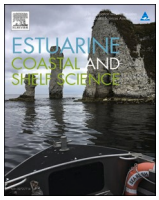
Estuarine, Coastal and Shelf Science 323 (2025) 109418

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



Interannual variability in the reproductive cycle of Manila clam, (*Ruditapes philippinarum*) influenced by environmental and parasitic factors on the west coast of Korea

Thatchaneshkanth Subramaniam [a,c], Jeong-Hwa Kim [a], Kwang-Sik Choi [a,*], Hyun-Sung Yang [b,**]

[a] Department of Marine Life Science (BK21 FOUR), Jeju National University, 102 Jejudaehakno, Jeju, 63243, Republic of Korea
[b] Tropical and Subtropical Research Center, Korea Institute of Science and Technology (KIOST), Jeju, 63349, Republic of Korea
[c] Department of Animal Science, Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, Kilinochchi, 44000, Sri Lanka

ARTICLE INFO

Keywords:
Annual gametogenesis
Manila clams
Ruditapes philippinarum
Parasitic infection
Coastal ecosystems

ABSTRACT

Extensive studies have shown that geographic variation, primarily driven by environmental factors, affects the reproductive cycle duration, gametogenesis, and spawning periods of the Manila clam, *Ruditapes philippinarum*. From February 2008 to December 2010, we monitored the annual reproductive cycle of Manila clams on a tidal flat in Garorim Bay, located on Korea's west coast. In adult males, spermatogenesis began between December and February, reaching a peak (90 %) by March and April. Partial spawning in males occurred from May to July, coinciding with seawater temperatures rising from 11.2 to 20.0 °C. In females, oogenesis commenced between January and February, while spawning began in June, reaching a peak activity level of 36 %.A brief 3-month spawning period was recorded in 2009, while in other years, spawning extended for 4–5 months. The condition index (CI) of the clams ranged from 78 to 139, and fluctuations in CI during the spawning season indicated multiple spawning peaks in males, while females exhibited a single peak. The intensity of the protozoan parasite *Perkinsus olseni* infection ranged from 0 to $1.4x10^5$ cells per gram of gill tissue, reflecting a low infection level compared to clams from other tidal flats on the west coast. Overall, variations in reproductive stages and CI at the study site appeared to be primarily influenced by seasonal fluctuations in water temperature and food availability, with negligible impact from parasite infection.

1. Introduction

Understanding the reproductive biology of commercially important bivalve species is essential for enhancing production and developing sustainable management strategies (Gosling, 2003). Extensive studies on the reproductive biology of the Manila clam across its distribution range indicate that the latitudinal gradient affects the duration of the reproductive cycle and the timing of gametogenesis and spawning (Drummond et al., 2006; Moura et al., 2018; Sugiura and Kikuya, 2022; Uddin et al., 2012). In temperate regions, seasonal variations in environmental factors such as temperature, salinity, and food availability drive the cyclical nature of reproduction. Optimal nutritional conditions and rising temperatures in late winter and early spring promote growth and gametogenesis (Navarro and Iglesias, 1995). On the west coast of Korea, gametogenesis in Manila clams begins in February, with the first spawning females observed in July, coinciding with the rapid increase in water temperature and the spring phytoplankton bloom (Park and Choi, 2004; Uddin et al., 2010, 2012; Jang et al., 2018).

The Manila clam (*Ruditapes philippinarum*) follows an annual reproductive cycle that includes gametogenesis, one or more spawning events (Uddin et al., 2012; Park and Choi, 2004), and a period of gonad reconstitution. Marine bivalves initiate gametogenesis in response to rising seawater temperatures (Navarro et al., 1989; Subramaniam et al., 2021). Within their thermal tolerance range, elevated temperatures enhance basal metabolism by improving oxygen binding and transport, respiration, excretion, and overall organ function (Fearman and

This article is part of a special issue entitled: The Manila Clam published in Estuarine, Coastal and Shelf Science.

* Corresponding author.
** Corresponding author.
E-mail addresses: skchoi@jejunu.ac.kr (K.-S. Choi), hsyang@kiost.ac.kr (H.-S. Yang).

https://doi.org/10.1016/j.ecss.2025.109418
Received 22 September 2024; Received in revised form 16 June 2025; Accepted 25 June 2025
Available online 25 June 2025

Moltschaniwskyj, 2010). Consequently, gametogenesis is an energy-intensive process that requires a continuous supply of nutrients to support the biosynthesis of reproductive materials, including proteins, lipids, and carbohydrates essential for gamete development. Sufficient food availability is critical for clams to meet the high metabolic demands of gametogenesis, which directly impacts their reproductive success and overall fitness (Navarro et al., 1989; Uddin et al., 2012; Lee et al., 2020).

While the west coast of Korea has been reported to exhibit a shorter spawning period in Manila clams, several studies suggest that variations in the spawning period are primarily driven by spatiotemporal differences in water temperature, food availability, and potentially parasite burden (Drummond et al., 2006; Park et al., 2006; Uddin et al., 2010, 2012). In particular, several studies have reported that the protozoan parasite *P. olseni* causes negative effects on the growth and reproduction of Manila clams in tidal flats along the west coast of Korea. *P. olseni* infects the gills and mantle tissue of clams, leading to tissue degradation, inflammation, and impaired physiological functions (Park and Choi, 2001; Park et al., 2006; Yang et al., 2012; Lee et al., 2021). If environmental changes are responsible for such variations in the reproductive cycle, similar fluctuations should be observed within the same population when exposed to comparable environmental conditions over time (Navarro et al., 1989). Understanding these environmental influences is therefore essential for predicting and managing the reproductive cycle dynamics of Manila clams across different regions, as well as within the same region over extended study periods.

The condition index (CI), defined as the ratio of dry flesh weight to dry shell weight, with the resulting value multiplied by 1000 to allow standardized comparisons. This widely used metric serves as a reliable indicator of the physiological condition and reproductive status of Manila clams (Lucas and Beninger, 1985; Uddin et al., 2012; Subramaniam et al., 2024). A sudden drop in CI value suggests that significant biological effort has been expended, either for maintaining energy under poor environmental conditions or disease or in releasing gametes (Beninger and Lucans, 1984; Park et al., 2006; Uddin et al., 2012; Subramaniam et al., 2024). Therefore, as an indicator of reproductive activity, the CI provides valuable information about the gonadal state of the clam (Laruelle et al., 1994; Drummond et al., 2006; Toba et al., 2007). Similarly, the highest observed CI corresponds to a peak in ripeness, following nutrient accumulation in preparation for spawning (Drummond et al., 2006; Park and Choi, 2004; Uddin et al., 2012). Furthermore, Uddin et al. (2012) reported that the CI, which aligned with the gonad somatic index (GSI), adequately explains the spawning peaks in Manila clams. According to their findings, Manila clams initiate spawning when the GSI, which represents the ratio of gonad weight to total body weight, exceeds 20, and other environmental parameters, such as water temperature, are favorable.

In this study, we monitored interannual variation in Manila clam populations over three years to understand the relationship between reproductive stages and environmental changes in the Naeri tidal flat on the west coast of Korea. The west coast of Korea is known for its favorable environmental and geographical conditions for Manila clam fisheries. Therefore, monitoring the annual reproductive cycle of Manila clams is essential for evaluating the long-term suitability and sustainability of the clam fishery in this area. We hypothesize that annual variation in the reproductive patterns of Manila clams may be attributed to shifts in environmental conditions and parasitic infections in the Naeri tidal flat. Examining annual variations in the reproductive cycle will enhance our understanding of the critical environmental parameters influencing the reproduction of Manila clam on the west coast of Korea.

2. Materials and methods

2.1. Sampling efforts and environmental conditions

Manila clams were collected from a tidal flat in Garorim Bay, located on the western coast of Korea (Fig. 1). A total of 1400 clams, with shell lengths (SL) ranging from 27.0 to 35.6 mm, were collected monthly between February 2008 and December 2010 (Table 1). After collection, the clams were transported to the laboratory, where their shell lengths were measured in millimeters, and the soft tissue was weighed in grams. Monthly sea surface temperature (SST) and chlorophyll-*a* levels at the study site were obtained from the Giovanni online data system, available through NASA GES DISC (https://giovanni.gsfc.nasa.gov/) (Fig. 2). Monthly averaged time series data for SST were obtained from the MODIS-Aqua sensor at a spatial resolution of 4 km. Chlorophyll-α concentration data were derived from the NASA Ocean Biogeochemical Model (NOBM), which provides outputs at a spatial resolution of 0.67° latitude by 1.25° longitude for the study area. The SST exhibited seasonal variations, ranging from 3.1 °C in winter to 24.9 °C in summer. Similarly, chlorophyll-*a* levels, used as an indicator of food availability for the clams, fluctuated throughout the study period. Generally, chlorophyll-*a* concentrations ranged from 2.3 to 6.4 μg/L, with occasional peaks up to 14.8 μg/L between July and August 2009.

2.2. Histology

In the laboratory, a thin slice (2–3 mm thick) was excised dorso-ventrally from the midsection of each clam, encompassing the gills, mantle, foot, and digestive system, for histological analysis. The remaining tissue was weighed, freeze-dried, and stored at −70 °C. The shells were dried and weighed to calculate the condition index, defined as the ratio of dry tissue weight to dry shell weight ((CI = dry shell weight/dry tissue weight)x1,000). Dorso-ventral tissue sections were fixed in Davidson's fixative, dehydrated in a graded series of alcohols, and embedded in paraffin. Approximately 5 μm-thick sections were then cut from the paraffin block, deparaffinized, stained with Harris's hematoxylin and eosin Y, and examined under a compound light microscope. The reproductive phases of the Manila clams were classified into six stages: (1) resting, (2) early developing, (3) late developing, (4) ripe, (5) partially spawned, and (6) spent (Uddin et al., 2012). The frequency distribution of these reproductive stages was analyzed. Additionally, histological slides were examined to assess the prevalence of common parasites in the clams.

2.3. Perkinsus olseni infection intensity and prevalence

A section of excised gill tissue from each clam was placed into a 15 ml conical tube containing 5 ml of Ray's fluid thioglycollate medium (RFTM, Ray, 1966), supplemented with antibiotics (nystatin 200 units/ml, chloramphenicol 100 ng/ml). The gill tissues were incubated in the dark at room temperature for one week. After incubation, the tissues were digested in 2 M NaOH at 60 °C, following the method described by Choi et al. (1989). The 2 M NaOH solution was then removed by washing with filtered seawater, and the *P. olseni* hypnospores were re-suspended in a known volume of phosphate-buffered saline (PBS, pH 7.6). Hypnospore counts in the subsamples were determined using a hemocytometer. Finally, the infection intensity was quantified as the number of *P. olseni* cells per gram of gill tissue, and the prevalence was expressed as the percentage of infected clams for each month of sampling.

2.4. Statistical analysis

The significance of monthly variations in the mean values of CI and *P. olseni* infection intensity from 2008 to 2010 was evaluated using one-way analysis of variance (ANOVA), followed by Duncan's Multiple

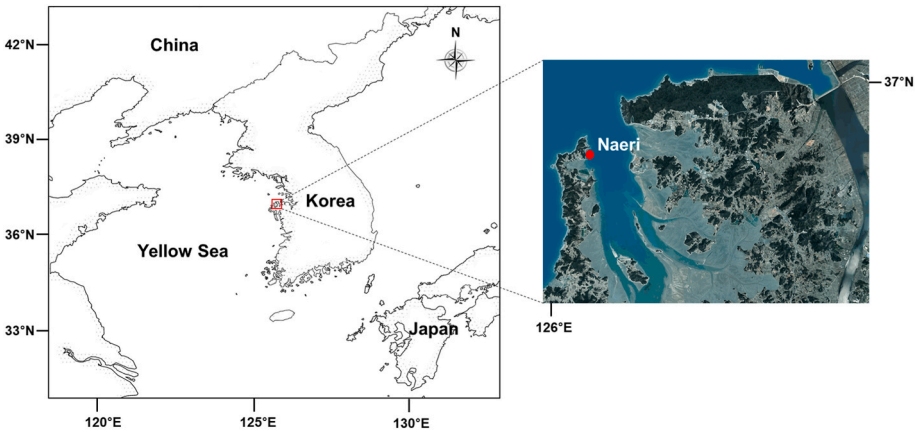


Fig. 1. Location of sampling site, Naeri tidal flat in the Garorim Bay on the West Coast of Korea.

Table 1
Summary of sampling efforts from 2008 February to 2010 December. The values represent the monthly mean ± the standard error. N, number of clams used in the study; SL, shell length in mm (SL); WTW, wet tissue weight in grams (WTW).

Year	Month	N	SL (mm)	WTW (g)
2008	F	40	35.6 ± 0.3	1.755 ± 0.051
	M	40	33.3 ± 0.3	1.450 ± 0.047
	A	40	32.8 ± 0.2	1.359 ± 0.047
	M	40	34.5 ± 0.3	1.978 ± 0.092
	J	40	34.1 ± 0.3	1.820 ± 0.079
	J	40	35.2 ± 0.3	2.507 ± 0.074
	A	40	35.6 ± 0.2	1.893 ± 0.048
	S	40	35.3 ± 0.3	1.887 ± 0.054
	O	40	33.4 ± 0.3	1.386 ± 0.041
	N	40	28.6 ± 0.2	0.880 ± 0.029
	D	40	29.1 ± 0.4	0.925 ± 0.063
2009	J	40	27.0 ± 0.3	0.680 ± 0.025
	F	40	29.3 ± 0.4	0.883 ± 0.058
	M	40	29.3 ± 0.2	0.892 ± 0.019
	A	40	28.7 ± 0.5	0.841 ± 0.059
	M	40	30.5 ± 0.4	1.117 ± 0.064
	J	40	28.4 ± 0.5	0.997 ± 0.058
	J	40	28.8 ± 0.4	0.917 ± 0.061
	A	40	32.6 ± 0.4	1.737 ± 0.058
	S	40	33.0 ± 0.2	1.332 ± 0.036
	O	40	34.5 ± 0.3	1.350 ± 0.039
	N	40	33.4 ± 0.3	1.265 ± 0.036
	D	40	32.8 ± 0.3	1.154 ± 0.035
2010	J	40	33.2 ± 0.4	1.117 ± 0.039
	F	40	33.6 ± 0.2	1.240 ± 0.029
	M	40	33.4 ± 0.2	1.499 ± 0.035
	A	40	32.5 ± 0.2	1.399 ± 0.050
	M	40	34.7 ± 0.3	1.982 ± 0.089
	J	40	33.3 ± 0.2	1.583 ± 0.068
	J	40	34.3 ± 0.2	1.833 ± 0.053
	A	40	33.8 ± 0.3	1.599 ± 0.068
	S	40	35.6 ± 0.3	1.710 ± 0.052
	O	40	31.2 ± 0.2	1.083 ± 0.022
	N	40	34.7 ± 0.3	1.688 ± 0.063
	D	40	35.3 ± 0.3	1.671 ± 0.051

Range Test for post hoc comparisons. To ensure a robust statistical analysis, the infection intensity data were subjected to rank transformation prior to testing. All statistical analyses were performed using the SAS software package (SAS Institute Inc., 2019; USA), with the level of significance set at $P < 0.05$.

3. Results

3.1. Biometry and CI

The monthly mean CI varied throughout the study period, ranging from 78 to 131 in male clams (Fig. 3) and from 77 to 150 in female clams (Fig. 4). Male clams exhibited a gradual increase in CI from winter to late spring, with multiple peaks observed during the summer months, followed by a gradual decline. Similarly, female clams showed a comparable trend, though their CI consistently peaked between May and July, followed by a significant decrease in the subsequent month ($P < 0.05$) (Fig. 4).

3.2. Reproductive cycle of R. philippinarum at the Naeri tidal flat

The monthly changes in the frequency distribution of reproductive stages in male clams, based on histological analysis, are summarized in Fig. 3. Spermatogenesis begins between December and February, with minimal engagement (10 %), gradually increasing to a peak of 90 % in the early developing stages by March and April. Partial spawning in male clams occurred between May and July, with up to 90 % of males actively engaged in spawning, coinciding with seawater temperatures rising from 11.2 to 20.0 °C, and extending through October. Fig. 4 illustrates the monthly fluctuations in the reproductive stages of female Manila clams. Oogenesis begins between January and February, with minimal engagement (7.4 %), coinciding with seawater temperatures of 3.5–4.7 °C. The proportion of females in the early developing stages increases steadily, reaching 100 % by March and April. Spawning begins in June, with a maximum of 36 % of females spawning, corresponding to seawater temperatures of 14.6–15.9 °C, and continues until October. Although male clams typically begin gametogenesis earlier than females, the spawning of both sexes was nearly synchronized throughout the study period.

3.3. Perkinsus olseni infection intensity and prevalence

The intensity and prevalence of *P. olseni* infection fluctuated seasonally throughout the study period (Fig. 5). The monthly average infection intensity ranged from 0 to 1.4 × 10^5 cells per gram of gill tissue, while the prevalence varied between 0 and 95 %. Lower infection intensities were generally observed from late fall to mid-spring, with higher intensities occurring from late spring through summer. Notably, in 2009, *P. olseni* infection intensity and prevalence were significantly lower ($P < 0.05$) compared to 2008 and 2010. In addition to *P. olseni*, histological analysis identified the presence of *Cercaria* sp., Rickettsia-like organisms (RLO), and encysted metacercaria in a small percentage of clams (less than 2 %).

4. Discussion

In this study, we investigate the interannual reproductive cycles and spawning pulse of Manila clams through histological analysis and

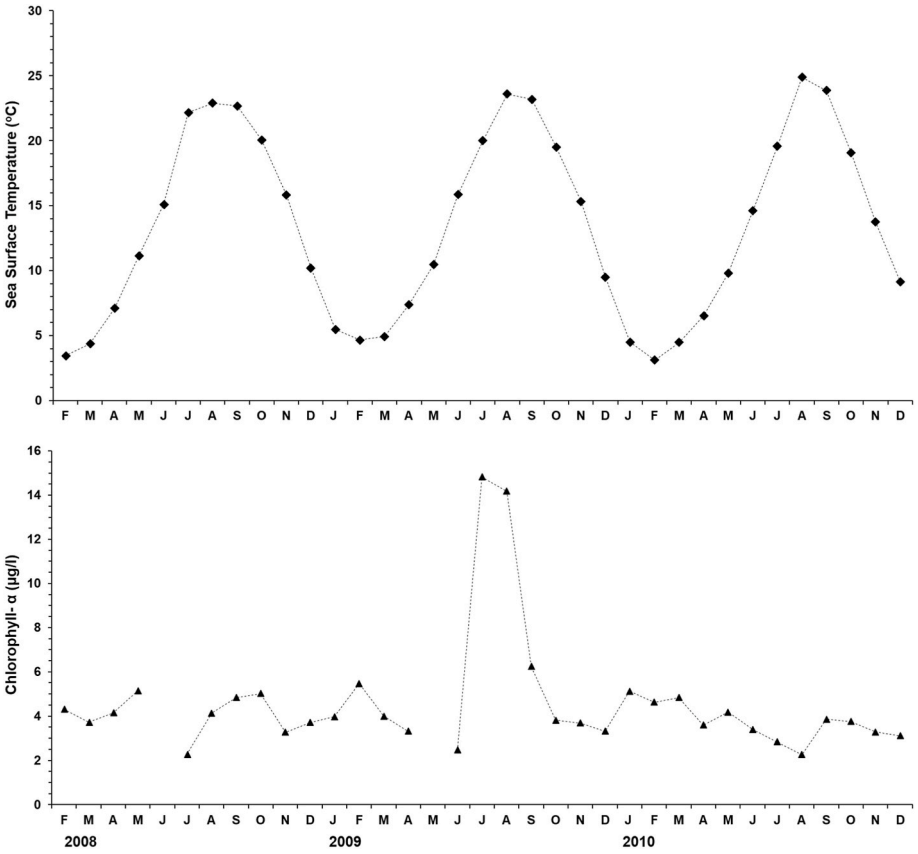


Fig. 2. Seasonal changes in the sea surface temperature (SST) and chlorophyll-*a* concentration in the study sites from 2008 February to 2010 December. The monthly chlorophyll-*a* concentration at the study sites was acquired from the Giovanni online data system, accessible through NASA GES DISC (https://giovanni.gsfc.nasa.gov/).

changes in the CI. The reproductive cycle of Manila clams encompasses several stages: gametogenesis, gamete development and ripening, spawning, and gonad redevelopment (Drummond et al., 2006; Uddin et al., 2012). The timing and duration of each stage exhibit significant variation, influenced mainly by environmental factors (Gosling, 2003; Uddin et al., 2012). Sea surface temperature plays a crucial role, as it affects the rate of gonadal development (Park and Choi, 2004; Uddin et al., 2012; Kim et al., 2017). Previous studies have reported that *R. philippinarum* on the west coast of Korea may initiate oogenesis when the water temperature exceeds 4 °C (Park and Choi, 2004; Uddin et al., 2012). However, the initiation temperature for gametogenesis in Manila clams can vary with geographical location, ranging from 5 to 14 °C (Robert et al., 1993; Drummond et al., 2006; Dang et al., 2010). This variation underscores the influence of environmental factors on the same bivalve species in different locations, highlighting the importance of studying the gametogenesis of Manila clams across various environments to understand how these factors impact their reproductive processes (Toba and Miyama, 1995; Gosling, 2003).

In this study, the first spawning of male clams was observed in early May (12.5 %) in 2008, July (90.5 %) in 2009, and June (22.7 %) in 2010. Male clam spawning continued until September in 2008 and 2009, while in 2010, it extended to October. For female clams, initial spawning occurred in June, with 36.8 % in 2008, 10 % in 2009, and 22.2 % in 2010. The spawning period in 2009 was notably short, lasting only from June to August, whereas it spanned from June to September in 2008 and extended to October in 2010. Although SST during the onset of spawning were similar each year, a surge in chlorophyll-*a* levels during the summer of 2009 may have shortened the spawning period for Manila clams. According to Hasegawa et al. (2014), fecundity and CI were higher at sites with greater food availability. However, histological analysis showed that gametogenic development was also completed at sites with lower food supply, suggesting that factors like SST influence mone on gonad maturation. Slow maturation, affected by SST or food supply, can lead to an extended spawning period. Similarly, According to Hofmann et al. (1992), minor changes in water temperature or a 2 to 4-week shift in plankton bloom timing can significantly impact bivalve spawning, as the reproductive process is energy-intensive and depends on available food supply, stored energy reserves, or both. On Korea's west coast, studies have reported varying spawning periods, ranging from June to October (Park and Choi, 2004; Yang et al., 2012; Uddin et al., 2010, 2012). These variations in spawning periods and the initiation of gametogenesis can be attributed to spatiotemporal differences in SST, food availability, and parasitic infections (Toba and Miyama, 1995; Park et al., 2006; Dang et al., 2010; Genez et al., 2015).

The major spawning pulse can also be inferred from monthly changes in the condition index (CI), as the massive release of gametes during spawning results in significant declines in both tissue weight and CI shortly afterward. In male clams, the monthly mean CI exhibited several peaks during the spawning period, indicating multiple spawning pulses. In contrast, the monthly mean CI of female clams reached its annual peak between May and July, followed by a significant decline at the end of the spawning season. Similar patterns have been observed on the west coast of Korea, where multiple spawning pulses in Manila clams were confirmed by fluctuations in gonad somatic index (GSI) values from May to August (Park and Choi, 2004; Uddin et al., 2012). The CI is closely linked to seasonal fluctuations in the biochemical composition of clams, and the observed reductions in growth and prolonged spawning under varying environmental conditions can be attributed to seasonal changes in biotic factors influencing the clams' physiological and nutritional status (Beninger and Lucans, 1984; Baek et al., 2014).

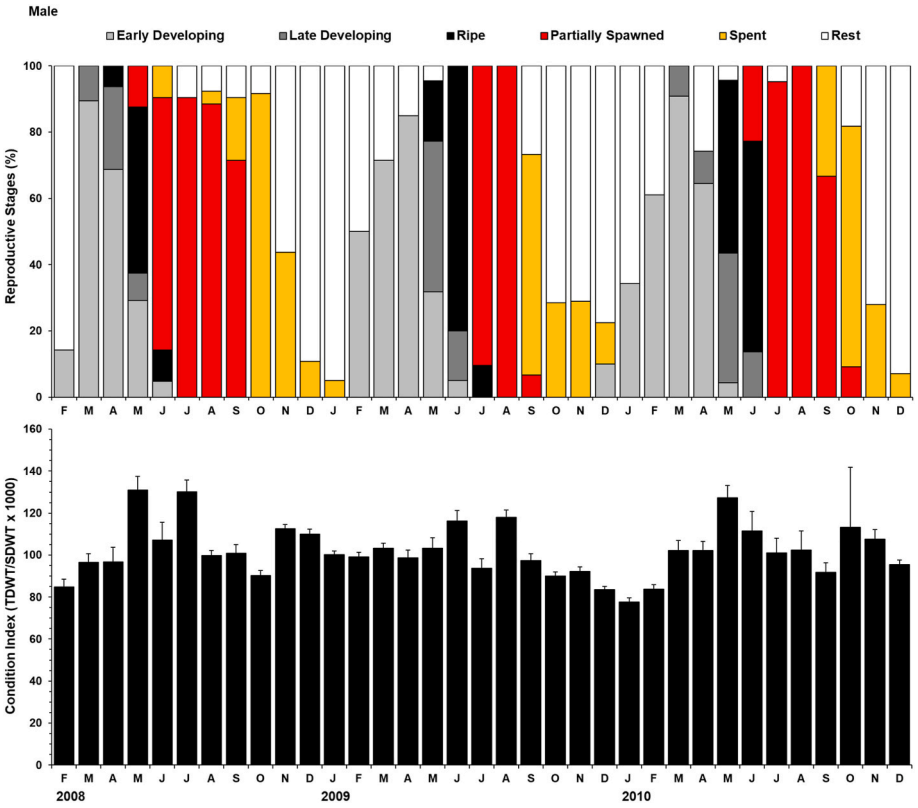


Fig. 3. Distribution of gametogenic stages and condition index (CI) of the male *Ruditapes philippinarum* collected from 2008 February to 2010 December.

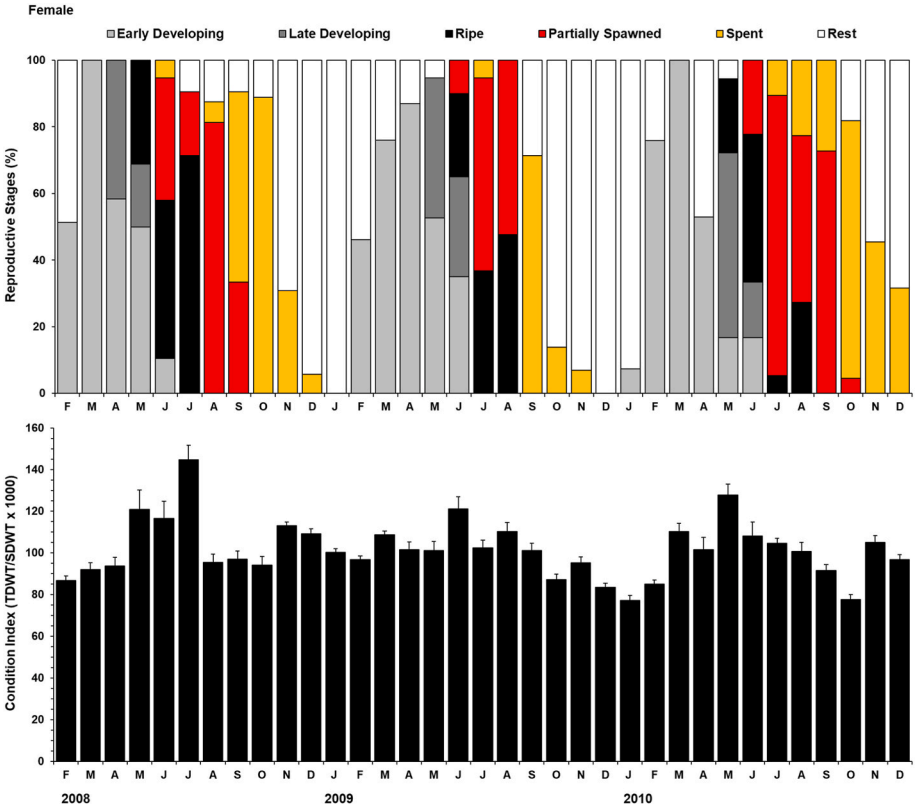


Fig. 4. Distribution of gametogenic stages and condition index (CI) of the female *Ruditapes philippinarum* collected from 2008 February to 2010 December.

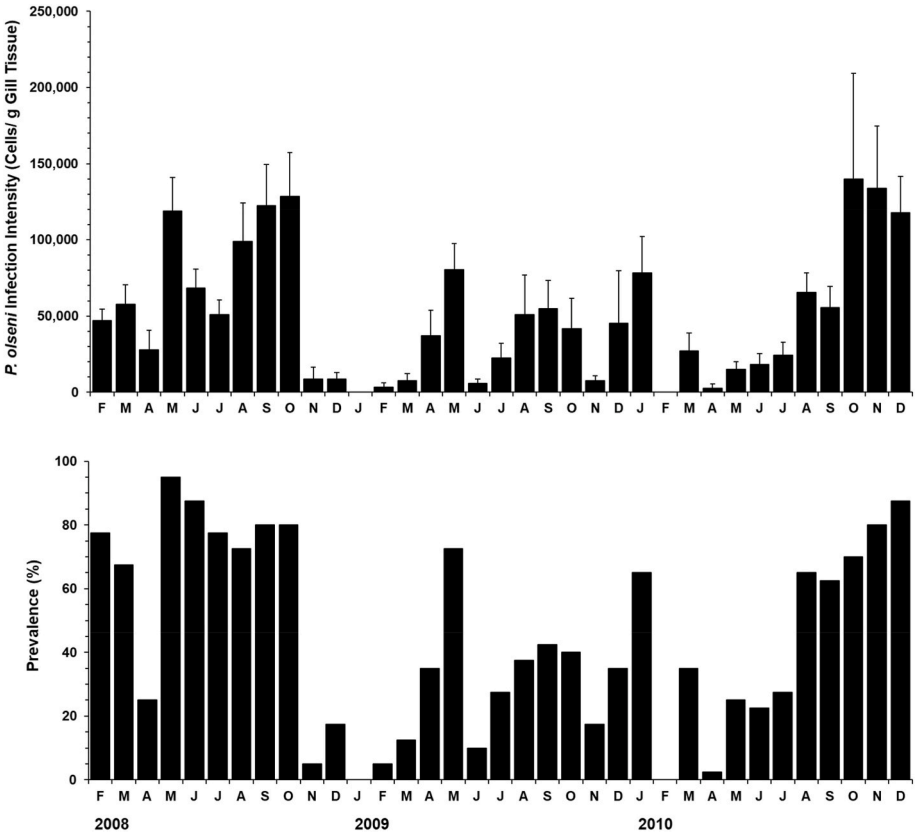


Fig. 5. Monthly mean *Perkinsus olseni* infection intensity (cells per gram in gill) and the infection prevalence in *Ruditapes philippinarum* collected from 2008 February to 2010 December.

The intensity and prevalence of *P. olseni* infection exhibited seasonality at the study site, though the pattern varied throughout the study period. Notably, both infection intensity and prevalence were comparatively lower in 2009. Compared to other studies on the west coast of Korea, the infection intensity observed in our study was markedly lower (Park and Choi, 2001; Lee et al., 2021; Yang et al., 2021; Subramaniam et al., 2024), suggesting that the physiological impact of *P. olseni* infection on Manila clam's reproduction might be less severe in our study area. Despite generally lower infection rates, we observed notable differences in the reproductive patterns of the clams that appeared to synchronize with the intensity of *P. olseni* infection. Specifically, in 2009, the clams experienced a shorter spawning period compared to 2008 and 2010. This shortened spawning period could be attributed to a combination of lower infection intensity and higher food availability in 2009. Study revealed that despite similar levels of *P. olseni* infection intensity, previous studies have shown significant differences in the GSI of Manila clams, suggesting that the combined effects of SST, food availability, and infection intensity are critical factors influencing both GSI and CI, thereby shaping reproductive effort (Subramaniam et al., 2025). Many studies have reported that *Perkinsus* infection can lead to energy depletion in bivalves, resulting in reduced growth and extended spawning period (Park et al., 2006; Lee et al., 2021; Yang et al., 2021). Our findings align with these reports, indicating that while lower infection levels may mitigate physiological impacts resulting in a short and peak spawning period, the presence of *P. olseni* still influences reproductive dynamics in Manila clams.

5. Conclusion

We observed minor variations in reproduction, particularly in gametogenesis and the spawning period, influenced by prevailing environmental conditions. This study provides valuable insights into the annual reproductive cycle of Manila clams over three years, emphasizing the impact of environmental factors such as SST and food resources. Notably, Manila clams from Naeri exhibited lower *P. olseni* infection intensity throughout the study period compared to other sites on the west coast of Korea, shedding light on the complex ecological dynamics of marine bivalve populations in dynamic coastal ecosystems.

CRediT authorship contribution statement

Thatchaneshkanth Subramaniam: Writing – original draft, Visualization, Formal analysis, Data curation. **Jeong-Hwa Kim:** Visualization, Methodology, Formal analysis, Data curation. **Kwang-Sik Choi:** Writing – review & editing, Validation, Supervision, Software, Project administration, Funding acquisition, Conceptualization. **Hyun-Sung Yang:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hyun-Sung Yang reports financial support was provided by Korea Institute of Ocean Science & Technology. Kwang-Sik Choi reports financial support was provided by National Research Foundation of Korea. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are indebted to the staff of the Shellfish Research and Aquaculture Laboratory at Jeju National University for their help in the field

sampling and laboratory analysis. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number 2019R1A6A1A03033553). This study was also partly supported by the Korea Institute of Ocean Science & Technology Project (PEA0301).

Data availability

Data will be made available on request.

References

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. https://doi.org/10.1016/j.marpolbul.2013.10.050.

Beninger, P.G., Lucans, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). J. Exp. Mar. Biol. Ecol. 79, 19–37. https://doi.org/10.1016/0022-0981(84)90028-5.

Choi, K.S., Wilson, E.A., Lewis, D.H., Powell, E.N., Ray, S.M., 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of thioglycollate method. J. Shellfish Res. 8, 125–131.

Dang, C., de Montaudouin, X., Gam, M., Paroissin, C., Bru, N., Caill-Milly, N., 2010. The Manila clam population in Arcachon Bay (SW France): can it be kept sustainable? J. Sea Res. 63, 108–118. https://doi.org/10.1016/j.seares.2009.11.003.

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. https://doi.org/10.1016/j.aquaculture.2005.10.052.

Fearman, J., Moltschaniwskyj, N.A., 2010. Warmer temperatures reduce rates of gametogenesis in temperate mussels, *Mytilus galloprovincialis*. Aquaculture 305, 20–25. https://doi.org/10.1016/j.aquaculture.2010.04.003.

Genez, P., Onal, U., Gezen, M.R., 2015. Comparison of gametogenci 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, 337–345. https://doi.org/10.2983/035.034.0216.

Gosling, E., 2003. Reproduction, settlement and recruitment. In: Gosling, E. (Ed.), Bivalve Molluscs: Biology, Ecology and Culture. Blackwell Publishing, Oxford, UK, pp. 131–168.

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, 739–746. https://doi.org/10.2983/035.033.0308.

Hofmann, E.E., Powell, E.N., Klinck, J.M., Wilson, E.A., 1992. Modeling oyster populations III. critical feeding periods, growth, and reproduction. J. Shellfish Res. 11, 399–416.

Jang, H.K., Kang, J.J., Lee, J.H., Kim, M., Ahn, S.H., Jeong, J.-Y., Yun, M.S., Han, I.-S., Lee, S.H., 2018. Recent primary production and small phytoplankton contribution in the Yellow sea during the summer in 2016. Ocean Sci. J. 53, 509–519. https://doi.org/10.1007/s12601-018-0017-z.

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, 91–101. https://doi.org/10.1007/s12601-017-0003-x.

Laruelle, F.J., Guillou, J., Paulet, Y.M., 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *Ruditapes philippinarum* on intertidal flats in Brittany. J. Mar. Biol. Assoc. U. K. 74 (2), 351–366. https://doi.org/10.1017/S0025315400039382.

Lee, H.-M., Kim, H.-J., Park, K.-I., Choi, K.-S., 2020. Enhanced growth, gonad maturation, and low-level parasite infection in juvenile Manila clam *Ruditapes philippinarum* cultured in subtidal cages on the south coast of Korea. Aquaculture 526, 735410. https://doi.org/10.1016/j.aquaculture.2020.735410.

Lee, H.-M., Park, K.-I., Yang, H.-S., Choi, K.-S., 2021. Negative impacts of *Perkinsus olseni* infection in Manila clam *Ruditapes philippinarum* observed from tidal flats in anmyeondo Island on the west coast of Korea during the post-spawning period. Ocean Sci. J. 56, 307–316. https://doi.org/10.1007/s12601-021-00024-0.

Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44, 187–200. https://doi.org/10.1016/0044-8486(85)90243-1.

Moura, P., Vasconcelos, P., Pereira, F., Chainho, P., Costa, J.L., Gaspar, M.B., 2018. Reproductive cycle of the Manila clam (*Ruditapes philippinarum*): an intensively harvested invasive species in the Tagus Estuary (Portugal). J. Mar. Biol. Assoc. U. K. 98 (7), 1645–1657. https://doi.org/10.1017/S0025315417001382.

Navarro, E., Iglesias, J.I.P., 1995. Energetics of reproduction related to environmental variability in bivalve molluscs. Haliotis 24, 43–55.

Navarro, E., Iglesias, J.I.P., Larrañaga, A., 1989. Interannual variation in the reproductive cycle and biochemical composition of the cockle *Cerastoderma edule* from Mundaca Estuary (Biscay, North Spain). Mar. Biol. 101, 503–511. https://doi.org/10.1007/BF00541652.

Park, K.-I., Choi, K.-S., 2004. Application of enzyme-linked immunosorbant assay (ELISA) for studying of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca: bivalvia) I. quantifying eggs. Aquaculture 241, 67–687. https://doi.org/10.1016/j.aquaculture.2004.08.017.

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. https://doi.org/10.1016/S0044-8486(01)00619-6.

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. https://doi.org/10.1016/j.aquaculture.2005.06.003.

Ray, S.M., 1966. A review of the culture method for detecting *Dermocystidium marinum* with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc. 54, 55–69.

Robert, R., Trut, G., Laborde, J.L., 1993. Growth, reproduction and gross biochemical composition of the Manila clam *Ruditapes philippinarum* in the Bay of Arcachon, France. Mar. Biol. 116, 291–299. https://doi.org/10.1007/BF00350019.

Subramaniam, T., Lee, H.-J., Jeung, H.-D., Kang, H.-S., Kim, C.-W., Kim, H.-S., Cho, Y.-G., Choi, K.-S., 2021. Report on the annual gametogenesis and tissue biochemical composition in the gray mussel, *Crenomytilus grayanus* (Dunker 1853) in the subtidal rocky bottom on the east Coast of Korea. Ocean Sci. J. 56, 424–433. https://doi.org/10.1007/s12601-021-00042-y.

Subramaniam, T., Cho, Y.-G., Lee, H.-M., Kim, J.-H., Shin, J.-S., Kwon, M.G., Jang, G.I., Choi, K.-S., 2024. 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. Ocean Sci. J. 59, 1. https://doi.org/10.1007/s12601-023-00125-y.

Subramaniam, T., Hong, H.-K., Park, K.-I., Cho, Y.-G., Choi, K.-S., 2025. Effects of *Perkinsus olseni* parasitism and environmental conditions on the gonad maturation and reproductive effort of female Manila clam (*Ruditapes philippinarum*) on the west and south coasts of Korea. J. Invertebr. Pathol., 108385. https://doi.org/10.1016/j.jip.2025.108385.

Sugiura, D., Kikuya, N., 2022. Cycle of reproduction, oocyte atresia, and maturation size of the Manila clam *Ruditapes philippinarum* on a pebble beach in Mutsu Bay, Northern Japan. Invertebr. Reprod. Dev. 66, 8–15. https://doi.org/10.1080/07924259.2021.1999337.

Toba, M., Miyama, Y., 1995. Influence of temperature on the sexual maturation in Manila clam, *Ruditapes philippinarum*. Suisan zoshoku 43, 305–314. https://doi.org/10.11233/aquaculturesci1953.43.305.

Toba, M., Yamakawa, H., Kobayashi, Y., Sugiura, Y., Honma, K., Yamada, H., 2007. Observations on the maintenance mechanisms of metapopulations, with special reference to the early reproductive process of the Manila clam *Ruditapes philippinarum* (Adams & Reeve) in Tokyo Bay. J. Shellfish Res. 26 (1), 121–130. https://doi.org/10.2983/0730-8000(2007)26[121:OOTMMO]2.0.CO;2.

Uddin, M.J., Yang, H.-S., Kim, H.-J., Hong, J.-S., Cho, M., Choi, K.-S., 2010. Seasonal changes in *Perkinsus olseni* infection and gametogenesis in Manila clam, *Ruditapes philippinarum*, from seonjedo Island in Incheon, off the west coast of Korea. J. World Aquacult. Soc. 41, 93–101. https://doi.org/10.1111/j.1749-7345.2009.00337.x.

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. https://doi.org/10.1016/j.aquaculture.2012.07.006.

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, 685–690. https://doi.org/10.2983/035.031.0312.

Yang, H.-S., Cho, Y.-G., Shin, J.-S., Park, H.-S., Choi, K.-S., 2021. Pathology survey of the Manila clam *Ruditapes philippinarum* from Hwangdo tidal flat in Cheonsu Bay on the west coast of Korea. Ocean Polar Res. 43, 365–370. https://doi.org/10.4217/OPR.2021.43.4.365.