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Perkinsus olseni infection in juvenile and small Manila clam *Ruditapes philippinarum* on the west coast of Korea surveyed in 2008 and 2010

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ABSTRACT

The recent decline in Manila clam landings in Korean waters is, in part, linked to a high level of infection by the protozoan parasite *Perkinsus olseni*. In this study, we first surveyed the infection status of protozoan parasite *P. olseni* in the juvenile and small Manila clams using Ray's fluid thioglycollate medium assay (RFTM). A total of 578 clams of shell length (SL) ranging 9.2 to 29mm were collected from 9 tidal flats in Gyeonggi Bay, the coast of Taean, Garorim Bay, and Gomso Bay in January and November 2008 and March 2010. The smallest clam (SL 9.2mm) of the survey was originated from Sungam in November 2008, which was infected by *P. olseni* with the infection intensity of 570, 000 cells/g tissue. RFTM revealed that the juveniles (10-15 mm SL) in Gomso Bay are infected by *P. olseni* with a mean infection intensity of 491,000 cells/g tissue, suggested that *P. olseni* infection in the juveniles can be initiated as early as a few months after the settlement. The juvenile (SL 20-25 mm) and small clams (SL 25-30 mm) in Hwangdo Island were heavily infected by *P. olseni*, with the mean infection intensity of 1.751×10^6 and 4.830×10^6 cells/g tissue, respectively. The survey confirmed that most of the juveniles and small clams in major clam beds on the west coast were infected by *P. olseni*, and it is believed that *P. olseni* infection is not limited to the adult clams.

Key words: *Perkinsus olseni*, *Ruditapes philippinarum*, juvenile, infection intensity, west coast of Korea

INTRODUCTION

The Manila clam *Ruditapes philippinarum* is one of the most commonly cultured marine mollusks on the west coast of Korea, where the size of the juvenile clams ranging 15-25 mm in SL is sowed on sandy-mud intertidal beaches as seeds, then they are harvested 2 to 3 years later. Currently, tidal flats in Incheon Bay, Garorim Bay, Anmyeondo Island in Taean coast, and Gomso Bay on the west coast of Korea are served as Manila clam culture grounds, as the tidal flats are licensed to the local shellfish unions or private growers. The annual Manila clam landings in Korea have been declined for the past decade, and mass mortality of the clam in late summer is found to be closely linked to the decline. Recent studies have reported that the mass mortalities of Manila clam in Taean coast is, in part, caused by high level of protozoan parasite *Perkinsus olseni*, a pathogen responsible for mass mortality of Manila clams in Japan and Europe (Park *et al*., 2006; Pretto *et al*., 2014, Nam *et al*., 2018; Waki *et al*., 2018). Several studies have reported spatio-temporal variation of *P. olseni* infection in Korean waters, while the surveys are limited to the adult clams size over 30 mm in SL (Kang *et al*., 2015; Ngo *et al*., 2004; Yang *et al*., 2012). Accordingly, *P. olseni* infection status in the juvenile

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or small clams in Korean waters is poorly known. Recent studies carried out in Japan revealed that high level of *P. olseni* infection is responsible for mass mortality of the juveniles, as well as the recent decline in Manila clam landing in Ariake tidal flat in the southern Kyusu, Japan (Waki and Yohinaga, 2013; Waki *et al*., 2018).

In this study, we first surveyed *P. olseni* infection among the juveniles, and small Manila clams size smaller than 30 mm distributed in the major clam culture grounds on the west coast of Korea. Accordingly, the present study reports first reports *P. olseni* infection status among the juvenile and small clams, which is crucial in the management of Manila clam aquaculture.

MATERIALS AND METHODS

1. Sampling effort

Figure 1 shows the sampling sites located on the west coast of Korea. In January and November 2008, we collected clams from 5 tidal flats in Incheon Bay, including Sunjedo Island (Sunje), Sungam, Naeri in Youngheung Island (Naeri), Jonghyun at Daebudo Island, and Bakmiri in Hwasung. Hwangdo and Padori tidal flats in Taean coast were also surveyed in January and November 2008, and Hajeon tidal flat in Gomso Bay. In March 2010, juvenile clams were also collected from Jungwang tidal flat in Garorim Bay (Table 1). For the survey, we selected different size classes of Manila clams as shell length (SL) ranging 10 to 15 mm, 16 to 20 mm, 21 to 25 mm and 26 to 29 mm (Table 1). A total of 578 clams were collected from 9 sites on the west coast of Korea to determine *P. olseni* infection intensities. According to Chung *et al*. (1994), Manila clams of SL smaller than 10 mm were estimated to be approximately 4 month old, while the clams of 10-15 mm SL were 8 months, clams of 16-20 mm SL were 12 months, and the 25-30 mm SL were approximately 2 years old.

2. Ray' Fluid Thioglycollate Medium Assay (RFTM)

We applied the Ray's FTM assay followed by the Choi's 2M NaOH digestion technique to determine the

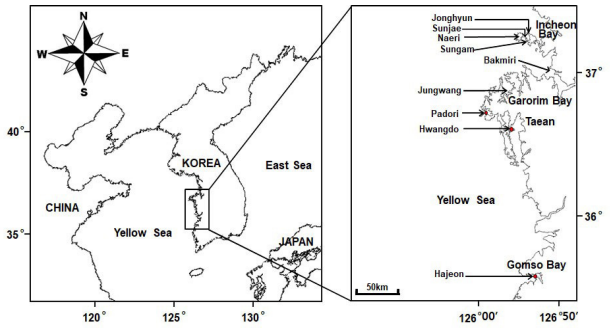

Fig. 1. Sampling sites on the west coast of Korea.

P. olseni infection intensity in each clam. After measuring the SL (i.e., the longest axis of the shell), clams were opened, and the whole tissue was removed and measured the wet weight (TWT) to mg using an electronic balance. The whole tissue was added in a conical tube containing 10 ml of FTM fortified with antibiotics (Park and Choi 2001; Park *et al*., 2006; Yang *et al*., 2012). The tubes were incubated at a dark condition at room temperature (25 °C) over a week. After the incubation, the tubes were centrifuged and the FTM was discarded and 5 ml of 2M NaOH was added into each tube and incubated in the water bath (50 °C) for one hour to digest the tissue. The number of *Perkinsus* hypnospore cells in the tube was then counted using a blood cell counter under a light microscope. *P. olseni* infection intensity of each clam was finally expressed as the number of *P. olseni* cells per gram TWT (cells/g TWT).

RESULTS AND DISCUSSION

Table 1 summarizes the survey results. In January 2008, a total of 146 juvenile or small clams of SL ranging from 15 to 29 mm were collected from the five sites in Incheon Bay. At Sungam tidal flat, the smallest clam (15.0 mm SL) exhibited the infection intensity as 716,000 cells/g TWT. At Bakmiri tidal flat, the mean infection intensity of small clams (25-29 mm SL) was recorded as 1,287,000 cells/g TWT, which was somewhat comparable to the intensity determined from the adult clams previously from other clam beds on the west coast (Park and Choi 2001).

In January 2008, a total of 57 clams were collected

Table 1. Summary of *P. olseni* infection in juvenile and small clams on the west coast of Korea. TWT, tissue wet weight

Sampling Period	Area	Site	N	Shell Length (SL, mm)	TWT (g)	*P. olseni* cells/g TWT	Highest
Jan 2008	Gyeonggi Bay	Sunje	18	25 > SL, Mean SL 26.2 ± 2.2	0.858 ± 0.201	627,057 ± 476,003	1,675,443
		Sungam	60	15 > SL > 20, Mean SL 17.4 ± 1.2	0.250 ± 0.051	242,125 ± 218,420	885,740
		Naeri	31	20 > SL > 25, Mean SL 22.7 ± 1.3	0.668 ± 0.123	269,172 ± 332,188	1,400,910
		Jonghyun	20	20 > SL > 25, Mean SL 24.2 ± 1.3	0.746 ± 0.132	813,916 ± 176,727	738,422
		Bakmiri	17	25 > SL > 30, Mean SL 25.6 ± 2.4	0.582 ± 0.130	1,287,352 ± 1,104,551	4,632,827
	Taean	Padori	20	25 > SL > 30, Mean SL 27.9 ± 1.6	0.847 ± 0.223	18,005 ± 35,751	115,545
		Hwangdo	12	20 > SL > 25, Mean SL 24.1 ± 1.9	0.930 ± 0.243	2,143,757 ± 1,061,917	4,155,673
		Hwangdo	25	25 > SL > 30, Mean SL 27.5 ± 1.0	1.366 ± 0.239	1,751,492 ± 921,169	4,530,068
	Gomso Bay	Gochang	17	10 > SL > 15, Mean SL 13.2 ± 1.5	0.113 ± 1.485	491,962 ± 457,781	1,407,866
		Gochang	17	15 > SL > 20, Mean SL 17.7 ± 2.2	0.275 ± 0.099	525,493 ± 649,751	1,844,139
Nov 2008	Gyeonggi Bay	Sunje	50	20 > SL > 25, Mean SL 21.3 ± 2.0	0.369 ± 0.107	378,107 ± 469,227	1,781,289
		Sungam	30	10 > SL > 15, Mean SL 14.1 ± 1.2	0.117 ± 0.029	941,199 ± 927,211	3,196,347
		Sungam	20	15 > SL > 20, Mean SL 16.7 ± 0.9	0.183 ± 0.029	698,834 ± 731,947	2,343,750
		Sungam	32	15 > SL > 20, Mean SL 19.2 ± 0.9	0.243 ± 0.036	620,010 ± 577,384	2,762,913
		Sungam	18	20 > SL > 25, Mean SL 22.9 ± 1.3	0.438 ± 0.094	588,927 ± 620,496	2,474,031
	Taean	Hwangdo	27	25 > SL > 30, Mean SL 18.9 ± 1.2	0.348 ± 0.059	4,783,860 ± 3,309,939	14,553,480
		Hwangdo	23	20 > SL > 25, Mean SL 22.4 ± 1.8	0.513 ± 0.064	4,830,725 ± 2,427,076	12,202,597
		Padori	50	20 > SL > 25, Mean SL 21.6 ± 1.5	0.437 ± 0.095	3,126 ± 6,273	26,136
Mar 2010	Garorim Bay	Jungwang	91	15 > SL > 20, Mean SL 17.7 ± 1.0	0.329 ± 0.109	1,348,155 ± 1,351,930	4,974,251

from Hwangdo Island in Anmyeondo Island and Padori in Taean coast. The small clams (20-25 and 25-29 mm SL) from Hwangdo Island showed a markedly high level of *P. olseni* infection, as 2,143,000 cells/g TWT and 1,751,000 cells/g TWT, respectively. In contrast, most of the juvenile and small clams in Padori tidal flat were not infected by *P. olseni*, or the mean infection intensity of the infected clams was low as 18,000 cells/g TWT.

A total of 34 juvenile clams ranging from 10-20 mm SL were collected from Hajeon tidal flat in Gomso Bay in January 2008. It was remarkable that the juvenile clams of SL ranging 10-15 mm (i.e., 4-8-month-olds) exhibited a high level of *P. olseni* infection with a mean of 491,000 cells/g TWT. An individual clam (SL 10.9 mm and TWT 0.059 g) collected from Hajenon tidal flat in January 2008 showed *P. olseni* infection intensity as 1,161,000 cells/g TWT, suggesting that the high level of infection is not limited to the adult clams of 2 to 3 years olds as previously reported from Gomso Bay (Park *et al*., 2006; Yang *et al*., 2012).

In November 2008, a total of 180 juvenile to small clams were collected from Sunjedo Island (Sunje) and Sungam in Incheon Bay. The smallest clam (9.2 mm SL, 0.120 g TWT) collected from Sungam tidal flat in November 2008 showed *P. olseni* infection intensity as 573,000 cells/g TWT. It was noticeable that the juvenile clams (10-15 mm SL) from Sungam tidal flat exhibited a high level of *P. olseni* infection as 941,000 cells/g TWT.

In November, *P. olseni* infection intensity of small clams (20-25 and 25-29 mm SL) collected from Hwangdo Island showed a markedly high level of infection with a mean of 4,783,000 cells/g TWT and 4,831,000 cells/g TWT, respectively. In contrast, the infection intensity of small clams in Padori tidal flat was very low, with a mean of 3,000 cells/g TWT.

In March 2010, we also collected juvenile Manila clams used as seeds (15-19 mm SL) for the clam culture from Jungwang tidal flat in Garorim Bay to determined *P. olseni* infection. A total of 91 seed clams were analyzed, and the mean infection intensity was recorded as 1,348,000 cells/g TWT (Table 1), suggesting that the seed clams were not free from *P. olseni* infection and the infection intensity was considered to be high.

Numerous studies have reported detrimental effects of the high level of *P. olseni* infection on the host organism, including slow growth, retarded reproductive maturation and decrease in the reproductive effort (Park *et al*., 2006; Villalba *et al*., 2004). Like *P. marinus*, *P. olseni* also has three life stages, including the vegetative trophozoite stage in the host tissue, hypnospore as a resting spore stage, and the zoospore as the motile flagellate stage and all of these stages are known to be infectious (Villalba *et al*., 2004). Previous studies carried out in Korea reported that most of Manila clams on the west coast are infected by *P. olseni*, while the infection intensity varies spatially and temporally. *P. olseni* favors high temperature and high salinity for their proliferation, as the infection intensity and prevalence often are positively correlated with the water temperature and salinity (Kang *et al*., 2015; Park *et al*., 2006, Villalba *et al*., 2004). Recent studies have reported that *P. olseni* is transmitted via feeding activity of the host organism, as juvenile Manila clams may acquire *P. olseni* particle from the ambient environment as they filter the seawater (Villalba *et al*., 2004; Park *et al*., 2010). According to Villalba *et al*. (2005), the juvenile carpet shell Tapes decussatus smaller than 20 mm in tidal flats in Ria de Arousa, Galicia Spain are free from *P. olseni*. In contrast, Waki *et al*. (2012) demonstrated *P. olseni* infection among the juvenile Manila clam (3-15 mm SL) by challenging the uninfected clams with *P. olseni* infected Manila clam tissues. Waki and Yoshinaga (2013) also demonstrated the lethal impact of *P. olseni* infection in the juvenile clams at laboratory conditions by challenging uninfected juvenile Manila clams by challenging with a high number of *P. olseni* zoospores. Currently, no studies have investigated *P. olseni* infection among juvenile Manila clams in Korean waters.

In this study, the highest *P. olseni* infection intensity was recorded from a juvenile clam (15.8 mm SL, 0.194 g TWT) collected from Hwangdo intertidal flat in November 2008. It is noticeable that the smallest juvenile clam surveyed in this study was 9.2

mm SL (0.120 g TWT) collected from Sungam in November 2008, while this juvenile clam showed its *P. olseni* infection intensity as high as 572,000 cells/g TWT. Such high infection intensity could be lethal to the juveniles, as Waki *et al*. (2012, 2013) demonstrated. In the Korean Manila clam industry, juvenile to small clams are often harvested and transported to other culture ground as the seeds, and some seed clams infected by *P. olseni* may transmit *P. olseni* to other areas if the infected clams are being transplanted as seeds.

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