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Histopathology status of black tiger shrimp (*Penaeus monodon*) in the conventional system in Bireuen, Aceh Province

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ABSTRACT

The purpose of this study was to identify the tiger shrimp *(Penaeus monodon)* histopatology status in traditional cultured ponds at Bireuen Regency, Aceh Province. The sampling was carried out by purposively random method from seven ponds and two shrimps were sampled from each pond. Several water quality parameters were checked like temperature, pH, salinity and ammonia. Result showed that black tiger shrimp (*Penaeus monodon*) which were cultivated in traditional ponds in the Bireuen Regency, Aceh Province were histopathologically changes. It was suspected the hepatopancreas changes were associated with viral infection like *Hepatopancreatica Parvovirus* (HPV) and *White Feces Disease* (WFD). Ammonia concentrations were found higher in some ponds like Alu Buya Village, Jangka Keutapang Village, Jangka Mesjid Village, Alu Kuta Village and Punjot Village. The management of regular feeding and water quality control is highly recommended to anticipate the viral potential attack in traditional shrimp pond farming at Jangka District, Bireuen Regency, Aceh Province.

Introduction

Indonesia is known for its richness and diversity of fishery biological resources (Muchlisin *et al.*, 2017) and crustaceans, especially shrimp commodities (Putra *et al.*, 2018; Putra *et al.*, 2019). Bireuen District is located in the middle of Aceh Province, known as a district that has adequate fishery resources (Muchlisin *et al.*, 2012) with the economic center of the golden triangle of Aceh economy because it is flanked by five districts/cities, namely Pidie Jaya District, Central Aceh, Bener Meriah, North Aceh, and the City of Lhokseumawe (Mangkuwinata, 2010). Administratively, this district has 17 sub-districts with 426,089 people and an area of about 1,901.21 km^2 (Rahmad, 2016). Bireuen Regency has 12 coastal sub-districts which have the potential for aquaculture ponds which have been utilized to have ± 4,945.64 hectares with a production rate of 7,605.95 tons/year. The sub-district that has the largest area of aquaculture ponds is the term sub-district (1,460 ha), while the sub-district that has a large pond is the Kuta Blang district (1.44 ha) (BPS, 2018).

Black tiger shrimp (*Penaeus monodon*) is one of Acehnese native shrimps that can be found on almost every eastern and western coast of Aceh province. The shrimp has triumphed in the 80s to 90s which ended with the collapse of the tiger prawn industry due to a viral disease. Moreover, viral diseases are known to have destroyed many of the world's shrimp farming industries in the 1990s. Shrimp virus disease outbreaks mostly occurred in the largest producing countries, such as in South America and Southeast Asia (Thailand, Indonesia) and China. The earliest shrimp disease encountered was by the White spot syndrome virus (WSSV) which originates from members of the *Nimaviridae* family and the genus Whispovirus, which has a large and closed ovaloid deoxybonucleic acid (DNA)

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genome with a tail like flagellum and helical nucleocapsid (Wongteerasupayal *et al.*, 1995). WSSV was first discovered in tiger prawns, but all cultivated penaeid shrimp are highly susceptible to viruses, with mass mortality generally approaching 80–100% in ponds within 3–10 days (Chou *et al.*, 1995; Lightner *et al.* 1998). Other shrimp virus diseases that are often found are Yellow head virus (YHV), Taura Syndrome virus (TSV) and IHHNV (Walker and Mohan, 2009). Several studies have been reported related to the growth of cultivated tiger shrimp in the Aceh region. Studies on the growth of tiger shrimps using supplements of gamat oil, sea grapes (*Caulerpa* sp) and eggshell flour have been carried out (Putra *et al.*, 2018; Safriani *et al.*, 2019; Putra *et al.*, 2019). The study of tiger shrimp parasites as reported by (Rosnizar *et al.*, 2018) on the identification and prevalence of ectoparasites in tiger shrimp (*Penaeus monodon*) based on the place of rearing. Akmal *et al.* (2015) have also conducted research on the effect of noni fruit flour at different doses to control *Vibrio harveyi* bacteria in post larvae of tiger shrimp (*Penaeus monodon*). Studies on shrimp virus infection in Aceh have also been carried out, namely on *Litopenaeus vannamei* shrimp in Peudada (Zulpikar *et al.*, 2016) and banana shrimp in Aceh Besar (Nurbariah and Khairurrazi, 2015). However, the study of black tiger shrimp virus disease identification in traditional ponds in Bireuen has never been carried out. This research aimed to investigate the histopathology status black tiger shrimp (*Penaeus monodon*) in Bireuen Regency. It is expected that the availability of information and identification of this histopathology status can become a consideration for a better future tiger shrimp management policy in Indonesia in general and in Bireuen Regency in particular.

Materials and Method

Location and research period

The research was conducted for 3 (three) months starting from December 2019 to March 2020. The sampling method was based on purposive sampling which was carried out in traditional tiger prawn ponds in Term District, Bireuen Regency (Figure 1). While the sample analysis was carried out at the Histopathology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University.

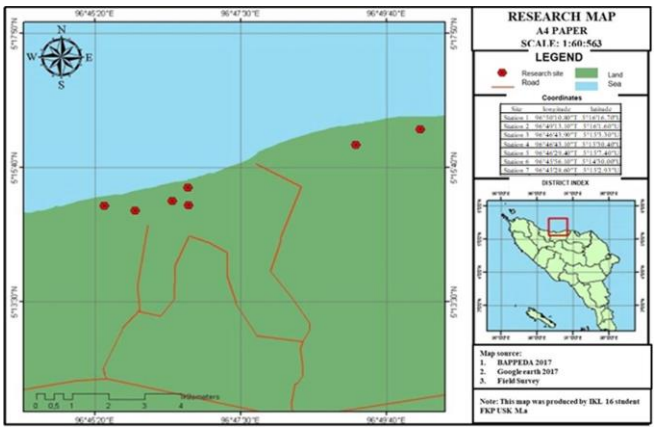

Figure 1. Sampling location.

Research procedure

The research procedure included two stages, namely collecting shrimp samples followed by the manufacture of histological preparations and measuring the environmental parameters of the cultivation area. Measurement of water quality is carried out for each sampling in the same pond area. Measurement of water quality parameters includes temperature measured with a thermometer, salinity using a refractometer, pH using a pH meter, DO measurement using a DO meter and ammonia test using a spectrophotometric facility during sampling. The procedure for making histological preparations in this study was carried out based on a modification from the previous procedure (Kitikiew *et al.*, 2013; Chen *et al.*, 2014; Putra *et al.*, 2012) which includes: fixation, dehydration and clearing, planting in paraffin (embedding), cutting, staining, mounting and observing.

Data analysis

The histopathological status of black tiger shrimp hepatopancreas were analyzed descriptively and presented in the form of pictures and narratives.

Results

The observations result regarding the histopathological status of tiger prawns in Bireuen districts are presented in the following figures.

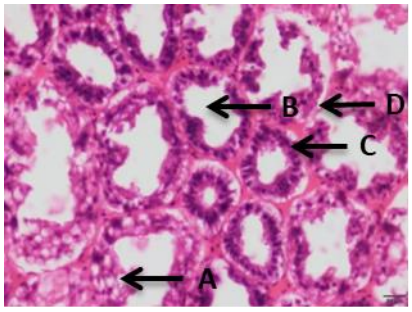

Figure 2. Histopathology of hepatopancreas of shrimp infected with HVP in pond 1, A

= tissue dehydration, B = tubular lumen, C = basophilic hypertrophy of intranuclear inclusion bodies and D = cell lysis; HE, 40X.

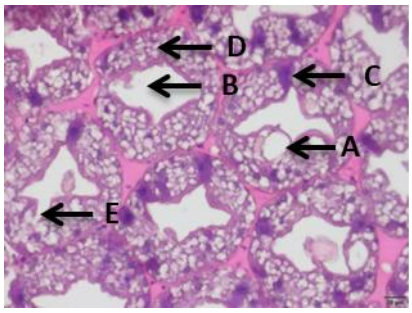

Figure 3. Histopathology of tiger prawn hepatopancreas infected with White Feces Disease (WFD) in pond 2: A = tissue dehydration, B = tubular lumen, C = inflammatory cells, D = tubular epithelial cells, E = cell lysis; HE, 40X.

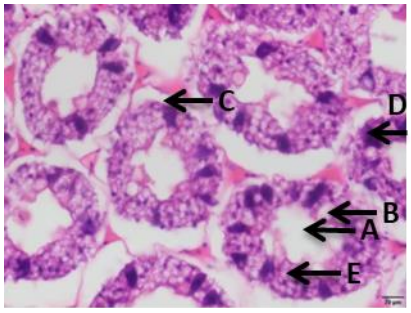

Figure 4. Histopathology of tiger prawn hepatopancreas infected with WFD in pond 3: A = Tubular Lumen, B = Tissue Dehydration, C = Cell Lysis, D = Inflammatory Cells, E = Tubular Epithelial Cells; HE, 40X.

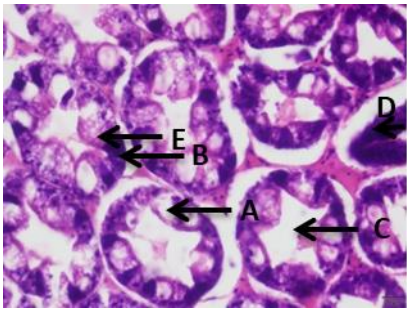

Figure 5. Histopathology of tiger prawn hepatopancreas infected with WFD in pond 4: A = Tissue Dehydration, B = Cell Lysis, C = Tubular Lumen, D = Inflammatory Cells, E = Tubular Epithelial Cells; HE, 40X.

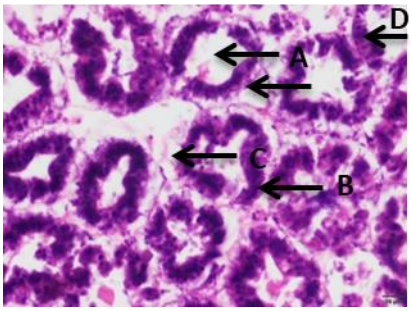

Figure 6. Histopathology of tiger prawn hepatopancreas infected with WFD in pond 5: A = Tubular Lumen, B = Inflammatory Cells, C = Tissue Dehydration, D = Cell Lysis; HE, 40X.

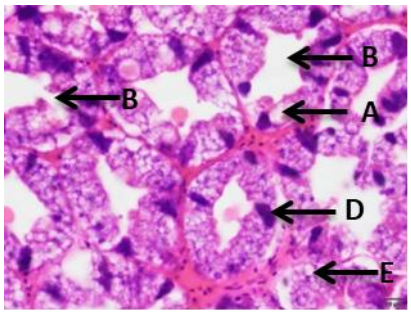

Figure 7. Histopathology of tiger prawn hepatopancreas infected with WFD in pond 6: A = Tissue Dehydration, B = Tubular Lumen, C = Cell Lysis, D = Inflammatory Cells, E = Tubular Epithelial Cells; HE, 40X.

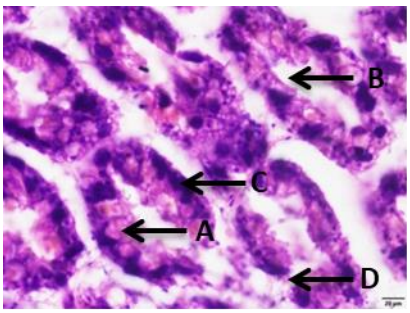

Figure 8. Histopathology of tiger prawn hepatopancreas infected with WFD in pond 7: A = Tissue Dehydration, B = Tubular Lumen, C = Inflammatory Cells, D = Cell Lysis; HE, 40X .

Table 2. Water quality parameters at the sampling location.

Pond	pH	Temperature (°C)	Salinity (ppt)	Amoniak (mg/L)
1 (Alu Buya)	8.36	33.6	24	0.06
2 (Tanoh Anoe)	7.66	34.7	25	0.00
3 (Jangka Alu Bie)	7.48	34.1	14.5	0.01
4 (Jangka Keutapang)	8.63	33.2	15	0.06
5 (Jangka Mesjid)	7.77	33.1	15.5	0.02
6 (Alu Kuta)	6.80	35.7	17	0.04
7 (Punjot)	7.59	35.2	15	0.05

Discussion

The cultivation of black tiger shrimp, *Penaeus monodon* in Bireuen Regency is generally dominated by traditional culture systems. Yusuf (2002) reported several districts that dominate black tiger shrimp culture in Aceh are Aceh Jaya, Aceh Besar, East Aceh including Bireuen districts. However, the development of black tiger prawn cultivation in Aceh is very stagnant and showing a downward trend. This is due to several factors, including the difficulty of finding quality broodstock that can produce good fry, lack of capital, poor shrimp culture technology, and concerns about disease attacks (Dahlia *et al.*, 2021). Until now, studies on black tiger shrimp disease are still routinely carried out. Moreover this is very important as a basis for information on disease status, distribution and prevention of shrimp disease.

Based on the observation result of hepatopancreatic condition in black tiger shrimp collected from several areas in Bireuen district (Figure 2-8), it significantly appeared that the hepatopancreas had tissue dehydration, basophilic hypertrophy, cell lysis and inflammatory cells. The intranucleus will appear normal in size at the time of the initial infection. It will get larger as the viral infection expanded. Changes in the distal tubular epithelial cells in the hepatopancreatic organ are evident. Madeali *et al.* (1998) and Karunasagar *et al.* (2009) have also reported that the nucleus of shrimp hepatopancreas cells will experience enlargement (hypertrophy) and even cell lysis when infected with *Hepatopancreatica Parvovirus* (HPV). Kasornchandra *et al.* (1998) have similarly stated that the intranuclear hypertrophy seen in cell tissue is different from the initial stage of viral infection. This is supported with the visual observation of the black tiger shrimp sample (*Penaeus monodon*) in Alue Buya Village (pond 1) were pale, the hepatopancreas was brown and the feces were white. As shown in Figure 2, the characteristics of the sample condition are in accordance with the characteristics of shrimp infected with HPV (Yanto, 2006). Inouye *et al.* (1992) also stated that the symptoms of HPV infection are not specific, but in some cases it appears that the hepatopancreas is brown, white feces and the shrimp turn pale.

The histological observation of black tiger shrimp hepatopancreas (*Penaeus monodon*) in Figure 2 showed that there was a change in the hepatopancreatic tissue. When compared to normal tissue as shown by Nazaruddin *et al.* (2014), it was suspected that the visual appearance of this tissue indicated that the shrimp was infected with a pathogen. The hepatopancreas infected shrimp shows inclusion bodies due to virus attack, cell lysis and fat degeneration that have similar characteristic of HPV infection and other shrimp viral diseases like IHHNV. Karunasagar *et al.* (2009) said that the positive shrimp infected with HPV then the hepatopancreatic cell nucleus will experience enlargement (hypertrophy), even cell lysis occurs. According to Wang *et al.* (1997) in the examination or detection of HPV found hepatopancreatic changes in the presence of inclusion bodies and cell lysis, but no visible inflammatory reaction. HPV disease is caused by parvovirus which contains small DNA with a diameter of 22-24 nm. In addition to attacking the hepatopancreas of shrimp, the virus sometimes attacks the gill organs and intestines and causes the body of the shrimp to turn pale and the hepatopancreas is brown, the excrement of the shrimp is white due to damage and decay and dysfunction of the hepatopancreas as the body's metabolic center (Inouye *et al.*, 1992).

There are several symptoms of HPV infection like whitish and atrophic hepatopancreas, anorexia, slow movement, tend to rise to the surface and the gills are infested with commensal organisms. Nazaruddin *et al.*, (2014) reported that changes in the hepatopancreas have been very visible in the presence of most cells showing round and basophilic inclusion bodies in the nucleus and located irregularly. In this study some changes were found in Figure 2 like tissue dehydration and lysis caused by HPV infection.

Another six observation pond was located in Tanoh Alue Village (pond 2), Jangka Alu Bie Village (pond 3), Jangka Keutapang village (pond 4), Jangka Mesjid village (pond 5), Punjot Village (pond 6) and Alu Kuta village (pond 7). The results of field observations of the six ponds showed that tiger shrimp had decreased appetite, white intestines and pale color. Based on the results of field observations, it was suspected that tiger shrimp (*Penaeus monodon*) was infected with *White Feces Disease* (WFD).

Histology observation of the hepatopancreas suspected to be infected with WFD as shown in Figure 3-8, there was a change from normal, namely there were inflammatory cells, cell lysis and tissue dehydration. According to Zhahrah *et al.* (2016) inflammatory cells occur due to disease or toxic agents that enter the tissue. Inflammatory cells are the tissue's immune response to *V. harvei* infection in vannamei shrimp. Inflammatory cells will go to the location of the bacterial infection, and then will fight against the infection.

Tissue dehydration in the hepatopancreas indicates disease in shrimp. Lightner (2008) reported that fat degeneration indicates a cell biochemical disorder caused by abnormal metabolism and toxic chemicals. The causes of fat degeneration are toxic substances, lack of oxygen or excess consumption of fat. Kasornchandra *et al.* (1998) expressed that the trigger factor for fat degeneration does not disappear; it can result in a more severe disruption of cell metabolism and result in cell necrosis and lysis. The above opinion supports the results of this study by finding changes in the histopathology of the tiger prawn hepatopancreas due to infection with WFD such as inflammation, cell lysis and tissue dehydration. The cause of shrimp infection with WFD is thought to be caused by water quality, excessive feed and high stocking density. WFD can have an impact on shrimp in the form of decreased appetite, stunted growth and mortality (Limsuwan, 2014).

The measurement of water quality was conducted by in-situ method. Water quality samples were obtained from seven sampling sites, namely Jangka Keutapang, Alue Buya, Tanoh Anoe, Jangka Alu Bie, Jangka Mesjid, Alue Kuta, and Punjot village which were in Term District, Bireuen Regency. The sampling location showed that the shrimp culture water quality parameters were still within the shrimp life tolerance range as shown in the Table 2.

The results of the ammonia level test in pond 1, pond 4, pond 5, pond 6, and pond 7 showed unfavorable results since they were more excessive compared to the tolerance limits for the quality standard of water quality of the Permen-KP (2016) and Boyd (1990) as shown at Table 2. High levels of ammonia were suspected of having leftover feed piled up at the bottom of the pond. Based on field observations, traditional pond farmers in the sampling areas provide garbage or organic waste to their ponds without paying attention to the impact of damage to water quality. The impact of high ammonia in a pond causes stress, poisoning and mass death. Pond 2 and pond 3 showed the results of the ammonia test according to the tolerance limits of the water quality standard (Table 2). Both ponds have not high ammonia content, it was assumed that the feed given was not excessive and the water was often changed. A lower pH was found at pond 6 when compared to the tolerance limit for water quality standards 7.5-8.5 (PERMEN-KP, 2016). Meanwhile, a higher pH found at pond 4. It is indicated that the pH has its fluctuation level. The pH fluctuation in shrimp culture may cause shrimp's loss appetite, stress, and slow growth.

Conclusion

Based on the initial histopathological study, it was detected some changes in the shrimp hepatopancreas like brownish colour, hyperthropy, cell lysis and tissue dehydration that associated with some viral diseases like HPV and WFD infection. In term of ammonia level, ammonia concentrations were found higher in some ponds like in Alu Buya Village, Jangka Keutapang Village, Jangka Mesjid Village, Alu Kuta Village and Punjot Village. The management of regular feeding and water quality control is highly recommended to anticipate the viral potential infection in traditional shrimp pond farming at Bireuen Regency, Indonesia.

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