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Gills and swimming leg histopathologies in pacific white shrimp (Lithopenaeus vannamei) from ponds exposed to the immunogenic membrane proteins of Zoothamnium penaei

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Abstract. Parasites are one of the causes of decreased commodity selling value in shrimp. Acute levels of parasite infestation can result in mass mortality and economic loss. Parasite penetration causes considerable damage by crushing the tissue consistency, causing irritation. The purpose of this study was to observe the tissue abnormality level caused by the ectoparasites that occurred in the gills and swimming leg tissue of vannamei shrimp. The samples were taken from brackish water ponds in Lamongan, East Java. The treatments used were samples without any treatment (control) and those exposed to the immunogenic protein membrane of Zoothamnium penaei (immunostimulant) at 3 and 5 mg/l. The shrimp were reared for 7 days, and then tested to identify the histopathological damages in Balai Karantina Ikan Kelas I, Juanda, Surabaya. The results showed that the control sample presented with enlarged gill tissue nucleus of a basophilic color compared to the normal cells. This happened because there was an infection of White Spot Syndrome Virus (WSSV). The shrimp’s swimming legs also showed some tissue damage, such as necrosis, inflammation, and basophilic mass. The 3 mg/l doze of immunostimulant showed some necrosis damage on the gills and swimming legs, while the 5 mg/l doze showed tissue degeneration and necrosis in the gills, as well as basophilic mass damages in the swimming legs.

1. Introduction
Shrimp are one prospective fishery commodity. One type of shrimp that is being cultivated in aquaculture is the vanamei shrimp. Vaname shrimp are relatively easy to cultivate and breed, making this species an important commodity cultivated in several countries around the world. The vaname shrimp weight is able to increase by more than 3 g each week in a high stocking density [1]. The high consumption of the community makes these organisms experience a very rapid and increased level of production.

However, there are a variety of obstacles, one of which is disease attacks in the form of bacteria, viruses or parasites. Diseases may cause the onset of slow growth, decreased aquaculture production, mass mortality and heavy losses for the fish farmers. This obstacle should be handled as fast as possible to prevent decreased cultivation activity, as mass mortality can happen in shrimp. This study used shrimp samples taken from some of the intensive brackish water ponds in Lamongan, East Java, Indonesia. The samples were examined microscopically to observe the infestation of ectoparasites on
the vaname shrimp and to discover the tissue damage before and after being given treatments of immunostimulants with a one-week interval.

2. Materials and methods

2.1. Materials
Container tank, aerator, siphonized hose, net, pH paper, thermometer, DO meter, test kit, beaker glass, section equipment (scissors, pinset, and scalpel), microtome, tissue cassette, object glass, light microscope, stereo microscope, plastic pot for sample fixation, cover glass, hot plate, base mold, tissue processor, the gills and swimming legs of vaname shrimp post-larvae (50), Davidson fixative solution, xylene, alcohol 70%, alcohol 80%, alcohol 90%, and alcohol absolute 96%, paraffin, Haematoxillin Eosin (HE) coloration, and immunostimulants in 3 mg/l (P13) and 5 mg/l (P15) concentrations.

2.2. Methods
The first group included shrimps that had not been given any treatment, while the second and third treatment groups were shrimp exposed to the immunogenic protein membrane at 3 mg/l (P13) and 5 mg/l (P15) concentrations respectively. The shrimp samples were taken from Instalasi Pengolahan Air Payau, Lamongan, Jawa Timur, Indonesia. The shrimp samples were reared for 7 days. The shrimp samples were then dissected for further histopathological analysis. This activity was followed with soaking the shrimp’s rear portion in the Davidson fixative solution. This was done as a post-mortem prevention, including hardening the agar cell for an easier cut, killing any pathogenic microorganisms, and increasing different refraction index on tissue component, as well as increasing the protoplasm affinity against some of the coloration substances. The gills and swimming legs were dissected and further kept in the fixative for 48 h and processed using histological techniques. The dehydration process was necessary, as it is the process of pushing out the water from the tissue. The tissues were dehydrated in a graded alcohol series (70%, 90%, and 100%) for 60 min each. The fixated samples were clearer, as the samples were soaked from the dehydration, resulting in a purified solution. This step aimed to make the tissue become transparent. The tissues were treated with xylene for 60 min twice and the paraffin blocks were made using the tissue embedding system. The tissue sections (4–5 µm) were taken and stained with haematoxylin and eosin. The stained tissue sections were mounted in DPX and observed under the light microscope.

3. Results and discussion

3.1. Result
Table 1 represents the ectoparasite examination of the PL 50 shrimp taken from brackish water ponds in Lamongan, East Java, Indonesia.

| No | ∑Parasite | Zoothamnium | Vorticella |
|----|-----------|-------------|-----------|
| 1  | 20        | 7           | 13        |
| 2  | 52        | 41          | 11        |
| 3  | 83        | 52          | 31        |
| 4  | 42        | 21          | 21        |
| 5  | 46        | 21          | 25        |
| 6  | 35        | 22          | 13        |
| 7  | 69        | 29          | 40        |

The water quality during the rearing period was also controlled intensively. Table 2 shows the average water quality level.

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Table 2. Water quality

| Time | Temperature | pH | DO   | Salinity |
|------|-------------|----|------|----------|
| H-1  | 28.1        | 7  | 6.74 | 17.20    |
| H-2  | 28.1        | 7  | 5.18 | 17.12    |
| H-3  | 28.1        | 7  | 6.50 | 17.14    |
| H-4  | 27.9        | 7  | 6.27 | 18.00    |
| H-5  | 27.7        | 7  | 6.61 | 17.10    |
| H-6  | 28.1        | 7  | 6.38 | 18.00    |
| H-7  | 27.7        | 7  | 6.71 | 18.00    |

The histopathological sample was taken twice. The first sample was made from the gills and swimming legs of the shrimp from the control (P0) treatment pond, while the second and third samples were made from the gills and swimming legs of the shrimp from the ponds with 3 (P13) and 5 (P15) mg/L immunostimulant concentration. The histopathological figures can be seen below (Figure 1).

Figure 1. Histopathological sample; A. Gill without treatment (P0); B. Swimming leg without treatment (P0); C. Gill exposed with 3 mg/l immunostimulant concentration (P13); D. Swimming leg
exposed with 3 mg/l immunostimulant concentration (P13); E. Gill exposed with 5 mg/L immunostimulant concentration (P15); F. Swimming leg exposed with 5 mg/L immunostimulant concentration (P15).

3.2. Discussion
The native examination result of the shrimp samples discovered many ectoparasite infestation incidents. The types of ectoparasite found in the shrimp samples were mostly recorded as Zoanthamnium sp. and Vorticella sp. These ectoparasites were observed to have infested the swimming legs and gills with a quite high prevalence, making it necessary to conduct molecular and histopathology tests. The virus sample was reportedly found to be WSSV infecting the vaname shrimp in the brackishwater ponds in Lamongan, East Java, Indonesia. This virus presence was tested using PCR with a positive result. The shrimp infected with WSSV suffer from discoloration caused by enlarged chromatophore cuticle occurrences. Chromatophores on shrimp are one of the defense systems in shrimp [2]. The hepatopancreas organ change ranged from 28.57% - 57.14%, which was also caused by WSSV virus infection. [3] mentioned that WSSV infection in shrimp causes hepatopancreas discoloration ranging from reddish-brown into enlarged yellow pale. The shrimp cells infected by WSSV suffered mass amounts of damage (necrosis) in their antenna, antenulla, rostum, periopod, pleiopod, and tail, reaching 20% damage total.

The tissue damage in P0 showed enlarged basophilic color gill compared to the normal tissue. The nucleus cell swelling (hypertrophy) was the result of virion piling development in the nucleus (Moore and Poss, 1999). Further development led to the nucleus moving to the edge, resulting in cariolsis. Nucleus swelling hits the liquid, causing cell rupture due to the cell wall tolerance being exceeded. A broken cell nuclei causes death to the shrimp [4]. The cell nucleus, whether it was infected or not, showed a light reddish color like eosinophile, as a part of absorbing the eosin coloration. The cells that were severely infected showed a dark blue color if they were basophilic, absorbing the hematoxylin. The tissue damage on the swimming legs in P0 included necrosis, inflammatory, and basophilic masses. Necrosis is the uncontrolled death of cells or tissues in any animal. The incidence rate of necrosis was caused by an integrity loss in the plasma membrane. Necrosis was triggered by local inflammatory reactions [5]. P13 and P15 was the addition of an immunostimulant treatment in 3 and 5 ppm concentrations respectively. The water quality in the rearing tank was maintained in order to minimize the impact factor presence of the environment toward more severed tissue damage. The immunostimulant used was an immunogenice protein membrane isolated from Zoothamniumpenaei. An immunostimulant is a material that is able to increase the immune system components [6].

These materials were able to modulate and repair the immune system imbalance. P13 showed there to be histopathological damage found as necrosis, whereas P15 observed there to be cell degeneration and necrosis on the gill tissue, as well as basophilic masses on the swimming leg tissue. Cell degeneration in the tissue damage occurred due to a cell metabolism disorder, making the cell lose its normal structure and function. There was tissue damage on the shrimp samples after the immunostimulant supplementation was delivered. This was possible as there was a damage impact on the target organs during the disease attack before the immunostimulant was given, making it take a longer time to discover the effective level of the immunostimulant supplementation for elevating the vaname shrimp’s immune system. Based on the molecular analysis after the rearing period using PCR, it obtained negative results for WSSV. This condition led to further rearing and observations in order to better conclude on the effective impact of the immunostimulant given by repeated soaking every 7 days during the rearing period.

4. Conclusion
Based on the research results, it was concluded that there was tissue damage in the shrimp taken from the ponds. Tissue damage was also found in the shrimp that were maintained with the addition of immunostimulants. This is possible because the shrimp have been exposed to the disease while in their
original habitat. Besides that, it still takes time to find out the effect of the addition of immunostimulants on increasing the immune response in shrimp.

5. References

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