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ARTICLE

Spatio-Temporal Variation in *Perkinsus olseni* Infection Intensity in Manila Clam *Ruditapes philippinarum* in Anmyeondo and Cheonsu Bay Tidal Flats on the West Coast of Korea

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Abstract

The alveolate protist *Perkinsus olseni* infections impose considerable stresses on the host Manila clam *Ruditapes philippinarum*, often reducing their tissue weight due to the drain on energy resources. However, the relationship between condition factors and *P. olseni* infection exhibits inconsistency in different scenarios, as it is influenced by various factors such as environmental conditions, food availability, and reproductive conditions. To gain deeper insights, we surveyed Manila clams occurring on tidal flats in Anmyeondo Island, Cheonsu Bay to assess *P. olseni* intensity and its effects on the fitness of Manila clams. The Ray's fluid thioglycollate medium assay revealed that *P. olseni* infection in clams from inner Cheonsu Bay had considerably greater *P. olseni* intensities in May and September 2021 than those from outer Cheonsu Bay and reference sites ($P < 0.05$). Despite the high level of *P. olseni* infection, condition index (CI), a ratio of the flesh weight to the shell weight, in clams from inner Cheonsu Bay was also significantly higher than in clams from outer Cheonsu Bay and reference sites ($P < 0.05$). A noteworthy finding of this study was the positive association between *P. olseni* infection intensity and CI in clams, which suggests that despite the high parasite load, clams were able to maintain a higher CI, possibly due to the abundance of nutrients and organic matter present in the inner bay. The *P. olseni* infection intensities measured in September were significantly higher at most of the sampling sites in the inner and outer bay, which could be associated with the poor health condition of clams during a post-spawning period. These results highlight the complexity of bivalves' ecological relationships in Cheonsu Bay and offer new insights into their adaptability accompanied by other factors to parasite stressors.

Keywords Perkinsus · Condition index · *Ruditapes philippinarum* · Infection intensity · Manila clam

1 Introduction

Parasitic protists belonging to the genus *Perkinsus* have been responsible for mass mortalities among various commercially valuable bivalve species, including Manila clams, a highly prevalent species in the coastal regions in the northwest Pacific regions (Park and Choi 2001; Nam et al. 2018; Cho et al. 2022). Several studies have monitored *P. olseni* infections in Manila clams in tidal flats on the west coast of Korea, reporting spatiotemporal variation in the infection prevalence and intensities (Park et al. 2006; Yang et al. 2010, 2012, 2021; Lee et al. 2021). The heavy burden of *P. olseni* infection has been associated with retarded growth, limited immune competencies, and poor reproductive capabilities among clams in this region (Kang et al. 2006; Park et al. 2006). Generally, heavy *Perkinsus* infection effectively inhibits the release of reactive oxygen species from host hemocytes, produces proteases capable of digesting immune-related cells (La Peyre 1996), and hampers phagocytosis in clams. Consequently, the host's immune defenses are compromised for energy expenditure, rendering it even

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more susceptible to *Perkinsus* infection and facilitating the proliferation of the parasite (La Peyre 1996; Lee et al. 2021). Additionally, an elevated level of *P. olseni* infection can adversely affect crucial activities, including burrowing and feeding, resulting in mass clam mortalities during the summer (Nam et al. 2018; Waki and Yoshinaga 2018).

The CI stands as a fundamental parameter frequently employed in ecological and physiological investigations involving bivalves. It serves as a pivotal tool in assessing the overall health and physiological well-being of bivalves, such as clams (Uddin et al. 2010; Zeng and Yang 2020). CI of clams is defined as the weight ratio of the wet or dry flesh to the shell weight, offering valuable insights into the organism's overall condition, including its growth rate, reproduction, and susceptibility to environmental stressors or diseases (Watanabe and Katayama 2010; Uddin et al. 2012; Lee et al. 2020a, b; Zeng and Yang 2020). The CI is widely adopted and practically employed for monitoring the fitness and reproductive status of clams due to its convenience and efficiency (Lucas and Beninger 1985; Lee et al 2020a, b; Lee et al. 2021). Changes in the CI of clams typically resemble alterations in food availability (Park et al. 2020), biochemical accumulation (Choi et al. 1989), reproductive status (Uddin et al. 2010, 2012), and disease infection (Lee et al. 2020a, b; Yang et al. 2021). In Korea, the CI of Manila clams exhibits a distinct pattern, with an initial increase during the spring season, reaching its peaks in late spring, and experiencing a gradual decline during summer spawning when the water temperature remains above 20 °C (Park et al. 2006, 2020; Uddin et al. 2010). Subsequently, a lower CI is observed in post-spawning clams in late summer through winter, followed by a condition recovery in the subsequent spring, marked by the accumulation of energy reserves in pre-spawning clams, leading to higher CI in clams (Lucas and Beninger 1985; Park et al. 2006, 2020).

Surrounded by Anmyeondo Island on the west and the two artificial lakes of Bunamho and Ganwolho on the north, the semi-closed Cheonsu Bay encompasses numerous sandy mud tidal flats that serve as habitats for various burrowing marine organisms (Jung et al. 2014; Park 2020). Cheonsu Bay spans 40 km in length and 10 km in width, with an average depth of about 20 m (Lee et al. 2022). Its vast tidal flats, integral to the bay and Anmyeondo coastal surroundings, serve as extensive clam culture grounds (Lee et al. 2020a, b; Yang et al. 2021). Further, the environmental factors of Cheonsu Bay are significantly influenced by the inflow of freshwater from the Ganwolho and Bunamho lakes located in the upper reaches of Cheonsu Bay (Kim et al. 2005; Lee et al. 2022, 2023). Consequently, the water column contains various other food sources, including particulate organic matter, dissolved inorganic nitrogen, and dissolved inorganic phosphorus, collectively providing diverse food resources for Manila clams in the tidal flats. Additionally, the influx of nutrients and organic matter fosters phytoplankton growth in the water column, serving as a valuable food source for marine benthic organisms and enriching the bay's ecosystem while supporting its complex food web (Komorita et al. 2014; Lee et al. 2019a, 2019b, 2022).

This study reports the findings of a comprehensive survey conducted to assess the intensity of *P. olseni* infection and CI in Manila clams within Cheonsu Bay, situated along the west coast of Korea. The study investigates the complex relationship between environmental conditions and the CI, particularly in the context of high parasitic infection rates among the clam population.

2 Materials and Methods

2.1 Sampling Efforts

Manila clams were collected from tidal flats in Cheonsu Bay, a coastal region located on the west coast of Korea (Fig. 1). Ten sampling locations were strategically chosen to encompass four inner and four outer regions of Cheonsu Bay and two reference sites for comparative analysis (Fig. 1). A total of 600 Manila clams with an average shell length (SL) ranging from 33.8 to 41 mm were collected in May and September of 2021 (Table 1). The monthly Surface Seawater Temperature (SST) and chlorophyll-a level in inner and outer Cheonsu Bay and reference sites were obtained from the Giovanni online data system, accessible through NASA GES DISC (https://giovanni.gsfc.nasa.gov/) to approximate the food availability for clams. Giovanni supplies aggregated data extracted from MODIS, a satellite-based sensor capable of providing average monthly SST and chlorophyll-a measurements for both the designated geographical area and the duration of our study.

2.2 Histology

At the laboratory, each clam's SL, the longest axis of the shell, was measured in mm. The soft tissues were separated from the shells, wet weight of each tissue was recorded in mg. To determine reproductive condition, a 2 to 3 mm-thick transverse section was cut from the middle of the clam, including the gills, mantle, foot, and digestive system, and subsequently fixed in Davison's fixative for histology. The remaining flesh was freeze-dried to determine the dry tissue weight in grams. CI was established as a ratio of dry tissue weight to the dry shell weight [CI = (dry tissue weight/dry shell weight) × 1000].

The fixed clam tissues underwent a series of ascending ethanol dehydration and were embedded in paraffin. Approximately 5 µm-thick sections were cut from the paraffin block, deparaffinized, stained using Harris'

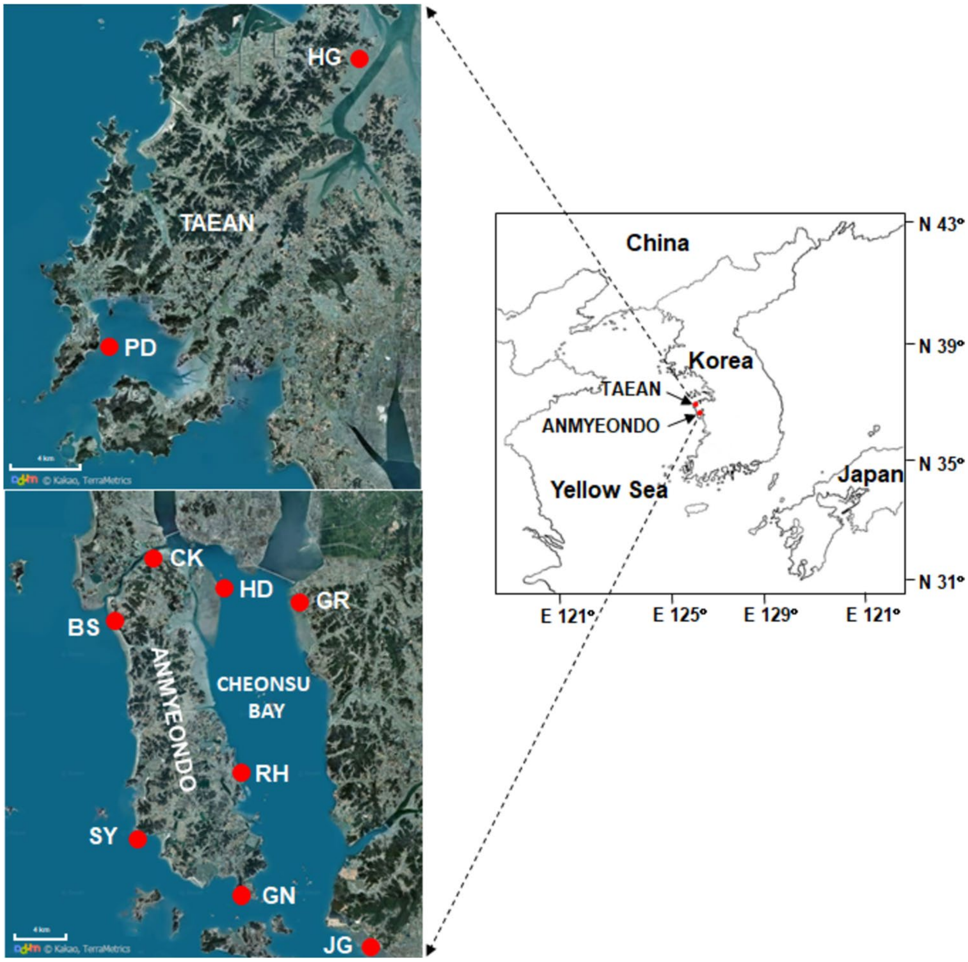


Fig. 1 Map showing the sampling sites. *Ruditapes philippinarum* (Manila clam) was collected from the west coast of Korea. Sampling encompassed inner Cheonsu Bay (*CK* Changgi-ri, *HD* Hwangdo, *GR* Gung-ri, *RH* Rahyang), outer Cheonsu Bay (*GN* Gonam, *JG* Jugyo, *SY* Sinya, *BS* Baegsajang), and reference sites (*HG* Hwalgog, *PD* Padori)

Table 1 Summary of sampling efforts

Sites	May 2021		September 2021	
	N	SL (mm)	*N*	SL (mm)
CK	30	38.2 ± 3.1	30	36.6 ± 3.6
HD	30	41.1 ± 3.8	30	39.2 ± 3.9
GR	30	36.4 ± 2.8	30	39.7 ± 4.5
RH	30	38.1 ± 2.3	30	37.8 ± 2.6
GN	30	36.0 ± 2.8	30	37.3 ± 2.8
JG	30	39.3 ± 2.5	30	39.2 ± 2.2
SY	30	35.6 ± 3.0	30	37.2 ± 3.3
BS	30	34.4 ± 1.9	30	33.8 ± 2.3
HG	30	35.6 ± 2.4	30	35.2 ± 2.2
PD	30	34.5 ± 1.4	30	35.2 ± 1.6

The values represent the mean and standard error

N numbers of clams used in the analysis, *SL* shell length in mm

hematoxylin and eosin Y, and examined under a compound light microscope. Manila clam reproductive phases were classified into six categories: (1) resting, (2) early developing, (3) late developing, (4) ripe, (5) spawning, and (6) spent. Subsequently, we assessed the frequency distribution of these distinct reproductive stages (Lee et al. 2020a, b; Yang et al. 2021).

2.3 *P. olseni* Infection Intensity

A piece of the excised gill tissue samples were taken from each clam and placed in a 10 ml conical tube containing 5 ml of Ray's fluid thioglycollate medium (RFTM, Ray 1966) fortified with antibiotics (nystatin 200 unit/ml, chloramphenicol 100 ng/ml). After 1 week of dark incubation at room temperature, the gill tissues were digested in 2 M NaOH at 60 °C (Choi et al. 1989). After removing 2 M NaOH solution by washing with filtered seawater, the *P. olseni* hypnospores were resuspended in a known volume of phosphate-buffered saline (PBS, pH 7.6), the number of hypnospores in a subsample was counted using a hemocytometer. Finally, the infection intensity was reported as *P. olseni* cells per gram of gill tissue.

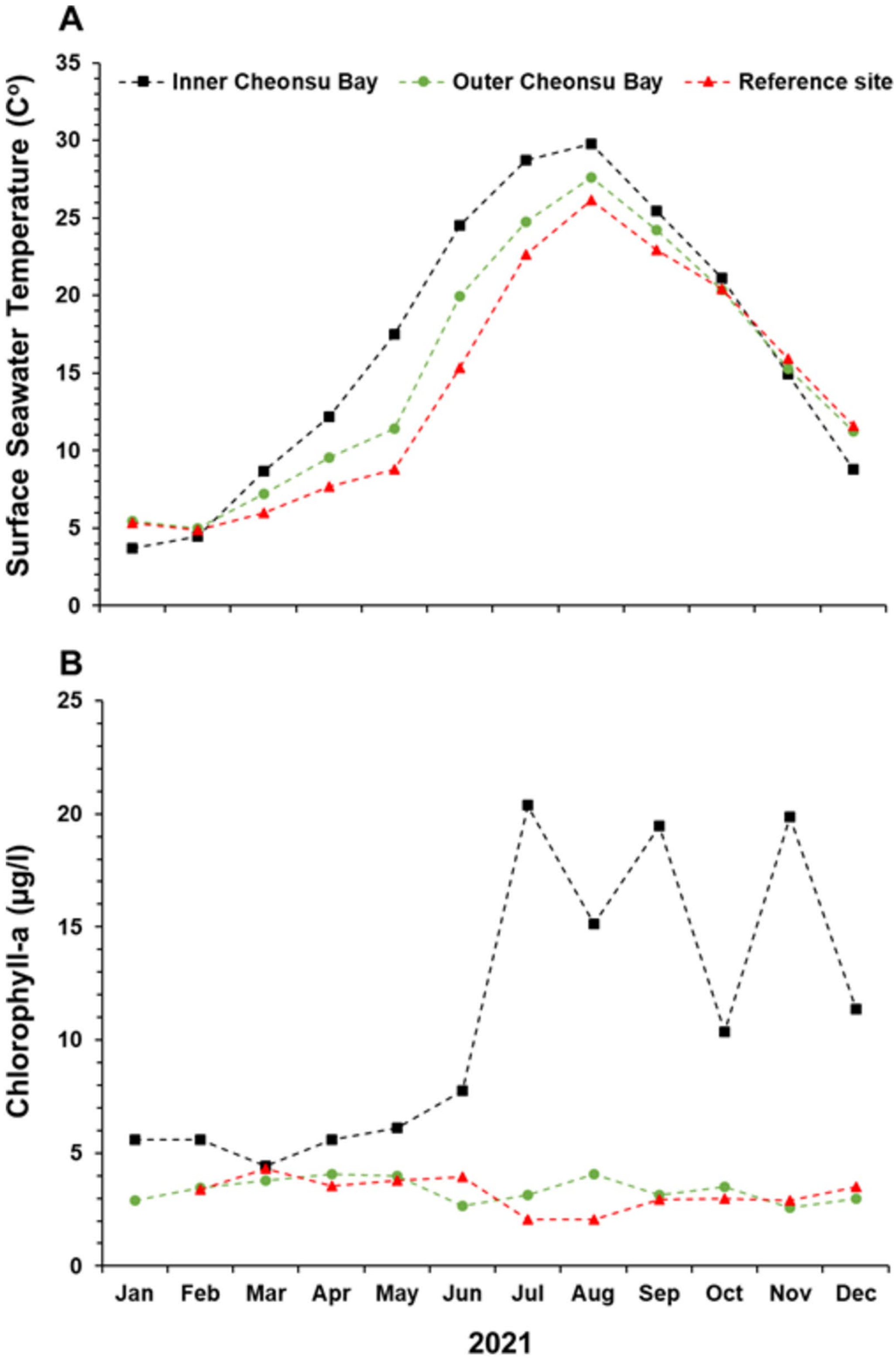


Fig. 2 Seasonal changes in the Surface Seawater Temperature (SST) (**A**) and chlorophyll-α concentration (**B**) in the inner Cheonsu Bay, outer Cheonsu Bay, and reference site in 2021

2.4 Statistical Analysis

The significance of variations in the mean values of CI and *P. olseni* infection intensity among the three distinct groups of clams, namely, those from inner Cheonsu Bay, outer Cheonsu Bay, and reference sites, was rigorously assessed using the one-way ANOVA followed by Duncan's Multiple Range Test. The *P. olseni* infection intensity data underwent a rank transformation in this analysis to ensure robust statistical evaluation. The statistical software package SAS (SAS Institute Inc; 2019 USA) was employed for this comprehensive analysis, with the predefined level of statistical significance set at $P < 0.05$.

3 Results

3.1 Environmental Conditions

Figure 2A illustrates the seasonal fluctuation in Sea Surface Temperature (SST) across all study sites. SST exhibited a gradual increase from less than 5 °C during winter to approximately 30 °C in summer. Notably, inner Cheonsu Bay showed an SST range of 3.7–29.8 °C while the reference site displayed a slightly narrower SST range (4.9–26.1 °C) compared to the inner and outer regions of Cheonsu Bay. In addition, seasonal variations in chlorophyll-a levels were also observed in inner Cheonsu Bay. However, no seasonal changes were observed in outer Cheonsu Bay and the reference sites (Fig. 2B). Specifically, inner Cheonsu Bay experienced a pronounced increase in chlorophyll-a from summer to winter, reaching levels of approximately 10–20 μg/l, in contrast to the lower concentrations (< 5 μg/l) observed at other study sites

3.2 Reproductive Condition

The histology revealed that in May, most clams were in the late developing and ripe stages, showing typical pre-spawning conditions. Similarly, in September, a predominant proportion of clams at the study sites were in the ripe and spent stages, indicative of the clams transitioning into the post-spawning condition (Fig. 3). In May, both inner Cheonsu Bay and outer Cheonsu Bay exhibited a similar pattern of reproductive stages, characterized by a minimal presence of the early developing phase and a substantial proportion of late developing and ripe stages. In contrast, clams in the reference sites had already commenced spawning, albeit at a smaller percentage (6–10%). In September, it was apparent that the reference sites exhibit a more advanced state of reproductive condition than inner and outer Cheonsu Bay.

3.3 *P. olseni* Infection Intensities

A graphical representation of the mean infection intensities of *P. olseni* across the various study sites within Cheonsu Bay is presented in Fig. 4. The ANOVA revealed a significant difference in *P. olseni* infection intensities between the inner and outer regions of Cheonsu Bay and the reference sites, regardless of the sampling period ($P < 0.05$). Notably, inner Cheonsu Bay showed the highest infection intensities, and the reference sites exhibited the lowest infection intensities, as confirmed by ANOVA ($P < 0.05$). In May, all study sites within the inner Cheonsu Bay displayed higher *P. olseni* infection intensities than the overall mean value (2.88×10^6 cells/g gills). Similarly, all study locations in the inner Cheonsu Bay also exhibited higher *P. olseni* infection intensities, exceeding the overall mean (4.47×10^6 cells/g gills) in September, except for HD in the upper bay. The infection intensities observed from the inner Cheonsu Bay in May and September were 15 to 20 times greater than the levels observed from the reference site. Except for site HD, all sites exhibited higher *P. olseni* infection intensities in September (post-spawned) compared to May (pre-spawned), with some sites displaying up to a threefold increase in *P. olseni* cell counts.

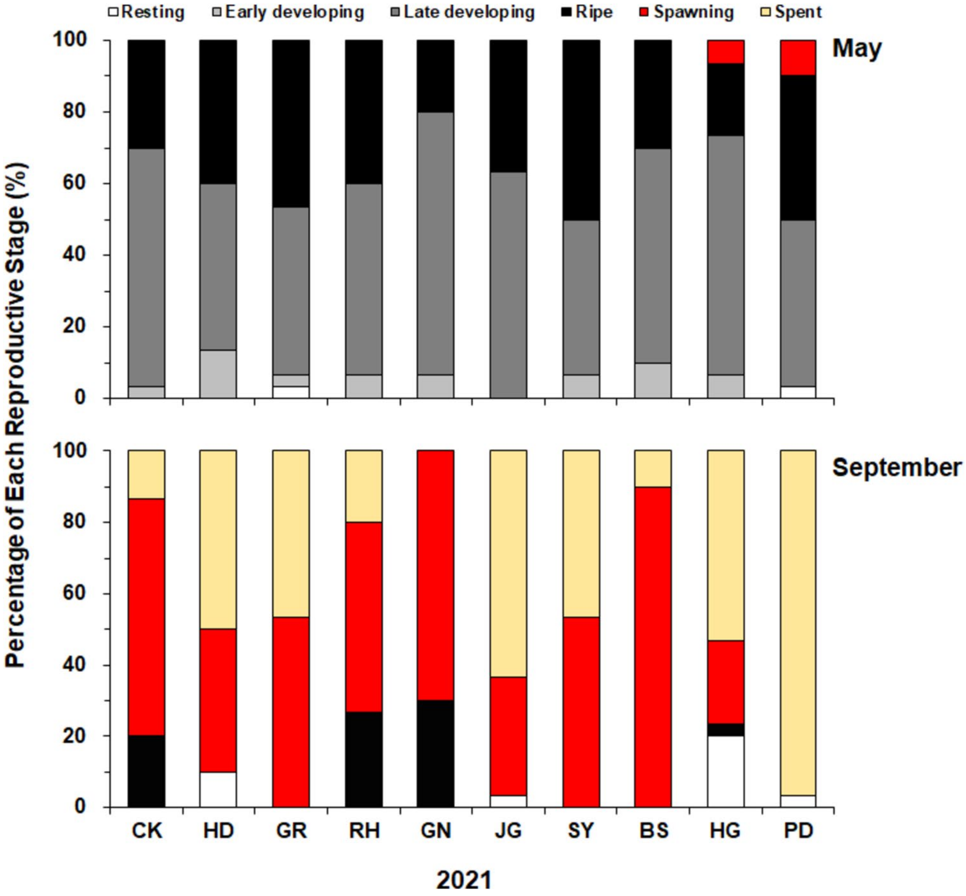
3.4 Condition Index

The mean CI for the various study sites encircling Cheonsu Bay is shown graphically in Fig. 5. Interestingly, despite having the highest *P. olseni* infection intensity in May and September, according to our result, clams from inner Cheonsu Bay had the noticeably highest CI (ANOVA, $P < 0.05$). Particularly noteworthy is that, in May, all study sites within inner Cheonsu Bay exhibited CI values exceeding the overall mean (190.2), while in September, the CI remained relatively stable across the locations. Additionally, in contrast to May, the CI of all study sites was lesser in September, with most sites exhibiting approximately half of the CI observed in the pre-spawned condition.

4 Discussion

Reproductive stage analysis provides valuable insights into how pre-spawning and post-spawning conditions of clams affect other criteria, such as CI, and parasitic infection further influence the proportion of reproductive stages (Gabbott 1983; Kang et al. 2000; Park et al. 2011; Lee et al. 2021). The pre-spawning period is the phase leading up to spawning, where bivalves typically undergo physiological changes and allocate significant energy resources to reproductive processes. In contrast, the post-spawning phase occurs after the release of gametes and is often characterized by altered

Fig. 3 Frequency distribution of different reproductive stages of *Ruditapes philippinarum* (Manila clam) gonads observed in May and September 2021



physiological conditions and energy investments (Gabbott 1983; Ren et al. 2003; Uddin et al. 2012). The delay or slow gonad maturation in bivalves can stem from various factors, including parasitic infections (Choi et al. 1989, 1994; Lee et al 2020a, b), limited food availability (Uddin et al. 2012), and suboptimal water temperatures (Park and Choi 2004; Uddin et al. 2010). Our current investigation shows that the reference sites exhibit a slightly advanced reproductive cycle compared to the other study sites. We hypothesize that the higher presence of parasitic infections in inner and outer Cheonsu Bay may contribute to this minor variation, as parasitic infections can diminish net productivity, which is crucial for both growth and reproduction in marine bivalves.

Numerous prior studies have extensively investigated *P. olseni* infection intensity from the west coast of Korea (Lee et al. 2021; Yang et al. 2021). However, this current study provides novel insights by revealing the highest recorded *P. olseni* infection intensity within the inner confines of Cheonsu Bay, which is distinguished as a semi-enclosed system, primarily owing to its restricted water circulation by the open sea (Lee et al. 2023). The life cycle of *P. olseni* comprises three infectious stages, with infectious cells released from infected hosts through feces, pseudo-feces, and decomposing tissues, eventually entering the sediment and potentially infecting new hosts during water column suspension and filtration or indirectly by the feeding activity (Villalba et al. 2004; Park et al. 2010; Wang et al. 2018). The findings of this study further substantiate the notion that post-spawned clams are significantly more susceptible to *P. olseni* infection when compared to their pre-spawned counterparts (Uddin et al. 2010). During the post-spawning season, accompanied by warm temperatures, the energy demands for clam growth intensify, resulting in a negative energy balance. Such adverse energy balance subsequently compromises the host's immune defenses following spawning, rendering it even more susceptible to *Perkinsus* infection and facilitating the proliferation of the parasite (Choi et al. 1989; da Silva et al. 2008; Flye-Sainte-Marie et al. 2009). Moreover, the elevated temperatures with high salinity experienced during the summer season favor the proliferation of *P. olseni* within clam populations (Figs. 2A and 4), typically resulting in a several-fold increase in prevalence and numbers in summer compared to the winter period (Lee et al. 2020a, b). Accordingly, increased temperature and higher salinity levels reduce the doubling time of *Perkinsus* cells. Moreover, the environmental conditions within the inner bay create a conducive environment for the accumulation of *Perkinsus* cells within both the clams and the sediment,

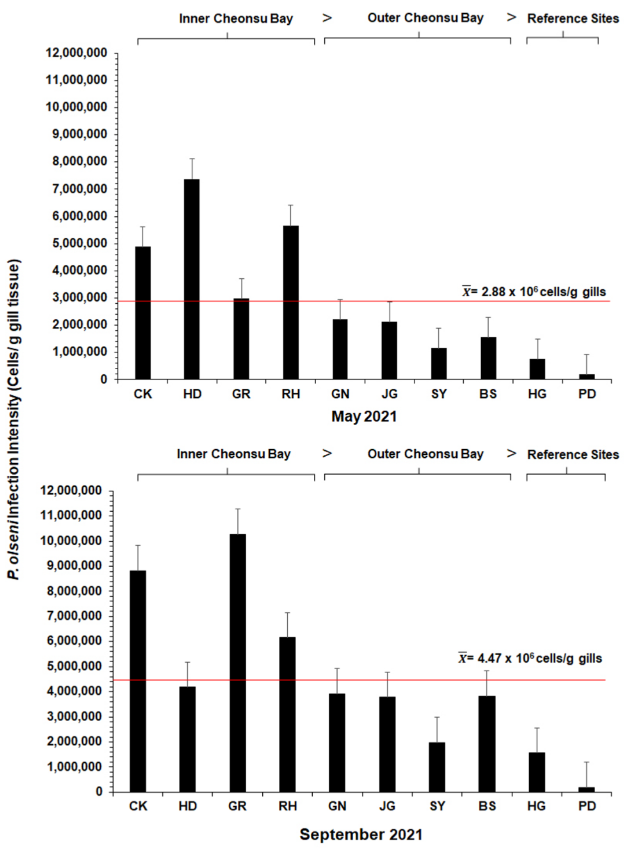


Fig. 4 Mean *P. olseni* infection intensity (cells/g gills) in *Ruditapes philippinarum* (Manila clams) across study sites in Cheonsu Bay reveals significant variations among inner Cheonsu Bay, outer Cheonsu Bay, and reference sites. Symbol > denotes statistically significant higher infection intensity among inner Cheonsu Bay, outer Cheonsu Bay, and reference sites (ANOVA, $P < 0.05$)

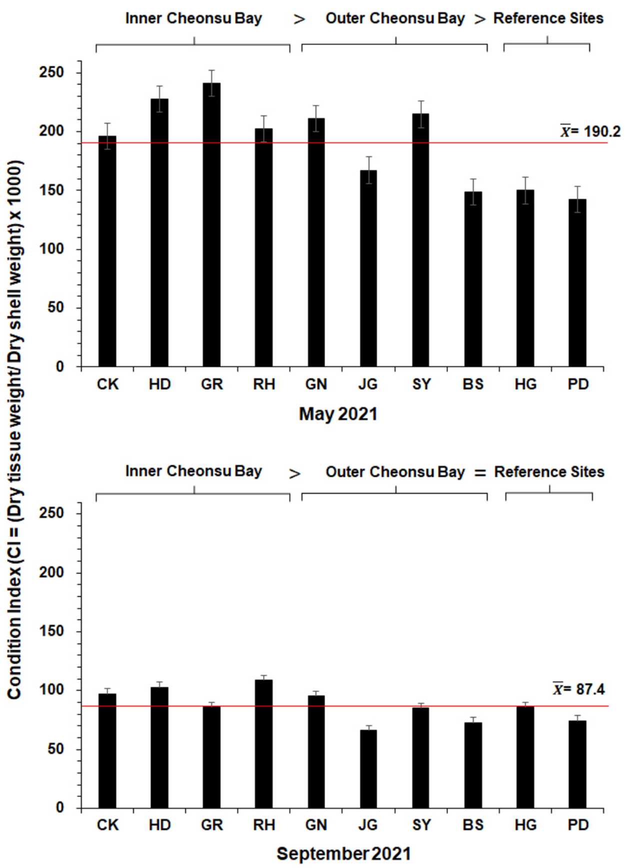


Fig. 5 Mean condition index (CI) of *Ruditapes philippinarum* (Manila clams) collected across the study sites in Cheonsu Bay. Symbol > denotes statistically significant higher CI among inner Cheonsu Bay, outer Cheonsu Bay, and reference sites (ANOVA, $P < 0.05$)

collectively contributing to elevated infection levels, particularly among post-spawned clams experiencing immune suppression (Park et al. 2010; Nam et al. 2018; Waki et al. 2018; Lee et al. 2020a, b).

In this study, we observed a high level of infection intensity coinciding with a higher CI in the inner Cheonsu Bay. Similar observations were documented by Yoshinga et al. (2010), indicating that even Manila clams with high CI exhibited high infection intensity starting in early summer. In marine bivalves, it is widely recognized that a higher parasite load can ultimately lead to a decline in fitness and higher energetic cost (Choi et al. 1989, 1994). However, when bivalves have access to an ample supply of nutrients from their surroundings, they can better manage the energy expenditures associated with parasitic infections. In such circumstances, they can allocate resources efficiently, not only to the parasites but also to maintain their overall CI, which statement supported that Manila clams may have successfully mitigated the physiological stresses induced by their heavy infection burden, possibly due to the ample food resources available, which could sufficiently compensate for the energetic expenses associated with the infection (Yang et al. 2012).

The elevated chlorophyll-a levels observed in inner Cheonsu Bay from summer to winter coincide with the influx of freshwater discharged from the artificial lakes Bunamho and Ganwolho in the upper Cheonsu Bay region (Kim et al. 2005; Lee et al. 2023). Simultaneously, freshwater discharge causes a substantial surge in dissolved inorganic nitrogen, raising concentrations by up to three to four times throughout the water column, coinciding with notably higher N/P ratios in Cheonsu Bay's seawaters, reaching two to seven times the typical values, particularly in the inner bay region (Lee et al. 2019a, b). Figure 2B provides a concise overview of chlorophyll-a level in 2021 from the inner Cheonsu Bay, outer Cheonsu Bay, and reference sites, which closely align with and validate the results previously reported by Kim et al. (2005), Lee et al. (2019a, b). Numerous studies have demonstrated that seasonal fluctuations in chlorophyll-a levels are matched with food availability, consequently enhancing growth, biochemical compositions and CI in marine

Table 2 Comparison of chlorophyll-α levels across different tidal flats

Location	Chlorophyll-*a* (μg/l)	References
Mohang, West Coast, Korea (36° 46′ N, 126° 7′ E)	0.8–5.1	Park et al. (2020)
Hajun, West Coast, Korea (35° 33′ N, 126° 30′ E)	3.2–10	Baek et al. (2014)
Begmiri, West Coast, Korea (37° 9′ N, 126° 39′E)	1.56–8.19	Uddin et al. (2012)
Bay of Arcachon, West Coast, France (44° 39′ N, 1° 8′ E)	1–27.2	Robert et al. (1993)
Akkeshi-ko, Eastern Hoakaido (43° 1′ N, 144° 51′ E)	1.3–5.5	Hasegawa et al. (2014)
Nakaumi Lake, Japan (35° 30′ N, 133° 12′ E)	1.4–51.4	Hirakiuchi et al. (2017)

bivalves (Park and Choi 2004; Baek et al. 2014; Hasegawa et al. 2014; Park et al. 2020). Corresponding with water temperature fluctuations, elevated chlorophyll-a levels are closely associated with the annual gametogenesis of clams (Uddin et al. 2012). For instance, Hofmann et al. (1992) demonstrated that even minor shifts in water temperature or slight variations in the timing of plankton blooms, as short as 2–4 weeks, can exert substantial impacts on the reproductive behavior of bivalves. Table 2 presents the chlorophyll-a levels in various bivalve culture grounds, providing valuable insights into the nutrient conditions and food availability across the different locations. According to previous studies, the tidal flats along the west coast of Korea displayed chlorophyll-a levels ranging from 0.8 to 10 μg/l, which were notably lower than those found in European waters, where levels reached up to 27 μg/l. Interestingly, Lake Nakaumi, which receives freshwater from Lake Shinji and several rivers and is connected to the Japan Sea through a single narrow channel, exhibited remarkably high chlorophyll-a levels, reaching up to 51 μg/l. Thus, the inner Cheonsu Bay exhibits meaningfully higher chlorophyll-a levels compared to other prominent bivalve culture grounds, signifying its potential for enhanced bivalve production within this region.

Inner Cheonsu Bay, characterized by substantial freshwater input, exhibited a substantial increase in particulate organic content, exceeding normal levels by more than twofold (Lee et al. 2023). Notably, Manila clams exhibit a remarkable capacity to utilize particulate organic matter significantly, with an astonishing utilization rate of up to 64% to support its high productivity (Komorita et al. 2014), which underscores the adaptability and resourcefulness of these clams in harnessing organic matter present in their environment to meet their nutritional needs (Mazzola and Sara 2001; Kedra et al. 2021). Intriguingly, it is observed that phytoplankton, typically considered a primary food source for filter-feeding bivalves like *Ruditapes philippinarum*, only contributes to approximately 28% of the clams' overall diet (Komorita et al. 2014). This observation suggests the presence of an abundance of organic matter in the surrounding water, which, in turn, enhances the overall food availability for these clams (Jonge and Beuselom 1992; Nakaoka 1992; Mazzola and Sara 2001; Komorita et al. 2014; Lee et al. 2019a, b). The clam's ability to diversify its dietary sources and rely significantly on the particulate organic matter further highlights its versatility in adapting to varying ecological conditions and resource availability. Therefore, we hypothesize that Manila clams may have effectively alleviated the physiological stresses imposed by their substantial infection burdens by consuming more food derived from organic matter and phytoplankton available in Cheonsu Bay, which appears capable of adequately compensating for the energetic costs associated with the infection.

In conclusion, our study revealed a compelling paradox within the inner Cheonsu Bay, where clams exhibit a notably higher CI despite experiencing a heavier *Perkinsus* infection burden in May and September. This intriguing phenomenon appears to be intricately linked to the increased food availability resulting from substantial water inflow, rich in organic matter. Moreover, this study provides valuable insights into the complicated interplay between *P. olseni* infection and food resources, shedding light on the complex ecological dynamics of marine bivalve populations in dynamic coastal ecosystems.

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Data availability The data supporting the findings of this study can be obtained from the corresponding author upon request.

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