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Research article

Comparative observation of the organ development and health of the tropical oyster *Crassostrea belcheri* (Sowerby, 1871) in hatchery and wild farming sites

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Abstract

The tropical oyster, *Crassostrea belcheri*, is an important, high-value seafood in aquaculture in Thailand. Understanding the development and physiological responses of *C. belcheri* in different rearing conditions is essential for sustainable production. This study compared the histological structures, mucus-secreting cell (MSC) distributions, and gonadal development of *C. belcheri* sampled from a hatchery (HAT) and three open-sea farming sites. The sampled oysters were classified into five categories based on shell height: 1.0–2.5 cm, 2.6–4.1 cm, 4.2–5.7 cm, 5.8–7.3 cm, and 7.4–8.8 cm. Size- and site-dependent differences in organ morphology were observed. In the largest HAT oysters, the average gill lamella length was 128.8 ± 7.3 µm; average mantle epithelium thickness 14.1 ± 1.0 µm and average digestive tubule diameter 86.5 ± 2.2 µm. All three morphometrics differed significantly across the four sites ($p < 0.05$). Four MSC morphotypes were observed. Gonadal development followed a protandric pattern, with male gametogenesis observed in oysters smaller than 5.8 cm and female oogenesis being dominant in larger individuals (> 5.8 cm). Oocyte maturation across sites was consistent rather than site dependent. Histopathological evaluation using the Health Assessment Index (HAI) revealed sublethal tissue alterations, including epithelial atrophy, digestive tubule regression and gill lamellar fusion, with a mean HAI score of 3.7 ± 1.2 for wild-farmed oysters compared with 1.8 ± 0.9 for HAT samples. These findings underscore strong associations between oyster size, environmental conditions and organ development, and the potential of MSC density and histopathological lesions as reliable biomarkers for aquaculture monitoring and health management in *C. belcheri*.

Keywords: Aquaculture health monitoring, *Crassostrea belcheri*, Histopathology, Mucus-Secreting Cells, Protandry.

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INTRODUCTION

The tropical oyster *Crassostrea belcheri* is an economically important bivalve in fisheries along the coast of Thailand (Sampantamit et al., 2020); however, overexploitation and environmental degradation have led to declining natural populations (Camp et al., 2015; Klinbunga et al., 2000; Sampantamit et al., 2020). The broad salinity tolerance of the species and high consumer demand have driven the expansion of hatchery-based seed production over recent decades (Chalermwat et al., 2003; Sagulsawasdipan et al., 2025; Tanyaros et al., 2008), but despite the commercial potential of *C. belcheri*, data concerning its organ development, tissue-level responses and health status across different rearing environments is limited (Sagulsawasdipan et al., 2025). The lack of biological data highlights the urgent need for relevant research to promote more sustainable aquaculture practices.

The shell height-based size distribution has long been used as a proxy for developmental status and ecological adaptation in aquatic invertebrates (Bourassa and Morin, 1995; Basset et al., 2004; Shin et al., 2005; Mouillot et al., 2006; Fey-Hofstede and Meesters, 2007; Reizopoulou and Nicolaidou, 2007; Trayanova, 2008). Studies of bivalves have demonstrated strong correlations between organ morphology, especially of the digestive gland, gills and mantle and environmental conditions, size classes and reproductive stages (Davenel et al., 2010; Loor and Sonnenholzner, 2016; Saavedra et al., 2017). Kongthong et al. (2023a) reported that the development of the digestive gland, gills and mantle of *Saccostrea cucullata* showed a positive correlation to the shell size distribution in the specific environmental context. The digestive gland of oysters is the organ primarily responsible for mucus production, feeding, locomotion and immune activities (Allam et al., 2013; Zhang et al., 2019), and while it is accepted that the mucus-secreting cells (MSCs) of bivalves are commonly located in epithelial tissues (Lopes-Lima et al., 2006; Lee et al., 2007; Espinosa et al., 2016; Joshy et al., 2022; Otegui et al., 2024), a recent study found that the digestive gland of *S. cucullata* presented the highest density of MSCs (Kongthong et al., 2023a). The morpho-histological identification of MSCs in *S. cucullata* revealed oval, cup-like, and pear-like shapes (Kongthong et al., 2023a), similar to those found in the bivalves *Solen grandis* Dunker (Xiuzhen et al., 2003), *Haliotis diversicolor* (Di et al., 2012), *Chaetopleura angulate*, and *Acanthochitona fascicularis* (Lobo-da-Cunha et al., 2022).

The internal organs of aquatic animals often show alterations, including shrinkage and tissue necrosis, as a result of exposure to hazardous chemicals and environmental stress (Hinton et al., 1992; Schrank et al., 1997). Histopathological assessment is a sensitive tool that can evaluate sublethal tissue changes that occur under laboratory conditions, environmental stress and aquaculture conditions (Adams et al., 1993; Winstead, 1995; Kang et al., 2000; Au, 2004; Kgo et al., 2006; Knowles et al., 2014; Gamain et al., 2016; Al-Hashem, 2017). Therefore, histopathological evidence can enhance our understanding of oyster health and environmental status (Kang et al., 2000). In the case of farmed *C. iredalei*, a histopathological assessment revealed alterations that included hyperplasia of digestive diverticula and the presence of granulocytomas, indicating increased mass mortality (Hong et al., 2017). However, the health of farmed *C. belcheri* has been studied comparatively only by Sagulsawasdipan et al. (2025), who found that *C. belcheri* in an enclosed rearing condition showed few histopathological alterations, indicating that the rearing condition was healthy.

To address the knowledge gaps mentioned above, the current study presents an integrated morphometric and histological comparison of *C. belcheri* across five shell height classes covering a range from 1.0 to 8.8 cm. Specimens were sourced from a hatchery and three coastal farming sites in Trang Province, southern Thailand. By evaluating organ structures, MSC characteristics, and gonadal development using standardized histological techniques and Health

Assessment Index (HAI) evaluations, the data from this study are expected to guide broodstock selection, oyster health monitoring, and inform sustainable aquaculture management.

MATERIALS AND METHODS

Hatchery-based oyster cultivation

Sexually mature *C. belcheri* specimens (designated as HAT) were sampled from the Marine Shellfish Breeding Research Unit, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya (RUTS), Trang Campus, Thailand. The selected specimens were reared at the same location under controlled hatchery conditions, as follows. Following induced spawning, fertilized eggs and free-swimming larvae were cultured in a flow-through seawater system equipped with a sand-filtration unit. Upon reaching the spat stage, oysters were allowed to settle on shell substrates and were subsequently reared under standardized environmental and nutritional conditions. Thirty sampled oysters with shell sizes ranging from 1.0 to 8.8 cm were randomly and finally used in this study. Thirty specimens were stratified into the following five shell size classes: 1.0–2.5 cm, 2.6–4.1 cm, 4.2–5.7 cm, 5.8–7.3 cm, and 7.4–8.8 cm (6 individuals per size class). All procedures were performed under the supervision of the Rajamangala University of Technology Srivijaya Animal Care and Use Committee under process number IAC 13-07-2025.

All samples were fed with a mixed algal diet containing *Chaetoceros calcitrans* and *Tetraselmis suecica* at a concentration of 6% (dry algal weight to dry meat weight). Water quality parameters, including temperature, salinity, and dissolved oxygen (DO), were monitored throughout the experiment using a YSI ProDSS multiparameter meter. The accurate calibration services were performed by a specialist company.

Field sampling and study sites

Wild-farmed *C. belcheri* specimens were collected from three open-sea farming sites in Trang Province, Thailand: Laem Makham (LM), Ban Laem (BL), and Libong island (LI) (Figure 1). All sites are shared and located close to dense mangroves, but LI is also surrounded by urban development. Thirty specimens were stratified into five shell size classes analogous to the HAT specimens. Live oysters were transported in aerated containers to the Oyster Laboratory at RUTS for further processing and analysis.

Morphometric and histological analysis

Morphometric measurements and histological examinations were conducted at the Histology Laboratory, Medical Science Academic Service Centre, Faculty of Medical Science, Naresuan University (accredited lab ID: 2-0100-0004-8). Shell height, defined as the maximum distance between the anterior and posterior shell margins, was measured using a precision Vernier caliper with an accuracy of ±0.01 mm, following the Aquatic Invertebrate Histology Manual (Howard et al., 2004).

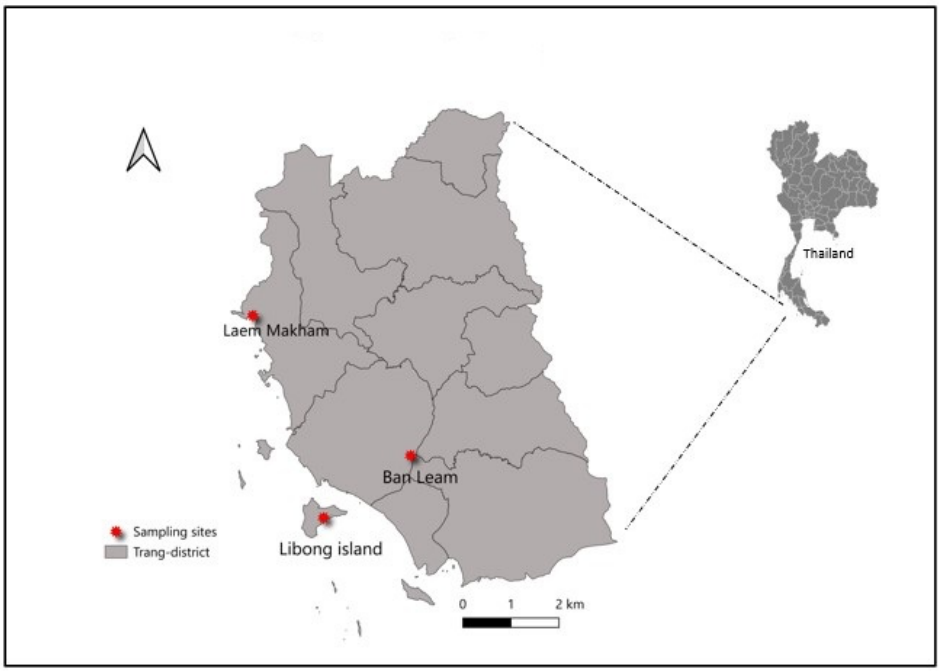

Figure 1 Map of representative sampling sites in Thailand including three wild farming (Laem Makham (LM), Ban Leam (BL), and Libong island (LI)).

Oysters were euthanized according to established guidelines for aquatic invertebrates (Leary et al., 2013). Tissue samples were fixed in 10% neutral buffered formalin for 24 h at room temperature and subsequently transferred to 70% ethanol for storage. Shells were decalcified using a standard decalcifying solution before paraffin embedding. Tissue sections were cut to a thickness of 4 µm using a rotary microtome. Two histological staining techniques were applied: hematoxylin and eosin (H&E) for general tissue morphology, and periodic acid-Schiff (PAS) for the detection of mucopolysaccharides (Kongthong et al., 2023b). Evaluated histological parameters included gill lamellae length, mantle epithelial thickness, digestive tubule diameter (Yonge, 1928), gonadal development stage (Kandeel et al., 2013; Morad et al., 2018), and type and density of MSCs (Kongthong et al., 2023a). Histological images were captured using a Panoramic Viewer system (3DHISTECH, Hungary) under a light microscope. The HAI for each oyster was determined based on the methodology described by Adams et al. (1993) to assess the health status of individual specimens. The HAI values were calculated as HAI = (1 × SI) + (10 × SII) + (100 × SIII), where SI, SII, and SIII represented the number of observed changes at stages I, II, and III, respectively. The obtained HAI values were subsequently used to analyze correlations with the spatial distributions of the oysters.

Statistical analysis

All quantitative data were displayed as means ± standard error (SE). Differences in morphometric parameters, MSC density, and gonadal development stages across shell size classes and between rearing environments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$. All statistical analyses were performed using GraphPad Prism and R software (version 5.0).

RESULTS

Water quality parameters across sampling sites

Water quality parameters, including pH, temperature, salinity, and dissolved oxygen (DO), were measured at HAT, LM, BL, and LI (Table 1). Water pH at the studied areas ranged from 7.18 ± 0.02 at HAT to 7.67 ± 0.33 at LM. Temperature was highest at BL (31.67 ± 1.20 °C) and lowest at HAT (27.13 ± 0.55 °C). Salinity levels were highest at HAT (26.67 ± 0.67 ppt) and lowest at LB (9.00 ± 1.53 ppt). DO concentrations ranged from 4.50 ± 0.58 mg/L at BL to 5.83 ± 0.67 mg/L at LB. Additionally, both temperature and salinity differed significantly among sampling sites ($p < 0.05$).

Table 1 The water quality parameters at the hatchery, Laem Makham, Ban Laem, and Libong Island sampling sites.

Water parameters	Hatchery	Laem Makham	Ban Leam	Libong island
pH	7.18±0.02	7.67±0.33	7.33±0.17	7.5±0.29
Temperature (°C)	27.13±0.55	31.67±0.33[a]	31.67±1.20[a]	29.67±0.33
Salinity (ppt)	26.67±0.67	23.00±1.16[b]	15.67±1.86[a]	9.00±1.53[abc]
Dissolved Oxygen (DO, mg/L)	5.43±0.24	5.50±0	4.50±0.58	5.83±0.67

Note: Significant differences between sampling sites (a vs HAT, b vs Ban Leam, c vs Laem Makham, $p < 0.05$

General histological features

Transverse histological sections of *C. belcheri* revealed the internal organization of key visceral organs, including the digestive gland, gills, mantle and gonads (Figures 2A–2F). The gill was positioned ventrally/laterally to the visceral mass, comprised of four pairs of ciliated filaments supported by connective tissue. Each filament contained multiple lamellae lined with ciliated columnar epithelium, facilitating respiration and particle filtration (Figures 2A-2E).

The mantle lined the inner shell surface and consisted of dorsal, ventral, and posterior lobes. The outer epithelial fold was smooth and separated from the inner folds by a periostracal groove (Figures 2A-2D). Mantle tissue was supported by underlying connective tissue, which also anchored the adjacent digestive gland and gonads.

The digestive gland was centrally located within the large and complex visceral mass surrounding the stomach. Composed of branched digestive tubules lined with secretory and absorptive epithelial cells (Figures 2A–2F), it was embedded in connective tissue that linked it to the mantle and gonadal tissues.

Gonadal tissues were distributed throughout the connective matrix of the visceral mass. In males (Figures 2A–2C), the gonads appeared as lobular structures composed of germ cells at various stages of spermatogenesis. In females (Figures 2D–2F), follicular oocytes and mature oocytes were found within organized ovarian follicles. The presence of a germinal epithelium lining indicated active gametogenesis in both sexes.

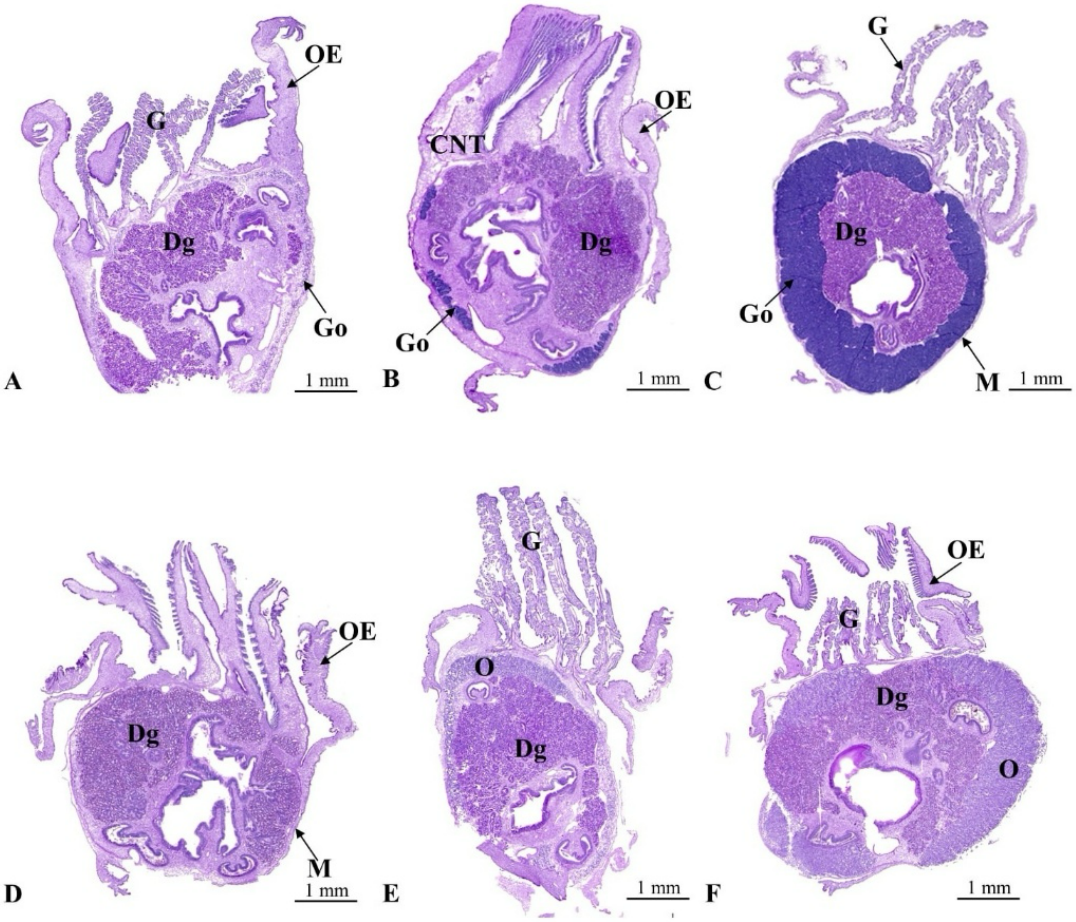

Figure 2 Transverse histological sections of *Crassostrea belcheri* showing the general histological organization of key internal organs, including the gills (G), digestive gland (Dg), gonads (Go), and ovary (O); (A-C) male gonads at various development stages, (D-F) female gonads with developing oocytes. The mantle (M), outer mantle epithelium (OE) and connective tissue (CNT) are shown.

Shell size distribution and associated organ development

HAT oysters displayed greater average shell sizes and more advanced organ development than oysters from the open-sea farming sites. The digestive gland appeared as a basophilic mass comprising multiple blind-ending diverticula connected to the stomach by primary and secondary ducts (Figure 3A). Digestive tubules were lined with columnar epithelial cells with basophilic cytoplasm. In contrast, ducts bore tall columnar cells with microvilli (Figure 3B). Tubule diameter significantly increased with shell size, from 45.42 ± 1.10 µm in the smallest oysters (1.0–2.5 cm from LM) to 86.49 ± 2.20 µm in the largest (HAT) ($F = 97.20$, $p < 0.01$) (Figure 3C).

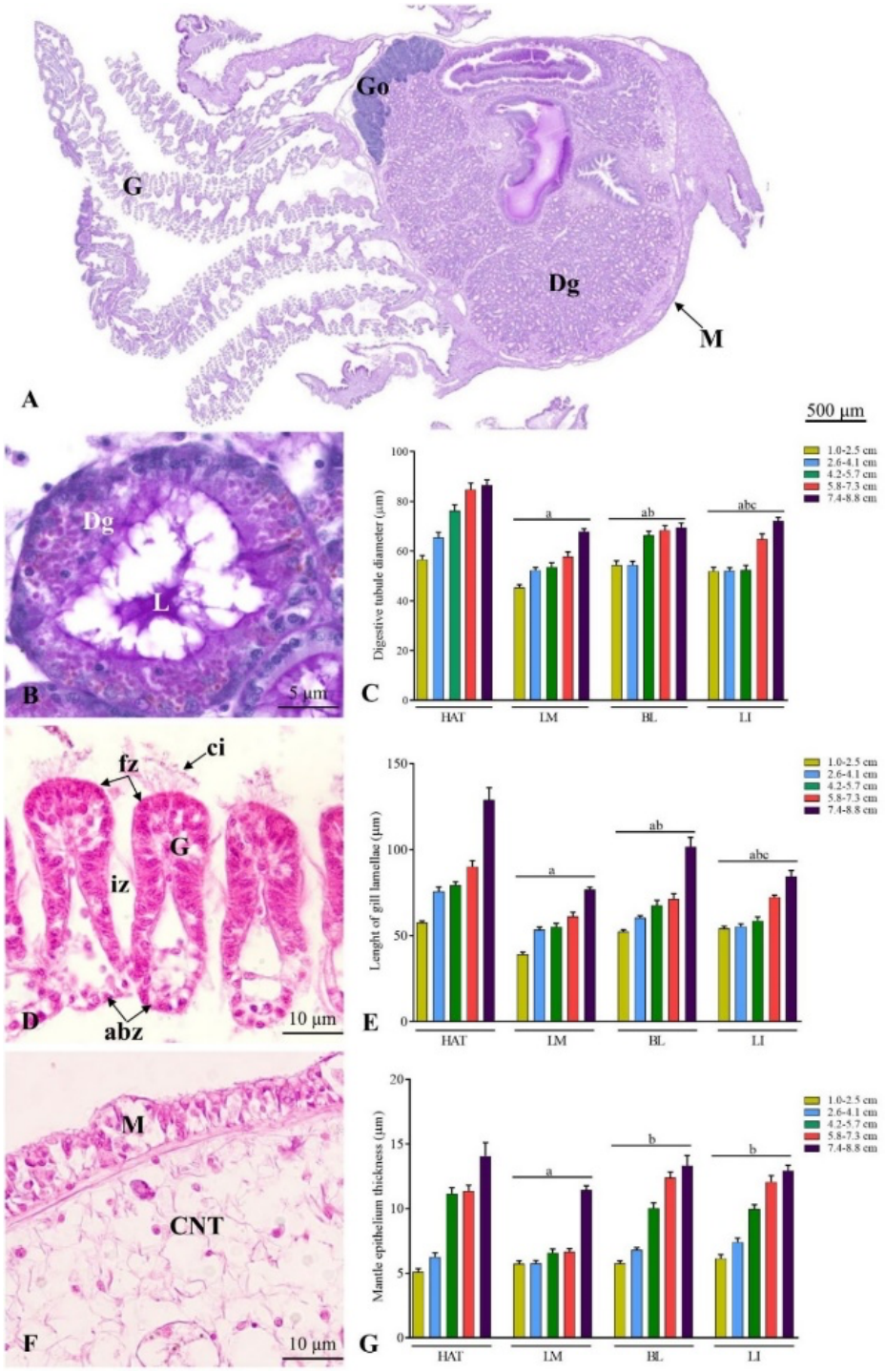

Figure 3 Histological structures and morphometric comparisons of the digestive gland, gill, and mantle epithelium in *Crassostrea belcheri* across shell height classes and four sites. (A) Cross-section showing digestive gland (Dg), gonad (Go), gill (G), and mantle (M); (B) Digestive tubule with visible lumen (L); (C) The chart shows the mean digestive tubule diameter of oysters sampled from four locations; (D) Gill lamellae showing frontal zone (fz), intermediate zone (iz), abfrontal zone (abz), and cilia (ci); (E) The chart shows mean gill lamellae length; (F) Mantle epithelium and connective tissue (CNT); (G) The chart shows mean mantle epithelium thickness. In all charts, significant differences ($p < 0.05$) among shell height classes are indicated by different letters (a, b, c).

The gill structure (ctenidia) was V-shaped and located among the visceral mass (Figure 3A). Each filament comprised three zones: frontal (ciliated columnar cells), intermediate (non-ciliated absorptive cells with acid polysaccharides), and abfrontal (hemolymph vessels, mixed ciliated and absorptive cells with neutral polysaccharides) (Figure 3D). Gill lamellae length increased significantly with shell size, from 39.16±1.34 µm (1.0–2.5 cm from BL) to 128.8±7.25 µm (7.4–8.8 cm from HAT) ($F = 101.3$, $p < 0.01$; Figure 3E).

Mantle epithelium, comprising outer and inner epithelial layers separated by connective tissue (Figures 3A, 3F), exhibited increased thickness with shell size from 5.11±0.25 µm to 14.08±1.04 µm ($F = 39.17$, $p < 0.01$; Figure 3G).

Mucus-secreting cell distribution

MSC distributions in *C. belcheri* were assessed in digestive tubules, gill filaments, and mantle epithelium using PAS staining. MSCs stained positively for neutral polysaccharides and exhibited four distinct morphotypes: circle, cup-like, pear-like, and stick-like (Figures 4A–4C).

MSC density varied by tissue type, shell size, and sites. Overall, the sampled oysters from the natural farm sites (LM, BL, LI) had higher MSC densities than HAT oysters, particularly in the gills (Figures 4D–4G).

In digestive tubules, the highest MSC density (11.13 ± 5.31 cells) occurred in 7.4–8.8 cm oysters from LM, while the lowest (0.38 ± 0.24 cells) was recorded in 2.6–4.1 cm oysters from HAT (Figure 4E). Differences among sites were significant ($F = 4.805$, $p < 0.05$).

Gill filaments displayed the highest overall MSC densities. The peak (10.00 ± 3.97 cells) was observed in the abfrontal zone of 5.8–7.3 cm oysters from LM, whereas HAT oysters showed consistently lower densities (2.00 ± 0.04 to 4.88 ± 1.86 cells) across all size classes (Figure 4F).

The density of MSCs in the mantle epithelium was lower than in the gills. The highest density (5.13 ± 1.53 cells) occurred in 5.8–7.3 cm oysters from HAT, and the lowest (1.63 ± 0.85 cells) in 7.4–8.8 cm oysters from BL (Figure 4G). Sites-based differences in MSC density in the mantle were statistically significant ($F = 4.250$, $p < 0.05$).

An increasing MSC trend with shell size was noted in digestive tubules, particularly at BL, while trends in gill and mantle tissues varied. MSC densities in the largest size class (7.4–8.8 cm) varied by site, with LM and BL showing relatively high values, while HAT exhibited a lower density in this class.

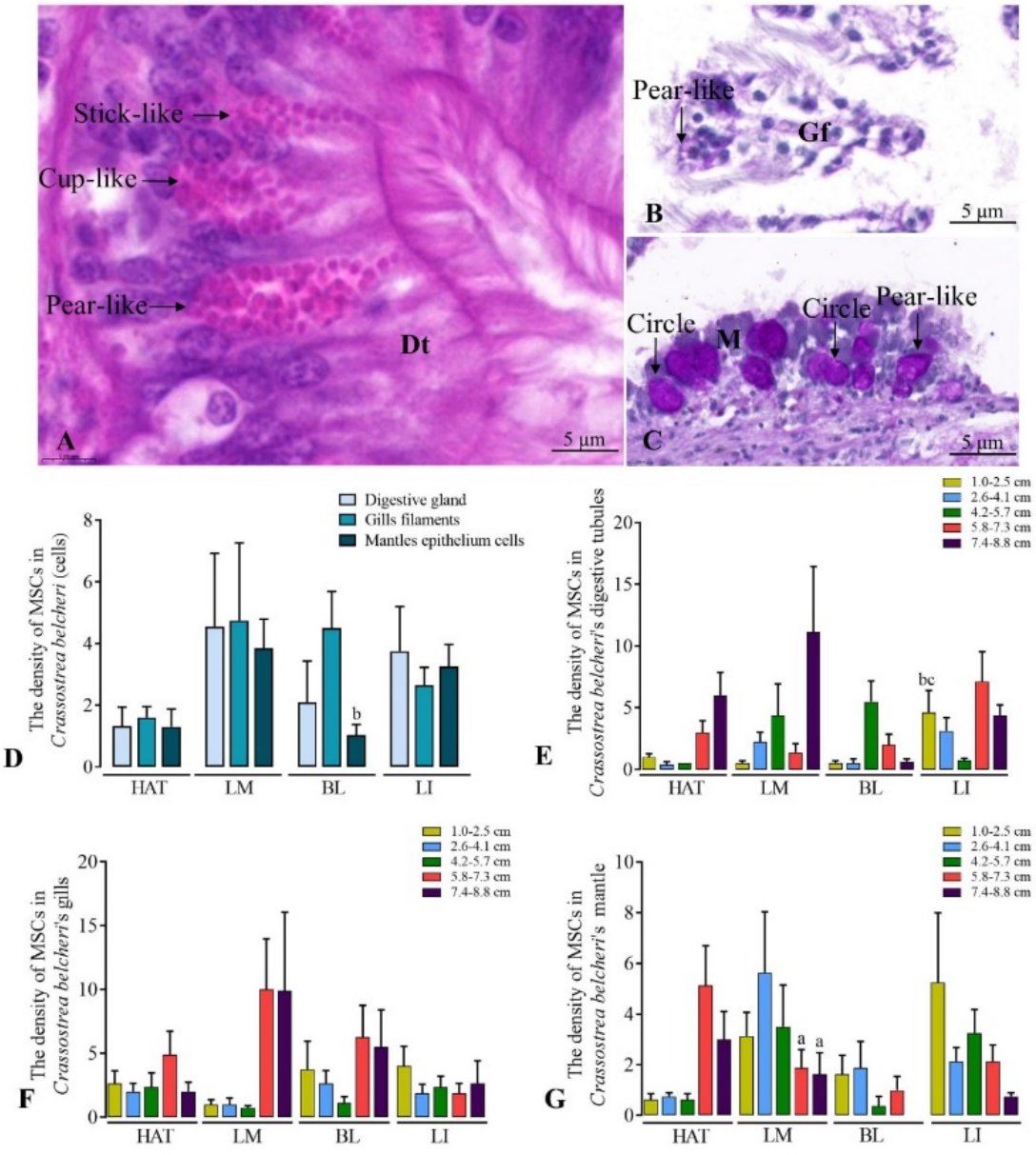

Figure 4 Histological distribution and density of mucous-secreting cells (MSCs) in the digestive tubules, gill filaments, and mantle epithelium of *Crassostrea belcheri* across five shell size classes and four sites (HAT, LM, BL, and LI). (A–C) MSCs identified by periodic acid-Schiff (PAS) staining in the digestive tubules (Dt), gill filaments (Gf), and mantle epithelium (M), show four distinct morphologies indicated by arrows. (D) The chart shows overall MSC densities in three types of tissue across all four sites. (E–G) The charts show MSC densities in digestive tubules (E), gill filaments (F), and mantle epithelium (G) across shell size classes and sites. Different letters indicate statistically significant differences ($p < 0.05$).

Gonadal development in relation to shell size distribution and sites

In males, the gonads were enclosed in a dense connective tissue capsule as well as the testicular wall (Figures 5A-5B). They contained organized testicular compartments (Figures 5A-5B). Spermatogenesis progressed through distinct cellular stages, beginning with spermatogonia, followed by primary spermatocytes, secondary spermatocytes, spermatids, and culminating with mature spermatozoa (Figures 5C–5E). The distribution and abundance of these cell types varied by shell size and sites. Early-stage spermatogonia were predominant in 1.0–2.5 cm oysters from LM (mean = 31.67±4.37 cells), whereas mature spermatozoa peaked in 5.8–7.3 cm oysters from HAT, indicating a positive correlation between shell size and reproductive maturity. Female gonads were first observed in oysters ≥ 5.8 cm, particularly at LM and BL. Oogenesis was classified into four stages: oogonia, pre-vitellogenic, vitellogenic, and post-vitellogenic oocytes (Figures 6A–6J). Follicular oocytes were observed within ovarian follicles; developing oocytes were found in the follicular lumen (Figures 5C–5E).

Mean oocyte counts per oogenic stage were as follows: oogonia (21.67 ± 5.24 cells), pre-vitellogenic oocytes (17.67 ± 0.88 cells), and vitellogenic oocytes (21.00 ± 2.08 cells). These stages were most frequently found in oysters from LM and BL. No significant differences in oogenic stage distribution were found among sites ($F = 2.503$, $p > 0.05$, Figure 6K).

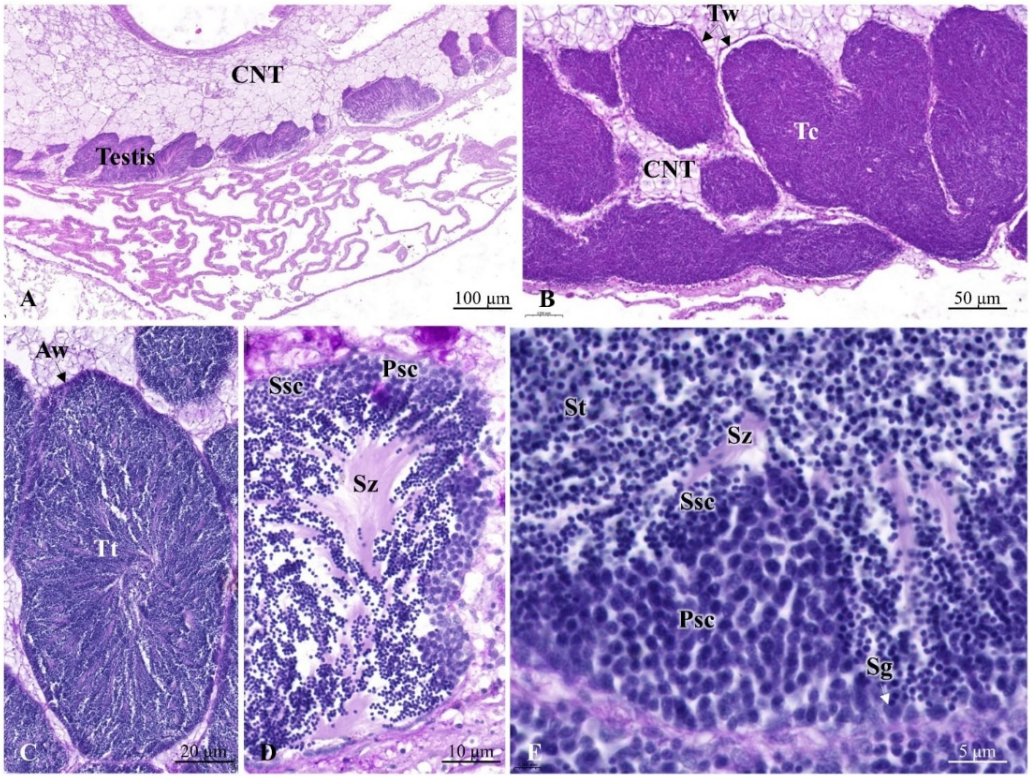

Figure 5 Light micrographs show the histological structure of the testis and spermatogenic stages in the male gonad of *Crassostrea belcheri*. (A) General view of the testis; (B) Testicular compartment (Tc) enclosed by connective tissue (CNT) and testicular walls (Tw); (C) Transverse section of testicular tubules (Tt); (D) Presence of primary spermatocytes (Psc), secondary spermatocytes (Ssc), and spermatozoa (Sz); (E) Higher magnification showing spermatogonia (Sg), primary spermatocytes (Psc), secondary spermatocytes (Ssc), spermatids, and spermatozoa (Sz) within seminiferous tubules (St).

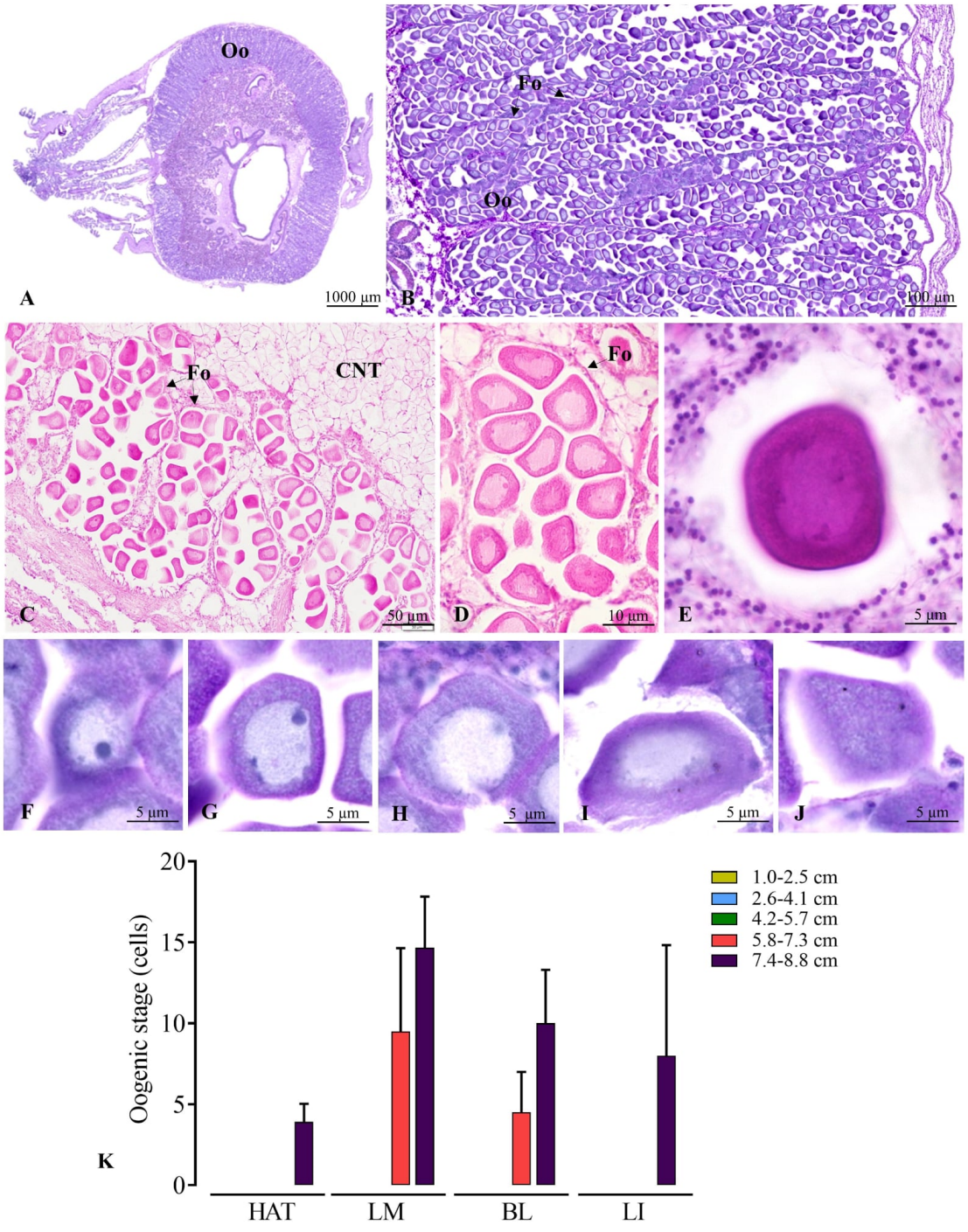

Figure 6 Histological structure of the ovary shows oogenic stages in female gonads of *Crassostrea belcheri* across different shell size classes and sites. (A–B) General view and organization of the ovary with developing oocytes (Oo) and follicular oocytes (Fo); (C–E) Cross-sections of ovarian follicles surrounded by connective tissue (CNT); (F–J) Oocyte developmental stages: oogonia, pre-vitellogenic, early vitellogenic, vitellogenic, and post-vitellogenic oocytes, respectively; (K) The charts show mean numbers of oogenic cells for each shell size class across all four sites.

Health assessment index

Histopathological assessment revealed sublethal lesions indicative of environmental stress. Oysters from BL exhibited the highest lesion severity across all tissues (Figures 7A-7C), as reflected by significantly higher HAI scores ($p < 0.05$; Figures 7D–7F).

In the digestive gland, histological changes included epithelial atrophy, digestive tubule regression, and necrosis, with significantly higher lesion scores at LM and BL than at HAT ($p < 0.05$; Figure 7D).

Gill tissue lesions included lamellar fusion, hemocytic infiltration and mucocyte proliferation, which were most pronounced in LM oysters (Figure 7B). Lesion severity in gills was significantly higher at BL than at HAT and LM ($p < 0.01$, $p < 0.001$; Figure 6E).

In mantle tissues, large lipid vacuoles and elevated myocyte density were observed, particularly in BL and LI oysters (Figure 7C). Mantle lesion scores were significantly greater at BL and LM than at HAT ($p < 0.05$, $p < 0.01$; Figure 6F).

The relationships between environmental factors (salinity and temperature) and the HAI scores of oyster organs from different sites were analyzed. The water quality parameters showed a strong and statistically significant correlation between the tissues analyzed and the environmental factors at all four sites ($p < 0.01$, $p < 0.001$, $p < 0.0001$; Figures 8A-8G).

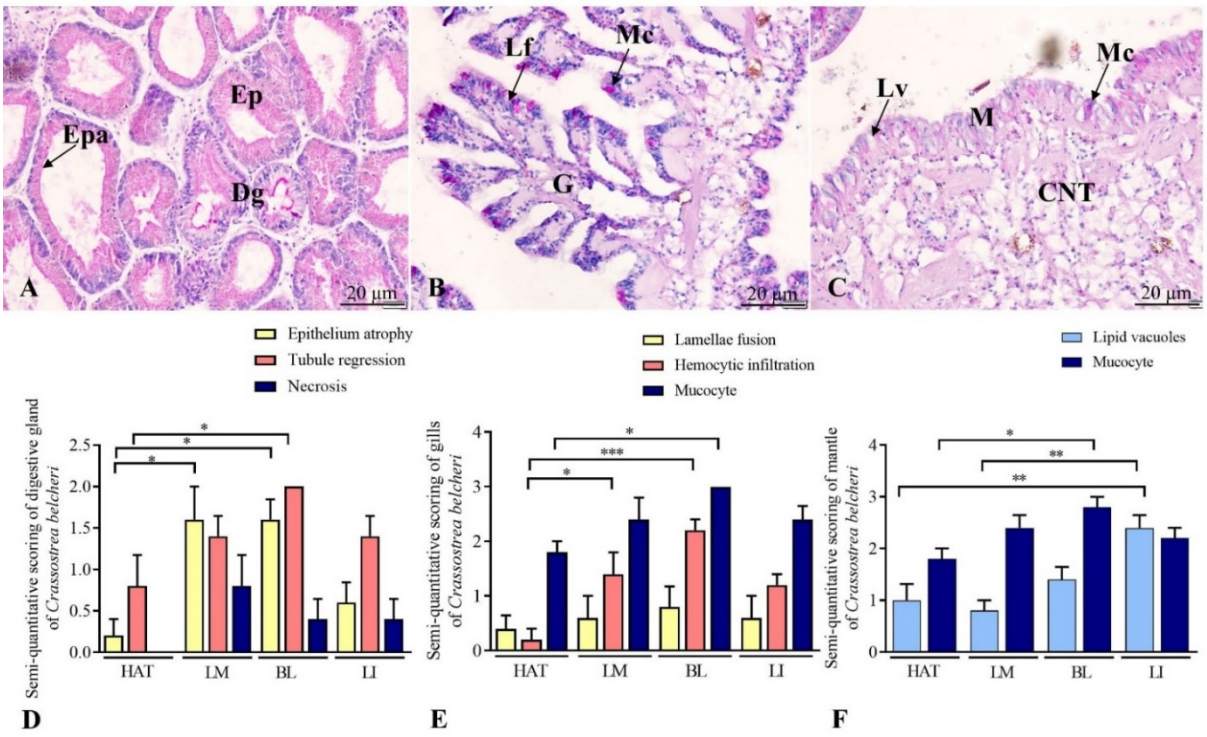

Figure 7 Histopathological alterations and semi-quantitative scoring of tissue lesions in digestive gland, gills and mantle of *Crassostrea belcheri* across different sites. (A–C) Representative micrographs of tissue lesions: (A) Digestive gland showing epithelial atrophy (Epa), tubule degeneration (Dg), and necrosis (Ep); (B) Gill with lamellar fusion (Lf), hemocytic infiltration (G), and mucocyte presence (Mc); (C) Mantle tissue displaying lipid vacuoles (Lv) and myocytes (Mc) adjacent to connective tissue (CNT). (D–F) Semi-quantitative Health Assessment Index scoring of lesions in digestive gland, gill and mantle tissues. Asterisks indicate statistically significant differences among sites (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

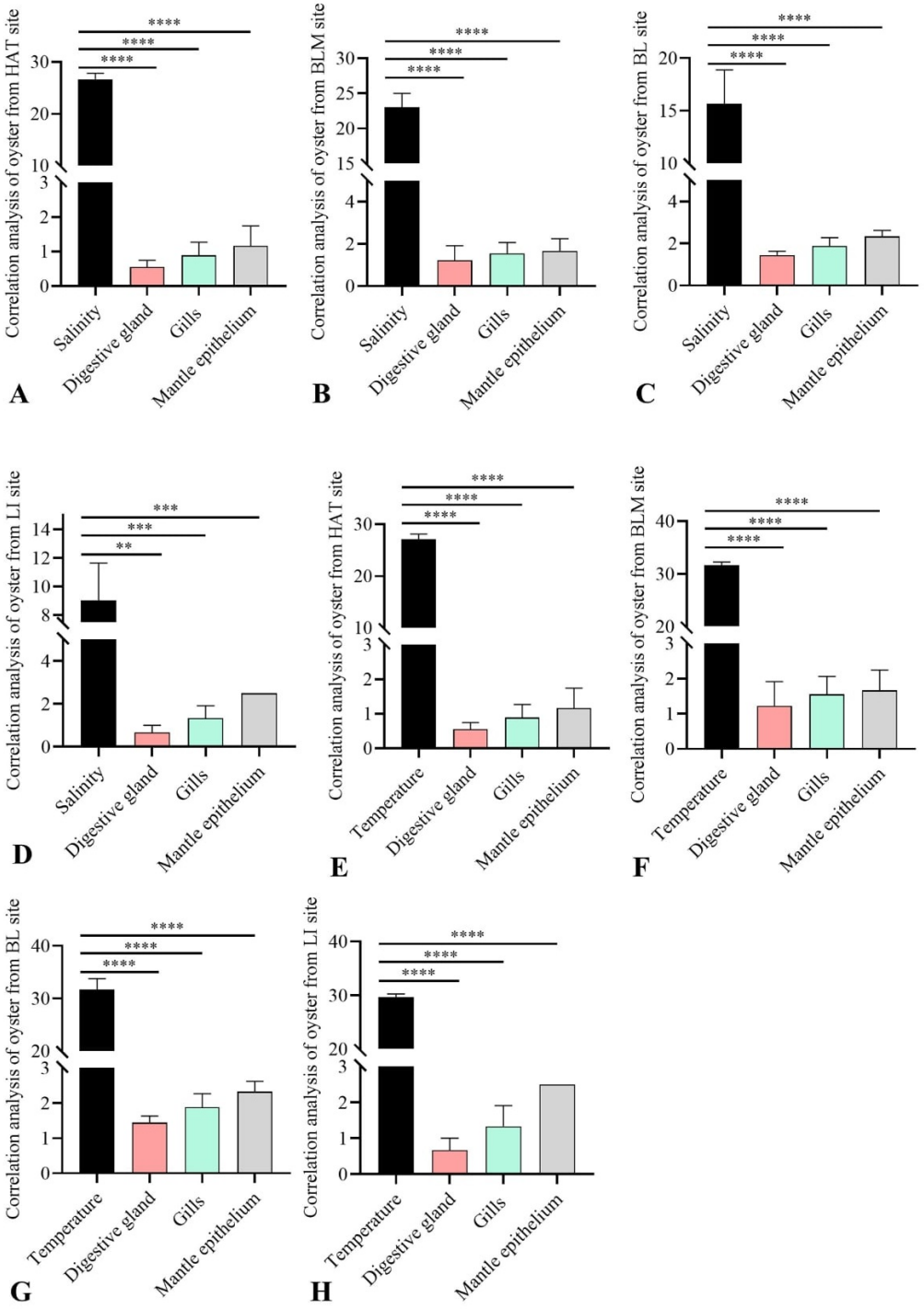

Figure 8 The analysis of correlations between water quality parameters and the Health Assessment Index scores of hatchery-reared and wild-farmed *Crassostrea belcheri* focused on the data acquired from digestive gland, gills, and mantle epithelium. Statistical analyses were conducted to examine the associations between salinity levels and organ health at four sites: (A) The hatchery (HAT), (B) Laem Makham (LM), (C) Ban Laem (BL), and (D) Libong island (LI). The influence of temperature on organ health was assessed at the same sites: (E) HAT, (F) LM, (G) BL, and (H) LI. Asterisks indicate statistically significant differences among sites (**p < 0.01, ***p < 0.001, ****p < 0.0001).

DISCUSSION

The present study provided an integrative assessment of the morphometric, histological, reproductive, and pathological characteristics of *C. belcheri* collected from four coastal locations in Thailand. The findings underscore the influence of site-specific environmental conditions on oyster growth, tissue integrity, reproductive development and overall health status.

Histological observations clearly identified key organs, including the digestive gland, gills, mantle and gonads, that revealed a conserved internal organization across all sites**.** The anatomical features observed in *C. belcheri* were consistent with those reported in other bivalves, such as *Limnoperna fortunei* (Freitas et al., 2022) and *Pinctada margaritifera* (Jabbour-Zahab et al., 1992). The well-developed gill lamellae and mantle folds highlighted their essential functions in respiration, filtration, and shell secretion. The central positioning of the digestive gland and its anatomical integration with reproductive tissues emphasize the physiological coordination between digestion and gametogenesis.

MSCs were distributed throughout the gills, digestive gland, and mantle epithelia, exhibiting circle, cup-like, pear-like, and stick-like morphologies. All MSCs stained positively with PAS, indicating secretion of neutral polysaccharides and glycoproteins, as commonly found in *S. cucullata* (Kongthong et al., 2023). Notably, high densities of MSCs, particularly in 5.8–7.3 cm oysters from LM, suggested an enhanced mucosal response, potentially linked to increased food filtration activity or immune defense. These findings are consistent with previous studies by Espinosa et al. (2016) and Zannella et al. (2017), which documented the morphological plasticity of MSCs in response to environmental conditions (Espinosa et al., 2016; Zannella et al., 2017). Thus, we also propose that the characteristics of MSCs can serve as sensitive biomarkers of ecological stress or microbial exposure in coastal sites.

Gonadal development in *C. belcheri* was strongly correlated with shell size; early spermatogenesis was observed in oysters as small as 2.6 cm, while the development of spermatozoa first showed in oysters > 5.8 cm. Oogenesis with mature oocytes became evident only in individuals exceeding 5.8 cm. These findings, along with those from studies of *C. brasiliana*, *C. rhizophorae* and *C. gasar* (Ferreira et al., 2006; Rodríguez-Jaramillo et al., 2008; Gomes et al., 2014; Castilho-Westphal et al., 2015), suggest that female reproductive investment may require higher energy reserves or more stable environmental conditions. The identification of all spermatogenic stages from spermatogonia to mature spermatozoa across shell size classes further confirmed the progressive maturation process. Additionally, oocyte classification into oogonia, pre-vitellogenic, vitellogenic, and post-vitellogenic stages underscores the asynchronous nature of oogenesis. The lack of significant differences in oogenic stage abundance among sites suggested a relatively synchronized reproductive cycle, possibly buffered by internal regulatory mechanisms despite environmental variability. All specimens in this study had mature gonads, and this study can report for the first time an estimated shell size distribution of more than 5.8 cm for male and female oysters at first maturity.

Histopathological examination revealed substantial tissue alterations in oysters from BL, including digestive epithelial atrophy, tubule regression, gill lamellar fusion, and hemocytic infiltration**.** These lesions were associated with significantly higher HAI scores**,** indicating elevated physiological stress. The presence of abnormally dense mucocytes and myocytes further suggested chronic exposure to environmental stressors such as pollutants, hypoxia or temperature fluctuations. Comparable lesions have been documented in bivalves inhabiting contaminated estuarine environments (Costa et al., 2013). Similar findings have been reported by Latchere et al. (2018) and Sarinsky et al. (2005), who found that oyster growth was susceptible to water quality and habitat productivity (Present study; Latchere et al., 2018; Sarinsky et al., 2005). These results support the utility

of HAI as a biomonitoring tool and raise concerns regarding the environmental status of the BL site.

CONCLUSIONS

The comprehensive evaluation of *C. belcheri* from four sites in Thailand revealed site-specific differences in morphometric and histological development across shell size classes. Two primary developmental life stages - juvenile (<5.8 cm) and adult (>7.3 cm) were identified, with internal organ maturation most advanced in oysters from HAT. It is proposed that the male and female sampled oysters reached first maturity when their shell size was greater than 5.8 cm. Oysters exhibited marked histopathological alterations, indicated by elevated HAI scores. The alterations included epithelial atrophy and lamellar fusion and could be caused by the environmental situation at that site. Furthermore, the results provide valuable baseline data to inform sustainable aquaculture practices, particularly with regard to site selection and the appropriate developmental stage at which to start cultivation. Moreover, the continued application of histopathological and MSC density-based assessments could improve early detection of environmental degradation and contribute to the conservation of oyster populations and the long-term health of coastal ecosystems in Thailand.

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Kitiya Kongthong: Methodology (supporting); Formal analysis (supporting); Writing – review & editing (supporting).
Narit Thaochan: Formal analysis (supporting); Writing – review & editing (supporting). **Sirilak Dusitsittipon:** Formal analysis (supporting); Writing – review & editing (supporting).
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