



## Histopathological characteristics of striped catfish (*Pangasianodon hypophthalmus*) in challenge with *Edwardsiella ictaluri*

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### ABSTRACT

The present study attempted to understand the histopathological index of striped catfish (*Pangasianodon hypophthalmus*) in challenge with *Edwardsiella ictaluri*, the causative agent of bacillary necrosis of *Pangasius*. Total of 355 healthy striped catfish juveniles was challenged with *E. ictaluri* (25 control; 330 infected). After the challenge, a total of 355 samples of the trunk kidney, liver, and spleen were collected at five different time points including just prior to infection, 24, 48, 264 and 312 hours post-infection (hpi) to measure the represented tissue damage of the fish. Results showed that many areas of the diseased liver, kidney, and fish organ tissues have been congested and hemorrhaged early at 24 hpi. The number of macrophage centers in the kidney and spleen increased at 24 - 48 hpi. Tissue damage (multifocal hemorrhages; necrosis) increased sharply from the period 48 - 256 hpi. The increased number of dead fish throughout infection, particularly the period from 48 to 200 hpi. At 312 hpi, the histopathological index was the highest, so the mortality rate was the highest during the experimental period.

## 1. INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors in the world including Vietnam. The Vietnamese fisheries production in the first six months of the year was estimated at 4,196.8 thousand tons, which increased by 2.5% over the same period last year (VASEP, 2022). Striped catfish (*Pangasianodon hypophthalmus*), a freshwater species cultured mainly in the Mekong Delta region in Southern Vietnam, is an important aquaculture species of high economic value. The popularity of striped catfish is due to its relatively fast growth rate, high flesh quality, tolerance to low oxygen and crowding, as well as its ability to adapt well to various cultural systems. It also presents an efficient feed conversion rate and accepts manufactured feed (Nguyen & Do, 2010; De Silva

et al., 2010; De Silva & Nguyen, 2011). In 2021, the production of striped catfish in Vietnam reached 1.56 million metric tons and a turnover of approximately 1.61 billion USD (VASEP, 2022). There has been a phenomenal shift from extensive to intensive culture of carp and catfishes in the last three decades. Intensive aquaculture offers an increased opportunity for spreading infectious diseases at all stages of production. There are many factors responsible for periodical disease outbreaks in aquaculture such as microorganisms (54.59% bacterial, 22.6% viruses, 3.1% mycotic) and parasitic agents (19.4%) (Dhar et al., 2014). Among the bacterial pathogens, *Pangasianodon hypophthalmus* (*P. hypophthalmus*) is most susceptible to *Aeromonas* spp. and *Edwardsiella ictaluri* (*E. ictaluri*) causing motile aeromonas

septicemia (MAS) and bacillary necrosis (BNP) respectively (Subagja et al., 1999; Ferguson et al., 2001). Especially, BNP caused by *E. ictaluri* is considered to be the most serious disease occurring in farmed striped catfish in Vietnam. This disease has had an increasing impact over the last ten years and has been reported to cause 50-90% mortality of stocks during a single outbreak (Nguyen, 2014).

Histopathological changes have been widely used as biomarkers to evaluate the health of fish exposed to contaminants (Thophon et al., 2003). Gill, kidney, and liver are responsible for vital functions, such as respiration, excretion, and the accumulation and biotransformation of xenobiotics in the fish. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows for examining these specific target organs (Gernhofer et al., 2001). Furthermore, the pathological changes discovered in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Laurén, 1990; Mamun et al., 2022).

There have been many research works on BNP, but these studies only focus on describing clinical symptoms, isolating and describing the causative agent (Ye et al., 2009; Crumlish et al., 2010). At the same time, there are no studies on comparative data on histopathological characteristics of *P. hypenthalmus* against *E. ictaluri*. Therefore, the principal aims of this study were to understand the differences in histopathological changes of two family groups of striped catfish in liver, kidney, and spleen tissues. Findings from this study are expected to provide more information about the disease manifestations at the cellular level on the internal organs of selective breeding striped catfish resistant to BNP disease caused by *E. ictaluri*.

## 2. MATERIALS AND METHOD

### 2.1. Research design

#### 2.1.1. Bacteria

Bacterial strain *Edwardsiella ictaluri* Gly09M isolated from BNP catfish in An Giang province in 2009 and preserved in the bacterial collection of the Research Institute for Aquaculture No.2 (RIA2) was used in this research. The LD<sub>50</sub> was  $4.9 \times 10^3$  CFU/0.2 mL/fish for striped catfish 15 - 25g (Le et al., 2013). The bacteria were restored and re-isolated from healthy striped catfish three times prior challenge.

#### 2.1.2. Experimental fish and challenge test

A total of 355 healthy striped catfish juveniles (15 – 20 g) were procured from National Feeding Center for Southern Freshwater Aquaculture located in Tien Giang province. The fish were acclimatized to laboratory conditions for 15 days. All individuals in each family were stocked in 90 L tanks at the stocking density of 0.26 fish/L. The cohabitant method was used in this challenge described in detail by Nguyen et al. (2022). The cohabitant fish ( $16.6 \pm 6.1$  g/fish) was firstly infected with the bacteria *E. ictaluri* by injection at a dose  $10^6$  CFU/0.2 mL per fish. Two days after the injection, the cohabitant fish were released into the tanks to rear together with the experimental fish, accounting for 35% of the total number of fish in each tank. After two days of communal rearing, suspension of *E. ictaluri* was added to the experimental tanks to retain the bacterial density ( $10^5$  CFU per 1 mL of water) for disease infection. The experiment was conducted throughout 312 hours post-infection (hpi).

### 2.2. Histopathological analysis

Lethargic fish were collected and dissected for white spots on internal organs (liver, kidney, and spleen) at the time points: before infection, 24, 48, 264, and 312-hour post-infection (hpi). A total of 25 samples of each internal organ were collected before infection, 24, 48, 264 hpi, and at 312 hpi were ten samples in the infected group. In the control group, five samples at each time point were collected. The tissues were dehydrated in ethanol series and cleared through pure chloroform. After completing this process, the samples were embedded in paraffin wax (60–70°C melting point) and a prepared block for sectioning. The samples were cut through a microtome machine with around 5 µm thicknesses and fixed on slides. Then, the slides were kept overnight in the open air for drying. Finally, the slides were stained with Hematoxylin and Eosin and mounted in DPX for long-term preservation (Coolidge & Howard, 1979). The semi-quantitative histopathological changes were investigated as described by Quach (2017). The measurements were performed using a light microscope under 40X magnification through a calibrated lens, obtaining numeric values which represented tissue damage per metric unit and were subsequently converted into microns (Cercadillo-Ibarguren et al., 2010).

### 2.3. Statistical analysis

The data of the histopathological index of the liver, kidney, and spleen of striped catfish at each time point were tested. The one-way ANOVA with a significant level of 5% ( $p < 0.05$ ) was used to compare the mean values of the histopathological index at each time point. These analyses were performed on the SPSS statistical software to test the significant difference at probability  $p < 0.05$ . All

data were presented as mean  $\pm$  standard errors of the mean (SE) in the actual unit of measurement.

## 3. RESULTS AND DISCUSSION

### 3.1. Clinical signs and gross pathology

Within 3 to 4 days post-exposure, clinical signs commonly associated with *E. ictaluri* infection were observed in the fish in the test challenge (Figure 1).



**Figure 1. Clinical signs and gross pathology of striped catfish after infection**

*White lesions were presented in the anterior kidney (K), spleen (S) liver (L) were enlarged*

At the time before infection, the fish showed symptoms: swimming fast, responding quickly to noise, smooth body with no signs of injury, no sores on the body, and no bleeding in the fins, or gills. Fish started to die after 5 days of cohabitation. At 48 hpi, small focal circumscribed haemorrhagic lesions present on the skin were observed in the skin of fish infected with *E. ictaluri*. After 14-day post-infection (dpi), the mortality rate of fish increased sharply. Fish in the challenge test showed behavioral changes including erratic swimming in a spiral motion and stopped feeding prior to mortality. Internally, the affected fish's kidney and spleen showed white lesions of 1-2 mm in diameter. Later, white lesions also occurred in the liver of infected fish (Figure 1). In addition to abdominal dropsy and fluid in the peritoneal cavity, the abdomen was enlarged. The kidney and spleen were enlarged.

A comparison of the data provided in this study showed that the fish exposed to the bacterium showed similar behavioral and clinical signs to those described for both natural and experimental

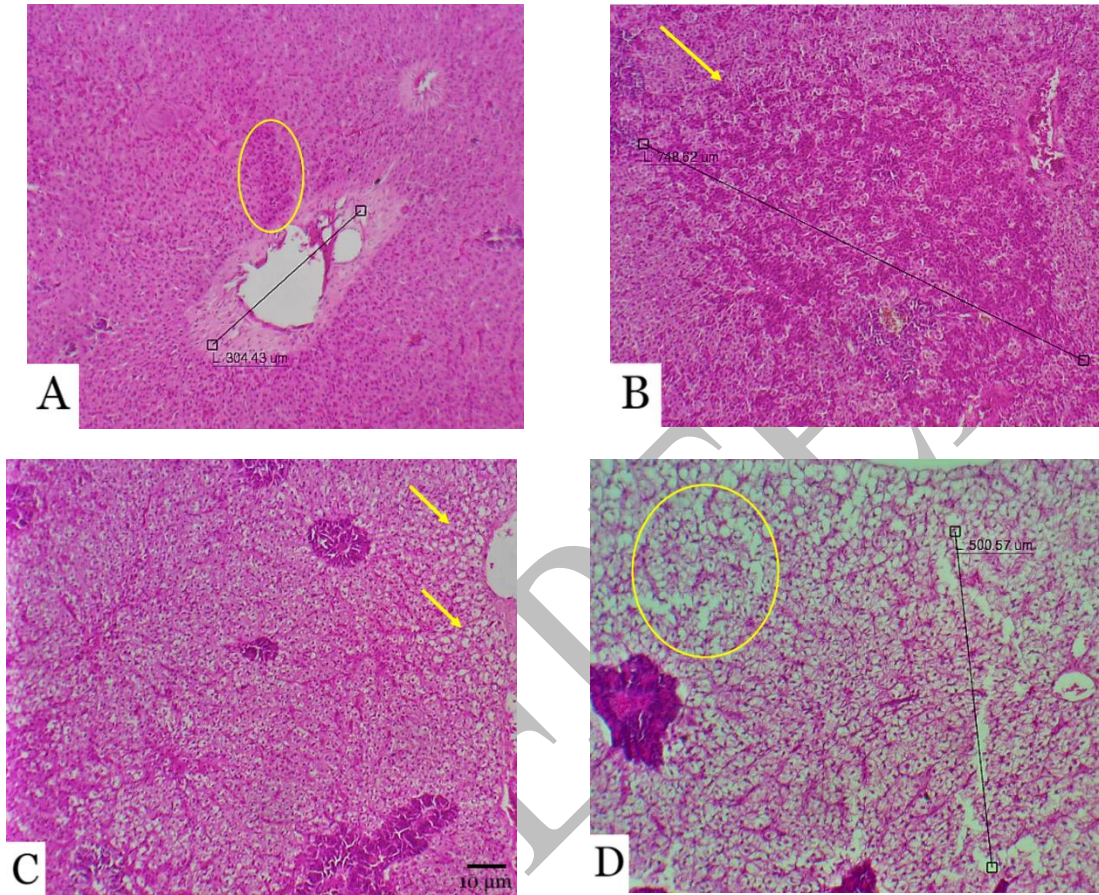
BNP infections (Ferguson et al., 2001; Crumlish et al., 2002; Crumlish et al., 2010). Bacterial recovery from the spleen and kidney samples of all infected striped catfish (both moribund and dead fish) produced bacterial colonies identified as *E. ictaluri*. The histopathology taken from moribund fish exposed to the bacteria and with gross clinical signs of BNP was also similar to that observed in the previous natural and experimental infections in BNP studies (Ferguson et al. 2001; Crumlish et al., 2002, 2010).

### 3.2. Histopathological index of liver

Some liver histopathological changes from the striped catfish sampled after the challenge were: (i) cell adhesions were destroyed; (ii) large areas of cellular necrosis and haemorrhage were present in the liver; (iii) many areas of hepatocytes were deformed leading to necrosis and loss of structure; (iv) multiple extensive areas of necrosis were observed in the liver (Figure 2). At 24 hpi, the liver began to hemorrhage slightly and increased sharply

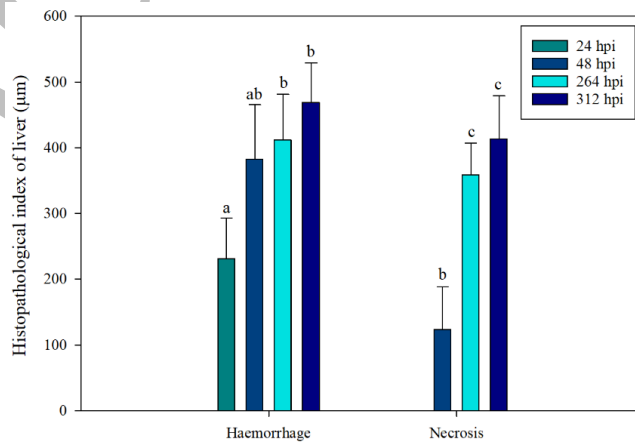
at 48 hpi (from  $231.93 \pm 60.94 \mu\text{m}$  to  $383.08 \pm 82.26 \mu\text{m}$ ). The differences were not statistically significant ( $p > 0.05$ ). Prolonged hemorrhage leads

to necrosis. At 312 hpi, the most necrotic liver tissue structure ( $413.40 \pm 65.49 \mu\text{m}$ ) resulted in fish death (Figure 3).



**Figure 2. Liver histopathological changes in the fish in the challenge**

(A) liver hemorrhages (circle) after 24 hours; (B) area tissues of multifocal hemorrhages after 312 hours; (C) liver tissues deformed after 48 hours (arrow); (D) necrosis of liver tissues (circle)



**Figure 3. Histopathological index of the liver during the challenge**

Values are Mean  $\pm$  SE, different letters indicate significant differences between treatments ( $p < 0.05$ ).

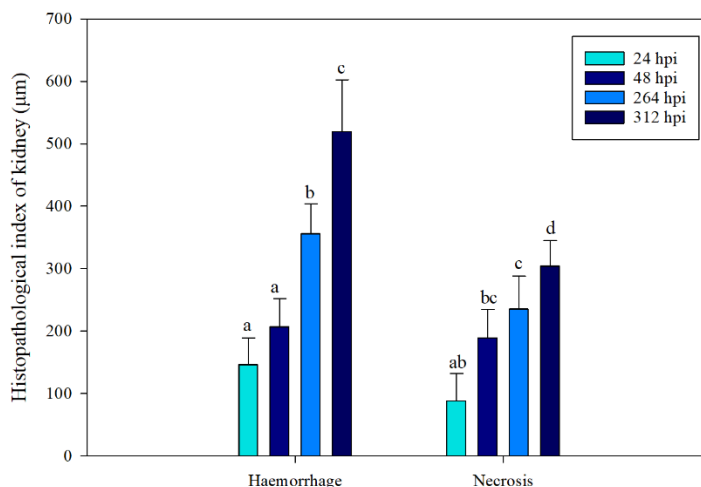
The liver is the largest of the extramural (outside the alimentary canal) organs. Fish liver served functions similar to those in mammals. Its functions included the assimilation of nutrients, detoxification, production of bile, and maintenance of the body's metabolic homeostasis including the processing of proteins, carbohydrates, lipids, and vitamins (Harder, 1975). The liver also played an important role in the synthesis of plasma proteins, like albumin, fibrinogen, and complement factors. According to Mahdy et al. (2021), the liver is encased within a fibroconnective tissue capsule and, unlike the mammalian liver, it contains no distinct lobulation, no apparent interlobular connective tissue stroma, and no typical portal triads. Rather, the most distinctive histological features of the liver include exocrine pancreatic tissue that surrounds all of the portal veins in mature striped bass (Kibenge et al., 2021), and an anastomosing bilaminar meshwork of hepatocytes that comprises the basic functional hepatic units (Patt & Patt, 1969). The parenchyma of the organ is contained within a thin capsule of fibroconnective tissue. The parenchyma itself is primarily composed of polyhedral hepatocytes typically with central nuclei. Glycogen deposits and fat storage often dissolved during the routine histological process, produce considerable histological variability. The hepatic structure normally varies (and considerably) in direct relationship to age, gender, available food, or temperature, and with endocrine influences strongly connected to the environmentally regulated breeding conditions. The liver tissue sections of diseased pangasius revealed multiple hemorrhages on the hepatic tissues along with numerous pyknotic nuclei, dilated sinusoids, vacuolization of cytoplasm, and cellular necrosis of hepatocytes. According to Ferguson (1989), the damage that takes place throughout the liver organization causes the liver to no longer function as detoxification, dialysis, protein metabolism, lipids, glucid, and bile secretion. From there, the virulence of bacteria that are not eliminated accumulates in the body and reduces resistance to pathogens combined with other adverse conditions that kill fish.

These findings are consistent with previous studies that show that *E. ictaluri* infection can cause

pathological lesions in different vulnerable fish species, including channel catfish (*Ictalurus punctatus*), brown bullheads (*Amieurus nebulosus*), ayu and zebrafish (*Danio rerio*) (Sakai et al., 2008; Areechon & Plumb, 2009; Hawke et al., 2013). Some pathological features have been studied such as infection ranging from edematous degenerating hepatocytes (Areechon & Plumb, 2009), to focal and multifocal hepatocytic necrosis, the presence of bacterial cells either in-between necrotic debris and within sinusoidal spaces (Sakai et al., 2008), or engulfed by infiltrating mononuclear cells (Iwanowicz et al., 2006).

### 3.3. Histopathological index of kidney

Some kidney histopathological changes from the striped catfish sampled after the challenge were: (i) multiple extensive areas of necrosis were observed particularly in the anterior kidney; (ii) haemorrhages were observed in the kidney; (iii) melanomacrophage centers (MMCs) appeared in kidney tissues; (iv) the glomerulus and the nephron were congested, bled, swelled and spread which leads to loss of kidney structure (Fig. 4). The histopathological index of fish through challenge time was compared in Fig. 5. At 24 hpi, the kidney appeared congestive and hemorrhagic phenomena ( $146.90 \pm 42.32 \mu\text{m}$ ). Congestive phenomena are considered the body's first response to pathogens due to special stimuli that cause capillaries to strengthen a larger-than-normal amount of blood to lead to inflammatory foci. Under the influence of bacterial toxins, blood capillaries rupture or due to increased capillary permeability, causing cells in the congestive region to escape mixed with organ blood cells causing hemorrhage. Prolonged hemorrhages from 24 to 48 hpi will cause the inflamed swollen kidney tissue to lose its structure and lead to necrosis. From there, the kidneys lose their function. The kidney had multifocal hemorrhages and necrosis increased with the experiment time. Necrosis of kidney tissues strongly increased at 48 – 312 hpi from  $189.41 \pm 45.20 \mu\text{m}$  to  $304.74 \pm 40.25 \mu\text{m}$ . The differences between the histopathological index of the kidney prior to and during the challenge were statistically significant ( $p < 0.05$ ).



**Figure 5. Histopathological index of the kidney prior to and during the challenge**

Values are Mean  $\pm$  SE, different letters indicate significant differences between treatments ( $p < 0.05$ ).

Kidneys play the most important part in maintaining the water-salt balance. The primary task of a freshwater fish kidney was to produce copious dilute urine to counteract the passive influx of water across the gills and integument. *E. ictaluri* also significantly harmed the trunk kidney. Renal histopathology revealed acute degeneration of hematopoietic tissues, and destruction of Bowman's space, glomerular necrosis, tubular necrosis, and infiltration of leukocytes were detected. Invasion and multiplication of pathogenic bacteria can damage the kidney and lead to glomerulonephritis. The trunk kidney would be vulnerable to hazardous byproducts of the bacterium since it is a filtering organ. According to Shah et al. (2017), granulomas are formed consisting of layers of white blood cells such as macrophages or lymphocytes that surround the area of necrotic tissue and damaged cells. These cells are not removed from the body but fibrosis and calcification form granulomas. This is also a characteristic histopathological manifestation in some fish with white spot disease. Huizinga et al. (1979) found extensive necrosis in the mid-kidney of largemouth bass (*Micropterus salmoides*) infected with *Aeromonas hydrophila*. Shotts et al. (1972) found that channel catfish that had been given injections of *Aeromonas hydrophila* had an increase in lymphoid cells and some necrosis of the epithelial cells lining their tubules in the trunk kidney. Mawdesley-Thomas (1969) also found generalized necrotic changes in the kidney of goldfish with furunculosis. Klontz et al. (1966) reported that the most significant histopathology of rainbow trout infected with *Aeromonas salmnicida*

was the change produced in the hematopoietic tissue. This hematopoietic tissue destruction was caused by *E. ictaluri* oligo-polysaccharide (O-PS) (Santander, 2014).

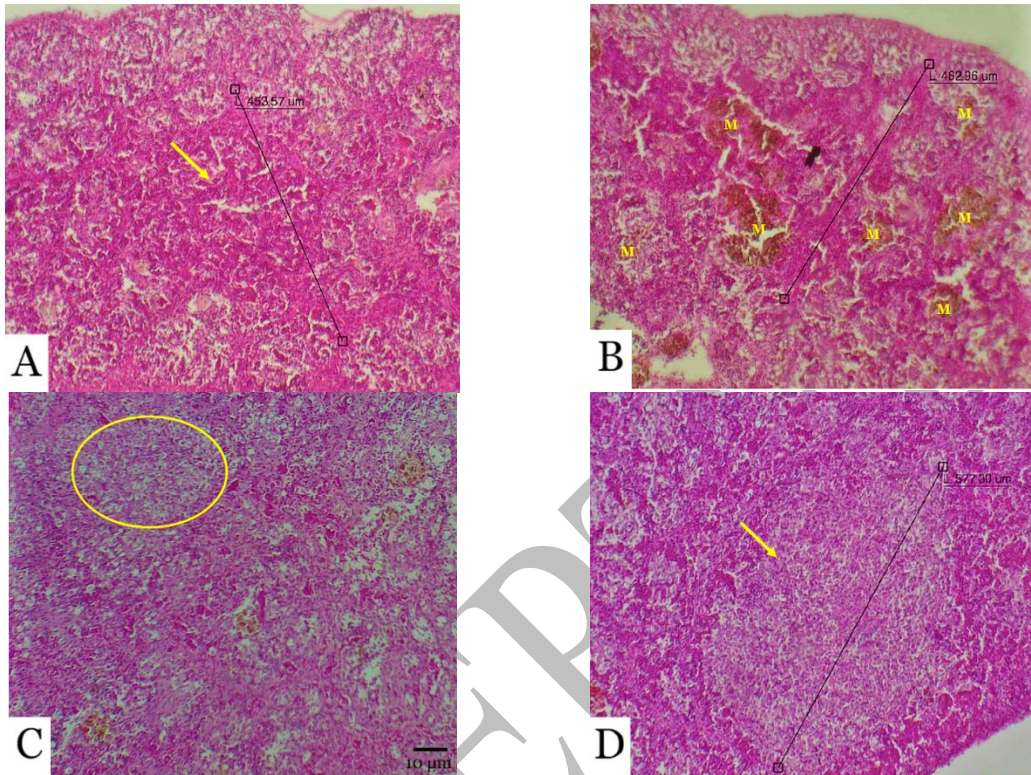
Similarly, in previous studies, Suanyuk et al. (2013) found in striped catfish (*Pangasianodon hypophthalmus*) and hybrid catfish, *Clarias macrocephalus*  $\times$  *Clarias gariepinus* when infected with *E. ictaluri* had hemorrhagic and granulomatous inflammation were observed in kidney, while mild to severe bacterial laden leukocytic cell infiltration were observed in channel catfish, zebrafish, hybrid catfish, and Nile tilapia (*Oreochromis niloticus*) (Soto et al., 2013; Suanyuk et al., 2013), the inflammatory cell infiltrates in zebrafish were composed of macrophages, neutrophils, eosinophilic granular cells, and fewer lymphocytes (Hawke et al., 2013). Variable degenerative changes turning into focal, multifocal or diffuse tubular, glomerular, and interstitial necrosis varying in intensity were evident in channel catfish, brown bullheads, Nile tilapia, zebrafish, and striped catfish (Petrie-Hanson et al., 2007; Soto et al., 2013).

### 3.4. Histopathological index of spleen

At 24 hpi, the spleen also showed extensive confluent areas of necrosis within the parenchyma. Large areas of haemorrhages were observed in the spleen. Extensive and prolonged bleeding leads to necrosis of the spleen. At the period 24 - 48 hpi, melano-macrophage centers appeared in spleen tissues (Figure 6). The histopathological index of the spleen through challenge time was compared in Figure 5. Areas of hemorrhages and necrosis

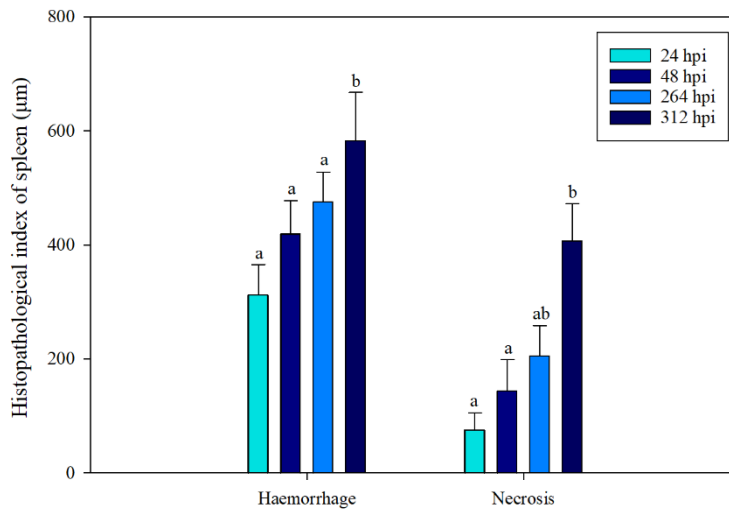
increased with the experiment time, quite early at 24 hpi ( $313.09 \pm 52.42 \mu\text{m}$ ;  $76.39 \pm 28.74 \mu\text{m}$  and reached  $582.84 \pm 84.84 \mu\text{m}$  (hemorrhages) and

$407.25 \pm 65.15 \mu\text{m}$  (necrosis). The differences between the histopathological index of the spleen at 312 hpi were statistically significant ( $p < 0.05$ ).



**Figure 6. Spleen histopathological changes in the fish after the challenge**

(A) hemorrhages of spleen tissues (arrow) at 24 hpi; (B) melano-macrophage centers (M) appeared in spleen tissues at 24 – 48 hpi; (C) necrosis of spleen tissues (circle); (D) area necrosis of spleen tissues spread out



**Figure 7. Histopathological index of spleen prior and during the challenge**

Values are Mean  $\pm$  SE, different letters indicate significant differences between treatments ( $p < 0.05$ ).

The spleen is the main erythropoietic tissue in fish. The spleen is covered by a thin, fibrous capsule with little evidence of contractile ability. The red pulp is an extensive, interconnecting system of splenic cords and sinusoid capillaries (open capillaries), consisting mainly of erythroid cells and thrombocytes, and usually comprises the majority of the splenic parenchyma (Dyková et al., 2022). The melanomacrophage (MM) is prevalent in the spleen. The MM is a phagocyte containing varying amounts of pigment, including melanin (black-brown), hemosiderin, ceroid or lipofuscin (yellow-pink to golden brown) localized in vacuoles. The MM and MMC were also found in the kidney and liver. The MMCs were thought to be a scavenger structure but their role in the immune system is ambiguous. Chronically stressed fish, including those that are unhealthy, tend to have more and larger MMC. The size and number of MMC also increase with fish age. Of all the organs inspected, the spleen had one of the worst injuries. Numerous researchers have noticed bacterial infection-induced cellular degeneration in the spleen (Mawdesley-Thomas, 1969; Ferguson & McCarthy, 1978; Huizinga et al., 1979; Liu et al., 2021). Wolke (1975) thought that severe hematopoietic and structural connective tissue necrosis of the spleen was the most prominent lesion. Since hematopoiesis is one of the functions of the spleen (Grizzle & Rogers, 1976), injury or damage to the spleen could cause the inability to produce erythrocytes and leucocytes resulting in anemia and leucopenia. Histopathology of the liver, kidney, and spleen of naturally infected fish was validated with severe pathologies such as macrophage aggregation with hepatitis, degeneration of hematopoietic tissues, and granulomatous splenitis (Loch et al., 2017; Mamun et al., 2022). This was consistent with clinical manifestations of fish, including reduced response and appetite or stopped eating.

Likewise, in previous work, splenic necrosis was evident in channel catfish, ayu, zebrafish, and striped catfish (Sakai et al., 2008; Hawke et al., 2013), vacuolations due to depletion of white pulp in channel catfish, brown bullheads, and hybrid

catfish (Iwanowicz et al., 2006; Jarboe et al., 1984; Suanyuk et al., 2013) and depletion of the red pulp (Blazer et al., 1985), changed erythrocyte distribution caused by hemorrhage (Areechon & Plumb, 2009), also Newton et al. (1989) and Baldwin and Newton (1993) denoted that the splenic parenchyma was moderately congested and the white pulp was hypocellular in channel catfish. Soto et al. (2013) found that macrophages and occasionally neutrophils containing bacteria were present within the hematopoietic tissue in channel catfish, ayu, zebrafish, and Nile tilapia.

According to Camp et al. (2000) the number of macrophage centers in fish increased significantly from 24 to 48 hpi, and multifocal hemorrhages and necrosis areas of the liver, kidney, and spleen increased slowly. MMCs in the kidney and spleen increased through time experiment. The reason can be explained by the mobilization of the immunological parameters in fish's immune response, which helped to increase survival in the early stage of infection. At this time MMCs decreased from yellow-brown to dark brown and the number was dense on the tissue. At 312 hpi, the histopathological index was highest, so the mortality rate was the highest during the experimental period.

#### 4. CONCLUSIONS

This study demonstrated that there were significant differences in the histopathological index, specifically damage tissue measurements (hemorrhages; necrosis) of striped catfish to bacteria *Edwardsiella ictaluri* from striped catfish. Many areas of the diseased liver, kidney, and fish organ tissues have congested, hemorrhaged, and necrotized. The number of macrophage centers in the kidney and spleen increased at the period 24 to 48 hpi. Tissue damage (multifocal hemorrhages; necrosis) increased sharply from the pre-infective stage, 24 hpi, 48 hpi and has the largest areas of damaged tissue at 312 hpi. Besides the assessment of the immune response, the histopathological index can also assess the impact of *E. ictaluri* through the duration of infection.

#### REFERENCES

- Areechon, N., & Plumb, J. A. (1983). Pathogenesis of *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*. *Journal of the World Mariculture Society*, 14(1-4), 249-260.
- Baldwin, T. J., & Newton, J. C. (1993). Pathogenesis of enteric septicemia of channel catfish, caused by *Edwardsiella ictaluri*: Bacteriologic and light and electron microscopic findings. *Journal of Aquatic Animal Health*, 5(3), 189-198.
- Camp, K. L., Wolters, W. R. & Rice, C. D. (2000). Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant



- families of channel catfish, *Ictalurus punctatus*. *Fish Shellfish Immunology*, 10(6), 475-487.
- Cercadillo-Ibarguren, I., España Tost, A. J., Arnabat Domínguez, J., Valmaseda Castellón, E., Berini Aytés, L., & Gay Escoda, C. (2010). Histologic evaluation of thermal damage produced on soft tissues by CO<sub>2</sub>, Er, Cr: YSGG and diode lasers. *Medicina Oral, Patología Oral y Cirugía Bucal*, 15(6), 912-918.
- Coolidge, B. J., & Howard, R. M. (1979). *Animal histology procedures of the pathological technology section of the National Cancer Institute, 2nd edition*. National Institutes of Health, Bethesda. 209 pages.
- Crumlish, M., Pham, T. C., Koesling, J., Vu, T., V., & Gravningen, K. (2010). Experimental challenge studies in Vietnamese catfish, *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. *Journal of fish diseases*, 33(9), 717-722.
- Crumlish, M., Tu, D. T., Turnbull, J. F., Nguyen, N. T. N., & Ferguson, H. W. (2002). Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases*, 25(12), 733-736.
- De Silva, S. S., & Nguyen, P. T. (2011). Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. *Reviews in Aquaculture*, 3(2), 45-73.
- De Silva, S. S., Ingram, B. A., Nguyen, P. T., Bui, T. M., Gooley, G. J., & Turchini, G. M. (2010). Estimation of nitrogen and phosphorus in effluent from the striped catfish farming sector in the Mekong Delta, Vietnam. *Ambio*, 39(7), 504-514.
- Dhar, A. K., Manna, S. K., & Thomas Allnutt, F. C. (2014). Viral vaccines for farmed finfish. *Virusdisease*, 25(1), 1-17.
- Dyková, I., Žák, J., Blážek, R., Reichard, M., Součková, K., & Slabý, O. (2022). Histology of major organ systems of Nothobranchius fishes: short-lived model species. *Journal of Vertebrate Biology*, 71(21074), 1-50.
- Fanta, E., Rios, F. S. A., Romão, S., Vianna, A. C. C., & Freiburger, S. (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and environmental safety*, 54(2), 119-130.
- Ferguson, H. W. (1989). *Systemic pathology of fish. A text and atlas of comparative tissue responses in diseases of teleosts*. Iowa State University Press.
- Ferguson, H. W., & McCarthy, D. H. (1978). Histopathology of furunculosis in brown trout *Salmo trutta* L. *Journal of Fish Diseases*, 1(2), 165-174.
- Ferguson, H. W., Turnbull, J. F., Shinn, A., Thompson, K., Tu, D. T., & Crumlish, M. (2001). Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases*, 24(9), 509-513.
- Gernhöfer, M., Pawert, M., Schramm, M., Müller, E., & Triebkorn, R. (2001). Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *Journal of Aquatic Ecosystem Stress and Recovery*, 8(3), 241-260.
- Grizzle, J. M., & Rogers, W. A. (1976). *Anatomy and histology of the channel catfish*. Auburn, Ala. : Auburn University, Agricultural Experiment Station, 100 pages.
- Harder, W. 1975. *Anatomy of fishes. E. Schweizerbart'sche Verlagsbuchhandlung (Nagele v. Obermiller)*. Stuttgart, West Germany, 744 pages.
- Hawke, J. P., Kent, M., Rogge, M., Baumgartner, W., Wiles, J., Shelley, J., L., Savolainen, C., Wagner, R., Murray, K., & Peterson, T. S. (2013). Edwardsiellosis caused by *Edwardsiella ictaluri* in laboratory populations of zebrafish *Danio rerio*. *Journal of aquatic animal health*, 25(3), 171-183.
- Hinton, D. E., & Lauren, D. J. (1990) Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: *Biomarkers of Environmental Contamination*, edited by J.F. McCarthy and L.R. Shugart Lewis Publishers, Boca Raton, FL, pp. 17-57.
- Hinton, D. E., Snipes, R. L., & Kendall, M. W. (1972). Morphology and enzyme histochemistry in the liver of largemouth bass (*Micropterus salmoides*). *Journal of the Fisheries Board of Canada*, 29(5), 531-534.
- Huizinga, H. W., Esch, G. W., & Hazen, T. C. (1979). Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacepede). *Journal of Fish Diseases*, 2(4), 263-277.
- Iwanowicz, L. R., Griffin, A. R., Cartwright, D. D., & Blazer, V. S. (2006). Mortality and pathology in brown bullheads *Amieurus nebulosus* associated with a spontaneous *Edwardsiella ictaluri* outbreak under tank culture conditions. *Diseases of Aquatic Organisms*, 70(3), 219-225.
- Jarboe, H., Bowser, P., & Robinette, H. (1984). Pathology associated with a natural *Edwardsiella ictaluri* infection in channel catfish (*Ictalurus punctatus* Rafinesque). *Journal of Wildlife Diseases*, 20(4), 352-354.
- Kibenge, F. S., & Strange, R. J. (2021). Introduction to the anatomy and physiology of the major aquatic animal species in aquaculture. In *Aquaculture Pharmacology* (pp. 1-111). Academic Press.
- Klontz, G. W. (1966). Bacterial diseases of the Salmonidae in the Western United States: pathogenesis of furunculosis in rainbow trout. *American Journal of Veterinary Research*, 27, 1455-1460.
- Le, H. P. (2013). Summary report on scientific and technological results of the project "Improving the efficiency of using inactivated vaccines through protein heat shock". Research Institute For Aquaculture No.2, 165 pages.

- Liu, C., Ma, J., Zhang, D., Li, W., Jiang, B., Qin, Z., ... & Wang, Q. (2021). Immune Response and Apoptosis-Related Pathways Induced by *Aeromonas schubertii* Infection of Hybrid Snakehead (*Channa maculata* ♀ × *Channa argus* ♂). *Pathogens*, 10(8), 997.
- Loch, T. P., Hawke, J. P., Reichley, S. R., Faisal, M., Del Piero, F., & Griffin, M. J. (2017). Outbreaks of edwardsiellosis caused by *Edwardsiella piscicida* and *Edwardsiella tarda* in farmed barramundi (*Lates calcarifer*). *Aquaculture*, 481, 202-210.
- Mahdy, O. A., Abdelsalam, M., Abdel-Maogood, S. Z., Shaalan, M., & Salem, M. A. (2022). First genetic confirmation of *Clinostomidae metacercariae* infection in *Oreochromis niloticus* in Egypt. *Aquaculture Research*, 53(1), 199-207.
- Mamun, A. A., Nasren, S., Rathore, S. S., & Mahub Alam, M. M. (2022). Histopathological Analysis of Striped Catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) Spontaneously Infected with *Aeromonas hydrophila*. *Jordan Journal of Biological Sciences*, 15(1), 93 – 100.
- Mawdesley-Thomas, L. E. (1971). Neoplasia in fish: a review. *Current topics in comparative pathobiology*, 1, 87-170.
- Newton, J. C., Wolfe, L. G., Grizzle, J. M., & Plumb, J. A. (1989). Pathology of experimental enteric septicaemia in channel catfish, *Ictalurus punctatus* (Rafinesque), following immersion-exposure to *Edwardsiella ictaluri*. *Journal of Fish Diseases*, 12(4), 335–347.
- Nguyen, N. P. (2014). *Environmental factors affecting the pathogenesis of Edwardsiella ictaluri in striped catfish Pangasianodon hypophthalmus (Sauvage)* (master's thesis). University of Stirling.
- Nguyen, P. T., & Do, O. T. H. (2010). Striped catfish aquaculture in Vietnam: a decade of unprecedented development. In *Success stories in Asian aquaculture* (131-147). Springer, Dordrecht.
- Nguyen, V. T., Tran, P. H., Kim, O. T. P., Nguyen, S. V., Trinh, T. T. & Nguyen, N. H. (2022). Accuracies of genomic predictions for disease resistance of striped catfish to *Edwardsiella ictaluri* using artificial intelligence algorithms. *G3*, 12(1), 1 - 13.
- Patt, D. I., & Patt, G. R. (1969). *Comparative vertebrate histology*. Harper and Row, New York, New York, USA.
- Petrie-Hanson, L., Romano, C. L., Mackey, R. B., Khosravi, P., Hohn, C. M., & Boyle, C. R. (2007). Evaluation of zebrafish *Danio rerio* as a model for enteric septicemia of catfish (ESC). *Journal of Aquatic Animal Health*, 19(3), 151-158.
- Quach, T. V. C. (2017). *The current status antimicrobial resistance in Edwardsiella ictaluri and Aeromonas hydrophila cause disease on the striped catfish farmed in the Mekong Delta* (Doctoral thesis). Can Tho University.
- Sakai, T., Yuasa, K., Ozaki, A., Sano, M., Okuda, R., Nakai, T., & Iida, T. (2009). Genotyping of *Edwardsiella ictaluri* isolates in Japan using amplified-fragment length polymorphism analysis. *Letters in applied microbiology*, 49(4), 443-449.
- Santander, J., Kilbourne, J., Park, J. Y., Martin, T., Loh, A., Diaz, I., Rojas, R., Segovia, C., DeNardo, D., & Curtiss III, R. (2014). Inflammatory effects of *Edwardsiella ictaluri* lipopolysaccharide modifications in catfish gut. *Infection and immunity*, 82(8), 3394-3404.
- Shah, K. K., Pritt, B. S., & Alexander, M. P. (2017). Histopathologic review of granulomatous inflammation. *Journal of clinical tuberculosis and other Mycobacterial Diseases*, 7, 1-12.
- Shotts, E. B., Gaines, J. L., Martin, L., & Prestwood, A. K. (1972). *Aeromonas*-induced deaths among fish and reptiles in an eutrophic inland lake. *Journal of the American Veterinary Medical Association*, 161(6), 603-607.
- Soto, E., Illanes, O., Revan, F., Griffin, M., & Riofrio, A. (2013). Bacterial distribution and tissue targets following experimental *Edwardsiella ictaluri* infection in Nile tilapia *Oreochromis niloticus*. *Diseases of Aquatic Organisms*, 104(2), 105-112.
- Suanyuk, N., Rogge, M., Thune, R., Watthanaphiromsakul, M., Champhat, N., & Wiangkum, W. (2013). Mortality and pathology of hybrid catfish, *Clarias macrocephalus* (Günther) × *Clarias gariepinus* (Burchell), associated with *Edwardsiella ictaluri* infection in southern Thailand. *Journal of Fish Diseases*, 37, 385–395.
- Subagja, J., Slembrouck, J., & Legendre, M. (1999). Larval rearing of an Asian catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae): Analysis of precocious mortality and proposition of appropriate treatments. *Aquatic Living Resources*, 12(1), 37-44.
- Thophon, S., Kruatrachue, M., Upatham, E. S., Pokethitiyook, P., Sahaphong, S., & Jaritkhuam, S. (2003). Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environmental pollution*, 121(3), 307-320.
- VASEP. (2022). *Striped catfish (Pangasianodon hypophthalmus) exports in Vietnam in 2021 with great efforts*. <https://vasep.com.vn/san-pham-xuat-khau/ca-tra/xuat-nhap-khau/xuat-khau-ca-tra-vietnam-nam-2021-voi-nhieuc-co-gang-ngoai-suc-tuong-tuong-23704.html>
- VASEP. (2022). *Vietnam fisheries production in the first 6 months of the year reached nearly 4.2 million tons*. <https://seafood.vasep.com.vn/why-buy-seafood/export-potentials/vietnam-fisheries-production-in-the-first-6-months-of-the-year-reached-nearly-4-2-million-tons-24928.html>
- Wolke, R. E. (1975). *Pathology of bacterial and fungal diseases affecting fish* (pp. 33-116). Madison, Wisconsin: University of Wisconsin Press.
- Ye, S., Li, H., Qiao, G., & Li, Z. (2009). First case of *Edwardsiella ictaluri* infection in China farmed yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, 292, 6-10.