A THESIS FOR THE DEGREE OF
MASTER OF SCIENCE

Assessment of temperature effects on the variation of reproductive cycle and parasites infection on Manila clam *(Ruditapes philippinarum)* in Shi-Heung-Ri beach on the east coast of Jeju Island

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A dissertation submitted in partial fulfillment of the requirement for the degree of Master of Science

2021. 08.
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Abstract

Post two decades since the first report of seasonal variations of *Perkinsus* and *Cercaria* and reproductive cycle of Manila clams distributed in Shi-Heung-Ri beach on the east coast of Jeju island, the climate change draws a query that whether these parameters alter in this area. Accordingly, the physiological condition of clams, *P. olseni*, *Cercaria* and other parasite infections were surveyed. From obtaining sea surface water temperature (SST) from KHOA database, the linear trend estimated that the mean SST fluctuation post 2 decades increased 1.07℃. Adult Manila clams were collected monthly from May 2019 to April 2020 and analyzed using gill RFTM (Ray's Fluid Thioglycollate Medium) assay (for *P. olseni*) and histology. The condition index of clams was highest in July (6.7±1.7) then decrease in August and September that correlated to the spawning peak. For RFTM assay, the dramatic increase in *P. olseni* infection intensity from October (2.1×10^5) to November (8.7×10^5) coincided with post-spawning condition of the clams. The prevalence and infection score of *P. olseni* was high with the mean of 89.7% and 3.0, respectively, without seasonal changes. The proportion of Rickettsia-like-organisms (RLOs) infection were highest in April (50.0%) and lowest in November (6.7%). Metacercaria *Parvatrema duboisi* mostly infected in the summer and autumn (16.7-43.3%) whilst *C. tapidis* and *C. pectinata* incidentally infested in some months with low prevalence (3.3 and 6.7%, respectively). To compare with the result in 2001-2002, Manila clams in the present study expressed poorer physiological condition (low CI and delay spawning season). Besides, the prevalence and infection score of *P. olseni* increased while the percentage of *C. tapidis* infestation decreased after 18 years. Thus, this study suggests that a slight increase in SST may exerted a great influence on the parasite infection dynamics although further investigation needs to be carried out to warrant this hypothesis.

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1. Introduction

Climate change effects can be different depending on the natural interaction between parasites/pathogens and hosts as well as the placement where it happens (Rowley et al., 2014). Thermal stress put together with the poor nutritional condition of the Manila clams in Korea prompted to the expansion of *Perkinsus olseni* trophozoites to cross defeating the host's immune response (Lee et al., 2020a). Increasing temperature is supposed to foster serious marteiliosis disease on both mussel and oyster (Fox et al., 2020) due to high mortality in both oyster (80 – 90%) and mussels (40 – 100%) (OIE, 2012). Cook et al., 1998 supposed that rising water temperature in the northern U.S favored the spread out of *Perkinsus marinus*- a southern parasite and related to this protozoa outbreak in the northeastern U.S. Moreover, they also suggested the extreme elevation temperature, but in short-time did not entirely trigger *P. marinus* compared to sustainable shift but less extreme. The transmission of parasites is, in the case of warmer conditions, possibly fostered and elevated their local diversity (Poulin, 2006). Long-term change of the climate significantly affected the prevalence and infection intensity of *P. marinus* in the Gulf of Mexico cause 60 to 80% oyster infection and the mortality in summer is regularly high (Powell et al., 1992). The climate model performed by Poulin, 2006 suggests that increasing a few degrees in environmental temperatures can expand cercarial emergence and the local impact of trematodes on intermediate hosts. These are considered as the general framework for most trematode-host interaction. The interaction between climate changes, in terms of ecology, and the host and diseases, however, are complicated to completely elucidate and interpret.

Besides oysters and mussels, a well-known marine bivalve, *Ruditapes philippinarum*, also called as short-necked clam or Manila clam, stands as a second critical part of aquaculture species, contributing to high commercial value and worldwide distribution (Cordero et al., 2017). This species plays as a vital marine commodity that influences the production of shellfish on the west coast in Korea (Mun et al., 2017). The

major natural territory of *R. philippinarum* is the sandy mud sediments of tidal flats and thus, this bivalve is partly liable for seawater purification by filtering suspended organic matter (Koo & Seo, 2020). Environmental and biological conditions are the major factors that influence the distribution of Manila clam in the intertidal zone (Gharbi et al., 2016; Kim et al., 2017; Yoon et al., 2013). Hence, in terms of natural conditions, water temperature, salinity, and prey species composition control the concordance habitat for this species (Kim et al., 2017).

For several decades, shellfish disease has been perceived as an obstacle to both the aquaculture industry and economic development by reducing shellfish production. Up to now, several diseases has been reported for Manila clam. Among them, *Perkinsus* is supposed to be associated with the diminishing in the clam population in Korea (Park & Choi, 2001). Through filtration activity in the water column, the clams are positively infected by *Perkinsus spp* via gill tissues (Cui et al., 2018). The study conducted by Waki & Yoshinaga, 2018 shows that *P. olseni* infected on Manila clam leading to suppressed growth, condition index, burrowing and filtration activity. In chronic cases, this results in systemic weakness and negative impacts on energy balance, causing host mortality. According to Soudant et al., 2013, *Perkinsus* progression are adjusted by the most two vital factors known as temperature and salinity. Many studies have been conducted to analyze the response of different *Perkinsus* life cycles via the various thresholds of temperature and salinity (Ahn & Kim, 2001; Park & Choi, 2001; Waki & Yoshinaga, 2015; La Peyre et al., 2008). The spread out of *P. olseni* among a host population is highly related to the expansion process of pre-zoosporangia, which leads to the production of the infective zoospore stage (Casas & La Peyre, 2013; Umeda et al., 2020). The optimal temperature for *P. olseni* is 19 – 28ºC and salinity is between 25 and 35ppt, moreover, this protozoa zoosporulated at salinity above 10ppt and between 15 – 32ºC (Soudant et al., 2013, Casas et al., 2002). Waki & Yoshinaga,

2013, performed an experiment that challenged *R. philippinarum* with *P. olseni* at different temperatures and confirmed that trophozoite propagation was more rapid at 28 and 30ºC.

In 2002, the first report of *Perkinsus* and *Cercaria tapidis* infection on Manila clams on the east coast of Jeju island was carried out (Ngo & Choi, 2004). The result showed that *Perkinsus* and *Cercaria* were the two major parasites observed in the clam population of the study area. The prevalence and infection of both species were low but restricted the gametogenesis of clams. Since this study, no more study is conducted to further analyze the parasites in this location. Post nearly two decades, it draws a query that whether the scenarios of global warming alters the reproductive cycle and parasites infection in this area. Accordingly, the physiological condition and parasites infection on Manila clams in Shi-Heung-Ri beach on the east coast of Jeju island, Korea were assessed. The finding of this study could serve as a part of baseline to promote further research about changes in the ambient conditions in ecosystem impact to the parasites-host interaction.

2. Material and Methods

2.1. Sampling effort

Thirty adult Manila clams were collected monthly from May 2019 to April 2020 at the intertidal zone of Shi-Heung-Ri beach (33º26'N, 126º55'E) on the east coast of Jeju island (Fig. 1). The substrate in Shi-Heung-Ri mainly contained tiny pebbles and coarse sand.

The sea surface water temperature (SST) in Shi-Heung-Ri at the sampling period was obtained from the Korea Hydrographic and Oceanographic Agency (KHOA) database. The fluctuation of SST was displayed in Fig. 2. The lowest temperature was 13.3ºC in February-2020 while it reached the peak of 24.3ºC in August-2019.

Since the lack of SST data in Shi-Heung-Ri from 2001 to 2020 on KHOA database, I selected the SST data in Seongsan (33°28'N, 126°56'E) that was 1km far away from the sampling site of this study. The available data on KHOA initiated from November 2003 to September 2020. The linear trend estimated that the mean SST fluctuation post 2 decades increased 1.07℃ ($y = 0.0053x + 16.912$) (Fig. 3). In 2004, the mean SST was 17.7℃ and varied from 12.3 to 24.9℃ in whole year. Until 2020, SST fluctuated in the range of 13.3 to 24.8℃ and the mean value was 17.8℃.

The Chlorophyll-a data was provided by Prof. Joon-Baek Lee (Department of Earth and Marine Sciences, College of Ocean Science, Jeju National University). The site observed these data was also Seongsan. The chlorophyll-a concentration was gathered at the depth of five to ten meters of seawater. Based on Fig. 2, the chlorophyll-a level strongly fluctuated in the range of 0.3 - 1.8 mg/m^3.

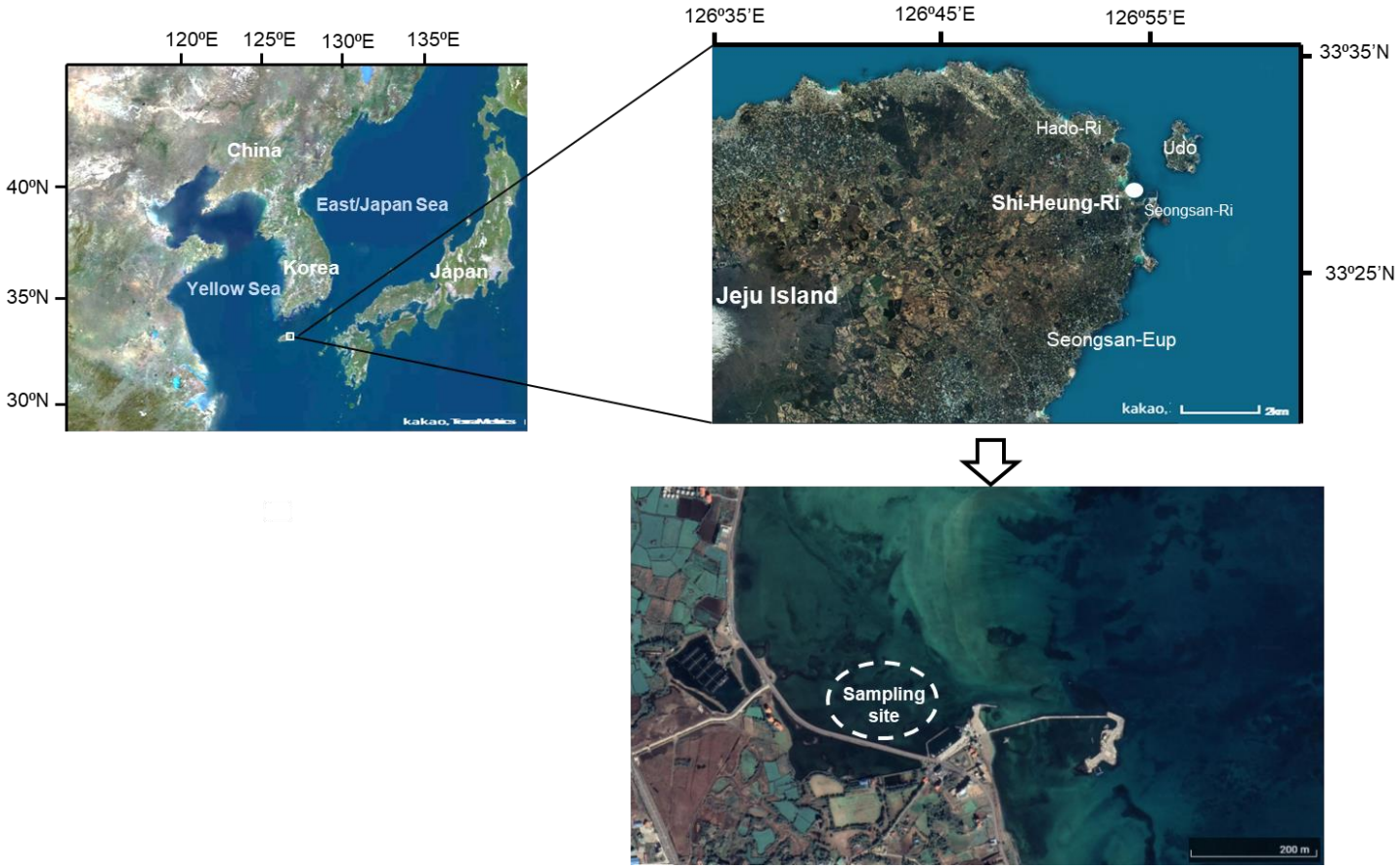

Fig. 1. Map shows the location of sampling site. Manila clams were collected in the intertidal zone of Shi-Heung-Ri beach, on the east coast of Jeju island, Korea.

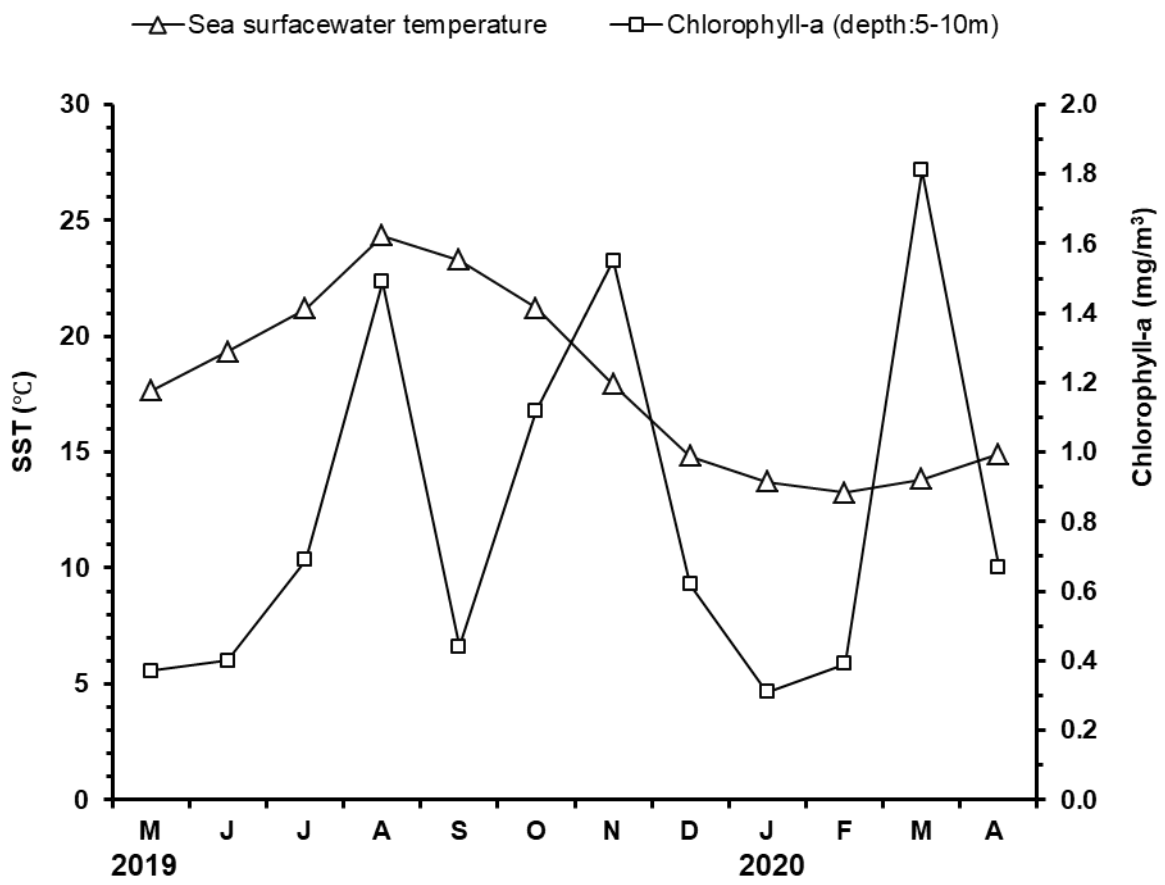

Fig. 2. Monthly sea surface water temperature (SST) and Chlorophyll-a concentration collected in sampling period.

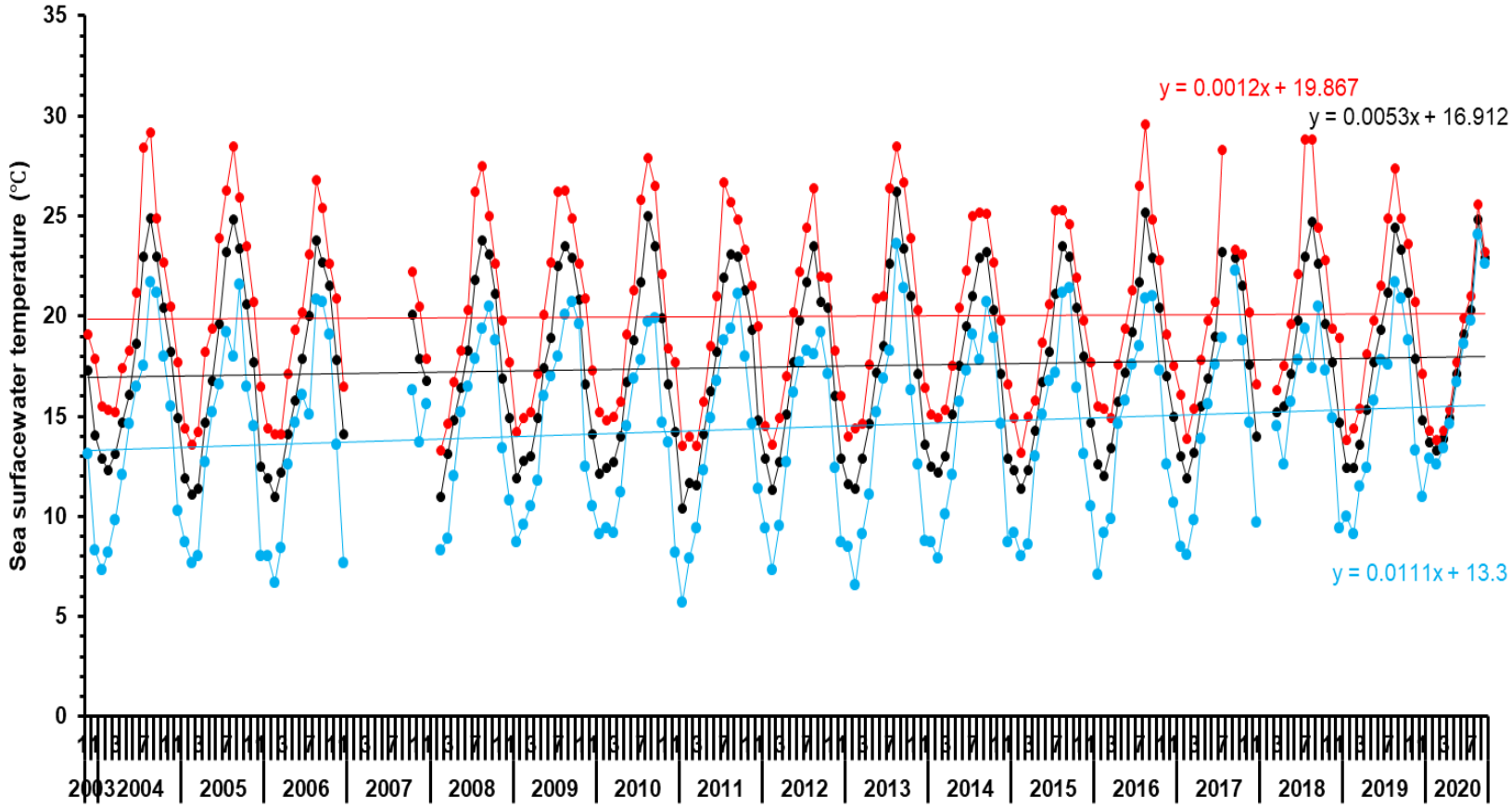

Fig. 3. The mean SST fluctuation gathered from November 2003 to September 2020 in Seongsan obtained from KHOA database. The red color indicates value of maximum SST. The black color shows Mean SST value and the blue color demonstrates minimum SST value.

2.2. Biological parameter and Condition index

Fresh clams were promptly transported to the laboratory for further experiment. At the laboratory, shell length (SL) (mm), shell height (SH) (mm), and shell width (SW) (mm) were determined by using electronic caliper. In this study, SL was defined as the longest axis of the shell while SH was measured from umbo to the edge of the shell, and SW was the thickness of the shell. The Tissue wet weight (TWWT) (g), Tissue dry weight (TDWT) (g) and Shell dry weight (SDWT) (g) were measured by electronic balance.

The condition index (CI) of individual clam in this study was estimated by three formulas as follow:

$CI = TWWT / SDWT$

$CI = (TWWT / SL \times SH \times SW) \times 10^5$

$CI = (TDWT / SDWT) \times 1000$

2.3. *Perkinsus olseni* infection intensity

One side gill from each clam was removed for Ray's fluid thioglycollate medium (RFTM) assay. The gill tissues were immersed in the 15mL conical tubes containing 5 mL RFTM supplemented with 30 µL antibiotics (nystatin 200 unit/mL and chloramphenicol 100 ng/mL) to induce hypnospores transformation. These tubes were preserved for 1 week in the dark at room temperature. Post incubation period, the gill tissues were digested using NaOH 2M and incubated at 60ºC in hot water bath for 1 hour, then centrifuge at 3000 rpm in 5 minutes. After discarding the supernatant and washing several times, 1X Phosphate-buffered saline (PBS) was added and using the hemocytometer to determine the number of *P. olseni* hypnospores by counting under the light microscope. The infection intensity was considered as the number of *Perkinsus* cells per gram of gill tissue weight.

2.4. Histology

To prepare for histological process, the transverse section including gonad, digestive gland, gill, mantle and foot was cut in the middle of body, then fixed in Davidson's solution in 24 hours and preserved in 70% ethanol. The sections were dehydrated by immersion in series of ethanol, clearing by xylene and embedded in paraffin. Paraffin blocks were sectioned at 7 μm by using a microtome. The section slides were stained with Harris' hematoxylin and eosin Y and evaluated under the light microscope.

The reproductive cycle of Manila clam was classified according to Park & Choi, 2004: resting, early developing, late developing, ripe, spawning and spent.

The *P. olseni* infection level was recorded according to the scale of Ngo & Choi, 2004 based on histological slides. The scale for evaluation was described as follow: 0 = none infection, 1 = *Perkinsus* limited to the gills and mantle, 2 = limited to the gills, mantle and digestive gland, 3 = found in the gills, mantle, digestive gland, gonad, 4 = found in all types of tissue, including the foot.

The trematode infestation was identified by their characteristic through histological observation. The metacercaria *Parvatrema duboisi* was recognized according to description of Yu et al., 1993, while *Cercaria tapidis* and *Cercaria pectinata* was specified following to Shimura et al., 1982.

The parasites prevalence (%) was expressed as the number of Manila clams that infected by parasites per total clams examined.

2.5. DNA amplification and cloning of *Cercaria pectinata*

To serve as DNA template for PCR amplification, 10 mg of freeze-dry tissue of Manila clams (N = 2) that infected with *C. pectinata* (observed by histology) were extracted using DNeasy Blood and Tissue kit (Quiagen, Germany). The primer used for this study was 18d (5'-CACACCGCCCGTCGCTACTACCGATTG-3') and 28cc (5'-

ACTCGCCGTTACTGAGGGAATCCTGGTTAG-3'). PCR reaction mixture contained 5μL 10× Ex Taq buffer, 4μL dNTP, 0.5μL of each primer, 0.25μL of Ex Taq DNA polymerase and 4μL DNA template in a total volume of 50μL. The amplification for cercaria DNA was performed under condition with denaturation in 5 min at 94℃, 25 cycles at 94℃ for 30 sec, 54℃ for 30 sec, and 72℃ for 1 min, and final extension at 72℃ for 5 min. The PCR products were electrophoresed on 1.2% agarose gel staining with ethidium bromide (EtBr) in 1× Tris Acetate EDTA (TAE) buffer. The result was visualized under long wavelength UV light and asserting product sizes based on a 100bp DNA ladder. To prepare for cloning, the positive PCR products were purified by using AccuPrep PCR/Gel purification kit (Bioneer, Korea) and ligated to pGEM T-Easy vector (Promega, USA). Then, the vectors were cloned by DH5α transformation, and the positive recombinant clones were sent to company for sequencing.

2.6. Statistical analysis

The variation monthly of sea surface water temperature, Biometry of clams, Mean of CI, *Perkinsus* infection intensity and Prevalence of parasites were calculated by using Microsoft Excel 2010.

3. Resuslts

3.1. Biometry and Condition index (CI)

The sampling time and biometry of Manila clams were illustrated in Table 1. Total 360 clams were collected from May 2019 to April 2020 for analysis. The shell length ranged from 31.9 ± 3.4 to 36.0 ± 2.8 mm and the tissue wet weight varied from 1.2 ± 0.3 to 1.9 ± 0.6 g.

In this study, the CI promptly rose from March to July that coincided with the gametogenesis of clams (Fig. 4). The highest value achieved in July before slightly declined in August and September that was spawning peak of clams. Thereafter, the value continued to remarkably drop in October. The lowest CI value among months was in November that considered as clam's post-spawning period.

Table 1. The sampling time, number of clams (N), mean ± standard deviation of shell length (SL) (mm) and tissue wet weight (TWWT) (g) of Manila clams used in this study.

Year	Month	N	SL (mm)	TWWT (g)
2019	May	30	34.4 ± 3.3	1.9 ± 0.6
	Jun	30	32.4 ± 4.9	1.7 ± 0.8
	Jul	30	32.9 ± 2.0	1.8 ± 0.4
	Aug	30	31.9 ± 3.4	1.8 ± 0.6
	Sep	30	33.4 ± 3.0	1.8 ± 0.5
	Oct	30	33.3 ± 3.9	1.6 ± 0.6
	Nov	30	36.0 ± 2.8	1.9 ± 0.5
	Dec	30	33.4 ± 2.5	1.4 ± 0.4
2020	Jan	30	33.3 ± 3.7	1.3 ± 0.5
	Feb	30	35.0 ± 2.3	1.5 ± 0.4
	Mar	30	31.9 ± 2.6	1.2 ± 0.3
	Apr	30	33.9 ± 2.9	1.7 ± 0.5

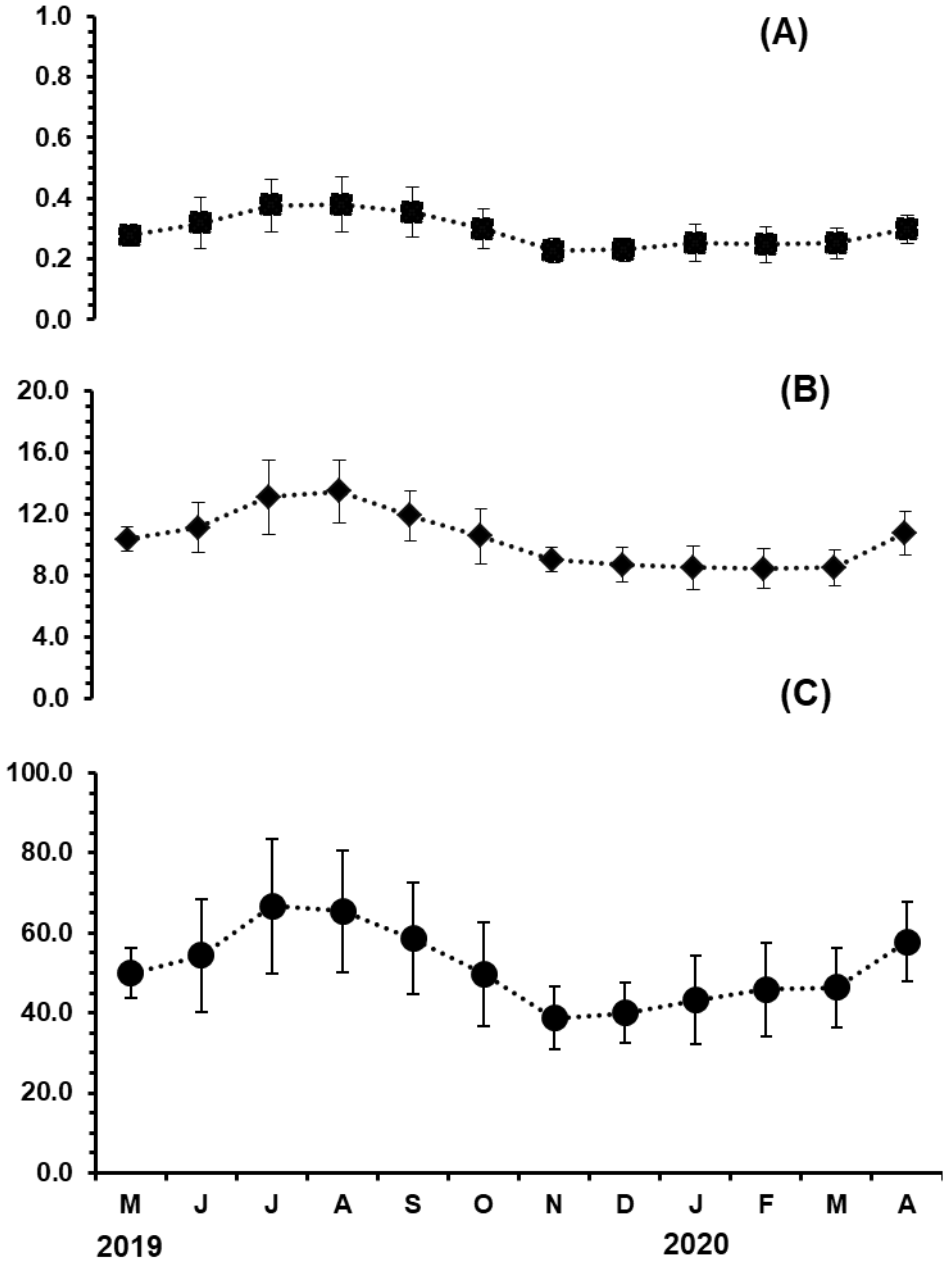

Fig. 4. The mean ± standard deviation of condition index (CI) of Manila clams in Shi-Heung-Ri. **(A)**. CI = TWWT / SDWT. **(B)**. CI = (TWWT / SL × SH × SW) × 10^5. **(C).** CI = (TDWT / SDWT) × 1000.

3.2. *Perkinsus olseni* infection intensity

Fig. 5 described the number of *P. olseni* hypnospore per gram gill tissue weight that was performed by incubating in RFTM. These values fluctuated from 212,080 ± 49,466 to 1,277,522 ± 283,545 cells. Between May and June, the infection intensity notably decreased to 467,997 ± 118,481 cells and the low number of *Perkinsus* cells maintained from June to October despite the high SST during this period. By contrast, November-2019 to April-2020 was the months that high proliferation of hypnospores was recorded despite lower SST compared to other months. Between October and November, the prezoosporangia cells suddenly extended from 212,080 ± 49,466 to 874,825 ± 152,688 cells per gram gill tissue weight and the hypnospore cells remained high level during the following period.

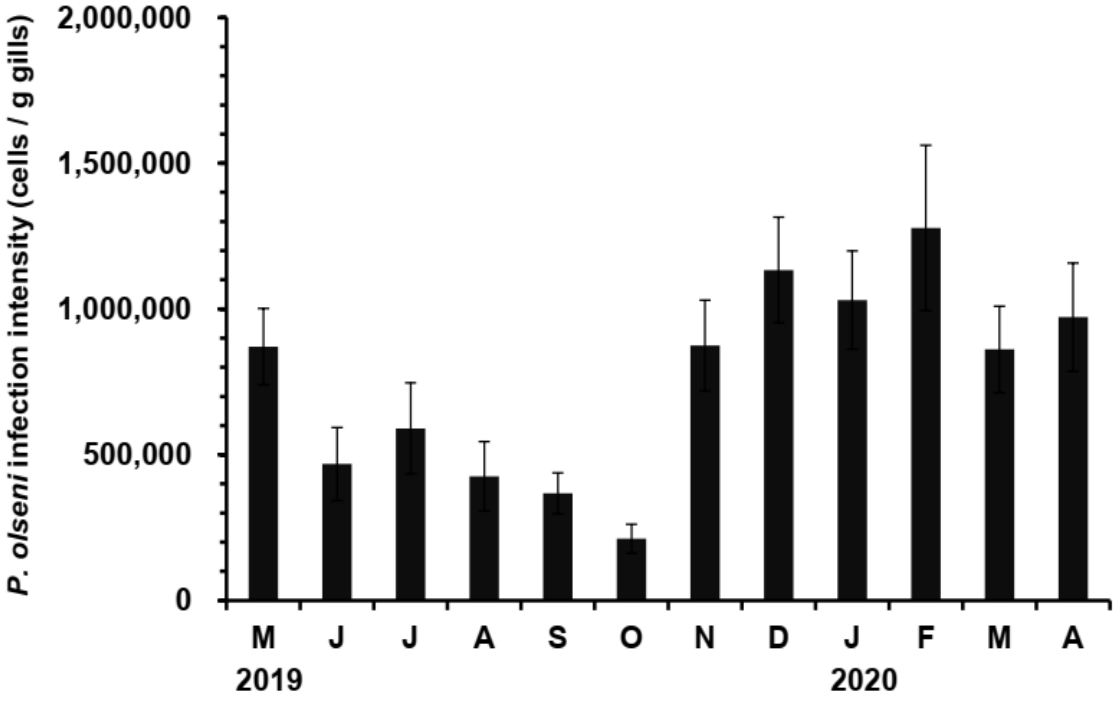

Fig. 5. The *Perkinsus olseni* infection intensity assessed by RFTM. The values were expressed as mean ± standard error (SE).

3.3. Histology

3.3.1. Reproductive cycle

The percentage of reproductive stage of Manila clams in each sampling month was described in Fig. 6. The gametogenesis initiated as soon as December (16.7%) but the dominant proportion was from March to June (63.0%) when the SST tended to increase. The first ripe clams were recorded in July at 13.3% and the percentage of this stage increased to 40.0% in August. The spawning peak of both male and female clams occurred in August and September when the SST reached the highest value (24.3 and 23.3℃, respectively). 56.7% of clams spawn in August while the proportion was 80.0% in September. Most of the clams with spent stage were observed in October (90.0%). November-2019 to February-2020 was the period of indifference sexual of clams. The result of the reproductive stage was associated with the condition index. At the ripe stage, the CI achieved the highest value and rapidly reduced in post-spawning time (October).

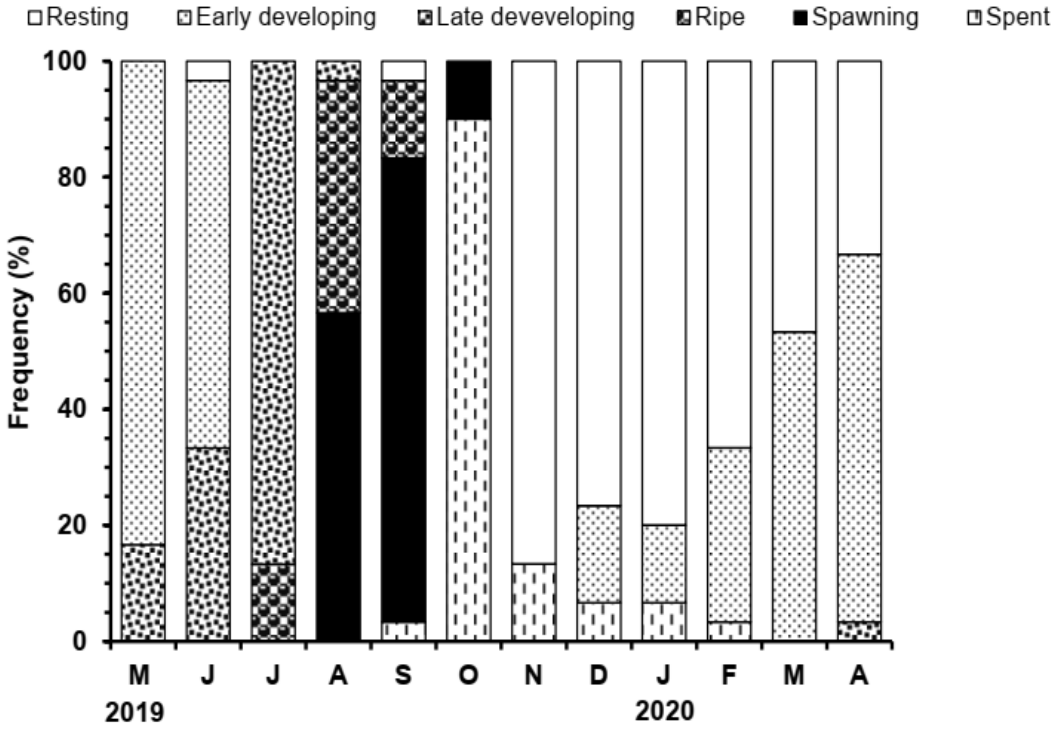

Fig. 6. Monthly variation in the percentage of reproductive development stages of Manila clams.

3.3.2. Parasites infection

Table 2 indicated the infection prevalence of *P. olseni* and other parasites during the period from May 2019 to April 2020. According to this table, *P. olseni*, *Rickettsia-like-organism* (RLOs) and *Parvatrema duboisi* were the main organisms observed on Manila clams with high infection proportion. Moreover, the appearance of two different trematode species known as *Cercaria tapidis* and *Cercaria pectinata* was notable. The prevalence of protozoa *P. olseni* was high most of the time while metacercaria *P. duboisi* mainly infected in the summer and autumn season. Besides, RLOs tended to occur year-round, however, their prevalence was not related to seasonal changes. *C. tapidis* and *C. pectinata* incidentally infested in some months with low prevalence in comparison with a high infection rate of trematode germ balls that observed in winter-spring time.

Table 2. Prevalence (%) of *Perkinsus olseni* and other parasites (Ricketsia-like-organism – RLOs, *Parvatrema duboisi*, *Cercaria tapidis*, *Cercaria pectinata* and germ balls) infection on Manila clam in Shi-Heung-Ri beach, Jeju island.

Year	Month	*P. olseni*	RLOs	*P. duboisi*	*C. tapidis*	*C. pectinata*	Germ balls
2019	May	96.7	23.3	16.7	0.0	0.0	0.0
	Jun	86.7	33.3	23.3	0.0	6.7	0.0
	Jul	93.3	30.0	46.7	0.0	0.0	0.0
	Aug	73.3	20.0	16.7	0.0	0.0	0.0
	Sept	76.7	36.7	43.3	3.3	0.0	0.0
	Oct	93.3	36.7	20.0	0.0	0.0	0.0
	Nov	90.0	6.7	3.3	0.0	0.0	6.7
	Dec	93.3	20.0	3.3	0.0	0.0	10.0
2020	Jan	93.3	26.7	3.3	3.3	0.0	0.0
	Feb	90.0	20.0	10.0	0.0	0.0	10.0
	Mar	96.7	23.3	10.0	0.0	0.0	3.3
	Apr	93.3	50.0	20.0	0.0	0.0	0.0

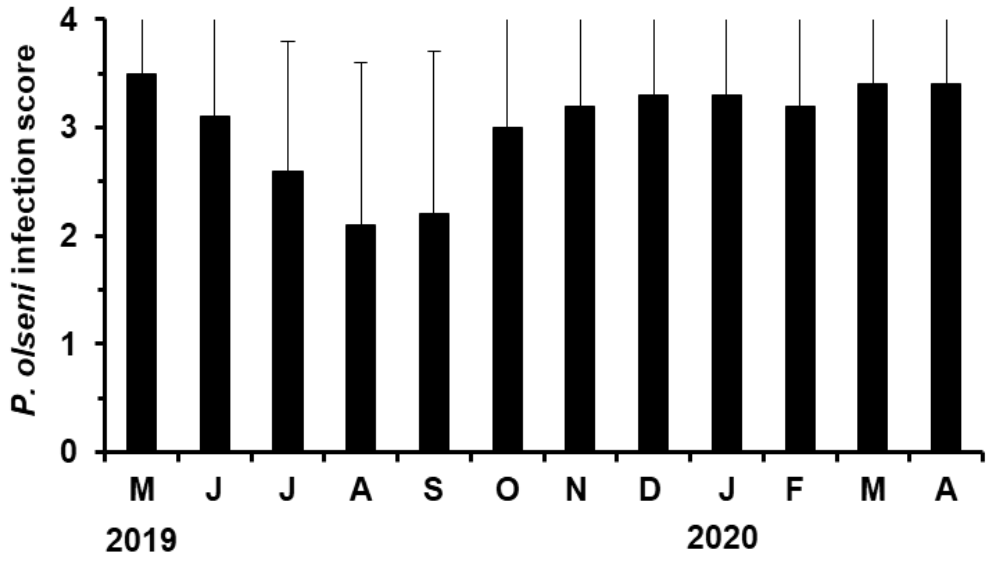

Fig. 7. Monthly change of *P. olseni* infection level scored by histological slides. The values were expressed as mean ± standard deviation.

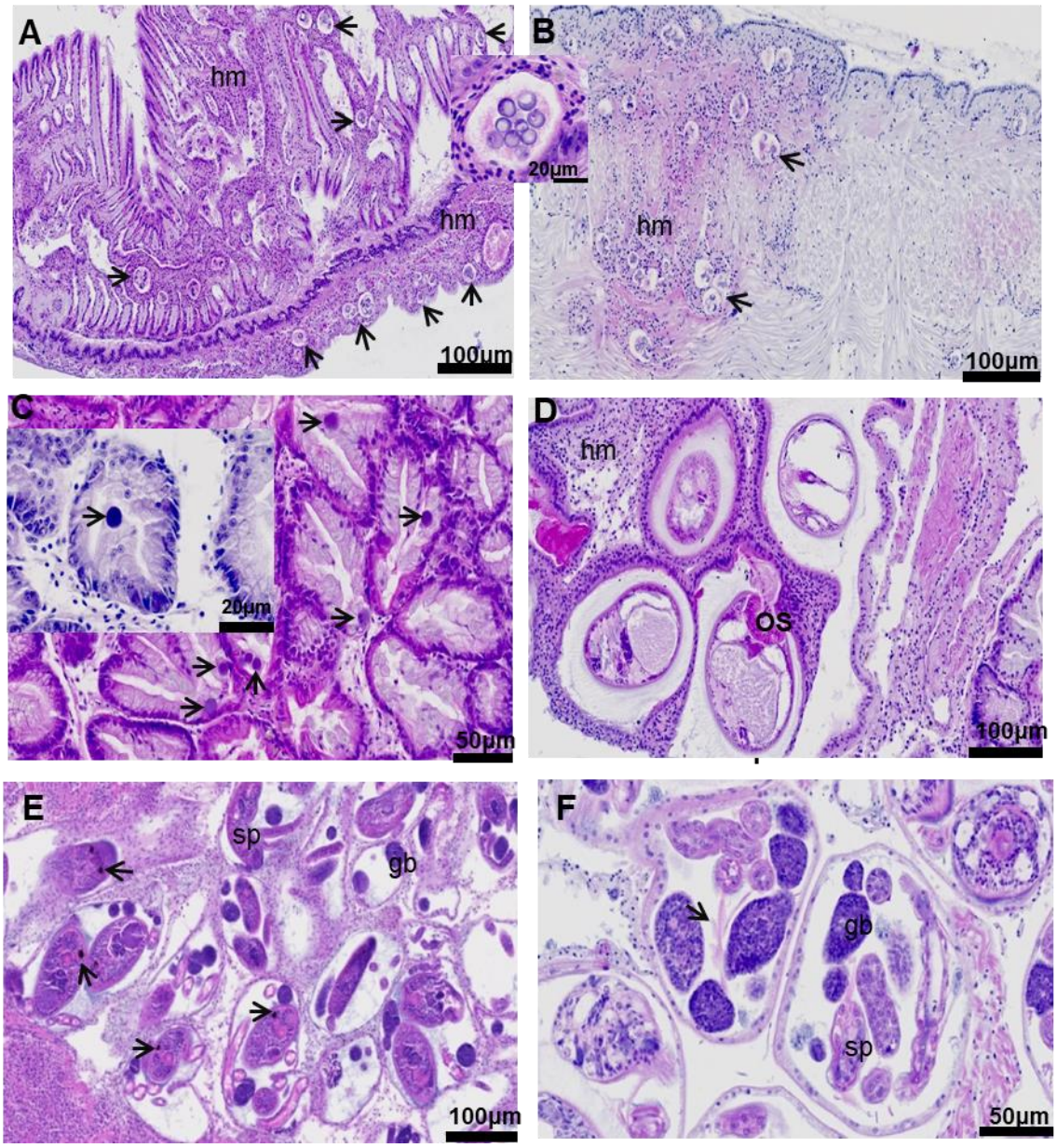

Fig. 8. Histological slides of Manila clams infected with parasites (H&E stain). **A.** Severe gill destruction by *P. olseni* cluster (arrow) and hemocyte infiltration (hm). **B.** *P. olseni* trophozoies (arrow) in the foot muscle tissue surrounded with hemocyte infiltration (hm). **C.** Rickettsia-like organism (RLOs) (arrows) in digestive epithilium without any response from the host immune. **D.** Cross section of mantle tissue displayed a number of *P. duboisi* with a strong oral sucker (OS). **E.** *C. tapidis* sporocyst contained young cercaria with two-eyespots (arrow), germ balls (gb) and sporocyst (sp) occupied in gonad. **F.** *C. pectinata* with setae tail (arrow), germ balls (gb) and sporocyst (sp) in the gonad.

Perkinsus olseni

The prevalence of *Perkinsus olseni* varied from 73.3% to 96.7% in the sampling period and without seasonal change. The percentage of this protozoa infection remained over 90.0% almost year-round, except in June, August and September that recorded at 86.7%, 73.3% and 76.7%, respectively (Table 2).

Histological slides expressed the average *P. olseni* infection score at 3.0 meaning that the trophozoite was found in almost organs as gills, mantle, digestive gland and gonad. However, the low infection score was mentioned during the ripe and spawning peak of clams in July, August and September (2.6, 2.1, 2.2, respectively) (Fig. 7). The shape of *P. olseni* trophozoite was spherical uninucleated cells with a large eccentric vacuole. Gills and mantle were two organs that displayed a high density of trophozoite infection. The severe infection with hemocyte infiltration resulted in lesion or structure deformation in the gills and increased the thickness of the mantle wall (Fig. 8-A). In some cases that Manila clams were heavily infected, the trophozoite was evenly spread out on the foot muscle. Hemocyte aggregation around the groups of *Perkinsus* cells led to inflammation and nodule formation on the foot muscle or connective tissue (Fig. 8-B).

Rickettsia-like-organism (RLOs)

Rickettsia-like-organisms (RLOs) was observed with the proportion varied from 6.7% to 50.0% (Table 2). Between October and November, the infection proportion suddenly reduced from 36.7% to 6.7% and then maintained at low prevalence before lifting to 50.0% in April. This species showed the sign of not being related to the season.

Rickettsia-like-organism was roundish basophilic inclusions that were detected in the digestive epithelium of Manila clam. No immune response from the host was observed around this organism (Fig. 8-C).

Parvatrema duboisi

Similar to *P. olseni*, *P. duboisi* also tended to appear throughout the sampling period

(Table 2). This metacercaria reached the highest prevalence in July and September (46.7% and 43.3%, respectively). The infection trend of this species dropped during October to January, from 20.0% to 3.3%. It seemed that the prevalence was higher in the summer and autumn period (16.7% - 46.7%) than in the winter and spring season (3.3% - 20.0%).

Through histological detection, *P. duboisi* had an oval shape with large oral sucker. *P. duboisi* mainly attached to the mantle cavity that was not seen in other organs. By using a strong oral sucker, this metacercaria prompted to mantle hyperplasia (Fig. 8-D). The immune response of the host was rare but sometimes was observed with hemocyte encapsulation around the metacercaria in mantle tissue.

Cercaria

In this study, *Cercaria tapidis, Cercaria pectinata* and trematode germ balls were randomly noticed in a few months with low prevalence. The infection incidence of *C. tapidis* was 3.3% that was solely observed in September-2019 and January-2020. *Cercaria pectinata* was observed in June at 6.7%. The prevalence of trematode germ balls varied from 6.7% to 10.0% that was mainly recorded in November-2019, December-2019, and February-2020 (Table 2).

Our sequencing displayed 99.6% identity with *Bacciger bacciger* (Accession number KJ633828.1) that has the cercaria stage named as *Cercaria pectinata*.

The result showed most of the gonad infected by three growth stages of trematode including sporocysts, cercaria and germ balls. In this study, two trematode species were described in Fig. 8-E and 8-F. *Cercaria tapidis* was recognized by two apparent eyespots and a long straight tail. By contrast, no eyespot was found in *Cercaria pectinata*, but this species had a tail with long thin spines. Although cercaria used the clam gonad as a crucial organ for nutrition, in cases of heavy infection, trematode also expanded its population in the digestive gland or foot muscle. Gonad castration and deterioration were the general demonstrations of cercaria impact on the host tissue through histological diagnosis.

4. Discussion

Condition Index (CI) is considered as a parameter to assess the health status of marine bivalves. By comparison between CI values of Manila clams in this survey, Japan and other locations with similar formula, Table 3 indicated the lower CI of clams in Shi-Heung-Ri than other areas. According to Uddin et al., 2013, despite relating to many factors as the gametogenic cycle, food availability, infection and disease, the CI tended to be compatible with the annual gametogenic cycle. The result of the present study agreed with the above conclusion by showing the increase of CI value linked to developing stages of the gonad in clams and at the ripe stage, the CI achieved the highest values. Table 4 summarized the gonad maturation and spawning peak of Manila clams in different regions. The spawning peak of clams in this study was in August and September that consider as later than the result of Ngo & Choi, 2004. Usually, Manila clams in Korea spawn from July to August in Incheon Bay (Uddin et al., 2010) or June to August in Gomso Bay (Park & Choi, 2004). The gonad maturation of clams in this result was longest (5 months) among other regions (2 - 4 months). Despite warming trend of SST in the survey area, the period for egg and sperm releasing in these clams still manifested slowly in comparison to other locations. Hence, we suppose that food availability plays a more critical role in sharing responsibility for gametogenesis than temperature (Delaporte et al., 2006; Delgado & Camacho, 2005; FAO, 2009). Fig. 9 showed the chlorophyll-a concentration at several sites along the coast of Korea and Jeju island. The mean concentration of chlorophyll-a around Jeju island was much lower than the concentration in the coastal mainland. The evidence from these reports seemed to support the interpretation that the clams in Jeju filter a smaller amount of food that may result in slow growth with low CI value and delay the spawning peak.

The report of Park & Choi, 2001 in Chepu and Gomso bay on the west coast of Korea displayed high value in both *Perkinsus* prevalence (87 – 100%) and mean infection intensity (1,077,628 cells/individual) in the winter period. At that time, the surface seawater

temperature was low as 3.1ºC resulted in the hypothesis that *Perkinsus sp*. persists well in winter. In general, this species was considered having higher metabolic activity than *P. marinus* and less affected by low temperature (La Peyre et al., 2008). To be linked with this finding, the trend of *P. olseni* infection intensity in present study was also higher from winter to spring despite lower SST than other seasons. This result may explain that the dramatic increase in *Perkinsus* infection intensity coincides with the post-spawning condition that exhausts the host's energy prompting to be more vulnerable with *P. olseni*. Moreover, high *P. olseni* infection appeared to prolong the resting stage of clams (Uddin et al., 2010). Spawning is a progress that requires energy potentially leading to immune depression and higher susceptibility to pathogenic infection. Manila clams post-spawning expressed the decline in both circulating granulocytes and phagocytosis capacity (Hong et al., 2014; Soudant et al., 2004) since the tissue protein levels in post-spawning clams were significantly less than those of pre-spawning clams, and thus, Manila clams post-spawning may not retain enough energy handling for cell immune responses (Hong et al., 2014). The restriction of Manila clams in energy budget and immunity generated appropriate conditions for *Perkinsus* growth (Park et al., 2006). Yang et al., 2012 agreed that the spawning of clams in Gomso Bay depleted the clams that weakened the defense proficiency. The low level of food supply combined with the poor physiological condition of the hosts may lead to favor the extension of *P. olseni* in clams of this bay in the autumn and winter season. Thus, the authors hypothesized that seasonal change of infection intensity was regulated externally by the fluctuation of water temperature and salinity, and internally by the annual gonadal cycle of clams (Yang et al., 2012).

Jung, 2008, surveyed the hydrological conditions around Korean peninsula and confirmed that the SST has increased by 0.975℃ during 37 years from 1968 to 2005 and salinity decreased 0.229. Besides, the warming trend spatially distributed most prominent in the East and South Sea. The SST and sea level around Korea have supposed to rise at the rate

about two to three times than the global mean, but the East Seas and around Jeju Island have a higher rate than Yellow Sea (KMA, 2020, Kim et al., 2011). Thus, Jung, 2008 assumed that the SST around Korean peninsula may extend by 0.63℃ and 2.48℃ in 2030 and 2100, respectively. Hence, this study shows that the SST on the east coast of Jeju island slightly increase over a long-term of nearly 20 years. This trend may lead to the observed elevation of parasitism infected on Manila clams. According to Byers, 2020, three main results of higher temperature were (1) enhance the metabolism of parasites because most marine parasites and their hosts were ectothermic, thus high temperature means parasites feed faster and heavier on the hosts, (2) increase oxygen stress on hosts due to decreasing dissolved oxygen levels and (3) increase transmission pathway since complex, multi-host parasites life cycles extended their seasonal residency and activity. The difference between parasite and host metabolism may favor the parasite in spending energy at warm temperature faster than the host's assimilation of new energy, while the inverse case occurred at a colder temperature that means the energy assimilated by the host would be equal to or exceed parasite energy manipulation (Paull et al., 2015).

In this study, the prevalence of *P. olseni* was much higher (73.3-96.7%) than the report of Ngo & Choi, 2004 (6.0-86.0%). Moreover, the prevalence in 2001-2002 was high in winter to spring (28.0 – 86.0%) and low in summer (6.0 - 42.0%) while no seasonal changes in this present study. The reason for this trend is thought to be related to the slight increase in SST that leads to boost the virulence of this protozoa to the host. High water temperature generated prosperous conditions for *P. olseni* expansion by defeating the immune response of Manila clam thus decreased host resistance to this parasite (Nam et al., 2018). According to Matozzo & Marin, 2011, high seawater temperature degraded cell-mediated immune functions in marine bivalves such as total hemocyte count, phagocytosis, and lysozyme activity. Moreover, high water temperature was not only exhausted metabolic energy sources in bivalves but also attenuated their potential in overcoming external

pathogens (Paillard et al., 2004; Villalba et al., 2004). In case of severe infection with *P. olseni*, the energy consumption caused by this protozoa may further rise when the water temperature is high. As stated by Villalba et al., 2004, *P. olseni* infection led to negative effects such as slow growth and insufficiency of energy for successful gametogenesis since the reserve energy declines in the infected host. Temperature affects the propagation of trophozoites through the host tissues. Based on this, the temperature was suggested to increase infection intensity, zoospores production, disease transmission, and host death (Waki et al., 2018). The average infection level was 3.0 (*Perkinsus* was found in gills, mantle, digestive, and gonad) whilst the average infection level observed 20 years ago was 0.63 (*Perkinsus* infection was limited to gill tissues) (Ngo & Choi, 2004). The *P. olseni* infection level assessed by histology in this study displayed the severe infection in almost the sampling period. *P. olseni* was observed for the most of gill and digestive of Manila clams with hemocytes infiltration or inflammation response around mature trophozoite (Park et al., 2010). The results of Wang et al., 2018 indicated that the zoospore of this protozoa invaded the gill and labial palps as main portal entry organ that linked with transformation of zoospore into trophozoite post attacking gill leaflet.

The present study confirmed the occurrence of Rickettsia-like-organisms (RLOs) infecting on Manila clams (6.7 – 50.0%) on the east coast of Jeju island, that not yet reported prior in the research of Ngo & Choi, 2004 . This study emphasized the high prevalence of RLOs inclusions almost year-round except November (6.7%) and the infection rate seemed not to be related to the seasons. Hong et al., 2016, recorded RLOs presented in digestive gland epithelium of Manila clams on the west coast of Korea with the prevalence at 3.3 - 6.7%. Bhaby, 2018 concluded that the high prevalence of RLOs detected on mussels *Mytilus galloprovincialis* in Morocco was highlighted during summer (37 - 50%) and significantly correlated with seasonal variation. Since the mortality of blood clam, *Tegillarca granosa* in China peaked at 100% in the spring and autumn seasons when the water temperature was

over 16℃, this suggested that the RLOs pathogenicity might be linked with high temperature and salinity (Zhu et al., 2012). Based on the histology result of this study, RLOs was found mainly in the digestive epithelial with no host tissue reaction despite the high infection level of these colonies. As reported by Travers et al., 2015, RLOs was observed in the epithelial cells of the mantle, digestive gland, gills, and connective tissue of several bivalve species. Carballal et al., 2001 detected the basophilic colonies of RLOs on cockle *Cerastoderma edule* that was more converged in digestive tubules than secondary ducts. These intracellular colonies, though, did not cause any obvious damage to cockles and no host defense response. The low infection intensity of RLOs was regularly observed in mollusks and not be related to inflammation (Norton et al., 1993). By contrast, Ceuta & Boehs, 2012 suggested that the cells infected with RLOs showed hypertrophy and cell lysis in the digestive epithelium and the gills. Notwithstanding, it is ambiguous that the RLOs caused mortality in mollusk or this species is merely symbionts with absence or restriction adverse impact on the host. RLOs do not expose any damage to the host may grow rapidly under favorable conditions and high RLOs infection intensity in infected tissues could change or weaken the normal function of the tissues, finally resulting in disease development (Flores & Martınez, 2020). Climate changes and short-term intension of ocean temperature could consequent to significant alteration of host-parasite dynamics in abalones invaded by RLOs and prompted to exacerbate withering syndrome to abalone populations (Neuman et al., 2010). The proliferation of RLOs seemed to be associated with environmental factors or host physiology that bolstered this bacteria replication up to enough cells to trigger disease or tissue alteration (Flores & Martınez, 2020).

The Gymnophalid metacercaria *Parvatrema duboisi* was also the main parasite infesting on clams in this survey with high prevalence. On the west coast of Korea, 10% of metacercaria *P. duboisi* was observed in the mantle cavity of Manila clams (Hong et al., 2016). Sohn et al., 2017 reported 2 species of metacercaria known as *Himasthla alincia* and

Parvatrema spp infecting on Manila clams on the western coast of Korea with the prevalence at 30.0 - 80.0% and 6.7 - 100.0%, respectively. Despite occurring year-round, the metacercaria *P. duboisi* in this study tended to appear more in the summer and autumn seasons that similar to the result of Özer & Güneydaı, 2015. The morphological characteristic of metacercaria *P. duboisi* was first confirmed on Manila clams in Korea by Yu et al., 1993. The adult parasite had an oval shape, large oral sucker with lateral projection on the lip, absence of the ventral pit, single cluster of vitellaria, and the genital pore separated with a ventral sucker. The histological observation in the present study agreed with Ituarte et al., 2001 that metacercaria of Gymnophallid mostly infested between the mantle and the valve, either free or surrounded by host tissue. According to Ching, 1995, this parasite may not cause damage if the invaded location was between the shell and mantle below the hinge. Conversely, aggregation of hemocytes was observed when clams *Leukoma theca* was parasitized by *Parvatrema* in the mantle epithelium by the oral sucker (Montenegro et al., 2021). The rise of Gymnophallid trematode prevalence may become harmfully to dominant mollusk populations by castrating and consuming many organs of their intermediate hosts as well as change the host behavior to make them easier to be detected by their predators (Huntley et al., 2014).

Trematodes had complicated life cycle in bivalves that served as intermediate hosts and carnivorous fishes were the final hosts (Shelley et al., 1988). The result of this study displayed the prevalence of *Cercaria tapidis* was 3.3% in a few months, that much lower than the survey of Ngo & Choi, 2004 before (2.0 - 12.0%). Besides *C. tapidis*, *Cercaria pectinata* was confirmed in the sampling period and not yet reported before despite low infestation proportion (6.7%). In addition, the infection of these cercaria was recorded without a relationship to seasonal changes. The reason for the sudden absence of these trematodes despite temperature elevation is suspected according to Morley & Lewis, 2015 that temperature increase was inappropriate for trematode infection in ectothermic hosts. The

temperature may put a direct restriction on trematode infectivity and the alteration in host susceptibility could shape variation in parasite establishment under different thermal regimes. In opposite, Selbach & Poulin, 2020, believed that increasing temperature has positively defected to cercaria transmission, however, these affect varied differently depending on trematode species and their host-searching ability. On the west coast of Korea, *C. tapidis* was recorded in gonad tissue with a low prevalence (6.7%) (Hong et al., 2014). Lee et al., 2001 observed *C. tapidis* in the Manila clams on the southern coast of Korea with 9.7% of prevalence. However, no host reactions appeared although the gonad was nearly full of sporocysts and cercaria. The prevalence of *C. pectinata* infested on Manila clams in Japan was related to seasonal change with a low infection rate from May to August (0.0 - 1.2%) and high from September to March (2.0 - 3.6%) (Shimura et al., 1982) . By contrast, the infection rate of *C. pectinata* recorded by Chun & Lee, 1976 on hard clam (*Meretrix lusoria*) on the western coast of Korea was low from February to May (2.0 - 7.2%) and high from June to November (20.2 - 29.6%). Shimura et al., 1982, investigated marine cercariae infected Manila clams in Japan and confirmed the morphological specificity as *C. pectinata* was a yellowish, non-oculate, trichocercous, fellodistomid cercaria whilst *C. tapidis* was an oculate cercaria with a long tail of five times as body. Both cercaria developed in sporocyst in the host gonad. Ramón et al., 1999 suggested that the sporocyst of *Bacciger bacciger* reduced clam reproduction by castration effect. The sporocyst employed storage substances (glycogen) of intertubular tissue resulting in delaying in the gametogenesis cycle. In heavily infested with *B. bacciger* of other species of bivalve, the foot and visceral mass were full of sporocysts while the first organ to be infected and destroyed was gonad. The hemocyte encapsulations sometimes happened around the parasites that seemed to be in a degenerative process. Despite the usually low prevalence infection population, the effect of this trematode on the host's reproductive capacity was serious with 50 to 75% gonad replaced by the parasite or completed castration (Shelley et al., 1988). The study of Ceuta &

Boehs, 2012, conducting on mussels *Mytella guyanensis* showed that the trematode in the sporocyst stage had a high potential for hindering defense mechanisms and devastating tissue.

5. Conclusion

The low condition index and slow reproductive development in clams were believed to be more associated with the low level of food in the water column rather than seawater temperature elevation. The dramatic increase of *Perkinsus olseni* infection intensity during winter and spring seasons could be coincided with the post-spawning condition that exhausts Manila clam's energy prompting to be more vulnerable with this protozoa. *Perkinsus olseni* prevalence and the infection score determined in this study were significantly higher than the levels measured in 2001-2002 without seasonal change. The high prevalence of Rickettsia-like-organisms and *Parvatrema duboisi* was notable. *P. duboisi* mainly infected in the summer and autumn season while RLOs tended to occur year-round. Trematode *Cercaria tapidis* and *Cercaria pectinata* displayed low prevalence and caused severe gonad castration on infected clams but no impact to the reproduction of clam populations. Thus, this study suggests that a slight increase in the mean sea surface water temperature may exerted a great influence on the parasite infection dynamics although further investigation needs to be carried out to warrant this hypothesis.

Table 3. Comparison of condition index in the present study and other locations from different countries.

CI formula	Site	Value	References
CI = Tissue wet weight / Shell dry weight	Jeju island, Korea	0.2 – 0.4	This study
	Jugyo tidal flat, Korea	0.56	Lee et al., 2020b
	Padori tidal flat, Korea	0.42	
	Seonyu-do Island , Korea	0.55 – 0.70	Nam et al., 2018
	Goheung, Korea	0.336 – 0.516	Hong et al., 2014
	Jeju island, Korea	0.297 – 0.529	Uddin et al., 2013
	Haeju, Korea	0.445 – 0.554	Yang et al., 2010
	Ariake Bay, Japan	0.404 – 0.594	Park et al., 2008
CI = (Tissue wet weight / SL × SH × SW) × 10^5	Jeju island, Korea	8.5 – 13.5	This study
	Jeju island, Korea	8.6 – 14.8	Ngo and Choi, 2004
	Hokkaido, Japan	14.5 – 19.6	Hasegawa et al., 2014
	Kaneda, Tokyo Bay, Japan	7 – 13	Hamasaki et al., 2014
	Uminokoen, Tokyo Bay, Japan	13 - 20	
	Sanbanse, Tokyo Bay, Japan	10 - 23	
	Kisarazu, Tokyo Bay, Japan	7 - 19	Toba et al., 2007
	Futsu, Tokyo Bay, Japan	7 - 25	
CI = (Tissue dry weight /Shell Dry weight) × 1000	Jeju island, Korea	38.7 – 66.7	This study
	Arcachon bay, France	38.6 – 78.6	Dang et al., 2013
	Arcachon bay, France	50 – 125	Robert et al., 1993
	Brittany, France	50 – 142	Laruelle et al., 1994
	Marmara Sea, Turkey	70-180	Colakoglu and Palaz, 2014
	Drumcliff Bay, Ireland	50-90	Drummond et al., 2006
	Dungloe Bay, Ireland	60 - 100	
	Eilat, Israel	60 - 110	Shpigel and Spencer, 1996

Table 4. The gametogenesis time (grey color) and spawning peak (dark color) of Manila clams *(R. philippinarum)* compared among different regions.

Location	Study period	Month	Temperature (°C)		References
		J F M A M J J A S O N D	Range	Spawning	
Jeju island, Korea	May 2019 – Apr 2020		13.3–24.3	24.9–31.2	This study
Jeju island , Korea	May 2001 – Apr 2002		10–27	23–28	Ngo and Choi, 2004
Incheon Bay, Korea	Apr 2003 – Jun 2004		10–25	22-25	Uddin et al., 2010
Gomso Bay, Korea	Mar 1999 – Feb 2000		7–28	24–28	Park and Choi, 2004
Matsukawa-ura, Japan	Mar 2005 – Oct 2005		8.7–21.0	19.1–21.0	Kanazawa and Sato, 2008
Jiaozhou Bay, China	May 2004 – Apr 2005		2.1–25.6		Ren et al., 2008
Drumcliff Bay, Ireland	Feb 2003 – May 2004		10–19	14–19	Drummond et al., 2006
Tagus Estuary,Portugal	Sep 2013 – Dec 2015		14–23	19–23	Moura et al., 2017
Lagoon of Venice, Italy	Jul 2000 – Jul 2001		6–22	20–22	Meneghetti et al., 2004
Cardak Lagoon, Turkey	Jan – Dec 2011		9–22	15–22	Genez et al., 2015

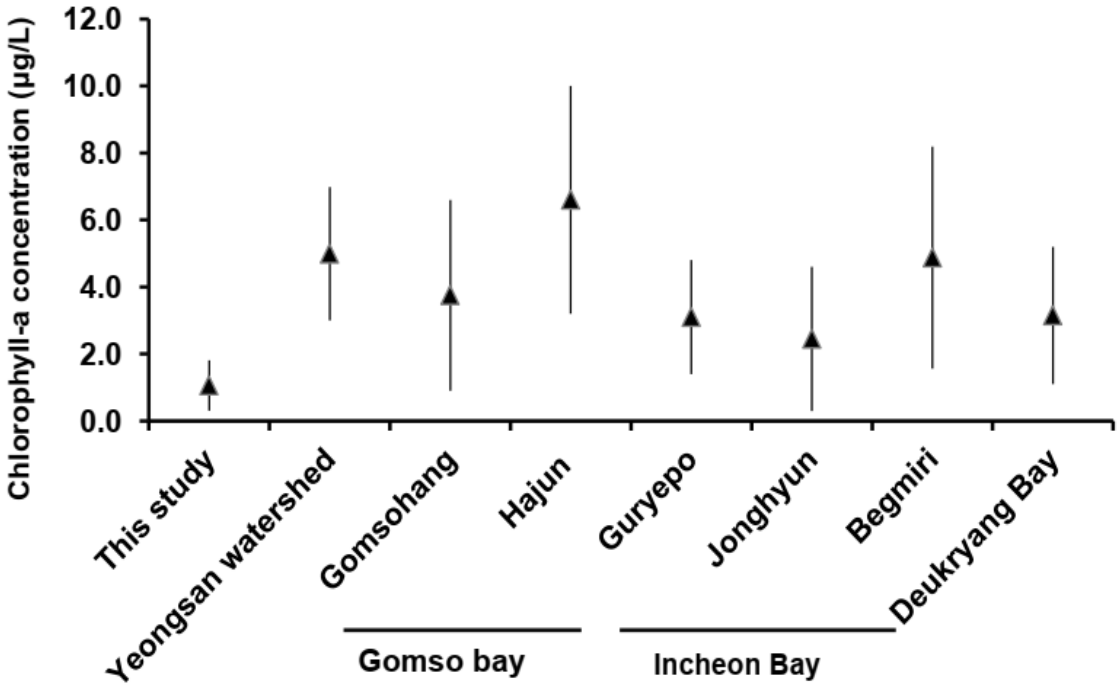

Fig. 9. Comparison of Chlorophyll-a concentration between this study, some areas in Jeju island and other sites in mainland of Korea. The references for this figure are as follow:

Jeju island: This study.

Korea mainland: Yeongsan watershed (Mamun et al., 2018), Gomso Bay: Gomsohang and Hajun (Baek et al., 2014) , Incheon Bay : Guryepo and Jonghyun (Mondol et al., 2015) , Begmiri (Uddin et al., 2012) , Deukryang Bay (Lee et al., 2015).

References

Ahn, K.J., & Kim, K.H., 2001. Effect of temperature and salinity on in vitro zoosporulation of *Perkinsus sp*. in Manila clams *Ruditapes philippinarum.* Dis. Aquat. Organ. 48, 43–46.

Aouini, F., Trombini, C., Volland, M., Elcafsi, M., Blasco, J., 2018. Assessing lead toxicity in the clam *Ruditapes philippinarum*: Bioaccumulation and biochemical responses. Ecotoxicol. Environ. Saf. 158, 193-203.

Baek, M.J., Lee, Y.J., Choi, K.S., Lee, W.C., Park, H.J., Kwak, J.H., & Kang, C.K., 2014. Physiological disturbance of the Manila clam, *Ruditapes philippinarum*, by altered environmental conditions in a tidal flat on the west coast of Korea. Mar. Pollut. Bull. 78, 137–145.

Bhaby, S., 2018. Histological analyses in seasonal variability of pathological impact on *Mytilus galloprovincialis* (Lamarck , 1819) of Moroccan North Atlantic. J. Histol. Histopathol. Res. 2 (1), 17-21.

Byers, J.E., 2020. Marine parasites and disease in the Era of global climate change. Annu. Rev. Mar. Sci. 13 (1), 397-420.

Carballal, M.J., Iglesias, D., Santamarina, J., Ferro-Soto, B., & Villalba, A., 2001. Parasites and pathologic conditions of the cockle *Cerastoderma edule* populations of the coast of Galicia (NW Spain). J. Invertebr. Pathol. 78 (2), 87–97.

Casas, S.M., Villalba, A., & Reece, K.S., 2002. Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). I. Identification of the aetiological agent and in vitro modulation of zoosporulation by temperature and salinity. Dis. Aquat. Org. 50 (1), 51–65.

Casas, S.M., & La Peyre, J.F., 2013. Identifying factors inducing trophozoite differentiation into hypnospores in *Perkinsus* species. Eur. J. Protistol. 49 (2), 201–209.

Ceuta, L.O., Boehs, G., 2012. Parasites of the mangrove mussel *Mytella guyanensis*

(Bivalvia : Mytilidae) in Camamu Bay , Bahia , Brazil. Braz. J. Biol. 72 (3), 421–427.

Ching, H.L., 1995. Evaluation of characters of the digenean family Gymnophallidae. Can. J. Fish. Aquat. Sci. 52 (S1), 78–83.

Chun, S.K., Lee, J.B., 1976. Studies on the *Cercaria pectinata* infected in the hard clam, *Meretrix Lusoria*. Bull. Korean Fish. Soc. 9 (1), 35-42. In Korean with English abstract.

Chung, O.S., Lee, H.J., Sohn, W.M., Park, Y.K., Chai, J.Y., & Seo, M., 2010. Discovery of *Parvatrema duboisi* and *Parvatrema homoeotecnum* (Digenea: Gymnophallidae) from migratory birds in Korea. Korean J. Parasitol. 48 (3), 271-274.

Colakoglu, S., Palaz, M., 2014. Some population parameters of *Ruditapes philippinarum* (Bivalvia, Veneridae) on the southern coast of the Marmara Sea, Turkey. Helgol. Mar. Res. 68, 539–548.

Cook, T., Folli, M., Klinck, J., Ford, S., & Miller, J., 1998. The relationship between increasing sea surface temperature and the Northward spread of *Perkinsus marinus* (Dermo) Disease Epizootics in oysters. Estuar. Coast. Shelf Sci. 46, 587–597..

Cordero, D., Delgado, M., Liu, B., Ruesink, J., & Saavedra, C., 2017. Population genetics of the Manila clam *(Ruditapes philippinarum*) introduced in North America and Europe. Sci. Rep. 7**,** 39745.

Cui, Y.Y., Ye, L.T., Wu, L., & Wang, J.Y., 2018. Seasonal occurrence of *Perkinsus spp.* and tissue distribution of *P. olseni* in clam (*Soletellina acuta*) from coastal waters of Wuchuan County, southern China. Aquaculture 492, 300-305.

Dang, C., de Montaudouin, X., Binias, C., Salvo, F., Caill-Milly, N., Bald, J., Soudant, P., 2013. Correlation between perkinsosis and growth in clams *Ruditapes* spp. Dis. Aquat. Org. 106, 255–265.

Delaporte, M., Soudant, P., Lambert, C., Moal, J., Pouvreau, S., & Samain, J.F., 2006. Impact

of food availability on energy storage and defense related hemocyte parameters of the Pacific oyster *Crassostrea gigas* during an experimental reproductive cycle. Aquaculture 254, 571–582.

Delgado, M., & Camacho, A.P., 2005. Histological study of the gonadal development of *Ruditapes decussatus* (L.) (Mollusca : Bivalvia) and its relationship with available food Sci. Mar. 69 (1), 87-97.

Drummond, L., Mulcahy, M., & Culloty, S., 2006. The reproductive biology of the Manila clam, *Ruditapes philippinarum*, from the North-West of Ireland. Aquaculture 254, 326–340.

FAO. 2009. *Ruditapes philippinarum*. In Cultured aquatic species fact sheets.

Flores, R.C., & Martınez, J.C., 2020. Rickettsiales-like organisms in bivalves and marine gastropods : a review. Rev. Aquac. 12 (4), 2010–2026.

Fox, M., Christley, R., Lupo, C., Moore, H., Service, M., & Campbell, K., 2020. Preventing and mitigating farmed bivalve disease: a Northern Ireland case study. Aquacult. Int. 28 (6), 2397–2417.

Genez, P., Onal, U., & Gezen, M.R., 2015. Comparison of gametogenic cycles of the endemic European carpet shell clam (*Ruditapes decussatus*) and the introduced Manila clam (*Ruditapes philippinarum*) from a temperate coastal Mediterranean lagoon in the Dardanelles, Turkey. J. Shellfish Res. 34 (2), 337–345.

Gharbi, A., Farcy, E., & Wormhoudt, A.V., Denis, F., 2016. Response of the carpet shell clam *(Ruditapes decussatus)* and the Manila clam *(Ruditapes philippinarum)* to salinity stress. Biologia. 71/5: 551—562.

Hamasaki, K., Yusaku, I., & Shuichi, K., 2014. Reproduction of an alien *Ruditapes* clam (Bivalvia: Veneridae) on recreational clam-gathering grounds in Tokyo Bay, Japan. Molluscan Res. 34(1), 54–61.

Hasegawa, N., Sawaguchi, S., Unuma, T., Onitsuka, T., & Hamaguchi, M., 2014. Variation in

Manila Clam (*Ruditapes philippinarum*) fecundity in eastern Hokkaido, Japan. J. Shellfish Res. 33 (3), 739–746.

Hégaret, H., da Silva, P.M., Wikfors, G.H., Lambert, C., De Bettignies, T., Shumway, S.E., Soudant, P., 2007. Hemocyte responses of Manila clams, *Ruditapes philippinarum* with varying parasite, *Perkinsus olseni* severity to toxic-algal exposures. Aquat. Toxicol. 84 (4), 469-479.

Hirakiuchi, H., Semura, H., & Hori, R., 2017. Reproductive cycle of the Manila Clam, *Ruditapes philippinarum*, in Lake Nakaumi, Japan. Japanese Journal of Benthology, 71, 64–69. In Japanese with English abstract.

Hong, H.K., Donaghy, L., Park, H.S., Choi, K.S., 2014. Influence of reproductive condition of the Manila clam *Ruditapes philippinarum* on hemocyte parameters during early post-spawning period. Aquaculture 434, 241-248.

Hong, H.K., Donaghy, L., Kang, C.K., Kang, H.S., Lee, H.J., Park, H.S., Choi, K.S., 2016. Substantial changes in hemocyte parameters of Manila clam *Ruditapes philippinarum* two years after the Hebei Spirit oil spill off the west coast of Korea. Mar. Pollut. Bull. 108, 171-179.

Hong, H.K., Jeung, H.D., Kang, H.S., & Choi, K.S., 2020. Seasonal variations in the hemocyte parameters, gonad development, energy storage and utilization of the giant honeycomb oyster *Hyotissa hyotis* (Linnaeus 1758) in Jeju Island off the south coast of Korea. Aquac. Rep. 17, 1002992.

Huntley, J.W., Fürsich, F.T., Alberti, M., Hethke, M., & Liu, C., 2014. A complete Holocene record of trematode-bivalve infection and implications for the response of parasitism to climate change. Proc. Natl. Acad. Sci. U.S.A. 111 (51), 18150-18155.

Ituarte, C.F., Cremonte, F., & Deferrari, G., 2001. Mantle-shell complex reactions elicited by digenean metacercariae in *Gaimardia trapesina* (Bivalvia: Gaimardiidae) from the Southwestern Atlantic Ocean and Magellan Strait. Dis. Aquat. Organ. 48 (1), 47–56.

Jung, S.G., 2008. Spatial variability in long-term changes of climate and oceanographic conditions in Korea. J. Environ. Biol. 29 (4), 519-529.

Kanazawa, T., & Sato, S., 2008. Environmental and physiological controls on shell microgrowth pattern of *Ruditapes philippinarum* (Bivalve: Veneridae) from Japan. J. Molluscan Stud. 74, 89 –95.

Korea Hydrographic and Oceanographic Administration. http://khoa.go.kr/

Korea Meteorological Administration (KMA). 2020. Korean Climage Change Assessment Report 2020. ISBN 978-89-954715-9-3. 48 pages.

Kim, S.L., Kwon, S.H., Lee, H.G., & Yu, O.H., 2017. Effects of environmental and biological conditions on the recruitment and growth of the Manila clam Ruditapes philippinarum on the west coast of Korea. Ocean Sci. J. 52 (1), 91–101.

Kim, S.J., Woo, S.H., Kim, B.K., Hur, S.D., 2011. Trends in Sea surface temperature (SST) change near the Korean peninsula for the past 130 years. Ocean Polar Res. 33 (3), 281-290.

Koo, B.J., & Seo, J., 2020. Filtration rates of the manila clam, Ruditapes philippinarum, in tidal flats with different hydrographic regimes. PLOS one 15 (2).

La Peyre, M.K., Casas, S.M., Villalba, A., & La Peyre, J.F., 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.

Laruelle, F., Guillou, J., Paulet, Y.M., 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *R. philippinarum* on intertidal flats in Brittany. J. Mar. Biolog. Assoc. U. K., 74 (2), 351–366.

Lee, H.M., Kim, H.J., Park, K.I., & Choi, K.S., 2020a. 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.

Lee, H.M., Cho, Y.G., Jeung, H.D., Jang, M.S., Hwang, J.Y., Choi, K.S., 2020b. Are juvenile Manila clam *Ruditapes philippinarum* free from *Perkinsus olseni* infection in Korean waters? Ocean Sci. J. 55 (4), 573-579.

Lee, M.K., Cho, B.Y., Lee, S.J. Kang, J.Y., Jeong, H.D., Huh, S.H., & Huh, M.D., 2001. Histopathological lesions of Manila clam, *Tapes philippinarum*, from Hadong and Namhae coastal areas of Korea. Aquaculture 201, 199–209.

Lee, Y. J., Choi, K.S., Lee, D.S., Lee, W.C., Park, H.J., Choy, E.J., Kim, H.C., & Kang, C.K., 2015. The role of the adductor muscle as an energy storage organ in the pen shell *Atrina japonica* (Reeve, 1858). J. Molluscan Stud. 81 (4), 502–511.

Lim, N.L., Lee, H.M., Jeung, H.D., Noseworthy, R.G., Jung, S., & Choi, K.S., 2019. Early larval development and annual gametogenesis of the brooding oyster *Ostrea circumpicta* (Pilsbry, 1904) in the shallow subtidal benthic ecosystem in Jeju island, off the south coast of Korea. Zool. Stud. 58:e29.

Mamun, M., Lee, S.J., & An, K.G., 2018. Temporal and spatial variation of nutrients, suspended solids, and chlorophyll in Yeongsan watershed. J. Asia Pac. Biodivers. 11 (2), 206-216.

Matozzo, V., & Marin, M.G., 2011. Bivalve immune responses and climate changes: is there a relationship? Invertebr. Surviv. J. 8, 70–77.

Meneghetti, F., Moschino, V., & Da Ros, L., 2004. Gametogenic cycle and variations in oocyte size of *Tapes philippinarum* from the Lagoon of Venice. Aquaculture 240, 473–488.

Mondol, M.R., Keshavmurthy, S., Lee, H.J., Hong, H.K., Park, H.S., Park, S.R., Kang, C.K., & Choi, K.S., 2015. Recovery of wild Pacific oyster, *Crassostrea gigas* in terms of reproduction and gametogenesis two-years after the Hebei Spirit Oil Spill Accident off the West Coast of Korea. Cont. Shelf Res. 111, 333–341.

Montenegro, D., Romero, M.S., & Gonzalez, M.T., 2021. Morphological and molecular

characterization of larval digenean trematodes (*Parvatrema*: Gymnophallidae) and their pathological effects on the clam *Leukoma thaca* (= *Protothaca thaca*) (Bivalvia: Veneridae) (Molina, 1782) from northern Chile. Parasitol. Int. 80, 102238.

Morley, N.J., & Lewis, J.W., 2015. Thermodynamics of trematode infectivity. Parasitology, 142 (4), 585–597

Moura, P., Vasconcelos, P., Pereira, F.., Chainho, P., Costa, J.L., & Gaspar, M.B., 2017. Reproductive cycle of the Manila clam (*Ruditapes philippinarum*): an intensively harvested invasive species in the Tagus Estuary (Portugal). J. Mar. Biolog. Assoc. U.K., 1-13. .

Mun, S., Kim, Y.J., Markkandan, K., Shin, W., Oh, S., Woo, J., Yoo, J., An, H., & Han, K. (2017). The whole-genome and transcriptome of the Manila clam (*Ruditapes philippinarum*). Genome Biol. Evol. 9 (6), 1487-1498.

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. Parasites Vectors 11 (1) **:** 42.

Neuman, M., Tissot, B., & Vanblaricom, G., 2010. Overall status and threats assessment of black abalone (*Haliotis cracherodii* Leach, 1814) populations in California. J. Shellfish. Res. 29 (3), 577−586.

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.

Nie, H., Chen, P., Huo, Z., Chen, Y., Hou, X., Yang, F., Yan, X., 2017. Effects of temperature and salinity on oxygen consumption and ammonia excretion in different colour strains of the Manila clam, *Ruditapes philippinarum*. Aquac. Res., 48, 2778–2786.

Norton, J.H., Shepherd, M.A., Abdon-Naguit, M.R., & Lindsay, S., 1993. Mortalities in the Giant clam *Hippopus hippopus* associated with Rickettsiales-like organisms. J. Invertebr. Pathol. 62 (2), 207-209.

Özer, A., & Güneydaı, S., 2015. Seasonality and host-parasite interrelationship of *Mytilus galloprovincialis* parasites in Turkish Black Sea coasts. J. Mar. Biolog. Assoc. U.K. 95 (8), 1591–1599.

Paillard, C., Allam, B., & Oubella, R., 2004. Effect of temperature on defense parameters in Manila clam *Ruditapes philippinarum* challenged with *Vibrio tapetis.* Dis. Aquat. Org. 59, 249–262.

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, & Choi, K.S., 2004. Application of enzyme-linked immunosorbent assay for studying of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca: Bivalvia): I. Quantifying eggs. Aquaculture 241, 667–687.

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.I., Tsutsumi, H., Hong, J.S., Choi, K.S., 2008. Pathology survey of the short-neck clam R*uditapes philippinarum* occurring on sandy tidal flats along the coast of Ariake Bay, Kyushu, Japan. J. Invert. Pathol. 99, 212 – 219.

Park, K.I., Yang, H.S., Kang, D.H., & Choi, K.S., 2010. Density dependent growth and mortality of Manila clam *Ruditapes philippinarum* reared in cages in Gomso-Bay, Korea. Korean J. Malacol. 26 (1), 91-95.

Paull, S.H., Raffel, T.R., Lafonte, B.E., & Johnson, P.T.J., 2015. How temperature shifts

affect parasite production: Testing the roles of thermal stress and acclimation. Funct. Ecol. 29 (7), 941–950.

Poulin, R., 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132 (1), 143–151.

Powell, E.N., Gauthier, J.D., Wilson, E.A., Nelson, A., Fay, R. R., & Brooks, J.M., 1992. Oyster disease and climate change. Are yearly changes in *Perkinsus marinus* parasitism in oysters (*Crassostrea virginica*) controlled by climatic cycles in the Gulf of Mexico? Mar. Ecol. 13 (3), 243–270.

Ramón, M., Gracenea, M., & González-Moreno, O., 1999. *Bacciger bacciger* (Trematoda, Fellodistomidae) infection in commercial clams *Donax trunculus* (Bivalvia, Donacidae) from the sandy beaches of the western Mediterranean. Dis. Aquat. Organ. 35 (1), 37- 46.

Ren, Y., Xu, B., Guo, Y., Yang, M., & Yang, J., 2008. Growth, mortality and reproduction of the transplanted Manila clam *(Ruditapes philippinarum* Adams & Reeve 1850) in Jiaozhou Bay. Aquac. Res. 39, 1759-1768.

Rowley, A.F., Cross, M.E., Culloty, S.C., Lynch, S.A., Mackenzie, C.L., Morgan, E., Riordan, R.M.O., Robins, P.E., Smith, A.L., Thrupp, T.J., Vogan, C.L., Wootton, E.C., & Malham, S.K., 2014. The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea—a review. ICES J . Mar. Sci. 71 (4), 741–759.

Robert, R., Trut, G., Borden, J.L., 1993. Growth, reproduction and biochemical composition of the Manila clam *Ruditapes philippinarum* in the Bay of Arcachon, France. Mar. Biol. 116, 291-299.

Selbach, C., & Poulin, R., 2020. Some like it hotter: trematode transmission under changing temperature conditions. Oecologia 194 (4), 745–755.

Shelley, C.C., Glazebrook, J.S., Turak, E., Winsor, L., & Denton, G.R.W., 1988. Trematode

(Digenea: Bucephalidae) infection in the burrowing clam *Tridacna crocea* from the Great Barrier Reef. Dis. Aquat. Organ. 4, 143-147.

Shimura, S., Yoshinaga, T., & Wakabayashi, H., 1982. Three marine cercariae in the clam *Tapes philippinarum* from Lake Hamana, Japan: Morphology and level of infection. Fish Pathol. 17 (2), 129-137. In Japanese with English abstract.

Shpigel, M., Spencer, B., 1996. Performance of diploid and triploid Manila clams (*Tapes philippinarum*, Adams and Reeve) at various levels of tidal exposure in the UK and in water from fish ponds at Eilat, Israel. Aquaculture 141, 159-171.

Sohn, W.M., Na, B.K., Cho, S.H., & Lee, W.J., 2017. Prevalence and density of digenetic trematode metacercariae in clams and oysters from western coastal regions of the Republic of Korea. Korean J. Parasitol. 55 (4), 399-408.

Soudant, P., Paillard, C., Choquet, G., Lambert, C., Reid, H.I., Marhic, A., Donaghy, L., & Birkbeck, T.H., 2004. Impact of season and rearing site on the physiological and immunological parameters of the Manila clam *Venerupis (=Tapes,=Ruditapes) philippinarum.* Aquaculture 229, 401–418.

Soudant, P., Chu, F.E., & Volety, A., 2013. Host-parasite interactions: Marine bivalve molluscs and protozoan parasites, *Perkinsus* species. J. Invertebr. Pathol. 114 (2), 196–216.

Toba, M., Yamakawa, H., Kobayashi, Y., Sugiura, Y., Honma, K., & Yamada, H., 2007. Observation on the maintenance mechanisms of metapopulations, with special reference to the early reproductive process of the Manila clam *Ruditapes philippinarum* (Adam&Reeve) in Tokyo bay. J. Shellfish Res. 26 (1), 121–130.

Travers, M.A., Miller, K.B, Roque, A., & Friedman, C.S., 2015. Bacterial diseases in marine bivalves. J. Invertebr. Pathol. 131, 11-31.

Uddin, M.J., Jeung, H.D, Yang, H.S., Kim, B.K., Ju, S.J., & Choi, K. S., 2013. Quantitative assessment of reproductive effort of the Manila clam *Ruditapes philippinarum* in a

lagoon on Jeju Island (Korea) using enzyme-linked immunosorbent assay. Invertebr. Reprod. Dev. 57 (4), 316–324.

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 Seonjaedo Island in Incheon, off the West Coast of Korea. J. World Aquac. Soc. 41 (S1).

Uddin, M.J., Yang, H.S., Park, K.J., Kang, C.K., Kang, H.S., & Choi, K.S., 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, 25–32.

Umeda, K., Yang, X., Waki, T., Yoshinaga, T., & Itoh, N., 2020. The effects of environmental and nutritional conditions on the development of *Perkinsus olseni* prezoosporangia. Exp. Parasitol. 209, 107827.

Velez, C., Leandro, S., Figueira, E., Soares, A.M., Freitas, R., 2015a. Biochemical performance of native and introduced clam species living in sympatry: The role of elements accumulation and partitioning. Mar. Environ. Res. 109, 81-94.

Velez, C., Figueira, E., Soares, A., Freitas, R., 2015b. Spatial distribution and bioaccumulation patterns in three clam populations from a low contaminated ecosystem. Estuar. Coast. Shelf Sci. 155, 114 – 125.

Villalba, A., Reece, K.S., Ordás, M.C., Casas, S.M., & Figueras, A., 2004. Perkinsosis in molluscs: A review. Aquat. Living Resour. 17 (4), 411–432.

Waki, T., & Yoshinaga, T., 2015. Suppressive effects of low salinity and low temperature on *in vivo* propagation of the protozoa *Perkinsus olseni* in Manila clam. Fish Pathol. 50 (1), 16-22.

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.

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 (1), 29-39.

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.

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.

Yang, H.S., Park, K.J., Choi, K.S., 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 (2), 93-100.

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. J. Shellfish Res. 31 (3), 685–690.

Yoon, S., Abe, H., & Kishi, M.J., 2013. Responses of Manila clam growth and its food sources to global warming in a subarctic lagoon in Japan. Prog. Oceanorg. 119, 48-58.

Yu, J.R., Chai, J.Y., & Lee, S.H., 1993. *Parvatrema timondavidi* (Digenea; Gymnophallidae) transmitted by a clam, *Tapes philippinarum*, in Korea. Korean J. Parasitol. 31 (1), 7-12.

Zan, X., Xu, B., Zhang, Ch., Ren, Y., 2014. Annual variations of biogenic element contents of Manila clam (*Ruditapes philippinarum*) bottom-cultivated in Jiaozhou Bay, China . J. Ocean Univ. China (Oceanic and Coastal Sea Research) 13 (4), 637-646 .

Zhu, Z., Xu, T., He, Z., Wu, X., Wu, L., Meng, Q., & Huang, J., 2012. Rickettsia-like organism infection associated with mass mortalities of blood clam, *Tegillarca granosa*, in the Yueqing Bay in China. Acta. Oceanol. Sin. 31 (1), 106-115.

ACKNOWLEDGEMENT

Two years run over, I would like to express my sincere thank you to my supervisor, Professor Choi Kwang-Sik, who gives me the chance to study in Korea, gives me advice during my works, and inspires the scientific passion to me. The warm thank you is sent to Professor Jung Suk-Geun and Doctor Kang Do-Hyung, who spent time to revise and gave recommendation for my thesis. The other sincere thank you is for Assoc. Professor Ngo Thi Thu Thao, who encourages and advises me when I was an undergraduate student until I graduated.

Finishing the master course, I also would like to send my warmest thanks to the staff in Shellfish research and Aquaculture laboratory, without their guidance and support, I could not complete my course. Thanks to Dr. Hong Hyun-Ki, who is as the great brother with all students in the lab, to Dr. Kim Hyun-Joong, who often talks to me about scientific works and Vietnamese culture. The other thanks go to Dr. Lee Hye-Mi and Cho Young-Ghan oppa, who support the procedure for me in order to come to Korea and take care during my thesis. I really thank you Shin Jong-Seop oppa and Kim Jong-Hwa onni for guiding me in the technical works from the first day and treat me like a younger sister. I also say thank you to Kajino Nobuhisa (Jin-Su) oppa, who always pleased to help during my experiment, and Lee Kyeong-Tee oppa, who experiences the same period in the master course, shares, and sympathizes mutually. I will spend thanks to Cho Sang-Hyun oppa and Kim Yun-Hwa onni, who helps me during my works.

Last but not least, I greatly thank you to my family- my dear mother and father, my younger brother, who is the motivation for me to overcome difficulties and always support and encourage me in my life, especially my mother, who always love and make me become stronger. I also thanks to Vietnamese friends who share everything during my course and sympathy and help each other whether we are happy or sad.