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Spatio-Temporal Variation in the Reproduction, Condition Index, and Parasite Load of the Manila Clams *Ruditapes philippinarum* from Two Tidal Flats in Yeongheungdo Island on the West Coast of Korea

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ABSTRACT

The Manila clam *Ruditapes philippinarum* is an ecologically and economically important bivalve in Korean tidal flats. To examine seasonal patterns in reproduction and health, we monitored monthly changes in gonadal development, condition index (CI), trematode infection, and *Perkinsus olseni* infection intensity at two tidal flats on Yeongheungdo Island in the west. Histology indicated that gametogenesis initiated in early spring, peaked in late spring to early summer, and was followed by spawning during summer. The CI increased prior to spawning and declined during autumn and winter, reflecting reproductive effort and food availability. Histology also revealed that heavy infection by the flatworm *Cercaria tapidis* leads to gonad castration during the spawning period. Trematode infection exhibited moderate seasonal variation but did not mask reproductive patterns. *P. olseni* infection intensity, measured by gill assays, displayed a significant spatial variation, as the intensity was significantly higher at Naeri tidal flat ($p < 0.05$), although *P. olseni* infection showed no significant correlation with CI or gonad maturation at either site. These findings suggest that, under the natural environmental conditions observed, *P. olseni* infection did not exert measurable adverse effects on the condition or reproductive development of the clams. This study emphasizes the substantial impact of seasonal environmental factors on the reproductive cycles of *R. philippinarum*, while suggesting that *P. olseni* infections may not directly affect reproductive performance at the observed infection levels.

Keywords: *Ruditapes philippinarum*, gonad maturation, condition index, *Perkinsus olseni*, trematode prevalence, west coast of Korea

INTRODUCTION

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Widely distributed along the intertidal and shallow subtidal zones of the coastal Yellow Sea, the Manila clam (*Ruditapes philippinarum*) is one of the most commercially and ecologically valuable components of intertidal and shallow subtidal ecosystems (Humphreys *et al.*, 2025). Manila clams occur at high densities on sandy-mud tidal flats on the West Coast of Korea, supporting the local fisheries and aquaculture (Uddin *et al.*, 2010, 2012; Kim *et al.*, 2017; Lee *et al.*, 2020). As a suspension feeder, Manila clam also plays a key role in nutrient cycling, benthic-pelagic coupling, and the maintenance of coastal biodiversity (Suh and Shin 2013; Kang *et al.* 2016; Houki *et al.*, 2025a, b). Sustainable management of natural clam populations requires a clear understanding of their reproductive biology, as spawning periods, gonad development, and recruitment success directly influence sustainable clam production (Park *et al.*, 2006; Uddin *et al.*, 2012; Subramaniam *et al.*, 2025a).

Seasonal variation in *R. philippinarum* reproduction is closely linked to environmental factors, including seawater temperature, food availability, and salinity, which regulate gonad maturation and spawning intensity (Park *et al.*, 2004; Uddin *et al.*, 2012; Subramaniam *et al.*, 2025b; Liu *et al.*, 2025). Changes in gonad development across seasons are monitored using histology, as this technique offers detailed microscopic images of the gonad at both cellular and tissue levels (Uddin *et al.*, 2012; Subramaniam *et al.*, 2025a, b). In addition to histology, the condition index (CI), a ratio of clam tissue weight to shell weight, is widely used as a physiological indicator of reproductive status, providing a quantitative measure that closely aligns with histological observations of gonad development (Uddin *et al.*, 2010, 2012; Lee *et al.*, 2012; Subramaniam *et al.*, 2025b). Long-term and site-specific monitoring of CI and gonadal maturation can identify subtle differences in reproductive timing and intensity among populations, which may be related to microhabitat conditions (Subramaniam *et al.*, 2025b).

In addition to environmental factors, parasitism can significantly affect clam reproduction and health. The trematode *Cercaria tapidis* infects the gonads of *R. philippinarum*, causing parasitic castration and a decline in physiological condition (Ngo *et al.*, 2004; Cho *et al.*, 2022; Le *et al.*, 2024). Although the impact of trematodes on bivalve hosts is well known, few studies have explored their seasonal prevalence in relation to environmental conditions and reproductive cycles in Korean waters (Ngo *et al.*, 2004). The protozoan parasite *Perkinsus olseni* also disrupts the growth and reproduction of Manila clams, with severe infections leading to host mortality (Park *et al.*, 2006; Yang *et al.*, 2012, 2021; Lee *et al.*, 2021). According to Subramaniam *et al.* (2025a), female clams heavily infected with *P. olseni* also exhibited hemocyte infiltration, necrosis, and granulacytoma in the gonad, which led to decreased reproductive effort.

Manila clams occur in high densities on sandy-mud tidal flats in Gyeonggi Bay (= Incheon Bay), where they are farmed or harvested commercially (Park *et al.*, 2010; Uddin *et al.*, 2010, 2012). Gyeonggi Bay, located on the mid-western coast of Korea, is one of the most hydrographically active estuarine systems in the Yellow Sea (Lee *et al.*, 2023). This bay is a hypertidal estuary with tidal amplitudes reaching up to 8 meters, the largest along the Korean coast. Such significant tidal ranges generate strong semidiurnal currents and asymmetric flood-ebb flows, which influence the exchange of material between tidal flats and coastal waters (Kim *et al.*, 2018, 2023). The combination of strong tidal currents and abundant nutrients results in high primary productivity, providing a consistent food supply for Manila clams on tidal flats in Gyeonggi Bay (Lim *et al.* 2007; Kwon *et al.* 2014; Park *et al.* 2010; Koh and Khim 2014).

Located in the middle of Gyeonggi Bay, Yeongheungdo Island features well-developed tidal flats where Manila clams are harvested commercially. This study examines the monthly changes in gonad maturation, CI, and parasite loads in *R. philippinarum* populations from two tidal flats on Yeongheungdo Island in Gyeonggi Bay. By combining histological, physiological, and environmental data, we aim to understand the seasonal reproductive patterns of Manila clams and how trematode and *P. olseni* infections affect CI and reproductive development. The results provide essential information to support effective management and conservation of Manila clams in Gyeonggi Bay.

MATERIALS AND METHODS

1. Study sites and sampling

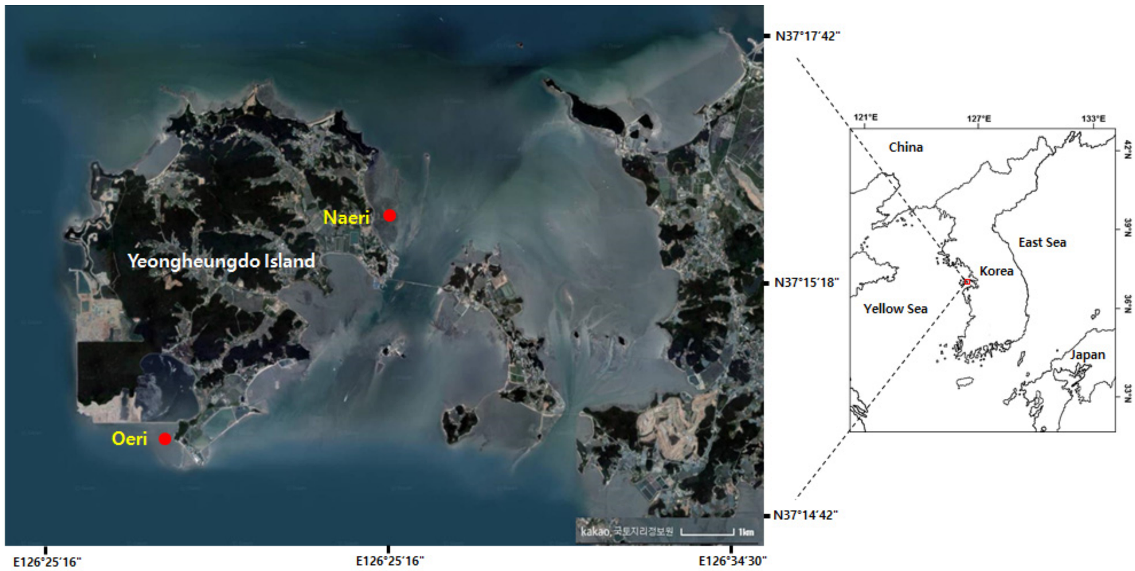

Fig. 1. The study site, the Naeri and Oeri tidal flats in Yeongheungdo Island on the West Coast of Korea.

Monthly sampling of *R. philippinarum* was conducted over 13 months from January 2007 to January 2008 at Naeri and Oeri intertidal flats in Yeongheungdo on the West Coast of Korea (Fig. 1). The Naeri and Oeri tidal flats are approximately 4 km apart. Forty adult clams with shell length (SL) over 30 mm were collected at each site during low tide. The monthly average seawater temperature at the tidal flats was obtained from the Giovanni online data system, available through NASA GES DISC (https://giovanni.gsfc.nass.gov/). Chlorophyll a levels as an indicator of the food availability for clams at Naeri and Oeri tidal flats were also referenced from Park *et al.* (2010), as they reported monthly changes in the environmental factors, including salinity, dissolved oxygen, and chlorophyll a levels recorded from Naeri and Oeri tidal flats from January to December 2007.

2. Histology and CI

After recording the SL, the longest axis of the shell was measured. The shell was then opened, and the body was removed and weighed to the nearest milligram. For histology, a transverse section taken from the mid-body was fixed in Davidson's solution for 24 h, dehydrated, embedded in paraffin, sectioned at 5 μm, and stained with Harris' hematoxylin and counterstained with eosin Y. Using a light microscope, the reproductive stage of each clam was categorized as indifferent (resting), early developing, late developing, ripe, partially spawning, or spent (Uddin *et al.*, 2010, 2012). Trematode infection in an individual clam was also identified through histology, and the prevalence of trematode infection was estimated as the percentage of infected clams among the total clams examined. The remaining tissue was weighed in mg, frozen at –60°C, and lyophilized to determine the dry weight. Consequently, the dry tissue weight of each clam used in the histology was estimated by multiplying the water percentage of the remaining tissue by its dry weight. CI of each clam was then calculated as CI = ((dry tissue weight in mg/dry shell weight) x 1,000) (Lee *et al.* 2020; Subramaniam *et al.*, 2024, 2025a, c).

3. *P. olseni* infection

One part of the gill tissue was excised from the clams used in the histology analysis to measure *P. olseni* infection. The gill tissue was weighed in mg and placed in a 10 ml conical tube containing fluid thioglycollate medium (FTM) fortified with antibiotics (Park *et al.*, 2006; Subramaniam and Choi, 2023; Subramaniam *et al.*, 2024). After 7 days of incubation, the tissues were dissolved in 2M NaOH, washed multiple times in phosphate-buffered saline (PBS, pH 7.6), and the number of *P. olseni* cells in the gill tissue was estimated by counting the cells with a blood cell counter (Ray, 1966; Choi *et al.*, 1989; Park and Choi, 2001). *P. olseni* infection was then expressed as the number of cells per gram of gill tissue. The percentage of clams infected with *P. olseni* among the total clams examined each month was reported as *P. olseni* infection prevalence.

4. Statistical analyses

The difference in the monthly mean of *P. olseni* infection intensity and confidence intervals (CI) of clams on the Naeri and Oeri tidal flats was analyzed using a paired t-test. Seasonal variations in *P. olseni* and trematode infections, as well as clam CI and reproduction, were examined employing Pearson's correlation coefficient analysis. For this analysis, the reproductive stage of each clam was classified into one of the following numerical scores: resting (i.e., indifferent) = 0, early developing = 1, late developing = 2, ripe = 3, partially spawning = 4, and spent = 5. The monthly gonad maturation index (GMI) was subsequently calculated based on the reproductive score, using the formula: GMI = ∑(reproductive stage score × percentage in stage) / 100.

RESULTS

1. Seasonal changes in reproduction and CI

The seawater temperature at the Naeri and Oeri tidal flats exhibited a distinct seasonal cycle, ranging from winter lows of approximately 2 to 3 °C in January to summer maxima of 24 to 26 °C in August (Fig. 2). This seasonal temperature variation significantly influenced the reproductive development of *R. philippinarum*. In the Naeri tidal flat, most clams remained in indifferent or early gametogenic stages during the coldest months (January to March), with 82 to 97% in the early or resting stages. As the temperature increased to 10 to 15 °C in April and May, gonads progressed into late and ripe stages, with 67% of clams in the late stage in April and 41%

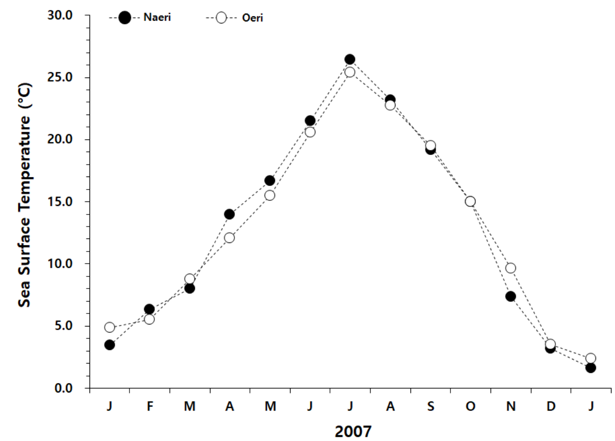

Fig. 2. Seasonal changes in the Surface Seawater Temperature (SST) at Naeri and Oeri tidal flats, obtained from NASA GES DISCND (https://giovanni.gsfc.nasa.gov/).

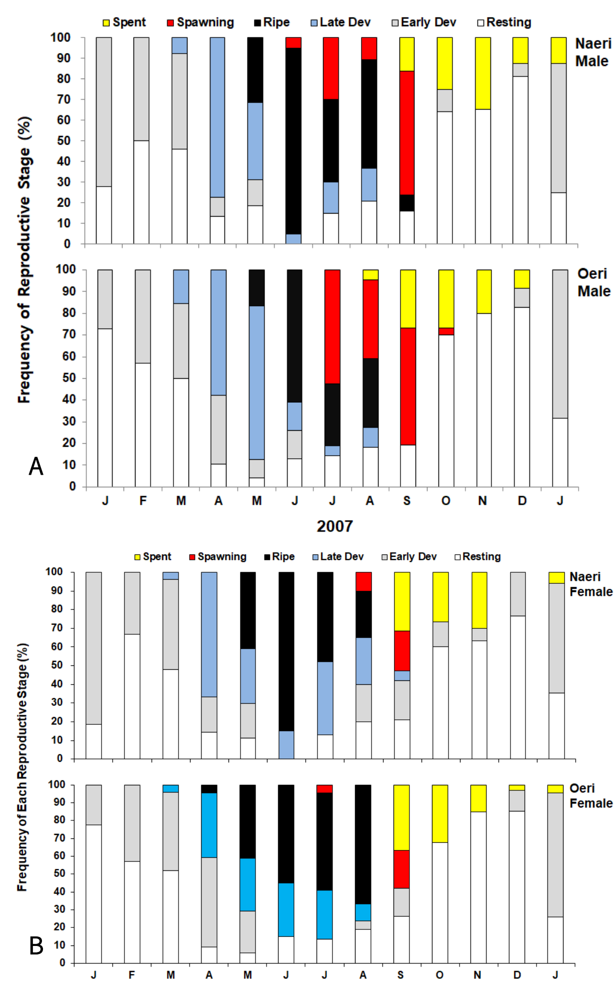

Fig. 3. The annual reproductive cycle of Manila clams on the Naeri and Oeri tidal flats measured by histology. **A:** Naeri tidal flat, **B:** Oeri tidal flat.

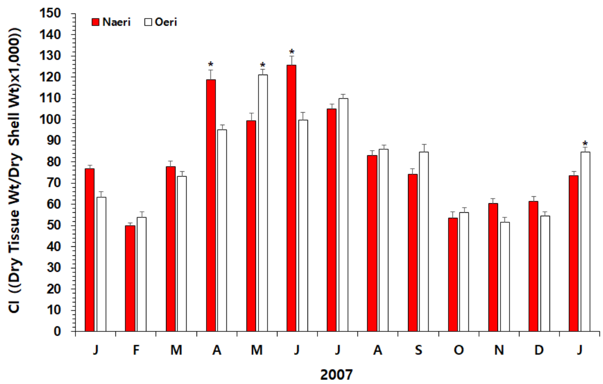

Fig. 4. Spatio-temporal variation in the condition index (CI) of *R. philippinarum* in Naeri and Oeri tidal flats during the course of study.

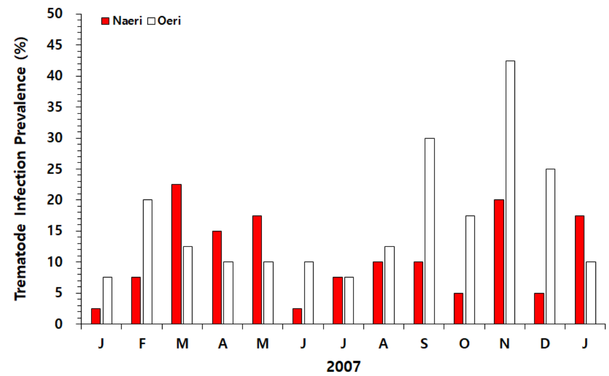

Fig. 5. The trematode infection prevalence monitored monthly from Naeri and Oeri tidal flats throughout the course of the study.

being already ripe by May. In June, 85% of the clams reached the ripe stage. Spawning occurred in August, when 10% of the clams were actively spawning, and by September, more than half of the population (53%) had entered spawning or spent stages (Fig. 3A and 3B).

In the Oeri tidal flat, a similar annual reproductive pattern was observed, although ripening occurred slightly earlier and with greater intensity. Spawning also took place in August and September, with 21% of females spawning in August and 37% having already spawned. As temperatures notably decreased in autumn (10 to 12 °C in October and December), clams on the Naeri and Oeri tidal flats exhibited regression to spent or resting stages, with more than 60 to 75% of clams reverting to early or indifferent gonads (Fig. 3A and 3B, lower graphs).

Seasonal variations in the dry-weight-based Condition Index (CI) closely correlated with reproductive cycles and temperature fluctuations (Fig. 4). At Naeri tidal flat, the CI recorded its minimum values in winter (49.8 to 77.8 during January and March), exhibited a rapid increase during gonad maturation, and attained its peak annual value (125.6) in June, coinciding with 85% of gonads being ripe. Subsequently, the CI decreased markedly following spawning (82.9 in August, when 10% of the population was spawning and 32% was spent) and continued to decline through autumn, reaching its lowest values post-gonad regression (53.6 in October).

In the Oeri tidal flat, winter CI values were similarly low (51.4 to 73.3), increased to 120.9 in May during peak reproductive readiness, and declined following spawning to 85.9 in September. By October, the CI had decreased to 56.1, aligning with 86% of the clams being in a spent state.

The t-test indicated that the annual mean CI of clams in Naeri (81.4) and Oeri (79.5) are not significantly different. However, the CIs recorded in the Naeri tidal flat in April and June are significantly higher than those in Oeri ($p < 0.05$). Additionally, the mean CIs of clams collected from the Oeri tidal flat in May and January 2008 were significantly higher ($p < 0.05$) than those in Naeri (see Fig. 5).

2. Parasite load

Fig. 5 displays the monthly prevalence of trematode infection in Manila clams. In the Naeri tidal flat, the prevalence ranged from 3% (January and June) to 23% (March). The prevalence of trematode infection in Manila clams in the Oeri tidal flat was somewhat higher than in the Naeri tidal flat, ranging from 8 to 43% (November). The prevalence of trematode infections exhibited distinct seasonality, with a high level during the post-spawning period in autumn.

The infection intensity of the protozoan parasite *P. olseni*, determined by the Ray's FTM (RFTM), showed an apparent seasonality in both tidal flats, with a different intensity (Fig. 6). In the Naeri tidal flat, the intensity ranged from 0.60 x 10^6 (September) to 2.51 x

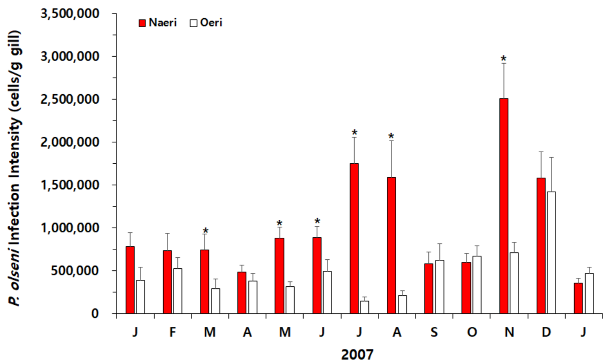

Fig. 6. Spatio-temporal variation in *P. olseni* infection intensity recorded monthly from Naeri and Oeri tidal flats on Yeongheungdo Island in Gyeonggi Bay.

10^6 cells/g gill (November). *P. olseni* infection intensities were consistently high, with maxima in July (1.75×10^6 cells/g gill) and August (1.59×10^6 cells/g gill). The infection intensity remained high in December (1.90×10^6 cells/g gill), despite seawater temperatures dropping to 3.2 °C. In the Oeri tidal flat, the *P. olseni* infection level varied monthly from 0.15×10^6 (July) to 1.42×10^6 cells/g gill (December). The infection intensities were notably higher in autumn, a period when most clams had either exhausted their reproductive resources or were in the resting stage following spawning. The paired t-test revealed that the average infection intensities recorded in March, May, June, July, August, and November from the Naeri tidal flat were significantly higher than those from the Oeri tidal flat ($p < 0.05$).

Table 1. The sampling efforts. N, number of clams used in the analysis, SL, shell length in mm, SD, standard deviation

Naeri		Oeri	
N	SL ± SD	N	SL ± SD
40	38.0 ± 3.4	40	35.6 ± 4.4
40	35.5 ± 2.2	40	39.9 ± 4.5
40	36.9 ± 3.8	40	36.7 ± 3.9
40	40.3 ± 3.7	40	39.0 ± 2.4
40	44.2 ± 2.9	40	35.5 ± 2.7
40	41.7 ± 3.6	40	36.3 ± 3.3
40	36.5 ± 1.8	40	38.5 ± 1.8
40	39.7 ± 2.4	40	35.9 ± 1.4
40	39.9 ± 2.4	40	38.1 ± 1.9
40	36.7 ± 3.4	40	37.9 ± 1.6
40	40.2 ± 2.0	40	38.2 ± 1.9
40	36.8 ± 1.9	40	38.2 ± 1.4
40	38.8 ± 1.9	40	38.5 ± 1.3

3. Effects of parasitic load on the clam reproduction and health

Table 2 shows the results of Pearson's correlation analysis. In the Naeri tidal flat, CI showed a positive correlation with temperature (r = 0.58) and a moderate correlation with GMI (r = 0.38), supporting the idea that seasonal warming promotes gonad maturation and condition. Parasite load (i.e., *P. olseni* and trematodes) exhibited a weak or no correlation with CI or GMI, indicating limited detectable effects. In the Oeri tidal flat, CI fluctuated seasonally in response to seawater temperature, exhibiting a strong positive correlation (r = 0.61). Unlike in the Naeri tidal flat, CI was negatively linked to both trematodes (r = –0.58) and *P. olseni* (r = –0.59), indicating significant adverse effects from the parasites. It was also observed that trematode infection and *P. olseni* infection had a moderate positive correlation (r = 0.58), suggesting a co-occurrence or common environmental factor.

Table 2. Pearson's correlation coefficients for the variables used in the analysis.

	Naeri tidal flat					Oeri tidal flat				
	SST	CI	Trematode nfection	*P. olseni* Infection	GMI	SST	CI	Trematode nfection	*P. olseni* Infection	GMI
SST	1.00	0.58	–0.20	0.15	0.02	1.00	0.58	–0.20	0.15	0.02
CI	0.58	1.00	–0.02	–0.12	0.38	0.58	1.00	–0.02	–0.12	0.38
Trematod e nfection	–0.20	–0.02	1.00	0.07	–0.20	–0.20	–0.02	1.00	0.07	–0.20
P. olseni Infection	0.15	–0.12	0.07	1.00	–0.07	0.15	–0.12	0.07	1.00	–0.07
GMI	0.02	0.38	-0.20	–0.07	1.00	0.02	0.38	–0.20	–0.07	1.00

Discussion

1. Annual reproduction and condition index

As previous studies have indicated, seasonal warming was the main factor affecting reproductive timing and condition in *R. philippinarum* on tidal flats along the West Coast of Korea (Park *et al.*, 2006; Uddin *et al.*, 2010, 2012). Female Manila clams in the Naeri and Oeri tidal flats progressed from indifferent/early stages to late and ripe conditions, and the CI increased in parallel, peaking immediately before spawning, as seawater temperature rose from winter minima (-2-3 °C) to summer maxima (-24-26 °C). The post-spawning decline in CI during late summer–autumn reflects energy expenditure on gamete release and subsequent catabolism, consistent with the reproductive cycles of temperate bivalves reported for Manila clams and related species (Uddin *et al.*, 2012; Ngo *et al.*, 2018; Park *et al.*, 2020; Subramaniam *et al.*, 2025b). In this study, the spring and early-summer increases in CI and ripeness coincided with elevated chlorophyll a levels, supporting the idea that an improved food supply promotes gametogenesis and somatic condition. Conversely, the autumn trough in CI aligns with reduced food availability and the spent/regression phase. According to Park *et al.* (2010), the chlorophyll a levels increased from 5.18 (March) to 13.17 (April) in the Oeri tidal flat and from 1.74 (March) to 7.83 (April) in the Naeri tidal flat during the time course of this study. In the Naeri tidal flat, low levels of chlorophyll a, a proxy for food availability for filter-feeding animals, persisted during late autumn and winter (October to February), ranging from 1.92 to 0.86, which coincided with the low CI levels.

Although chlorophyll a concentrations were consistently higher at the Oeri tidal flat than at the Naeri tidal flat throughout most of the year, the CIs of clams from the two tidal flats were not significantly different. This suggests that bulk phytoplankton biomass, as estimated by chlorophyll a, may not fully explain the quality or availability of food sustaining clam condition. Like other filter-feeding bivalves, Manila clams are known to utilize a wide range of suspended particles, including benthic microalgae, resuspended organic detritus, and microbial aggregates, in addition to phytoplankton (Watanabe *et al.*, 2009; Komorita *et al.*, 2014; Dias *et al.*, 2019; Houki *et al.*, 2025b). Therefore, it is believed that clams at the Naeri were supported by additional food sources, such as resuspended detrital organic matter from tidal sediments, which compensated for the lower chlorophyll a (Dang *et al.*, 2009; Zhao *et al.*, 2013; Both *et al.*, 2020)

2. Parasitic load

Bathige *et al.* (2025) reviewed parasites, both single-celled and multicellular, found in Manila clams, highlighting the adverse effects of *P. olseni* infection on the host. Several studies show that most Manila clams on the tidal flats and subtidal soft bottoms along the west and south coasts are infected with *P. olseni*, although infection levels vary widely (Park and Choi, 2001; Kang *et al.*, 2017, 2024; Subramaniam *et al.*, 2025c). Heavily infected Manila clams on the west coast exhibited decreased reproductive effort, delayed gonad development, and higher mortality rates (Choi *et al.*, 1989, 1994; Park *et al.*, 2006; Nam *et al.*, 2018; Lee *et al.*, 2021; Subramaniam *et al.*, 2025a).

Since the *P. olseni* infection intensity in Manila clams at the Naeri tidal flat was significantly higher than at the Oeri tidal flat (Fig. 7), it was expected that the CI of clams at Naeri would be lower due to the infection. The conflicting results observed in this study could be explained by either the infection intensity at Naeri not being high enough to affect the CI or by the food supply at Naeri being sufficient to outweigh the effects of the infection. A conflicting correlation was also observed between high *P. olseni* infection and high CI on the West Coast, as Subramaniam *et al.* (2024) reported a considerably high level of *P. olseni* infection and high CI in Manila clams on Hwangdo tidal flat in Anmyeondo, which was partly explained by a high level of food supply. Additionally, a high level of *P. olseni* infection and a high level of CI were also observed in Manila clams

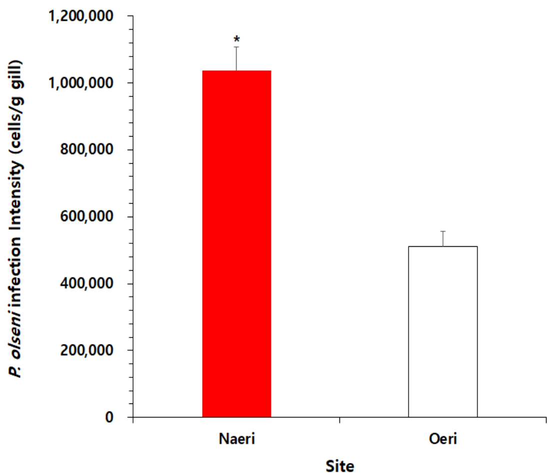

Fig. 7. The annual mean *P. olseni* infection intensity measured from the Naeri and Oeri tidal flats.

collected from the subtidal environment on the South Coast, which was attributed to better feeding conditions in this environment (Lee *et al.*, 2020; Subramaniam *et al.*, 2025c).

Larval trematode infection in Manila clams is also considered a factor that impairs host reproduction and growth (Park *et al.* 2008; Cho *et al.* 2022). Ngo and Choi (2004) reported gonad castration of female Manila clams caused by larval *C. tapidis* infection during the spawning period on a sand flat in Jeju Island. Histology revealed that in August, the infected female gonads were filled with larval trematodes, while uninfected clams had fully mature eggs in their follicles. The larval trematode infection in Manila clams on tidal flats in Gyeonggi Bay was also reported by Park *et al.* (2013), with an infection prevalence ranging from 5 to 12.5% in early spring. Using histology and species-specific PCR, Le *et al.* (2024) identified the larval trematode in Manila clams collected from 26 tidal flats in Gyeonggi Bay and Taean areas on the West Coast. According to Le *et al.* (2024), the larval trematode infection is tissue-specific: *C. tapidis* and *Bacciger bacciger* infect the gonad and visceral mass, *Parvatrema duboisi* targets the mantle, and the encysted form of the Himasthalid trematode resides in the foot. As observed in this study, the female gonads of female clams were often completely filled with *C. tapidis*, indicating that larval trematode infection hampers the growth and gonad development of the infected clams. Pearson's correlation analysis revealed a strong negative correlation between the CI and the parasite load (i.e., *P. olseni* and *C. tapidis* infection) at the Oeri tidal flat, suggesting the effects of parasitism on the growth and reproduction. It is also observed that *P. olseni* infection and trematode infection are positively correlated on the Oeri tidal flat, indicating that co-infection with these parasites may have contributed to the seasonal decline in the clam condition.

In conclusion, we observed seasonal changes in reproduction, CI, and parasite load of clams at Naeri and Oeri tidal flats in Yeongheungdo Island, Gyenggi Bay. It is believed that seasonal variations are closely linked to water temperature and food supply, which may influence the timing and health of Manila clam reproduction. The parasite burden also affected the health of the host clam, although the effects were site-specific.

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