MORPHOLOGICAL AND ENDOCRINE PROFILING OF OVARIAN DEVELOPMENT IN GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* REARED IN PINGTUNG, TAIWAN

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The giant freshwater prawn (*M. rosenbergii*) is a major aquaculture species in Asia, and reproductive efficiency directly determines hatchery productivity. Accurate ovarian staging is therefore critical for broodstock management and seed production. In this study, we established an integrated ovarian developmental staging framework combining gross morphology, histological characterization, quantitative oocyte proportion analysis, and Gonadosomatic Index (GSI). Female broodstock were maintained under controlled laboratory conditions. Ovaries were processed using standard paraffin histology and Hematoxylin–Eosin staining. Oocyte diameter and proportional composition were quantified using image analysis software ImageFocus Plus (Euromex; Duiven, The Netherlands). Six gross ovarian stages were defined based on color transition, size, and anatomical extension. Sampled specimens were categorized from Stage I (Transparent/Immature) to Stage VI (Dark Orange/Mature). Histological analysis revealed a clear progression from Oogonium (8-10 μm) to Mature oocytes (160-170 μm) characterized by heavy yolk granule accumulation (vitellogenesis). Quantitative proportional analysis provided objective classification of ovarian stages. While body weight showed no significant correlation with maturation stage, GSI (%) increased progressively from 0.11±0.03 in early stages to 4.05±0.88 at Stage VI. Endocrine regulation of vitellogenesis is mediated through the eyestalk-hepatopancreas-ovary axis. Vitellogenesis- inhibiting hormone (VIH) from the eyestalk X-organ/Sinus gland complex suppresses vitellogenin (Vg) synthesis. The reduction of inhibitory signaling stimulates both hepatopancreatic and ovarian Vg production, leading to subsequent yolk deposition within developing oocytes. The transition from previtellogenic (Stage III) to vitellogenic stages (Stage IV-V) corresponds with increased yolk granule accumulation and significantly elevated GSI values. This integrated staging system enhances broodstock selection accuracy and provides a practical and physiological reference model for crustacean reproductive research and hatchery management.



Figure 1. Procedural workflow for ovarian tissue sampling and gross morphological documentation in *Macrobrachium rosenbergii*. (A) The anatomical location of the ovary was initially identified, situated dorsally above the hepatopancreas within the cephalothorax. (B) The upper exoskeleton (carapace) was carefully excised using micro-dissection scissors along the lateral sides to expose the internal organs. (C) The intact ovarian tissue was meticulously dissected away from the surrounding connective tissues and hepatopancreas using fine forceps. (D) Gross morphological evaluation, measurement, and digital photographic recording were immediately performed to document the specific coloration, volume, weight and anatomical extension of the gonad prior to subsequent histological processing.

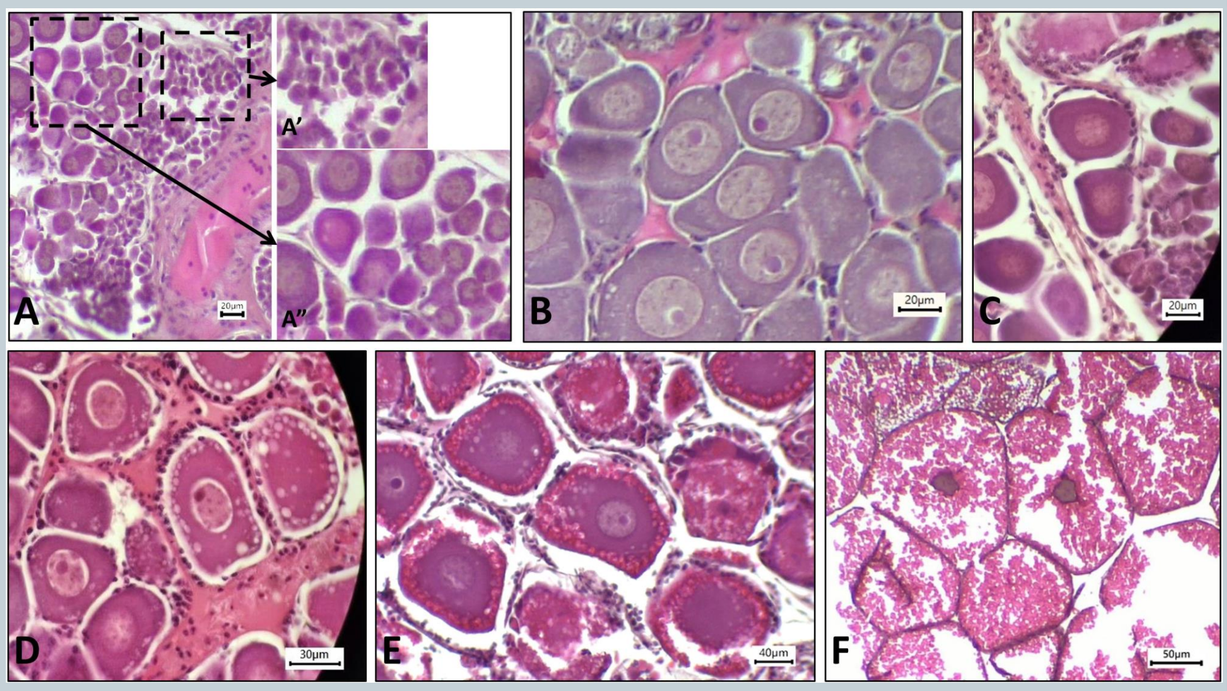


Figure 2. Histological characterization of oocyte differentiation and vitellogenic maturation stages (H&E staining).
(A) Overview of the early-stage ovary showing a germinal zone containing clusters of **(A') Oogonia**, characterized by irregular circular profiles with minimal cytoplasm and cluster-like aggregation near the ovarian center (diameter: 8-10 μm, and **(A") Chromatin-nucleolus stage oocytes**, distinguished by having the highest nucleo-cytoplasmic ratio among all stages without surrounding follicle cells (diameter: 15-20 μm). **(B) Early perinucleolus stage oocyte**, demonstrating a decreasing nucleo-cytoplasmic ratio and the initial recruitment of sparse, spindle-shaped follicle cells along the oocyte periphery (diameter: 40-50 μm). **(C) Late perinucleolus stage oocyte**, exhibiting a two-fold increase in size relative to the early phase, with a prominent nucleus occupying approximately half of the cell volume, completely encapsulated by a continuous monolayer of spindle-shaped follicle cells (diameter: 80-90 μm). **(D) Previtellogenic stage oocyte (Endogenous vitellogenesis)**, where follicle cells transition from spindle-shaped to capsule-like morphology with increased cell density, accompanied by the distinct appearance of lipid vacuoles (oil droplets) within the basophilic cytoplasm (diameter: approx 100 μm). **(E) Vitellogenic stage oocyte (Exogenous vitellogenesis)**, characterized by a highly compressed, dense follicular layer and extensive accumulation of eosinophilic yolk granules throughout the central cytoplasm (diameter: 120-140 μm). **(F) Maturation phase oocyte**, representing the final stage where the nucleus shrinks to a minimal central sphere (lowest nucleo-cytoplasmic ratio) and the expanded ooplasm is entirely packed with mature yolk granules (diameter: 160-170 μm). Scale bars are indicated individually within each panel.

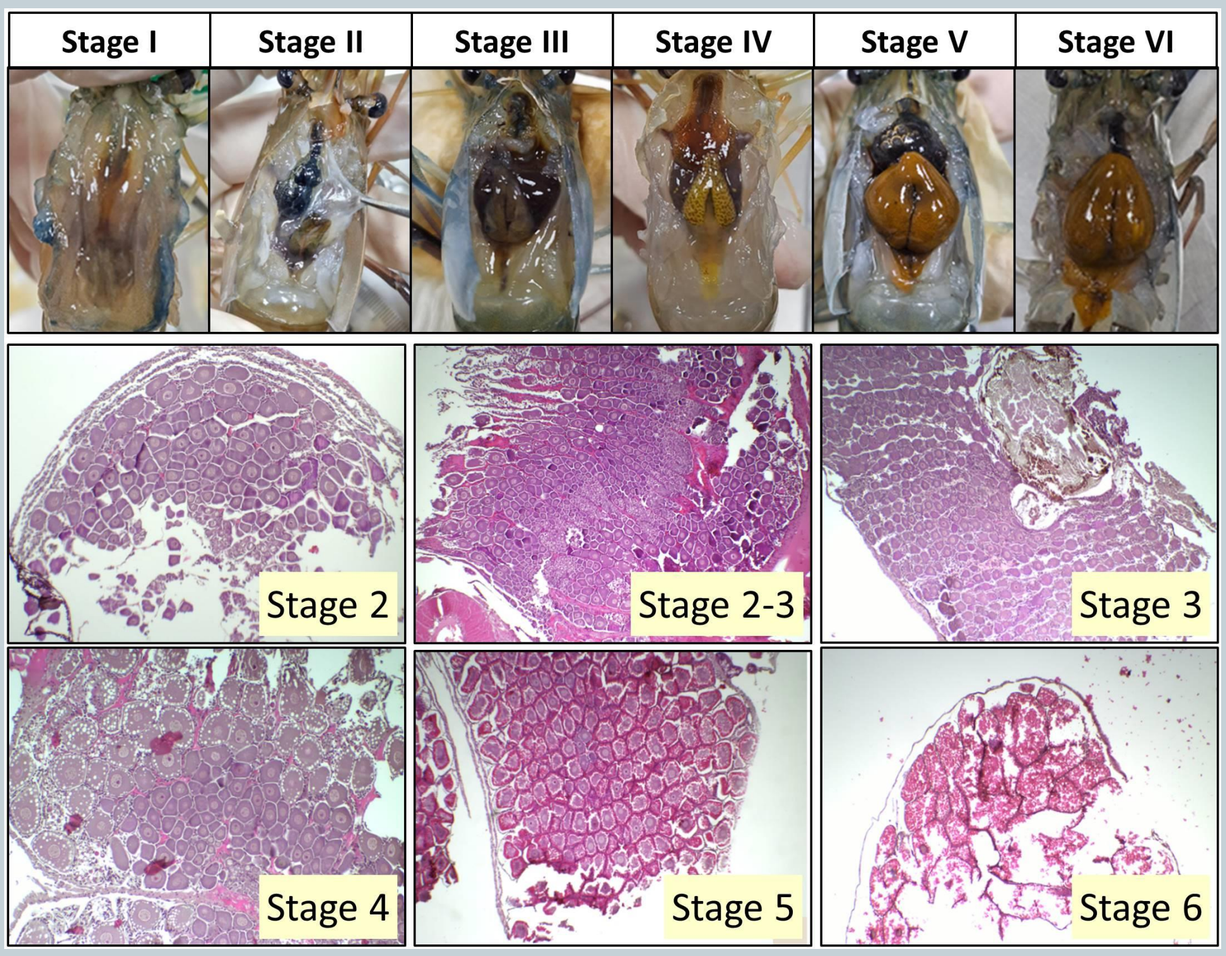


Figure 3. Gross morphological criteria of the six ovarian developmental stages (Stage I to Stage VI) correlated with quantitative histological compositions.
Stage I: Represents the initial immature phase characterized by a highly transparent, thin, and narrow ovarian band situated dorsally above the hepatopancreas. Germ cells are predominantly restricted to early-stage oogonia and chromatin-nucleolus oocytes.
Stage II: The ovary shifts from transparent to a semi-translucent, slightly matte appearance, showing early brownish hues near the central axis. Histologically, the tissue is predominantly occupied by early and late perinucleolus stage oocytes, with a minimal presence of residual chromatin-nucleolus oocytes.
Stage III: The ovary presents a distinct brownish-cream margin with a lighter central band, covering approximately half of the hepatopancreatic surface. Quantitative analysis shows that un-vitellogenic cells (oogonia, chromatin-nucleolus, and perinucleolus oocytes) account for 26% of the composition, while pre-vitellogenic oocytes (undergoing endogenous vitellogenesis) constitute the remaining 73%.
Stage IV: The gonad extends significantly along the longitudinal axis, moving anteriorly over the stomach and posteriorly toward the heart, covering roughly two-thirds of the hepatopancreas. The surface displays characteristic brownish spotting over a yellowish-tan base. Oocytes display a clear spatial arrangement proliferating outward from the innermost germinal zone to the peripheral layer. Quantitative distribution reveals 3.33% oogonia and chromatin-nucleolus cells, 29.07% perinucleolus oocytes, and 67.61% combined pre-vitellogenic, vitellogenic, and rare mature oocytes.
Stage V: The ovary expands massively, exhibiting a rounded profile that completely covers the hepatopancreas and reaches past the cardiac region. The tissue color matures into a deep orange-yellow interspersed with subtle dark patches. Cells are heavily packed with dense vitellogenin droplets.
Stage VI: Represents the fully mature, massive ovarian condition where the tissue heavily covers the stomach anteriorly and stretches posteriorly into the first abdominal segment. The color shifts to a distinct dark orange-red with prominent microscopic black speckles across the ovarian surface. Due to the extremely high lipid and yolk granule content, mature oocytes (160-170 μm) are highly fragile during sectioning, with intact cellular sections revealing an ooplasm completely filled with dense mature yolk granules.

Conclusion

- **Morphological Correlation:** A reliable 6-stage ovarian maturation framework for *M. rosenbergii* was successfully established by correlating gross external color with precise histological markers.
- **GSI as a Key Metric:** While body weight shows no significant correlation with reproductive readiness , GSI increases progressively from 0.11% to 4.05% and serves as an accurate indicator of maturity.
- **Endocrine Stage Transition:** The sharp increase in GSI and oocyte diameter (8 μm to 170 μm) between Stage III and Stage IV marks the critical endocrine switch from VIH inhibition to intense exogenous vitellogenesis.
- **Technical Optimization:** To overcome tissue detachment caused by high lipid and yolk content in mature (Stage VI) ovaries, implementing slide coating, alcohol pre-treatment, or adjusting section thickness is recommended.
- **Aquaculture Impact:** This integrated staging system provides hatchery managers in Pingtung, Taiwan, with a practical, scientifically grounded tool to optimize broodstock selection and spawning induction.