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Are Juvenile Manila Clam *Ruditapes philippinarum* Free from *Perkinsus olseni* Infection in Korean Waters?

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Abstract – As a suspension feeder, Manila clam *Ruditapes philippinarum* (A. Adams and Reeve 1850) plays a crucial role in the coastal soft bottom ecosystem in the temperate region, linking the benthic primary production to the upper trophic level. Manila clam density on tidal flats on the west coast of Korea has been declining for the past decades, and infection by the protozoan parasite *Perkinsus olseni* (Lester and Davis 1981) is one of the major causes for the decline. Recent studies carried out in Japan revealed that *P. olseni* induces mortalities of the juveniles in their natural habitats, which may lead to the recruitment failure and subsequent decline in the clam population. In this study, we surveyed *P. olseni* infection in juvenile Manila clam occurring on two tidal flats on the Taean coast. Ray's fluid thioglycollate medium assay (RFTM) revealed that *P. olseni* infection was not limited to the adult clams, and the juvenile and small-sized clams are also infected by *P. olseni*. As young as four-month-old juveniles from Jugyo tidal flat were infected by *P. olseni*, with the prevalence (i.e., percentage of the infected individuals) of 75.0% and the intensity of 7.77×10^5 cells g^{-1} wet tissue weight (WT). The adult Manila clams (SL > 30 mm) from Jugyo tidal flat showed a prevalence of 96.0%, and the intensity as 5.80×10^5 cells g^{-1} WT. The observed infection prevalence and intensity of the juvenile are somewhat comparable to those of the adult clams, suggesting that a high level of *P. olseni* infection in the juveniles may lead to mortality and a long term decline in the clam population density.

Keywords – *Perkinsus olseni*, *Ruditapes philippinarum*, Manila clam, juvenile, infection prevalence and intensity, RFTM

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1. Introduction

Manila clam *Ruditapes philippinarum* inhabits the temperate muddy and sandy intertidal and shallow subtidal, where the clams burrow into the sediment to maximum of 10 cm from the surface to avoid lethal risks such as predation and desiccation (Kurihara 2003; Toba et al. 2011; Takeuchi et al. 2015). On the west coast of Korea, Manila clam is one of the dominant species in the intertidal benthic community, where the density often exceeds 1,000 individuals per meter square (Park et al. 2013, 2018). Such a high density reported from the west coast is, in part, attributed to the characteristic high microalgal productivity in the tidal flats (Park et al. 2014; Kwon et al. 2020). Despite the favorable food conditions, Manila clam production on the west coast has declined for the past decades, and the recurring mass mortality is one of the major factors responsible for the decline (Park et al. 2006; Nam et al. 2018). The mass mortality events of Manila clams on the west coast often coincide with a high level of parasitism in the clams, especially the protozoan parasite *Perkinsus olseni* (Park et al. 2006, 2010; Lee et al. 2020).

Perkinsus olseni infects various marine bivalves and gastropods, and the infected hosts often show retarded growth and reproduction (see the review of Villalba et al. 2004 and Choi and Park 2010). Mortalities of adult Manila clams parasitized by a high level of *P. olseni* have been reported from several locations in the world, including the west coast of Korea (Pretto et al. 2014; Ruano et al. 2015; Nam et al. 2018). Recently, Waki et al. (2018) reported a high level of

juvenile clam mortality from a tidal flat in southern Japan, where the Manila clam density showed a long term decline. The survey indicated that the juveniles were infected by *P. olseni*, and they postulated that the mortality linked to the high level of *P. olseni* infection in the juvenile might prevent recruitment, resulting in the long term decline of the adult clam population in the study site. Park et al. (2018) reported that there is no recruitment of the juvenile clams in some tidal flats on the west coast of Korea, while the pathogenic condition of the juvenile remains unexamined.

While numerous studies have reported *P. olseni* infection among adult clams in various tidal flats in Korea waters, infection in the juveniles remains poorly known (Park and Choi 2001; Park et al. 2006; Uddin et al. 2010; Yang et al. 2012). Recently, Yang et al. (2019) first reported *P. olseni* infection cases in the juvenile and small clams on the west coast, although the data are somewhat insufficient to substantiate the infection. In the summer of 2016 and 2017, we investigated *P. olseni* infection in the juveniles in two tidal flats on the west coast, where previous studies reported a high level of infection. In this study, we report the survey results of *P. olseni* infection in juvenile clams.

2. Materials and Methods

Sampling effort

We selected Jugyo tidal flat as the study site to survey *P. olseni* infection in the juveniles. Jugyo tidal flat is located on the northern coast of Boryeong county on the west coast of Korea (Fig. 1). According to Park et al. (2018), Jugyo tidal flat consists of gravelly muddy sand. Kang et al. (2017) and Yang et al. (2019) reported that Manila clams in Jugyo tidal flat are heavily infected by *P. olseni*. As a control, we also visited Padori tidal flat in Geunso Bay, approximately 55 km north of Jugyo tidal flat, where the bottom sediment is characterized as gravelly muddy sand (Park et al. 2018; Jeon et al. 2019). Kang et al. (2017) and Yang et al. (2019) reported a low level of *P. olseni* infection in Manila clams in Padori tidal flat. In August 2016, 409 clams ranging from 8.5 to 42.5 mm in shell length (SL) were collected from Jugyo tidal flat (Fig. 1). In August 2017, we also gathered 407 clams ranging from 9.9 to 43.3 mm SL from Padori tidal flat for the analysis (Fig. 1).

Biometry

At the laboratory, SL (i.e., the longest axis of the shell) of

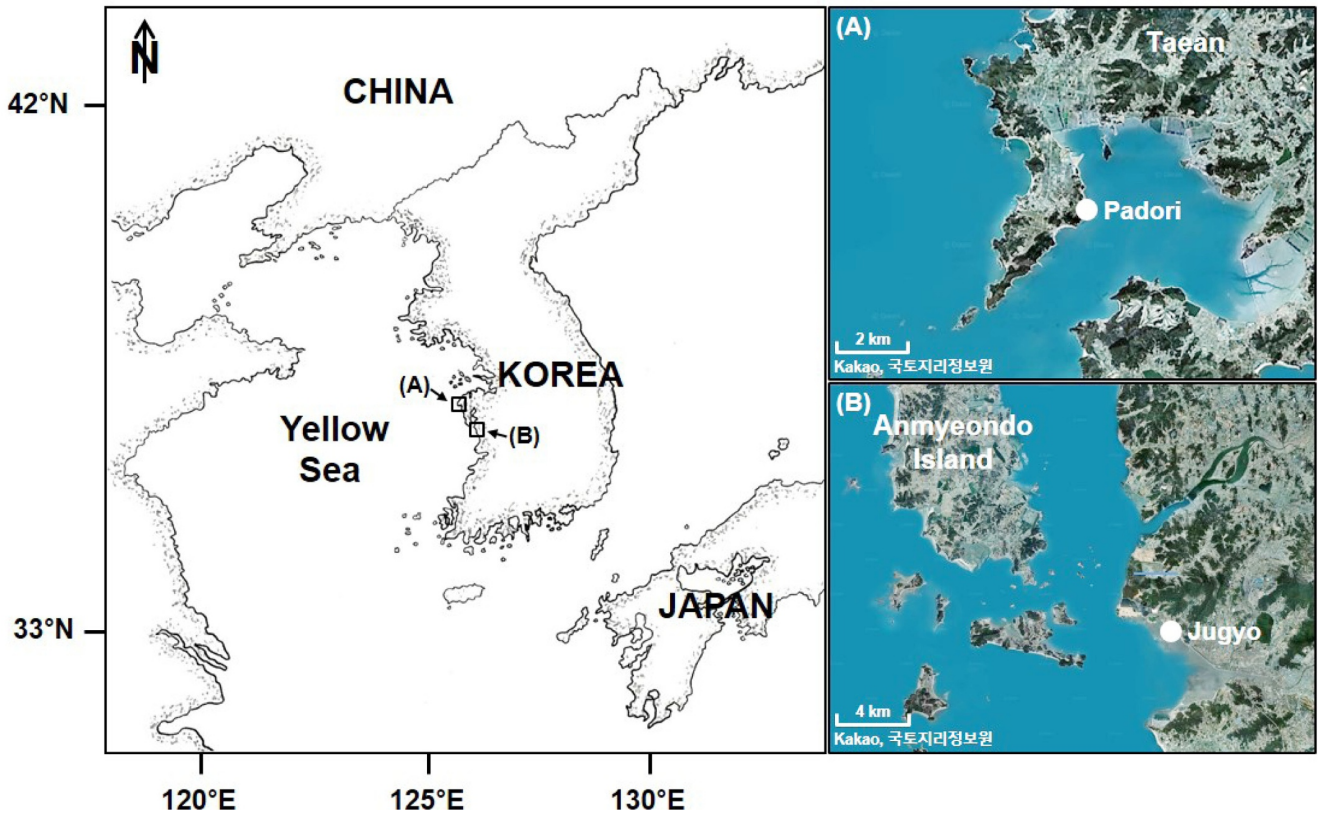

Fig. 1. Map showing the study sites showing Padori (A) and Jugyo (B) tidal flats on the west coast of Korea

each clam was measured to mm using an electronic caliper. The soft body was removed from the shell and measured to mg using an electronic valance. The weight of the shells was also measured to mg. Condition index (CI) was determined as the ratio of the wet tissue weight (WT) to the dry shell weight.

The age of each clam used in this study was determined by reference to the von Bertalanffy growth curves of *Ruditapes philippinarum* reported in Korea by Chung et al. (1994) and Choi et al. (2011). Based on SL, we grouped all the collected clams into six size classes, including three different classes of juvenile (7 to 10 mm, 10 to 15 mm and 15 to 20 mm), two different classes of small clams (20 to 25 mm, 25 to 30 mm) and a class of adult clams (30 to 45 mm). According to the growth curve, Manila clams smaller than 10 mm SL are four months old, 10 to 15 mm SL are nine months old, and 15 to 20 mm SL are 15 months old. Therefore, the juveniles we defined in this study are believed to be 4 to 15 months old, while the small clams are approximately 1.5 to 3 years old (Fig. 2).

P. olseni infection intensity analysis using RFTM assay

Ray's fluid thioglycollate medium assay (RFTM, Ray 1966) and NaOH digestion (Choi et al. 1989) were adapted to diagnose *P. olseni* infection. After measuring the weight, the whole tissue was placed in a conical tube containing 10 ml of FTM media fortified with antibiotics (nystatin 200 unit ml^{-1}, chloramphenicol 100 ng ml^{-1}). After a week of incubation in the dark at room temperature, the FTM was discarded, and all tissues were digested in 2M NaOH at 60°C for one hr. After several washing steps, the hypnospores developed in the media were resuspended in 3 to 10 ml of phosphate-buffered saline (PBS) and the number of cells was counted using a hemocytometer. Finally, the infection intensity of *P. olseni* was expressed as the number of prezoosporangia cells per gram of the wet tissue (cells g^{-1} WT).

Statistical analysis

The significance of variations of mean values among all CI and the infection intensities were verified using the non-parametric one-way ANOVA (Kruskal-Wallis test). For the analysis, CI and the infection intensity data were rank-

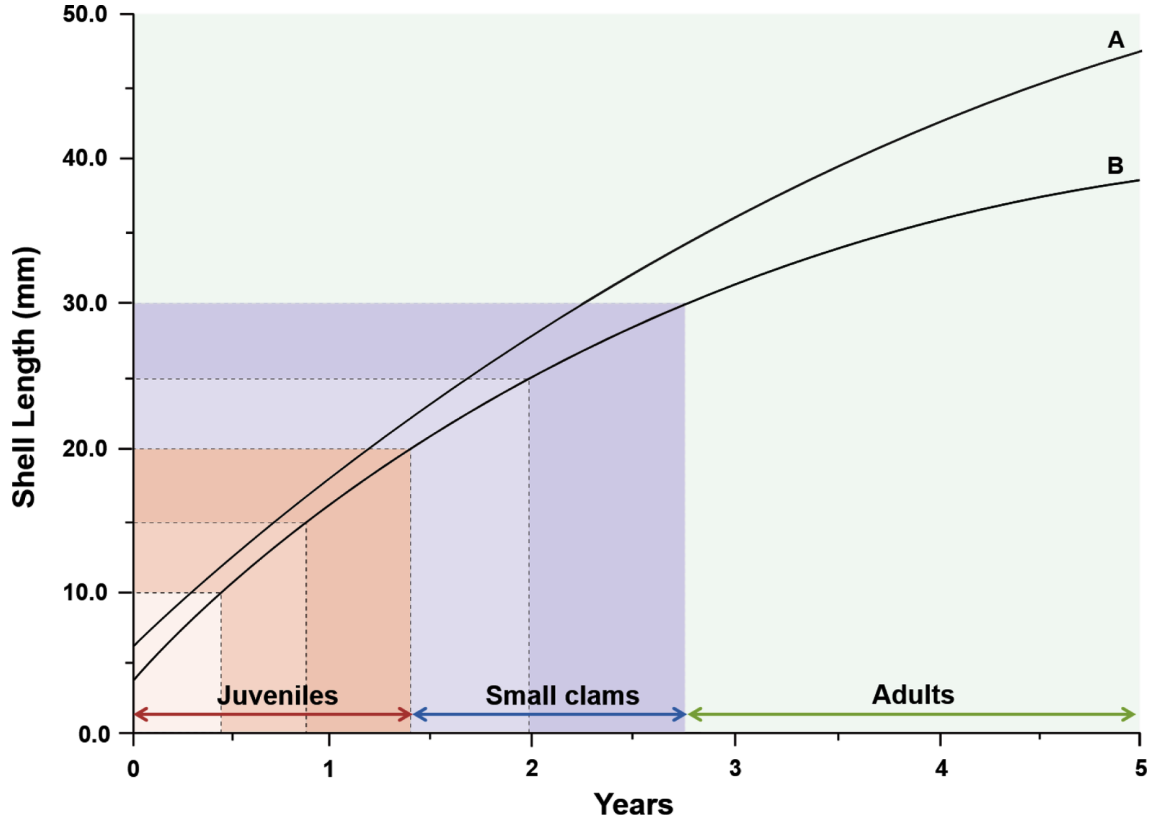

Fig. 2. von Bertalanffy growth curves of *Ruditapes philippinarum* used in this study. A, the growth curve estimated by Chung et al. (1994) and B, Choi et al. (2011)

transformed. SAS statistical package (SAS Institute Inc; 2019 USA) was used for the analysis with the statistically significant level at $p < 0.05$.

3. Results and Discussions

Table 1 summarizes the numbers, sizes, and CI of the clams collected from the two tidal flats. The mean CI of the clams from Jugyo tidal flat was significantly higher than those of clams from Padori, regardless of the size classes ($p < 0.05$). At Jugyo tidal flat, the mean CIs of juvenile clams (7−20 mm SL) ranged from 0.35 to 0.53, and the mean CI of the adult clams (30−45 mm SL) was significantly higher than the juveniles ($p < 0.05$).

Table 2 shows *P. olseni* infection prevalence and the intensity of the clams analyzed using RFTM. The infection prevalence (i.e., percentage of the infected clams) of Manila clams from Jugyo tidal flat ranged from 63.7% (15−20 mm SL) to 97.1% (25−30 mm SL). It was noticeable that the juvenile clams of 7−10 mm SL (N = 16) occurring on Jugyo tidal flat exhibited a high prevalence (75.0%), although the prevalence of the small and the adult clams was higher than that of the juveniles (81.6−97.1%).

The juvenile clams from Jugyo tidal flat also showed a high level of *P. olseni* infection intensity, ranging from 3.82×10^5 cells g^{-1} WT (15−20 mm SL) to 7.77×10^5 cells g^{-1} WT (7−10 mm SL). The infection intensities of the juveniles were somewhat comparable to the 1.5 to 3 years-old small and adult clams, as their infection intensities ranged from 4.58×10^5 cells g^{-1} WT (20−25 mm SL) to 5.80×10^5 cells g^{-1} WT (30−45 mm SL). The non-parametric ANOVA test indicated that the mean infection intensity of the adult clams

Table 1. Shell length (SL), wet tissue weight (WT), and condition index (CI) of *Ruditapes philippinarum* collected from Jugyo and Padori tidal flats

Site	Shell Length (SL, mm)	N	Mean SL (mm)	WT (g)	CI
Jugyo	7 ≤ SL < 10	16	9.3 ± 0.4	0.04 ± 0.03	0.35 ± 0.09
	10 ≤ SL < 15	180	12.8 ± 1.3	0.09 ± 0.03	0.47 ± 0.08
	15 ≤ SL < 20	91	16.5 ± 1.2	0.18 ± 0.06	0.53 ± 0.07
	20 ≤ SL < 25	38	22.4 ± 1.2	0.54 ± 0.12	0.52 ± 0.09
	25 ≤ SL < 30	34	27.1 ± 1.6	1.01 ± 0.21	0.57 ± 0.06
	30 ≤ SL < 45	50	35.0 ± 3.7	2.24 ± 0.72	0.56 ± 0.09
Padori	7 ≤ SL < 10	1	9.9	0.03	0.33
	10 ≤ SL < 15	92	13.4 ± 1.2	0.07 ± 0.02	0.39 ± 0.06
	15 ≤ SL < 20	113	17.5 ± 1.4	0.17 ± 0.05	0.42 ± 0.05
	20 ≤ SL < 25	77	22.3 ± 1.7	0.42 ± 0.13	0.41 ± 0.06
	25 ≤ SL < 30	59	27.2 ± 1.4	0.79 ± 0.16	0.40 ± 0.08
	30 ≤ SL < 45	65	35.1 ± 3.8	1.92 ± 0.69	0.42 ± 0.07

Table 2. *Perkinsus olseni* infection prevalence (Prev.) and intensities in the different size groups of Manila clams analyzed in this study. WT, wet tissue weight

Site	Shell Length (SL, mm)	N	Prev. (%)	Mean infection intensity (cells g^{-1} WT)	Highest infection intensity (cells g^{-1} WT)
Jugyo	7 ≤ SL < 10	16	75.0	$7.77 \times 10^5 \pm 8.83 \times 10^5$	3.07×10^6
	10 ≤ SL < 15	180	76.7	$5.54 \times 10^5 \pm 6.38 \times 10^5$	3.09×10^6
	15 ≤ SL < 20	91	63.7	$3.82 \times 10^5 \pm 5.63 \times 10^5$	3.81×10^6
	20 ≤ SL < 25	38	81.6	$4.58 \times 10^5 \pm 4.49 \times 10^5$	1.74×10^6
	25 ≤ SL < 30	34	97.1	$5.50 \times 10^5 \pm 4.35 \times 10^5$	1.51×10^6
	30 ≤ SL < 45	50	96.0	$5.80 \times 10^5 \pm 3.09 \times 10^5$	1.36×10^6
Padori	7 ≤ SL < 10	1	-	1.32×10^6	1.32×10^6
	10 ≤ SL < 15	92	4.3	$2.15 \times 10^4 \pm 1.48 \times 10^4$	1.31×10^6
	15 ≤ SL < 20	113	1.8	$8.31 \times 10^2 \pm 7.29 \times 10^3$	7.59×10^4
	20 ≤ SL < 25	77	39.0	$2.15 \times 10^4 \pm 4.72 \times 10^3$	2.08×10^5
	25 ≤ SL < 30	59	81.4	$3.72 \times 10^4 \pm 4.72 \times 10^4$	1.76×10^5
	30 ≤ SL < 45	65	90.8	$4.37 \times 10^4 \pm 5.41 \times 10^4$	2.50×10^5

is significantly higher than the juvenile and small clams ($p < 0.05$).

Perkinsus olseni infection prevalence of Manila clams from Padori tidal flat varied from 1.8% (15−20 mm SL) to 90.8% (30−45 mm SL), and the intensities ranged from 8.31 × 10^5 cells g^{-1} WT (15−20 mm SL) to 4.37 × 10^5 cells g^{-1} WT (30−45 mm SL). The paired *t*-test indicated that *P. olseni* infection intensity of Manila clams from Padori tidal flat is significantly lower than the intensities of Manila clams from Jugyo tidal flat, regardless of the size classes ($p < 0.05$).

Numerous studies have reported that a high level of *P. olseni* infection exerts sub-lethal effects on the hosts such as retarded growth and reproduction, and often a high level of *P. olseni* infection leads to the mortality of the parasitized clams (Villalba et al. 2004; Park et al. 2006; Pretto et al. 2014; Waki et al. 2012, 2018). In particular, the detrimental effects of *P. olseni* parasitism on juvenile Manila clam was confirmed experimentally in Japan, as *P. olseni* challenged juveniles showed a high level of mortality (Shimokawa et al. 2010; Waki et al. 2012; Umeda et al. 2013; Waki and Yoshinaga 2013, 2018). The experiments carried out in Japan indicated that the infection level exceeding 10^6 cells g^{-1} WT could be lethal to the juveniles (Shimokawa et al. 2010; Waki and Yoshinaga 2013, 2018). Waki et al. (2018) also reported that the recent decline in Manila clam density in Ariake Bay in Japan is linked to the mass mortalities of the newly recruited juveniles, where the juveniles exhibited a *P. olseni* infection intensity exceeding 10^6 cells g^{-1} WT.

RFTM assay carried out in this study indicated that more than 60.0% of the juvenile clams (4 to 15-month-olds) at Jugyo tidal flat are infected by *P. olseni*, and approximately one-half of the infected juveniles showed the intensity close to 10^6 cells g^{-1} WT (Fig. 3). Using RFTM assay, Yang et al. (2019) also estimated *P. olseni* infection intensities of juvenile clams ranging from 10 to 15 mm SL in Gomso Bay and Incheon Bay, and they reported the mean infection intensities of the juveniles as 4.91 × 10^5 cells g^{-1} WT (Gomso Bay, January 2008) and 9.41 × 10^5 cells g^{-1} WT (Sungam Incheon, November 2008). It was noticeable that the highest infection intensity of the juveniles reported by Yang et al. (2019) is exceptionally high, as much as 1.41 × 10^6 cells g^{-1} WT (Gomso Bay) and 3.20 × 10^6 cells g^{-1} WT (Sungam Incheon Bay). According to Waki and Yoshinaga (2013) and Waki et al. (2018), such a high level of infection can be lethal to the juveniles, since the level exceeds 10^6 cells g^{-1} WT. In the natural habitat of the clams, a high level of *P. olseni* infection may negatively impact activities such as burrowing and feeding, as well as depressing the cellular immune functions of the host (Waki and Yoshinaga 2018; Nam et al. 2018).

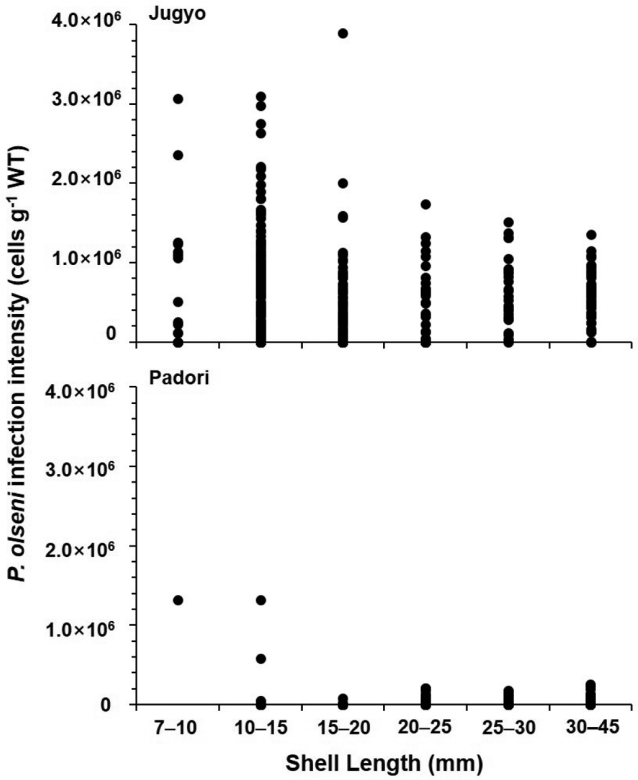

Fig. 3. *P. olseni* infection intensity of Manila clams in different size groups

The life cycle of *P. olseni* features three distinct stages including the trophozoite stage, a vegetative stage in the host tissue, the prezoosporangia (i.e., hypnospore) stage, a resting stage in the environment, and the motile zoospore stage where the parasite emerges from the prezoosporangia stage (see a review of Villalba et al. 2004). All these three life stages are known to be infectious (Villalba et al. 2004; La Peyre et al. 2008). The infectious *Perkinsus* cells are also released from an infected host via feces and pseudo-feces, and decomposing tissues of moribund hosts (Bushek et al. 2002; Villalba et al. 2004). *Perkinsus olseni* particles are also presented in the sediment and then transmitted to a new host via filtering of the infectious cells as they are resuspended in the water column (Park et al. 2010; Wang et al. 2018). In this study, the adult clams occurring on Jugyo and Padori tidal flat show a high *P. olseni* prevalence (90.8 to 96.0%), indicating that both tidal flats are *P. olseni* endemic areas. The observed high level of *P. olseni* infection in the juveniles at Jugyo tidal flat

can be explained by the active feeding activities of the juveniles, as they filter the infectious *P. olseni* cells in their environment (Wang et al. 2018; Waki and Yoshinaga 2018). In this study, we did not estimate the mortality of the juvenile clams caused by *P. olseni* in the study area, although RFTM assay indicated that the level of infection in some juveniles is considered to be lethal. For the proper management of Manila clam populations on the west coast, more studies on *P. olseni* infection dynamics and the related impacts on juvenile clams should be carried out to enhance the wild Manila clam population.

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References

Bushek D, Ford SE, Chintala MM (2002) Comparison of *in vitro*-cultured and wild-type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. Dis Aquat Organ **51**:217–225. doi:10.3354/dao051217

Choi KS, Park KI (2010) Review on the protozoan parasite *Perkinsus olseni* (Lester and Davis 1981) infection in Asian waters. In: Isimatsu A, Lie HJ (eds) Coastal environmental and ecosystem issues of the East China Sea. Nagasaki University, Nagasaki, pp 269–281

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

Choi YM, Yoon SC, Lee SI, Kim JB, Yang JH, Yoon BS, Park JH (2011) The study of stock assessment and management implications of the Manila clam, *Ruditapes philippinarum* in Taehwa river of Ulsan. Korean J Malacol **27**:107–114. doi:10.9710/kjm.2011.27.2.107 (in Korean)

Chung EY, Ryou DK, Lee JH (1994) Gonadal development, age and growth of the short necked clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae), on the coast of Kimje, Korea. Korean J Malacol **10**:38–54 (in Korean)

Jeon JH, Jeung HD, Park KJ, Lee KH, Ahn HM, Lee HJ, Choi YS, Song JH, Kang HW (2019) Effect of sand supplement and plowing on the occurrence of natural Manila clam (*Ruditapes philippinarum*) spats in the tidal flat of Geunso bay, Taean-gun, Chungnam province, on the west coast of Korea. Korean J Malacol **35**:125–135. doi:10/9710/kjm.2019.35.2.125 (in Korean)

Kang HS, Yang HS, Reece KS, Cho YG, Lee HM, Kim CW, Choi KS (2017) Survey on *Perkinsus* species in Manila clam *Ruditapes philippinarum* in Korean waters using species-specific PCR. Fish Pathol **52**:202–205. doi:10.3147/jsfp.52.202

Kurihara T (2003) Adaptations of subtropical Venus clams to predation and desiccation: endurance of *Gafrarium tumidum* and aboidance of *Ruditapes variegatus*. Mar Biol **143**:1117–1125. doi:10.1007/s00227-003-1158-9

Kwon BO, Kim H, Noh J, Lee SY, Nam J, Khim JS (2020) Spatiotemporal variability in microphytobenthic primary production across bare intertidal flat, saltmarsh, and mangrove forest of Asia and Australia. Mar Pollut Bull **151**:110707. doi:10.1016/j.marpolbul.2019.110707

La Peyre MK, Villalba SM, La Peyre JF (2008) Determination of the effects of temperature on viability, metabolic activity and proliferation of two *Perkinsus* species, and its significance to understanding seasonal cycles of perkinsosis. Parasitology **135**:505–519. doi:10.1017/S0031182008004150

Lee HM, Kim HJ, Park KI, Choi KS (2020) 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

Lester RJG, Davis GHG (1981) A new *Perkinsus* species (Apicoplexa, Perkinsea) from the abalone *Haliotis ruber*. J Invertebr Pathol **34**:181–187. doi:10.1016/0022-2011(81)90073-2

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 intertidal sandy mudflats on the west coast of Korea during hot summer. Parasite Vector **11**:1–7. doi:10.1186/s13071-018-2620-3

Park J, Kwon BO, Kim M, Hong S, Ryu J, Song SJ, Khim JS (2014) Microphytobenthos of Korean tidal flats: A review and analysis on floral distribution and tidal dynamics. Ocean Coast Manage **102**:471–482. doi:10.1016/j.ocecoaman.2014.07.007

Park KI, Choi KS (2001) Spatial distribution of the protozoan parasite *Perkinsus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. Aquaculture **203**:9–22. doi:10.1016/S0044-8486(01)00619-6

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. doi:10.1016/j.aquaculture.2005.06.003

Park KI, Yang HS, Kang HS, Cho M, Park KJ, Choi KS (2010) Isolation and identification of *Perkinsus olseni* from feces and marine sediment using immunological and molecular techniques. J Invetbr Pathol **105**:261–269. doi:10.1016/j.jip.2010.07.006

Park KJ, Heo S, Lee JH, Oh HN, Ryu SO (2018) Characteristics of

seed occurrence and inhabited environments of Manila clam, *Ruditapes philippinarum* beds in the west coast of Korea. Korean J Malacol **34**:17−29. doi:10.9710/kjm.2018.34.1.17 (in Korean)

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. doi:10/9710/kjm.2013.29.3.207 (in Korean)

Pretto T, Zambon M, Civettini M, Caburlotto G, Boffo L, Rossetti E, Arcangeli G (2014) Massive mortality in Manila clams (*Ruditapes philippinarum*) farmed in the Lagoon of Venice, caused by *Perkinsus olseni*. B Eur Assoc Fish Pat **34**:43−53

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

Ruano F, Batista FM, Arcangeli G (2015) Perkinsosis in the Manila clams *Ruditapes decussatus* and *R. philippinarum* in the northeastern Atlantic and Mediterranean Sea: A review. J Invetebr Pathol **131**:58−67. doi:10.1016/j.jip.2015.07.015

Shimokawa J, Yoshinaga T, Ogawa K (2010) Experimental evaluation of the pathogenicity of *Perkinsus olseni* in juvenile Manila clams *Ruditapes philippinarum*. J Invertebr Pathol **105**:347−351. doi:10.1016/j.jip.2010.08.007

Takeuchi S, Yamada F, Shirozu H, Ohashi S, Tamaki A (2015) Burrowing ability as a key trait in the establishment of infaunal bivalve populations following competitive release on an extensive intertidal sandflat. J Exp Mar Biol Ecol **466**:9−23. doi:10.1016/j.jembe.2015.01.011

Toba M, Ito M, Kobayashi Y (2011) Bedload transport of newly-settled juveniles of the Manila clam *Ruditapes philippinarum* observed in situ at Banzu tidal flat, Tokyo Bay. J Shellfish Res **30**:777−789. doi:10.2983/035.030.0318

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. doi:10.1111/j.1749-7345.2009.00337

Umeda K, Shimokawa J, Yoshinaga T (2013) Effects of temperature and salinity on the *in vitro* proliferation of trophozoites and the development of zoosporangia in *Perkinsus olseni* and *P. honshuensis*, both infecting Manila clam. Fish Pathol **48**:13−16. doi:10.3147/jsfp.48.13

Villalba A, Reece KS, Ordas MC, Casas SM, Figueras A (2004) Perkinsosis in molluscs: A review. Aquat Living Resour **17**:411−432. doi:10.1051/alr:2004050

Waki T, Shimokawa J, Watanabe S, Yoshinaga T, Ogawa K (2012) Experimental challenges of wild Manila clams with *Perkinsus* species isolated from naturally infected wild Manila clams. J Invertebr Pathol **111**:50−55. doi:10.1016/j.jip.2012.05.009

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 **153**:134−144. doi:10.1016/j.jip.2018.03.001

Waki T, Yoshinaga T (2013) Experimental challenges of juvenile and adult Manila clams with the protozoan *Perkinsus olseni* at different temperatures. Fish Sci **79**:779−786. doi:10.1007/s12562-013-0651-4

Waki T, Yoshinaga T (2018) Experimental evaluation of the impact of *Perkinsus olseni* on the physiological activities of juvenile Manila clams. J Shellfish Res **37**:29−39. doi:10.2983/035.037.0102

Wang Y, Yoshinaga T, Itoh N (2018) New insights into the entrance of *Perkinsus olseni* in the Manila clam, *Ruditapes philippinarum*. J Invertebr Pathol **153**:117−121. doi:10.10168/j.jip.2018.03.005

Yang HS, Lee HM, Choi KS (2019) *Perkinsus olseni* infection in juvenile and small Manila clam *Ruditapes philippinarum* on the west coast of Korea surveyed in 2008 and 2010. Korean J Malacol **35**:291−295. doi:10.9710/kjm.2019.35.4.291

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. doi:10.2983/035.031.0312