Histological Assessment of Blood Cockles (*Anadara granosa*) using Different Stains and Fixatives

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Abstract

Blood cockles (*Anadara granosa*) or locally known as 'kerang' are mollusc belonging to Bivalvia class. It is an important fisheries commodity and a delicacy in Malaysia. In RMK-9, one of the objectives of Malaysia's aquaculture industries is to maximize the production of bivalves up to 130,000 MT per year, which includes cockles, green mussels, clams and oysters. Cockles become one of the important bivalves cultured in Malaysia as it had very good market value in Malaysia, Thailand and Singapore. According to Phillips and Muttarasin (1985), 80% of the cockles marketed in Thailand were imported from Malaysia. In spite of the diversity and its economic importance, the knowledge of bivalves specifically cockle medicine should be developed in order to meet the requirement of diagnostic work which is necessary in determining the cause of diseases infecting the animals. Up to date, there is no comprehensive information of the histology of cockles. Thus, this study was undertaken to provide basic histological descriptions of normal and anomalies microstructures in *Anadara granosa* by using different fixatures and stains. Forty live cockles were divided into two groups; one group was fixed with Davidson solution and the other fixed with 10% buffered formalin. The tissues were then processed for 18 hours, and later embedded with paraffin wax and sectioned at 5 µm thickness. There were stained with four stains namely, Haematoxylin and Eosin, Masson's Trichome, Periodic Acid Schiff's and Van Gieson's stains. The sections were evaluated under a computer attached-compound light microscope at low and high magnification. The organs were assessed for microscopic structures staining affinity (Cox *et al.,* 2006). Haematoxylin and Eosin stain was considered the better stain to be used for mantle, foot complex and haemocytes tissues. Combination of Haematoxylin and Eosin and Periodic Acid Schiff's stains can be used to stain digestive system and palp. Gills could be stained with Masson's Trichome and Van Gieson's, while for gonads Masson's Trichome would be the preferred choice. Van Gieson's on the other hand, was the choice for foreign body detection. Gills of cockles could be divided into three parts: frontal, intermediate and abfrontal zone similar to *Mytella falcate* (David *et al*., 2008). The intermediate zone have a homogenous densely stained structure embedded in the filament which highly indicative that it provided supportive frame to the frontal zone.

The palp (Figure 2) consisted of ciliated cuboidal and columnar epithelia, which was postulated that this organ collected food particles from the gills, and helped in transferring to the mouth opening as in *Mercenaria mercenaria* (Eble, 2001). The haemocytes could be divided into agranular and granular types, similar to as reported by Cheng (1981). The mantle was postulated to play an important role in helping to move particles into the shell cavity due to the present of cilia along the epithelium cells

at the edges. The digestive gland appeared to be ciliated, as in *Mercenaria mercenaria*, thus its function might not as secretion gland solely, but its cilia helped to move food particles forward. The tall columnar epithelium of intestine was secured on thin basement membrane which was then directly connected to thick layer of connective tissue provided a "channel" for the hemocytes to reach the epithelium.

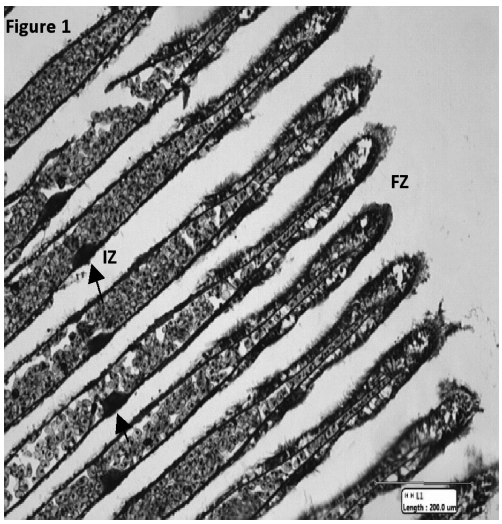

Fig. 1 The intermediate and frontal zone of gills filaments. The intermediate zone (IZ) with embedded structure (arrow). The frontal zone (FZ) consisted of tall columnar epithelium with cilia. Masson's Trichome, (×200).

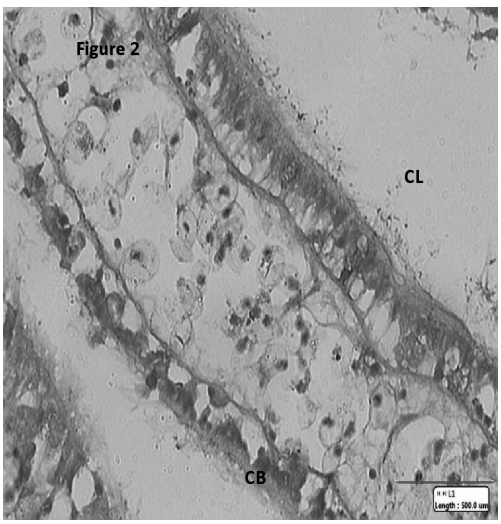

Fig.2 The palp filament which consisted of ciliated tall columnar epithelia with apex-located nuclei (CL) and ciliated cuboidal epithelia at the opposite side (CB). PAS, (×400).

The acini of male gonads were bulb-shaped, constructed of thick connective tissue and the gametes were seemed to fill up the lumen. This condition was similar seen in *Placopecten magellanicus* (Beninger and Pennec, 2006). In comparison to a study done by Broom (1983), the female gonads seen in this study were considered normal, healthy and not in spawning stage. The foot tissues were covered with tall columnar epithelium with large nucleus located at the base of the cells and rested on dense connective tissue. The muscle fibers were arranged multi directional which is suggestively due its important function in locomotion and burrowing.

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