Characteristics of Aeromonas hydrophila-infected Catfish (Clarias sp.)

H Kartikaningsih1,2,3*, Yahya1, F Z Rohman1 and A A Jaziri1,2,3

1 Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia
2 Bioseafood Research Group, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia
3 Halal Thoyyib Science Center, Universitas Brawijaya, Malang 65145, Indonesia

*Corresponding author: hartatikartikan@ub.ac.id

Abstract. Catfish production was affected by Aeromonas hydrophila. This study aims to investigate the effect of A. hydrophila infection on catfish (Clarias sp.). Physical properties (SEM and histology), morphology, and organoleptic test. The results of this study reported that the morphology of catfish infected with A. hydrophila had clinical symptoms, such as swelling on stomach and intestines were black on days 5 to 7, in the skin injured on day two and hemorrhagic on day 6-7, the quality of the meat decreased on the day 7. The organoleptic test indicated that the quality of the mucus, texture, and odor was reduced. Furthermore, the results of the histological test reported stretching of the muscle.

1. Introduction

The demand for catfish has increased every year, which causes catfish production to increase in Indonesia. According to Sitio et al., [1] catfish production increased by 35% per year (2010-2014). In 2010, production reached 270,600 tons and 900,000 tons in 2014.

During cultivation, the main problem with catfish production is disease infection. Infection of the disease can reduce fish health and cause death in fish, which can reduce catfish production [2] and results in financial losses to fish farmers. Diseases in fish can be caused by bacteria, viruses, or parasites, both ectoparasites, and endoparasites. One bacterial disease that attacks fish is caused by bacteria of the genus Aeromonas, including Aeromonas hydrophila and Aeromonas salmonicida. Aeromonas hydrophila is a Gram-negative, short rod-shaped, has polar flagella and is motile [3]. The disease attack from the bacteria often called Motile Aeromonas Septicemia (MAS), is a severe disease that can attack all types of freshwater fish in the tropics. This disease will become an epidemic when the condition of the fish decreases due to stress and decreased water quality [4].

A. hydrophila infected catfish shows the following signs: body-color becomes dark, skin becomes rough, and bleeding occurs, which will then occur ulcers (haemorrhagic). Also, swimming ability decreases and often gasps on the surface of the water because the gills are damaged which makes it challenging to breathe fish, bleeding in internal organs such as the liver, kidneys or spleen, slightly bloated stomach, fins are damaged and the host becomes whitish, and damaged eyes [2,5,6]

In determining fresh catfish, there are still many consumers who do not know how the difference between fresh fish and fish infected with A. hydrophila. Besides, lack of information related to the determination of fresh fish by looking at signs of A. hydrophila infection. Therefore, the need for guidelines to help consumers see the characteristics of fish infected with A. hydrophila. The purpose
of this study was to determine the effect of infecting *A. hydrophila* bacteria during seven days of cultivation on physical properties (SEM and histology), morphology, TPC, and organoleptic test of catfish (*Clarias* sp.).

## 2. Materials and method

### 2.1. Materials

Catfish was obtained from UPT PTPB (Technical Implementation Unit for Aquaculture Technology Development) in Malang, East Java, Indonesia. *A. hydrophila* isolates were obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya.

### 2.2. *A. hydrophila* infection in catfish

In the process of infecting *A. hydrophila* in catfish, which was done by immersion method. A total of 5 catfish put into the aquarium and then fed as much as ± 40 g based on the calculation of the length and weight of the fish. The samples were adapted for five days, then carried out an infection in catfish after 24 hours of the adaptation process using *A. hydrophila*, which had been cultured in TSB for 48 hours at 30ºC. Furthermore, fish were kept by feeding twice a day for seven days, and then the meat is tested.

### 2.3. Determination of morphological tests

In morphological testing (clinical symptoms), first, it must be paid attention to the condition of fish from day 1 to day 7, including the response to fish feed, swimming behavior, and condition of fish [8].

### 2.4. Determination of organoleptic test

Determining the level of freshness of fish could be done by organoleptic tests in terms of the condition of fish on panelists every day, from day 1 to day 7. It was considered such as texture, odor, meat, and mucus. An organoleptic method is a method of evaluation using only the human senses (sensory). This method is very fast, easy, and practical to assess, but its accuracy depends on the level of a trained person who carried it out [9].

### 2.5. Determination of TPC test

In the TPC test carried out on meat samples. First, in the sample of catfish, 1 gram of meat was taken and then mashed with mortar. Diluted samples were poured plate on NA. Incubated for 24 hours at 30ºC. Then, we observed the results [10].

### 2.6. SEM test

Catfish meat which has a thickness of 1x1 mm has been fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 hours. The sample was then rinsed with distilled water for 1 hour and dehydrated in ethanol with serial concentrations of 50%, 70%, 80%, 90% and 100% (v/v). Dry samples was mounted on a bronze stub and coated with gold (SPI-Module Sputter coater, West Chester, PA, USA). Specimens were observed with SEM (PHENOM G2 pro, Netherlands) at a 20 kV acceleration voltage.

### 2.7. Histological test

In histological testing, samples were cut 1x1 cm large, then soaked first in fixative material (40% formaldehyde) and allowed to be fixed. Next, the tissue was cut and placed in a cassette. The holder was placed in a basket on the automatic processor. The processor processed the tissue through a series of alcohol solutions with increasing concentrations, and then to a clarifying agent (xylene) and finally into a wax melt to complete the wax impregnation process (paraffin). In the process of embedding (embedding), hot wax was dripped into a mold containing tissue to form blocks. After that, the process of cutting (sectioning) where a single piece was taken then floated on the surface of the hot water, which aligns the folds and then transferred to the glass object. Finally done staining on the slide and mounting. Scan on 2013 German OLYMPUS BX 51 microscope.
3. Results and discussion

3.1. Morphological test of catfish

Morphology test conducted by the treatment of control sample and infected samples with *A. hydrophila* consisted of observing stomach contents, wound samples, and flesh samples. This morphological test aimed to determine clinical symptoms due to *A. hydrophila* infection in catfish. The result of a morphological test on catfish was as follows:

3.1.1. Stomach

Morphology of stomach content in the untreated catfish was not swelling (Figure 1A), while in Figure 1B-E, showed the infected samples with *A. hydrophila* on day 1 to day 4 were in the normal condition without swelling. In addition, Figure 1F-H, exhibited the intestines of catfish at day 5-7 infected with *A. hydrophila* were swelling and blackish color. There was swelling in the intestines of the fish (Figure 1F-H). This can be caused by *A. hydrophila* causing hemorrhagic fins, around the mouth, even exophthalmia, with swelling of the kidneys. *A. hydrophila* infection causes severe intestinal lesions, including fusion and accumulation of intestinal villi, and induces severe cell inflammation [4,11].

![Figure 1](image1.png)

**Figure 1.** Observations on stomach of catfish. (A) Normal tissue as control and (B-H) treated samples of catfish infected with *A. hydrophila* in the culture period were 1-7 days. Arrow indicated the stomach condition of catfish during treatments.

3.1.2. Observation of wounds
In Figure 2B, the samples infected with *A. hydrophila* on day 1 were in normal condition without injury. Figure 2C-F showed the condition of catfish at day 2 to day five infected with *A. hydrophila* has changed in skin color and appearance of wounds. Besides, Figure 2G-H, was a condition in catfish at day 6- infected with *A. hydrophila* showed ulcers on the skin (hemorrhagic).

![Images of catfish](image1.png)

**Figure 2.** Observations on wounds of catfish. (A) Normal tissue as control and (B-H) treated samples of catfish infected with *A. hydrophila* in the culture period were 1-7 days. Arrow indicated wounds condition of catfish during treatments.

There was a change in the color of the skin that starts to turn pale and the emergence of wounds on the fish's body (Figure 2C-F), while Figure 2G-H exhibited hemorrhagic on the body. Infection scars
found in fish will turn into ulcers, abdominal bloating, and skin color fading from gray to pale [4]. Fish infected with *A. hydrophila* have many different symptoms. Skin ulcers can occur anywhere in fish and are often surrounded by red tissue. Other organs that are often affected by this disease are the gills, kidneys, liver, spleen, pancreas, and skeletal muscles. Prajitno [2] states that catfish that are affected by *A. hydrophila* are usually morphologically or physiologically. *A. hydrophila* is the primary pathogen of septicemia, and the external symptoms of this disease are hemorrhagic spots on the body and cause epidemics of fish epidemics [11].

c) Observation on catfish meat

In the study of morphological meat conditions in catfish, obtained results in accordance with Indonesian National Standard, 01-2346. Figure 3A, the untreated catfish had very bright cuts, specific types, no milking along the spine, intact wall stomach meat. Infected catfish showed very bright, specific type of meat, no milking along the spine, intact abdominal wall (Figure 3.B-C). At days 3 and 4 infected catfish showed a brilliant, specific type of meat, no milking along the spine, intact abdominal wall (Figure 3D-E). Besides, Figure 3F-G, at days 5 and 6 infected with *A. hydrophila* had the characteristics of a slightly less brilliant, specific type of meat, no milking along the spine, the intact wall of the meat intact. While at day 7, showed the incision of the flesh (Figure 3. H).

![Figure 3](image)

**Figure 3.** Observations on catfish flesh. (A) Normal tissue as control and (B-H) treated samples of catfish flesh tissue in the culture period were 1-7 days. Arrow indicated the flesh condition of catfish during treatments.

The conditions above, Figure 3H, there was a change in the color of fish flesh. The change in behavior is followed by changes in morphology such as the back of the body of the fish to swell white and eventually turn red, and then fish meat becomes damaged. It is due to *A. hydrophila* infection [12].
3.2. Organoleptic test (Scoring)
Organoleptic test evaluation on samples according to SNI of fresh fish. Organoleptic tests included meat, mucus, and catfish texture.

3.2.1. Organoleptic test on flesh
In the flesh, testing consists of color and appearance, by giving a detailed assessment by looking at the score sheet table of fish in the range of values 1 to 9. Organoleptic test results showed that the control obtained an average value of 9 while the infected catfish meat \( A \ hydrophila \) on day 1 to day 6 decreased in the range of 8.8-7. However, these results were still allowed by standards of fresh fish. On the contrary, at day, the average meat value was 5.13, meaning that it was below the standard of fresh fish. This can be caused due to \( A. \ hydrophila \) infection in catfish.

3.2.2. Organoleptic tests on catfish mucus
Organoleptic test results on catfish mucus, where the control treatment obtained an average value of 9, while the meat of catfish infected with \( A. \ hydrophila \) at day1-7 showed a decrease in the range of 8.9-7, but was allowed by standards of fresh fish. Hyperaemia is mucus removal. Mucus released by fish can reach 2-2\(^{1/2}\) of the bodyweight of the fish and cover the entire body of the fish [17].

3.2.3 Organoleptic tests on the texture of catfish
Organoleptic test results on the texture of catfish showed that the control treatment obtained an average value of 8.8. In contrast, the catfish meat infected with \( A. \ hydrophila \) at day 1 to 7 gradually decreased in the range of 8.86-7. Organoleptic changes in fish can be caused by softening the texture of fish meat. The softening of the texture in fish meat occurs because of the breakdown of proteins into simpler compounds, namely polypeptides, amino acids, and ammonia, which can increase fish pH [9].

3.2.4. Organoleptic test on the smell of catfish
Results of the organoleptic test on the odor of catfish showed that the control treatment obtained an average value of 9. In contrast, the catfish meat infected with \( A. \ hydrophila \) at day 1 to 7 showed a decrease in the range of 8.4-7. Bacteriological decomposition in which fish protein will be decomposed by bacteria, which will produce toxic substances and odor. Fish that are still alive or not long after they die are still sterile, but after that, the bacteria found on the surface of the body or in the intestine will penetrate the meat [13].

3.3. TPC test for \( A. \ hydrophila \) in catfish meat
TPC test results obtained total bacteria on selective media (Rhimler Shotts) for meat samples in the control treatment obtained values of 0 cfu/mL. Whereas the treatment obtained the highest value of 1.60x10\(^{25}\) cfu/mL on the 7th day. The lowest value is obtained at 0 cfu/mL on days 4-6. For NA media, meat samples in the control treatment obtained the highest value of 8.63x10\(^{10}\) cfu/mL on the 7th day and the lowest of 3.26x10\(^{10}\) cfu/mL on day 1. Whereas in the treatment sample, the highest value was obtained 2.7x1026 cfu/mL at day 7, while the lowest value was obtained at 2.75x10\(^{11}\) cfu/mL at day 1. This study can be concluded that the analysis of the TPC test on meat for control treatment was not found \( A. \ hydrophila \).

In general, \( A. \ hydrophila \) grows in fresh water, especially waters that contain high organic matter. A. Hydrophila can attack all types of freshwater fish, and this type of disease is called MAS or also called Haemorrhagic septicemia. This bacterial attack is latent (prolonged), so it does not show symptoms of the disease even though it has been found on the body of the fish. This bacterial attack is only seen when the fish's body resistance decreases due to stress caused by a decrease in water quality, lack of feed, and inpropper handling [2].

3.4. SEM analysis of catfish samples
Catfish samples without treatment (control) showed that the fish did not experience damage to the body (Figure 4A). However, catfish infected with *A. hydrophila* at day 1 to 4 damaged, such as cracks and folds in muscle tissue (Figure 4B-E). While Figure 4F-H, at day 5 to 7 there has cracks in the folds of muscle tissue that were quite clear in samples of infected catfish. Ischemia or stopping of blood flow, which causes necrosis of muscle cells [15]. Damage of blood to blood vessels around the muscles can induce edema so that muscle fibers will appear to be sparse because the cavities between the fibers are filled with fluid. Other changes that occur in a fish muscle are degeneration and necrosis of cells. Fish muscle showed hollow color, nuclear migration, sarcoplasmic necrosis, localized edema, and muscle cell nuclei, which undergo karyokinesis and karyorrhexis [16].

![Figure 4](image)

**Figure 4.** SEM image on flesh tissue of catfish. (A) Normal tissue as control and (B-H) treated samples of catfish flesh tissue in the culture period were 1-7 days. Arrow indicated damages in the muscle tissues of catfish during treatments.

### 3.5. Histology test analysis

In histological observations that were used to determine the presence or absence of morphological damage in the flesh of catfish infected with *A. hydrophila*. This study used a scanner on the OLYMPUS BX 51 microscope with a magnification of 40 times (40 µm). Histological observations of catfish meat are shown in Figure 5. For the control sample, it was found that the sample had not been damaged where the muscle fibrous necrose was still tight (Figure 5A). While in Figure 5B-F, a sample infected with *A. hydrophila* at day 1-5 showed that there was damage in the presence of stretched necrose muscle fibers. While Figure 4G-H at day 6-7 showed the muscle fibrous necrose was very clearly visible, that is the formation of cracks.

![Figure 5](image)
Figure 5. Histological observation on flesh tissue of catfish. (A) Normal tissue as control and (B-H) treated samples of catfish flesh tissue in the culture period were 1-7 days. Arrow showed damage in the presence of stretched necrose muscle fibers.

4. Conclusion
In summary, the morphology of infected catfish showed clinical symptoms with swelling in the stomach and intestine became blackish at days 5-7, wounds on day 2, and hemorrhagic at days 6-7. The mucus, texture and odor of flesh have decreased daily. The number of bacteria was above Indonesian National Standard.

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