *et al.*, 2003; Berthe *et al.*, 2004; Villalba *et al.*, 2004). As parasites, these pathogens have a narrow range of host organisms, except *P. olseni* which has a wide range of host animals, including clams, mussels, abalones, and cockles in various regions of the world (Lister and Davis, 1981; Villalba *et al.* 2004; Carella *et al.*, 2023; Cho *et al.*, 2022, 2023).

Impacts of *P. olseni* parasitism in the host organisms include a deterioration in host growth and reproduction, cellular immune capacities, and mortality. Such negative impacts of *P. olseni* parasitism are closely linked to environmental parameters such as seawater temperature, salinity, food condition, and environmental pollution (see the review of Villalba *et al.*, 2004). Accordingly, numerous studies have reported the deleterious effects of *P. olseni* based on either field surveys or laboratory experiments. Waki and Yoshinaga (2018) experimentally infected juvenile Manila clams (5-9 mm in SL) with a high number of *P. olseni* zoospores (> 10^6 cells) and monitored the physiological responses of the heavily infected clams in a laboratory condition. Over 52 days of the experiment, the heavily infected juvenile clams demonstrated negative impacts, such as retarded growth, lowered filtration, and slow burrowing activities compared to the control clams (Waki and Yoshinaga, 2018). The hemocytes of Manila clam exposed to *P. olseni* zoospore in vitro also showed a significantly depressed phagosome acidification (Fuertes *et al.*, 2024). High levels of infection by *P. olseni* in Manila clam also depress gonad maturation and subsequent egg production. Using the Manila clam egg protein-specific antibodies in an indirect enzyme-linked immunosorbent assay (ELISA), Park *et al.* (2006) measured the egg mass of individual clams in an annual reproductive cycle. The histology-ELISA combined assay revealed that the reproductive maturation of the females of more heavily infected clams on the west coast of Korea took a longer period to become ready to spawn, and produced less quantity of eggs, demonstrating the negative impacts of the parasitism. Similarly, Ferandez-Boo *et al.* (2023) also demonstrated retarded gonad maturation and reproductive capacity of Manila clam artificially infected by *P. olseni*. Accordingly, the impacts of *P. olseni* parasitism on Manila clam could be more pronounced during the spawning and post-spawning period, when the water temperature and health condition of the host clam are more favorable to the parasite.

The infection intensity and prevalence of *P. olseni* in Manila clams during pre-spawning and post-spawning conditions in Korean waters were

comprehensively surveyed by Yang (2011). Using the RFTM assay, Yang assessed the infection levels in Manila clams collected from 23 sites across major tidal flats on the west and south coasts and estuaries on the east coast. The study found that infection intensities were significantly higher during the post-spawning period compared to the pre-spawning period in most locations, suggesting that Manila clams are more susceptible to *P. olseni* infection after spawning. Notably, the condition index (CI) of Manila clams measured during the post-spawning period in 2007 was considerably lower than the CI values recorded during the pre-spawning period in May, implying a combined impact of spawning and elevated infection levels.

Some of the sampling sites in the present study overlapped with those examined by Yang in the fall of 2007, allowing for a direct comparison of *P. olseni* infection levels and CI values. Clams from Pohang, a subtidal estuary, were free from *P. olseni* infection; none of the clams examined in 2007 or the present study showed the presence of *P. olseni* hypnospores in RFTM assays. Similarly, Park and Choi (2001) reported no *P. olseni* infection in Manila clams collected from the Hyeongsangang estuary in Pohang in May 2000. Additionally, clams from a brackish lagoon on the east coast, sampled in May 2000 (Yang, 2011), also showed no signs of infection in RFTM assays, suggesting that low salinity in seawater may protect clams from *P. olseni*, as the parasite thrives in high salinity and elevated water temperatures.

In this study, the highest *P. olseni* infection level was observed in clams from the Masan tidal flat on the south coast (4.872×10^6 cells/g gill), which aligns with Yang's (2011) findings of 4.135×10^6 cells/g gill. The underlying cause of such high infection levels in clams from the Masan tidal flat requires further investigation, although factors such as sediment composition and restricted water circulation in the semi-enclosed bay may contribute to the elevated infection levels.

In summary, this study surveyed *P. olseni* infection in populations of Manila clams (*Ruditapes philippinarum*) along the west, south, and east coasts of Korea during the post-spawning period in October 2013, using RFTM and histology. Infection intensities varied significantly among sites, ranging from no infection (e.g., Pohang and Imwon on the east coast) to over one million cells per gram of gill tissue in several locations. On the west coast, sites such as Seonjae, Hwangdo, and Gomso exhibited infection levels ranging from 1.0 to 4.3×10^6 cells/g gill, while sites on the south coast, including Wando, Yeosu, Tongyeong, and Masan, showed infection levels between 1.1 and 4.8×10^6 cells/g gill. The high infection prevalence and intensities observed in some tidal flats on the west and south coasts may be linked to elevated water temperatures and physiological stress during the post-spawning period. However, further detailed studies are needed to confirm this hypothesis.

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REFERENCES

Ahn, H.-M., Ki, H.-J., Jeong, H.-D., Lee, H.-J., Han, H.-K., Park, K.-J., Song, J.-H. (2016). Comparisonof growth, condition index and mortality of manila clam (*Ruditapes philippinarum*) between originated from China (Liaoning Dandong) and Chungnam (Taean) in Cochang tidal flats. *Korean Journal of Malacology,* **32**: 175-184.

Balseiro, P., Montes, J., Conchas, R.F., Novoa, B., Figueras, A. (2010) Comparison of diagnostic techniques to detect the clam pathogen *Perkinsus olseni*. *Diseases of Aquatic Organisms*, **90**: 143-151.

Berthe, F.C.J., Le Roux, F., Adlard, R.D., Figueras, A. (2004) Marteiliosis in molluscs: a review. *Aquatic Living Resources,* **17**: 433-448.

Carella, F., Tejedor, M.F., Villari, G., Andree, K.B., De Vico, G. (2023) The endoparasite *Perkinsus olseni* affecting the Mediterranean mussels (*Mytilus galloprovincialis*) in the Italian and Spanish waters: a new possible threat for mussel Aquaculture and wild animal population. *Frontiers in Marine Science*, **10**: 1116837.

Cho, Y.-G., Lee, H.-M., Hwang, J.Y., Jang, G.I., Kwon,M.G., Kim, B.S., Park, K.-L., Choi, K.-S. (2022) Molecular and histological identification of the protozoan parasite *Perkinsus olseni* in the blood cockle *Anadara kagoshimensis* (Tokunaga, 1906)

occurring on the south coast of Korea. *Aquaculture,* **561**: 738721.

Cho, Y.-G., Kim, J.-H., Choi, H.J., Jang, G.-I., Oh, Y.-G., Lee, S.-J., Hong, S.-Y., Kwon, M.-G., Choi, K.-S. (2023) First report of *Perkinsus olseni* infections in blood cockles *Tegillarca granosa* on the south coast of Korea. *Diseases of Aquatic Organisms,* **156**: 39-45.

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

Conchas, R.F., Santamarina, J., Lama, A., Longa, A., Montes, J. (2003) Evolution of Bonamiosis in Galicia (NW Spain). *Bulletin of the European Association of Fish Pathologists,* **23**: 265-272.

Fernandez-Boo, S., Duarte, C., Guevelou, E., Sousa, J., Freitas, R., Joaquim, S., Costas, B., Magalhaes, L., Matias, D., Cruz, A. (2023) Effects of the alveolate parasite *Perkinsus olseni* infection on sexual maturation and spawning efficiency of the clam *Ruditapes decussatus*. *Parasitology,* **150**: 321-328.

Fuertes, V.N.B., Watanabe, Y., Itoh, N. (2024) Differences in phagosome acidification of Manila clam hemocytes in response to two *Perkinsus* species with contrasting proliferation dynamics in the host: *P. olseni* and *P. mediterraneus*. *Journal of Invertebrate Pathology,* **207**: 108216.

Hou, W., Wei, X., Bai, W., Zheng, Y., Tan, Q., Liu, Z., Rong, B., Ge, C. (2023) Effects of Manila clam *Ruditapes philippinarum* culture on the macrobenthic community. *Frontiers in Marine Science,* **10**: 1084386.

Kim, J.H., Subramaniam, T., Nobuhisa, K., Shin, J.-S., Choi, K.-S. (2024) Monitoring *Perkinsus olseni* infection in Manila clam *Ruditapes philippinarum* on intertidal beaches in Jeju Island. *Korean Journal of Malacology,* **40**: 127-134.

Lavoie, M.-F., Mckindsey, C.W., Pearce, C.M., Archambault, P. (2016) Influence of intertidal Manila clam *Venerupis philippinarum* Aquaculture on biogeochemical fluxes. *Aquaculture Environment Interactions,* **8**: 117-130.

Le, T.C., Jeung, H.-D., Cho, Y.-G., Choi, K.-S. (2024) Survey of trematodes in Manila clam *Ruditapes philippinarum* on the west coast of Korea: A preliminary study. *Journal of Invertebrate Pathology,* **206**: 108172.

Lee, H.-M., Park, K.-I., Yang, H.-S., Choi, K.-S. (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 post-spawning period. *Ocean Science Journal,* **56**: 307-316.

Martini, A., Napolitano, R., Capoccioni, F., Martinoli, M., Tonachella, N., Aguiari, L., Piva, P., Rossetti, E., Pulcini, D. (2025) Prefacing the challenge-assessment of the environmental efficiency of Manila clam (*Ruditapes philippinarum*) production based on hatchery-produced and wild seed. *Aquaculture,* **595**: 741474.

Nam, K.-W., Jeung, H.-D., Song, J.-H., Park, K.-H., Choi, K.-S., Park, K.-I. (2018) High parasite burden increases the surfacing and mortality of the Manila clam (*Ruditapes philippinarum*) in intertidal sandy mudflats on the west coast of Korea during hot summer. *Parasite & Vector*, **11**:1–7.

Ngo, T.T.T., Choi, K.-S. (2004) Seasonal changes of *Perkinsus* and *Cercaria* infections in the Manila clam *Ruditapes philippinarum*from Jeju, Korea. *Aquaculture,* **239**: 57-68.

Park, K.-I., Choi, K.-S. (2001) Spatial distribution of the protozoan parasite *Perkinsus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. *Aquaculture* 203: 9-22.

Park, K.-I., Figueras, A., Choi, K.-S. (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, K.-J., Song, J.-H., Choi, Y.-S., An, K.-H. (2013) Changes in density and culture conditions of the Manila clam *Ruditapes philippinarum* on the west coast of Korea. *Korean Journal of Malacology,* **29**: 207-216. (in Korean with English abstract)

Pretto, T., Zambon, M., Civettini, M., Caburlotto, G.F., Boffo, L., Rossetti, E., Arcangeli, A. (2014) Massive mortality in Manila clams (*Ruditapehs philippinarum*) farmed in the lagoon of Venice, caused by *Perkinsus olseni*. *Bulletin of European Association of Fish Pathologists,* **34**: 43-53.

Ray, S.M. (1966) A review of the culture method for detecting *Dermocystidium marinum* with suggested modifications and precautions. *Proceedings of the National Shellfish Association,* **54**: 55-69.

Saurel, C., Ferreira, J.G., Cheney, D., Suhrbier, A., Dewey, B., Davis, J., Cordell, J. (2014) Ecosystem goods and services from Manila clam culture in Puget Sound: a modelling analysis. *Aquaculture Environment Interactions,* **5**: 255-270.

Subramaniam, T., Cho, Y.-G., Lee, H.-M., Kim, J.-H., Shin, J.-S., Kwon M.-G., Jang, G.-I., Choi, K.-S. (2024) Spatio-temporal variation in *Perkinsus olseni* infection intensity in Manila clam *Ruditapes philippinarum*in Anmyeondo and Cheonsu Bay tidal flats on the west coast of Korea. *Ocean Science Journal,* **59**: 1.

Uddin M.J., Yang, H.-S., Choi, K.-S., Kim, H.-J., Hong, J.-S., 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. *Journal of World Aquacult Society,* **41**: 93-101.

Villalba, A., Reece, K.S., Ordás, M.C., Casas, S.M., Figueras, A. (2004) Perkinsosis in molluscs: A review. *Aquatic Living Resources,* **17**: 411-432.

Waki, T., Yoshinaga T. (2018) Experimental evaluation of the impact of *Perkinsus olseni* on the physiological activities of juvenile Manila clams. *Journal of*

Shellfish Research, **37**: 29-39.

Yang, H.S. (2011) Annual variation of reproductive effort, biochemical composition and *Perkinsus olseni* infection in Manila clam, *Ruditapes philippinarum*, off the west coast of Korea. Ph.D. Dissertation, Jeju National University, pp 157.

Yang, H.-S., Park, K.-I., Donaghy, L., Adhya, M., Choi, K.-S. (2012) Temporal variation of *Perkinsus olseni* infection intensity in the Manila clam *Ruditapes philippinarum* in Gomso Bay, off the west coast of Korea. *Journal of Shellfish Research,* **31**: 685-690.