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## Histopathology of the Hepatopancreas of Pacific White Shrimp, *Penaeus vannamei* from None Early Mortality Syndrome (EMS) Shrimp Ponds

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### ABSTRACT

Hepatopancreas study of Pacific White shrimp, *Penaeus vannamei* was done on the shrimp samples from a commercial shrimp farm in Setiu, Terengganu. About six individual shrimps were collected randomly from eight different shrimp grow-out ponds for histopathology analysis at Universiti Malaysia Terengganu laboratory. Most of the hepatopancreas samples appeared to be healthy, however in the histopathology results showed signs of unhealthy hepatopancreas conditions including slough hepatopancreas tubules cells (SHP), degeneration of Tubules Lumen (TL), enlarged of the hepatopancreas nuclei cell and lack of B, F and R epithelial cells in the hepatopancreas tubules. The study shows that HP conditions can be the indicator to determine the conditions of the shrimp health status.

**Key words:** Histopathology, hepatopancreas, *Penaeus vannamei*, early mortality syndrome

### INTRODUCTION

Pacific white shrimp, *Penaeus vannamei* are well known as an important Penaeid species for commercial production (Danya and Jagadish, 2014; Alcivar-Warren *et al.*, 2007). *P. vannamei* is commonly cultured in Malaysia, China, Thailand and Taiwan. This species was introduced from the Pacific in 1995 which then being commercialized in Malaysia in 2001 resulted for the shrimp production increased up to 70% as compared to year 2000. The Malaysian shrimp production also increased up to 70,000 t with 80% comprising of *P. vannamei* in 2007. The production of *P. vannamei* in Malaysia however dropped from 70,000 mt in 2010 to 40,000 mt in 2011 due to the disease outbreaks of Early Mortality Syndrome (EMS) or also known as Acute Hepatopancreatic Necrosis Disease (AHPND) (Eduardo and Mohan, 2012a). In 2011, this disease has spread to Vietnam and Malaysia and was discovered in Thailand in year 2012 (Flegel, 2012). In China, shrimp farmer in Hainan, Guangdong and Fujian suffered about 80% in losses during the first half of production in 2011 (Panakorn, 2012). The same situation also occurred in Vietnam which the widest spreads of EMS disease was reported in Mekong Delta in Southern Vietnam. In other parts in Vietnam, also were reported about 330 million of *P. monodon* died and caused a loss of over USD 555,840.00 from farm culture in Bac Lieu and Tra Vinh and also lost of USD 69,480.00 from farm in Soc Trang (Mooney, 2012).

Hepatopancreas (HP) is one of the indicators in the shrimp body which can be used to identify the shrimp health condition. The HP was known as a large gland in shrimp which carries out functions of a liver and pancreas. It is essentially composed of branched tubule and different type of epithelial cell lining the tubules (Wu and Yang, 2011). Bautista *et al.* (1994) reported that HP and liver are very sensitive to different diet and water borne pollutant thus they are often used to monitor the effect of various toxicants. Generally, the shape and colour of the shrimp HP can be used as the indicator of the shrimp health condition and can also identify the severity of problems that affected the shrimp. The HP can be observed directly on the head under light source. The shrimp is known to be healthy with optimum appetite if the HP is in solid triangular shape with brown colouration. The shrimp have disturbance in its digestive system and infected with disease if the HP appeared to be shrinkage in size and HP has mix brown and blue colouration. Disease infection may affect the texture of the hepatopancreas making it less solid, mix texture between solid and slimy and has whitish blue colouration which caused the animal unable to feed. In the case of EMS infection, the affected shrimp also showed an abnormal hepatopancreas including shrunken, small, swollen and discoloured. Study done by Lightner *et al.* (2012) on infected *P. vannamei* found out the EMS trace limited to the hepatopancreas and they had discovered that lack of mitotic activity in E cells of hepatopancreas, dysfunction of central hepatopancreas B, F and R cells and massive sloughing of central HP tubule epithelial. Prachumwat *et al.* (2012) also identified dysfunction of the tubule epithelial cells by doing histopathology studies. According to Lightner (2012) from the clinical test done on EMS infected shrimp at the pond level had discovered that HP connective tissue capsule was pale to white in colour, significant atrophy of HP, black spot or streaks at the HP, HP does not squash easily between thumb and finger and mortality started as early as 10 days of stocking after the clinical sign where dead shrimp were found sinking at the bottom. Therefore healthy HP will illustrate the good condition of the shrimp in the pond level. Because of the important role of HP in shrimp survival, this study was carried out to determine the healthy condition of *P. vannamei* by examining the condition of their HP in a commercial farm level at Setiu, Terengganu through histopathology analysis.

## **MATERIALS AND METHODS**

Shrimp samples were collected from the local commercial shrimp farm at Setiu Terengganu latitude; longitude (5°33'47" N and 102°49'6" E). The size of each pond was 5000 m<sup>2</sup> and stocking density 500,000 shrimp per pond. Six shrimps were collected from eight randomly selected ponds at different Day Of Culture (DOC). The selected ponds were Pond (DOC32), Pond (DOC15), Pond (DOC102), Pond (DOC65), Pond (DOC 96), Pond (DOC 20), Pond (DOC 36) and Pond (DOC 27). The samples were transported back to Universiti Malaysia Terengganu (UMT) for histopathology analysis. The shrimp samples were firstly fixed in the Davidson's solution for two days. After that, the HP were extracted from the shrimp and were placed in cassette. The HP samples were placed in 70% alcohol before they were transferred into the tissue processor. The procedure for tissue processing were done as followed: Alcohol 70% for 1 h continued by alcohol 90%, alcohol 95%, alcohol 100%, ( xylene I, xylene II, xylene III) for clearing and wax (2 h) continued with another wax (2 h) for impregnation. After that the sample was embedded, cut and sectioned. Sectioned samples were dried on hot plate for 2 days (Fig. 1(a-d)). The samples were then stained using the hematoxylin and eosin procedure according to the

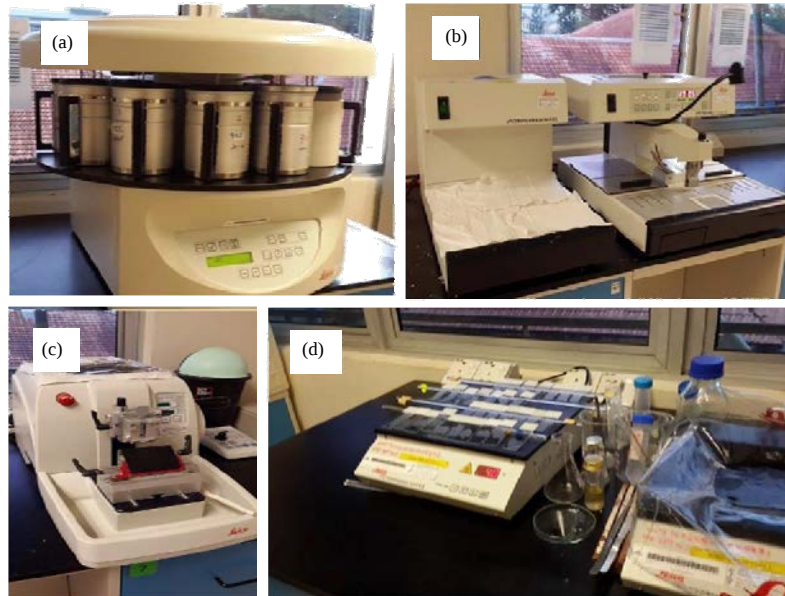


Fig. 1(a-d): Histology equipment used in the present study, (a) Tissue processor (16 h), (b) Embedding machine, (c) Cutting and sectioning machine and (d) Water bath and hot plate for slide samples drying

procedure as followed: Xylene I (5 min), continued by xylene II (5 min), 100% alcohol I (5 min), 100% alcohol II (5 min), 95% alcohol (2 min), 70% alcohol (2 min), running tap water (2 min), hematoxylin (10 min), running tap water (2 min), acetone acid (3 dip), running tap water (2 min), 2% potassium acetate (3 min), running tap water (2 min), eosin (5 min), 95% alcohol (5 min), 95% alcohol (5 min), xylene I (5 min), xylene II (5 min) and finished with dpx mounting. The slide samples were then analyzed using advance microscope *Nikon eclipse* 80i. The infected and uninfected HP tissue was identified under the microscope and labeled for further data analysis.

## RESULTS

Table 1 showed the HP conditions analyzed for eight pond using Advance microscope *Nikon eclipse* 80i. The result showed that most of the HP from 6 individual of shrimp for each pond were in healthy condition. However Pond (DOC 102) and Pond (DOC 32) did not show sign of healthy HP. The result showed that the HP became sloughing, karyomegally or enlarged of the HP nuclei, collapse of the hepatopancreas tubules, lack of B, F, R of HP epithelial cell and degeneration of the tubule lumens due to the karyomegally of the HP nuclei. Shrimp samples from Pond (DOC36), Pond (DOC27) and Pond (DOC14) showed healthy HP condition (Fig. 2-4). Healthy HP showed intact E, B, R epithelial cell, the Tubule Lumen (TL) and still rounded epithelial tubule instead of degraded or in necrosis conditions. Shrimp samples from Pond (DOC 102) (Fig. 5) and from Pond (DOC32) (Fig. 6) showed both healthy and unhealthy HP. Some of the tubule HP were collapse, sloughing of the hepatopancreas (SHP) tubules, lacking of epithelia cells (B, F, R cells), karyomegally of the HP nuclei and the degeneration of the Tubules Lumen (TL) were observed in the HP tissue.

Table 1: Hepatopancreas conditions analyzed from eight pond using advance microscope *Nikon eclipse 80i* (Six individual shrimp per pond)

| Ponds day of culture | Hepatopancreas conditions |                           |                              |                         |                                  |
|----------------------|---------------------------|---------------------------|------------------------------|-------------------------|----------------------------------|
|                      | HP sloughing              | Karyomegally of HP nuclei | Degeneration of tubule lumen | Collapse of the tubules | Lack of epithelial B, F, R cells |
| 32                   | ×                         | -                         | -                            | ×                       | -                                |
| 14                   | ×                         | ×                         | ×                            | ×                       | ×                                |
| 102                  | -                         | -                         | -                            | -                       | -                                |
| 65                   | ×                         | ×                         | ×                            | ×                       | ×                                |
| 96                   | ×                         | ×                         | ×                            | ×                       | ×                                |
| 20                   | ×                         | ×                         | ×                            | ×                       | ×                                |
| 36                   | ×                         | ×                         | ×                            | ×                       | ×                                |
| 27                   | ×                         | ×                         | ×                            | ×                       | ×                                |

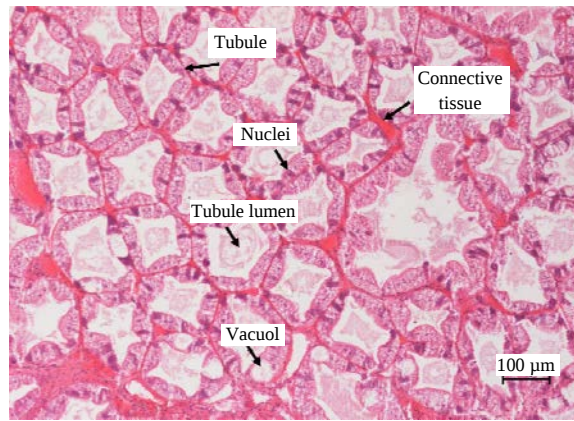


Fig. 2: Hepatopancreas section of healthy shrimp from Pond (DOC36) observed under 40× magnification using Advance microscope *Nikon eclipse 80i*



Fig. 3: Hepatopancreas section of healthy shrimp from Pond (DOC27) observed under 100× magnification using Advance microscope *Nikon eclipse 80i*

## DISCUSSION

The destruction of HP tissue observed maybe due to the disease affected the healthy shrimp. The present study showed the degeneration of the E, F, R epithelial tissue in HP and the collapse of the epithelial tubules in some of the shrimp HP. Study done by Lightner *et al.* (2012) on both *P. monodon* and *P. vannamei* affected shrimp, showed that the EMS trace limited to the hepatopancreas and that was described as lack of mitotic activity in E cells of hepatopancreas,

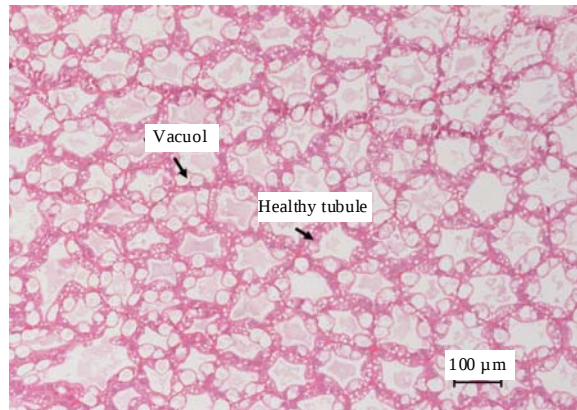


Fig. 4: Hepatopancreas section of healthy shrimp from Pond (DOC14) observed under 100× magnification using Advance microscope *Nikon eclipse 80i*

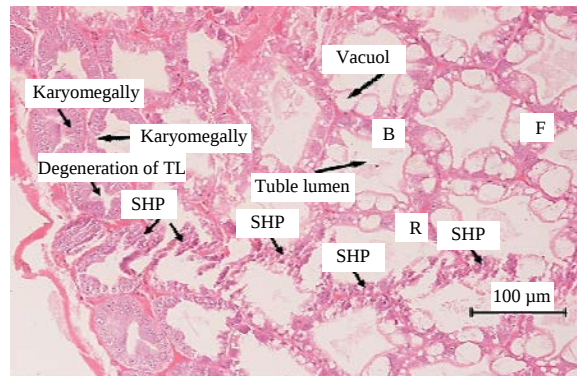


Fig. 5: Hepatopancreas section of unhealthy shrimp from Pond (DOC102), sloughing of hepatopancreas cell (SHP), enlarge of the hepatopancreas nuclei and degeneration of tubule lumen (TL) under 200× magnification using advance microscope

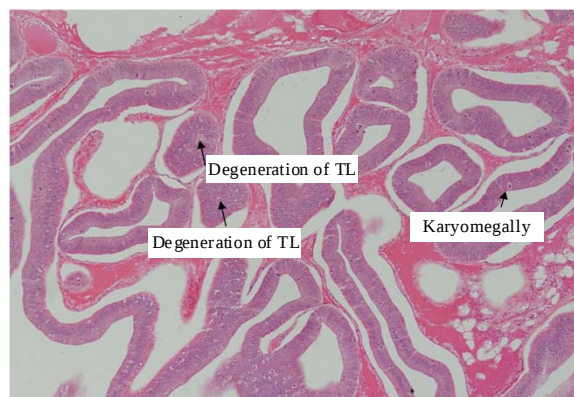


Fig. 6: Hepatopancreas of unhealthy shrimp Pond (DOC32), degeneration of TL and enlarged of the nuclei observed under 100× magnification

dysfunction of central hepatopancreas B, F and R cells, massive sloughing of central HP tubule epithelial cells and massive intertubular hemocytic aggregation followed by secondary bacterial infections. Similar histopathological result was also obtained by Prachumwat *et al.* (2012) which identified dysfunction of the tubule epithelial cells that progress from proximal to distal ends of HP tubules. This degenerative pathology strongly suggests a toxic etiology, but anecdotal information suggests that disease spread patterns may be consistent with an infectious agent. According to Lightner (2013), the cause of EMS was thought to be a microbial infection with the *Vibrio parahaemolyticus* as the causative agent. The name of this disease was then changed to AHPND (Acute Hepatopancreatic Necrosis Disease). Lightner (2013) has identified the EMS pathogen as a unique strain of a relatively common bacterium, *V. parahaemolyticus*, which had been infected by a virus known as a phage, caused it to release a potent toxin. A similar phenomenon occurs in the human disease cholera, where a phage makes the *Vibrio cholerae* bacterium capable of producing a toxin that causing cholera's life-threatening diarrhea. Lightner *et al.* (2014) also observed acute degeneration of the HP by initially decrease of R, B and F cell followed by marked reduction of mitotic activity in E cells, dysfunction of R, B and F cells and prominent karyomegally (enlarged nuclei) and sloughing into the HP tubule lumen. According to Eduardo and Mohan (2012b), in Malaysia, EMS was first reported in mid-2010 in the east coast states of Pahang and Johor resulting in a drop in *P. vannamei* production from 70,000 mt in 2010-40,000 mt in 2011.

## CONCLUSION

It can be concluded that HP condition can be the indicator to determine the condition of the shrimp. Histopathological analysis on the HP was able to identify the health of HP cells in the shrimp. All results from the analysis showed the healthy hepatopancreas detected in shrimp samples from Pond (DOC32), Pond (DOC15), Pond (DOC102), Pond (DOC65), Pond (DOC 96), Pond (DOC 20), Pond (DOC 36) and Pond (DOC 27) sampled. However, there were also some destruction in HP cells among the healthy HP in the shrimp samples from Pond (DOC 32) and Pond (DOC102). Overall the results showed that the HP of the shrimp samples were healthy which means that they are able to carry out healthy biological metabolisms in the shrimp bodies.

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