



Research Article

A STUDY ON TOXIN OF *Edwardsiella ictaluri* IN STRIPED CATFISH (*Pangasianodon hypophthalmus*) AND GROWTH PERFORMANCE AT THE FRY STAGE OF 45 DAYS OLD

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ABSTRACT

Bacteria Edwardsiella ictaluri is a major pathogenic bacterium threatening aquatic species and seriously affecting the aquaculture industry globally. Previous studies have focused on analyzing bacteria's pathological signs and virulence on pangasius fingerlings. However, research on the virulence and pathogenicity of Edwardsiella ictaluri in the striped catfish at the fry stage is still limited. Therefore, this study was carried out to evaluate the pathogenicity and virulence of Edwardsiella ictaluri bacteria on 45-day-old striped catfish (Pangasianodon hypophthalmus) through infection experiments. A total of 1,170 striped catfish were subjected to infection at concentrations of 0, 10³, 10⁴, 10⁵, and 10⁶ CFU/mL by the immersion method. After nine days of infection, the results showed that the cumulative mortality of striped catfish ranged from 3.08 - 100%. The lethal dose (LD₅₀) of E. ictaluri on striped catfish at the fry stage was 7.59 × 10⁴ CFU/mL. At all concentrations, the growth parameters (weight gain, percentage specific growth rate, daily weight gain, length gain, percentage specific growth rate length, daily length gain of striped catfish treatment) were reduced compared with the control group.

Keywords: Edwardsiella ictaluri; fry stage; LD₅₀; striped catfish; Pangasianodon hypophthalmus

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is Vietnam's main aquaculture species and is farmed mainly in the Mekong Delta (Phan et al., 2009). However, in recent years, pangasius farming has faced many difficulties, such as poor-quality seed, degraded environment, and many diseases which rapidly reduce the effectiveness of production (Ta et al., 2022). Previous studies showed that in striped catfish, diseases such as liver and kidney pus, hemorrhage, head edema, bacillary necrosis in pangasius, and parasites (Le, 2013).

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Bacillary Necrosis in Pangasius (BNP) was first recorded at the end of 1998 in An Giang, Dong Thap, and Can Tho provinces in striped catfish, then the disease gradually spread to neighboring areas (Ferguson et al., 2001). BNP disease has no specific external manifestations, and some fish bleed in the microscopic or whole body, the abdominal cavity contains a small quantity of thick fluid, and the internal organs, such as the liver, kidney, and spleen, have many white spots (Nguyen et al., 2004). *Edwardsiella ictaluri* (*E. ictaluri*) is the causative agent found by Crumlish et al., 2002). Currently, pyelonephritis is one of the common diseases in catfish (Tran et al., 2021). The disease appeared in all rearing stages (Dang & Nguyen, 2009). However, it was most prevalent in fish under four months of age, especially in the fry stage aged 21-30 days and juveniles 40-90 days old, with a high percentage of juveniles infected were 46% and 30%, respectively (Tran et al., 2021). Ly (2009) reported that the percentage of fish with pyelonephritis decreased gradually with increasing weight and did not see diseased fish at the stage of reaching a weight over 900 g.

In aquatic species, many infectious diseases have recognizable characteristics, such as clinical signs and increasing mortality (Plumb et al., 2011). In addition, the pathogenicity of bacteria depends on the number of bacteria, the concentration depending on the species infected, and factors such as virulence, route of infection, and environmental factors affecting pathogenicity (Plumb et al., 2011; Carraschi et al., 2012; Song et al., 2014). Infection testing studies are needed for each species to develop more effective prevention and treatment protocols for aquatic pathogens (Dias et al., 2016). There have been many studies on the virulence of *E. ictaluri* bacteria in some aquatic species (Nguyen et al., 2009; Ye et al., 2009; Crumlish et al., 2010) with many other infection methods together. However, these studies mainly focused on pathogenicity, LD₅₀ dose determination, and the growth effect of *E. ictaluri* in catfish at the juvenile stage. Research on striped catfish in the fry stage is still minimal. Therefore, the objective of the present study was to determine the effect and virulence of *E. ictaluri* bacteria on 45-day-old fry-stage striped catfish through LD₅₀ value and other growth parameters to provide information, theoretical basis, and, at the same time, serve as a basis for further studies to develop vaccines and treatment methods for pyelonephritis in catfish at the fry stage.

2. Materials and methods

2.1. Materials

2.1.1. Fish

The experimental fish was 45-day-old fry striped catfish (1 – 4 g/fish). A total of 1,170 striped catfish were procured from National Feeding Center for Southern Freshwater Aquaculture in Tien Giang province. Experimental fish are healthy, uniform, flexible, and have bright eyes.

2.1.2. Bacteria

The bacterial strain *Edwardsiella ictaluri* Gly09M was isolated from BNP catfish in An Giang province in 2009 and preserved in the Research Institute for Aquaculture No.2

(RIA2) bacterial collection was used in this research. The bacteria were restored and re-isolated from healthy striped catfish three times to the challenge.

2.2. Methods

2.2.1. Growing and diluting bacteria

E. ictaluri was recovered by inoculating sheep blood agar at 28°C after 24 - 48 hours to observe colony morphology and color to determine purity. *E. ictaluri* was proliferated by taking about 2 to 3 colonies into a sterilized 30 mL BHI (Nutrient Broth) container, shaking at 200 rpm for 24 hours. *E. ictaluri* was transferred to sterilized 15 mL falcon tubes and centrifuged at 4,000 rpm for 15 minutes. Discard the solution above and use sterile physiological saline (0.85% NaCl) to wash the bacteria (repeat 2-3 times). Determine the bacterial density by spectrophotometer with a wavelength of 550 nm and adjust the OD = 1 to correspond to a bacterial density of 1.2×10^9 CFU/mL (Dang et al., 2014).

Using a sterile pipette, aspirate 1 mL of 10^9 CFU/mL bacterial solution into a test tube containing 9 mL of physiological saline to obtain a solution with a bacterial density of 10^8 CFU/mL. Do the same to dilute the bacteria to the densities for the following experiment.

2.2.2. Fish management

All experimental fish were kept in 30 L composite tanks in Research Center for Aqua-Feed Nutrition and Fishery Post-Harvest Technology - Research Institute for Aquaculture No.2. Before stocking the fish, the aquarium was technically treated before adding water. Fish are bathed with diluted salt water with a concentration of 2 - 3% for 10-15 minutes to kill parasites and pathogens. Fish were fed commercial feed twice daily at 3-4% of body weight (Luu et al., 2022). The photoperiod was 12 hours light: 12 hours dark. Water parameters (pH, total ammonium concentration, and temperature) were frequently monitored, and the temperature was maintained at 26°C to avoid poor water quality that stresses fish.

2.2.3. Determine 50% lethal dose (LD₅₀) of *Edwardsiella ictaluri* on 45-day-old striped catfish

A total of 1,170 45-day-old striped catfish were inoculated with *E. ictaluri* by immersion for the duration of the experiment with treatments at doses of 0 CFU/mL (one non-immersion control and one control impregnated with BHI equivalent to the highest concentration of bacteria), 10^3 , 10^4 , 10^5 , and 10^6 CFU/mL. Each treatment was repeated three times with a density of 1 fish/0.4 L of water and maintained water temperature from 26 to 28°C. Record the daily number of fish deaths during 13 days post-infection (dpi). The lethal dose of 50% of experimental fish (LD₅₀) of bacteria was determined by the Probit equation (Finney, 1971) and verified by the formula of Reed and Muench (1938).

$$LD_{50} = 10^{a \cdot p \cdot d}$$

Where: $p \cdot d = (L\% - 50 / L\% - H\%)$, a: power at which bacteria cause fish death is lowest but more than 50%, H%: highest fish mortality rate but less than 50%, L%: lowest fish mortality rate but over 50%.

2.2.4. Determine the effect of *Edwardsiella ictaluri* on the growth performance of 45-day-old striped catfish

After being transferred to a 30 L tank, fish will be infected with *E. ictaluri* bacteria by soaking at 0, 10³, 10⁴, 10⁵, and 10⁶ CFU/mL concentrations. Each treatment was repeated three times with a density of 1 fish/0.4 L of water and maintained water temperature from 26 to 28° C. Daily observed pathological signs, recorded the number of dead fish, isolated *E. ictaluri* from the kidneys of lethargic fish and re-identified the bacteria by PCR method. Indicators of monitoring before and after infection (0, 3, 5, 7, 9-day post-infection (dpi)) according to the method of Tok et al. (2017):

1. Weight gain (WG) = (Final weight - initial weight).
2. Percentage specific growth rate (%SGR) = (Log_e final body weight - log_e initial body weight) ÷ Culture days x 100
3. Average daily weight gain (DLG) = (Final weight – initial weight) ÷ Days of culture
4. Percentage length gain (%WG) = (Final length– initial length) ÷ (Initial length) x100.
5. Percentage specific growth rate (%SGR_L) = (Log_e final body length - log_e initial body length) ÷ Culture days x 100
6. Average daily length gain (DLG) = (Final length – initial length) ÷ Days of culture

2.2.5. Data analysis

All study data are presented in Mean ± SE form and processed according to statistical probability algorithms using SPSS 26.0 software. The level of significance used to test the significant difference between treatments was 0.05 (p < 0.05, the difference was statistically significant) through one-way analysis of variance (ONEWAY ANOVA).

3. Result and discussion

3.1. Cumulative mortality

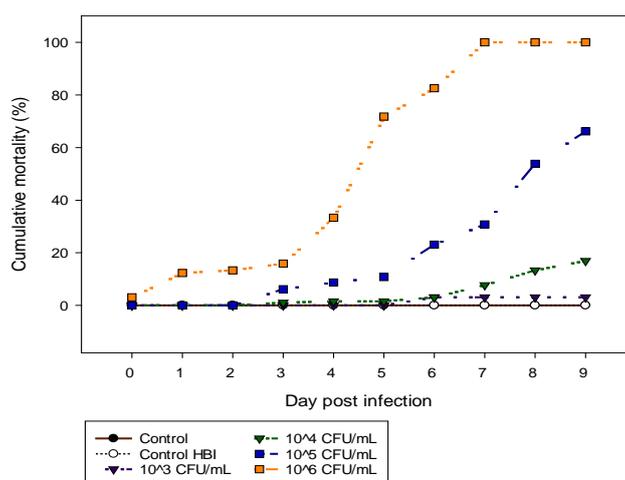


Figure 1. Cumulative mortality (%) of fry stage striped catfish in the exploratory experiment

The virulence and pathogenicity of the *E. ictaluri* Gly09M strain were determined through the soaked experiment at four concentrations: 10^3 CFU/mL, 10^4 CFU/mL, 10^5 CFU/mL, and 10^6 CFU/mL. The results showed that *E. ictaluri* Gly09M caused fish mortality from 3.08 to 100%, especially fish that died the most after five days of infection concentrated from day 5 to 9 (Figure 1). In the immersion challenge performed in this study, mortality rates proved to depend on the challenge dose received (Murray et al., 1992). Specifically, after nine days of infection, striped catfish in the treatment with 10^3 and 10^4 CFU/mL had a low mortality rate of 3.08% and 16.92%, respectively (Figure 1). Meanwhile, the treatment with *E. ictaluri* concentration of 10^5 CFU/mL had 66.15% cumulative fish mortality (Figure 1). Fish in the bacterial immersion treatment at 10^6 CFU/mL had the highest mortality rate of 100% (Figure 1). The fish in the physiological saline injection treatment had a mortality rate of 0%, and the fish were healthy and functioning normally.

Striped catfish in the experiments infected with pyelonephritis did not have specific external pathological signs. Most fish showed sluggish swimming on the water, separation from the school, slow response to noise, and quit eating. The internal organs, such as the liver, kidney, and spleen in the abdominal cavity of diseased fish, have many white spots. The diseased pangasius samples obtained were found to have many white spots on the internal organs, similar to those previously studied in striped catfish (Crumlish et al., 2002; Tu et al., 2010). The formation of white spots on the liver, kidneys, and pus is a form of the body's immune response to isolate and eliminate foreign substances entering the body, creating chronic inflammation (Labrie et al., 2008).

3.2. Determining the lethal dose of 50% (LD_{50}) of *E. ictaluri* Gly09M

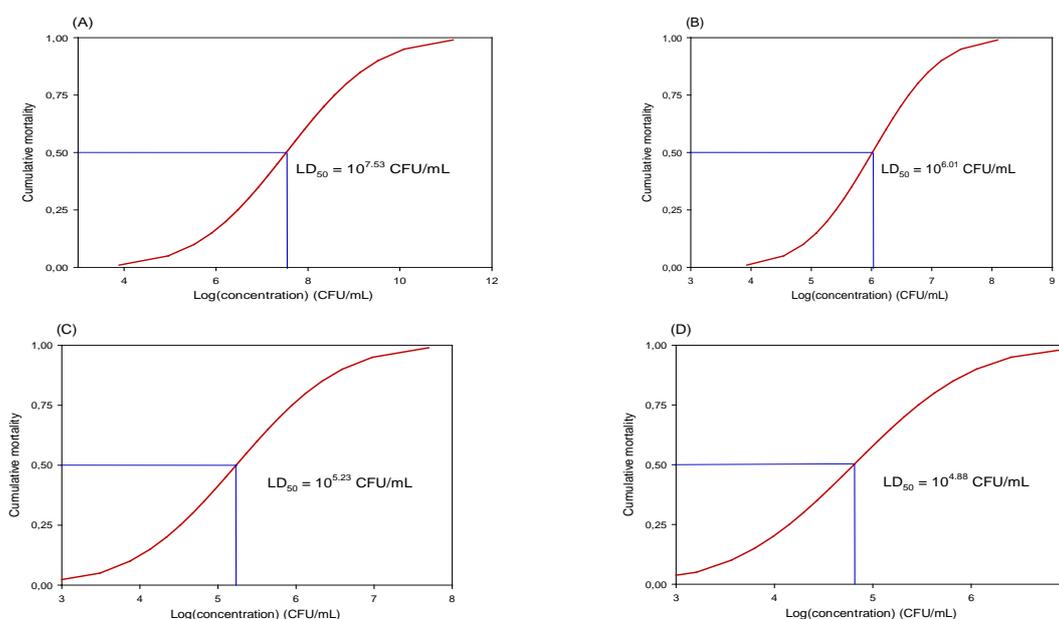
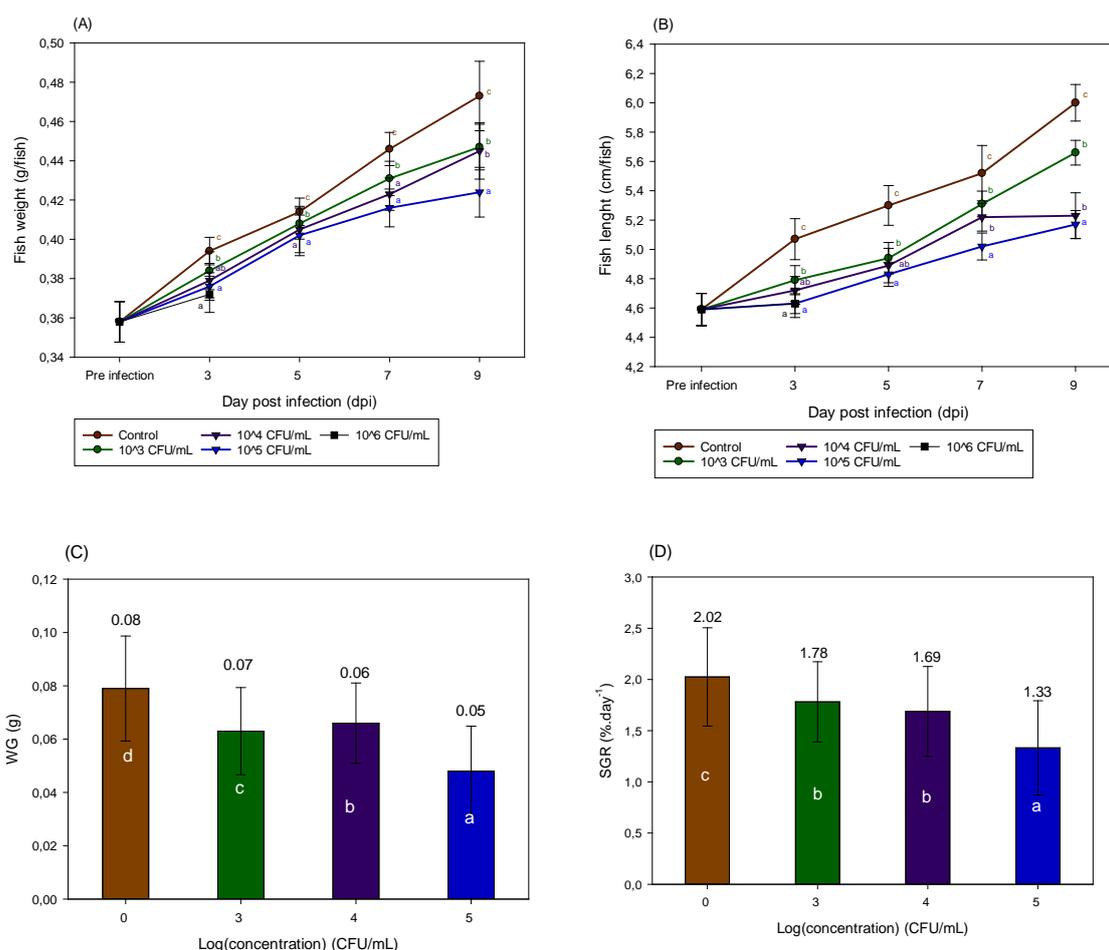


Figure 2. The lethal dose (LD_{50}) of striped catfish infected with *E. ictaluri* Gly09M at 3 dpi (a), 6 dpi (b), 7 dpi (c) and 9 dpi (d)

The LD₅₀ value of the bacterial strain decreased through the infection stages (from 10^{6.28} to 10^{4.88} CFU/mL) and at 9 dpi reached 10^{4.88} (~ 7.59 × 10⁴) CFU/mL (Figure 2). The results showed that the virulence of this bacterium was higher than that of *E. ictaluri* AG strain, which caused 53.3% death of fingerlings with an average weight of 10 g (Tran et al., 2014). The LD₅₀ of *E. ictaluri* was also determined in striped catfish as 7.5 - 10 g, which is 3.6 × 10⁵ CFU/mL (Nguyen & Dang, 2020). Research results are consistent with previous studies on striped catfish. The lethal dose in 45-day-old fry-stage striped catfish was lower than the lethal dose in fingerlings. Dang and Nguyen (2009) determined that the LD₅₀ value of *E. ictaluri* strains CAF-06-02 and CAF-06-03 in catfish was 1.4 × 10⁵ CFU/ml and 0.54 × 10⁵ CFU/ml.

3.3. The influence of *E. ictaluri* Gly09M on growth parameters

The influence of *E. ictaluri* Gly09M on growth parameters of 45-day-old fry-stage striped catfish was studied through the parameters of weight, length, WG, DWG, SGR, LG, DLG, SRGL presented in Figure 3.



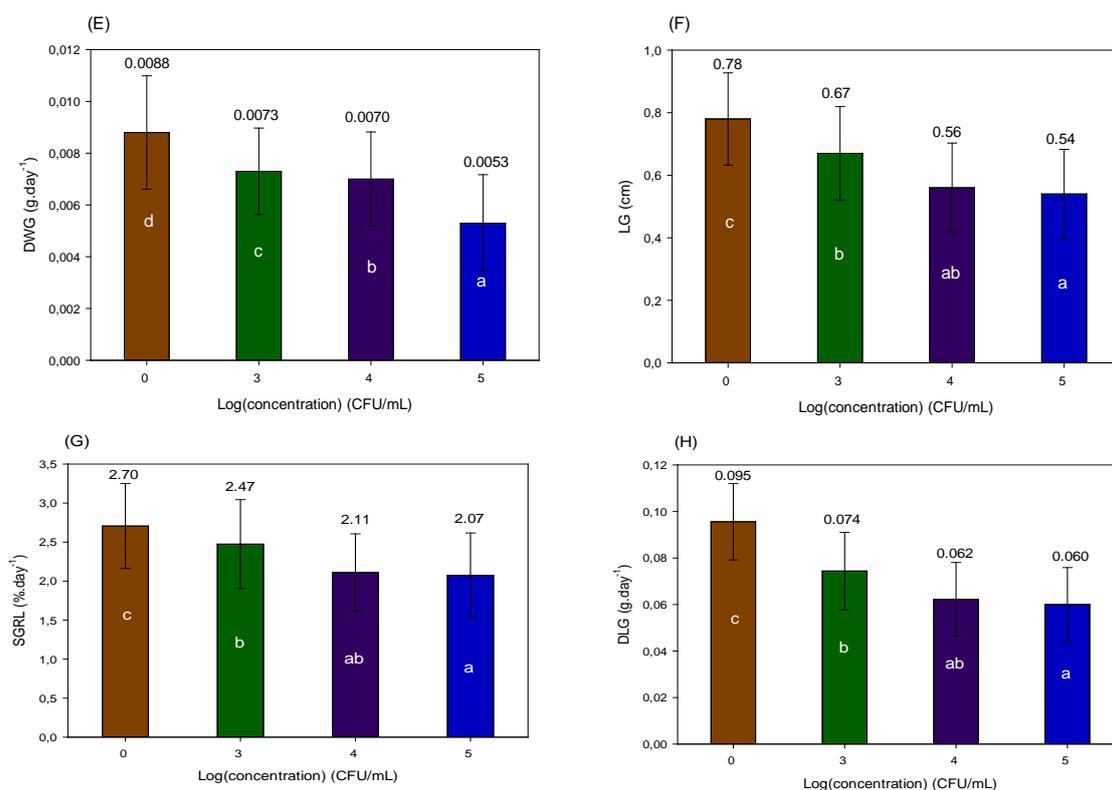


Figure 3. Growth parameters of 45-day-old fry-stage striped catfish at 9 dpi with *E. ictaluri* Gly09M with (A) weight; (B) length; (C) weight gain (WG); (D) percentage specific growth rate (%SGR); (E) daily weight gain (DWG); (F) length gain (LG); (G) percentage specific growth rate (%SGRL); (H) daily length gain (DLG) at different sampling times. Values are mean \pm SE; different letters indicate significant differences between treatments ($P < 0.05$)

The study results showed that *E. ictaluri* Gly09M inhibited striped catfish growth through the variation of growth indices in the bacterial immersion treatments compared with the control treatment (Figure 3). Specifically, the weight and length of fish in the control treatment (weight and length from 0.35 g, 4.59 cm to 0.47 g, 6.00 cm) over the days of infection increased more strongly than in the bacterial immersion treatments (weight and length ranged from 0.35 g, 4.59 cm to 0.42 - 0.45 g, 5.17 - 5.66 cm) (Figure 3). The weight and length of fish obtained at 9 dpi were different between the control and the rest of the treatments ($p < 0.05$); in the bacterial immersion treatment at concentrations of 10^3 and 10^4 CFU/mL, there was no significant difference between length and weight (because of $p > 0.05$). The growth parameters at 9 dpi in the control treatment were the highest and statistically different from the other treatments (WG: 0.08 g; %SGR: 2.02 %·day⁻¹; DWG: 0.0088 g·day⁻¹; LG: 0.78 cm; %SGRL: 2.70 %·day⁻¹; DLG: 0.095 g·day⁻¹) ($p < 0.05$) (Figure 3). Bacterial immersion at 10^5 CFU/mL showed the slowest growth (WG: 0.05 g; %SGR: 1.33 %·day⁻¹; DWG: 0.0053 g·day⁻¹; LG: 0.54 cm; %SGRL: 2.07 %·day⁻¹; DLG: 0.060 g·day⁻¹).

Growth parameters in the *E. ictaluri* treatments grew slower than the control immersion treatments, and the influence of *E. ictaluri* could explain some hematological and histological indices previously studied (Tran et al., 2021; Luu et al., 2022). Some studies have shown that the clinical status of the disease in catfish depends on the mode of infection with *E. ictaluri* (Newton et al., 1989). *E. ictaluri* enters the fish body in two ways: through the respiratory tract and the digestive tract. The bacteria travel through the nose, then into the olfactory nerve to the brain, and from the meninges to the skull and skin. In addition, *E. ictaluri* also enters the digestive tract by mouth to the intestinal mucosa and enters the bleeding causing bacteremia (Blazer et al., 1985). Immune parameters fluctuate during infection (red blood cells tend to decrease; white blood cells tend to increase) (Tran et al., 2021), affect and nutrient synthesis for fish and therefore retard growth. Luu et al. (2022) reported that at 48 hours post-infection, internal organs (liver, kidney, spleen) showed signs of hemorrhage leading to necrosis. From there, damage to the organs responsible for metabolism can cause slow growth or even death of fish.

There is currently little published information on the effects of *E. ictaluri* on striped catfish in the fry stage. This study studied the effects of *E. ictaluri* on some growth parameters. Research results are similar to previous studies on *E. ictaluri* causing disease in striped catfish in fingerlings. Studies show that *E. ictaluri* inhibits the growth of striped catfish (Bui et al., 2020; Luu et al., 2022). Growth indicators show signs of slowing down, which leads to fish stopping eating and dying.

4. Conclusions

The study's results showed that for the strain *Edwardsiella ictaluri* causing Bacillary Necrosis in Pangasius disease in striped catfish at 45 days of age, the cumulative mortality after nine days of the experiment at the concentrations ranged from 3.08 - 100%. The lethal dose (LD₅₀) of *E. ictaluri* was 7.59×10^4 CFU/mL. After nine days of infection, at all concentrations, growth parameters (weight gain; percentage specific growth rate; daily weight gain; length gain; percentage specific growth rate (length); daily length gain) of striped catfish were reduced compared to the control treatment. At the bacterial immersion concentration of 10^5 CFU/mL, the growth parameters decreased sharply, and there was a statistical difference compared with the other treatments ($p < 0.05$). In future studies, it is necessary to evaluate the effects of *E. ictaluri* bacteria on the striped catfish fry stage through immunological and histological parameters to develop preventive and curative measures for fish.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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NGHIÊN CỨU ĐỘC LỰC VÀ ẢNH HƯỞNG ĐẾN KHẢ NĂNG SINH TRƯỞNG CỦA VI KHUẨN *Edwardsiella ictaluri*

TRÊN CÁ TRA (*Pangasianodon hypophthalmus*) HƯƠNG GIAI ĐOẠN 45 NGÀY TUỔI
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TÓM TẮT

Edwardsiella ictaluri (*E. ictaluri*) là một loại vi khuẩn gây bệnh trên các loài thủy sản và ảnh hưởng nghiêm trọng đến ngành nuôi trồng thủy sản trên toàn cầu đặc biệt là cá tra. Các nghiên cứu trước đây về loài này chủ yếu tập trung phân tích dấu hiệu bệnh lý và độc lực của vi khuẩn trên cá tra giống. Tuy nhiên, việc nghiên cứu độc lực và khả năng gây bệnh của *E. ictaluri* trên cá tra ở giai đoạn cá hương còn hạn chế. Nghiên cứu này được thực hiện nhằm đánh giá khả năng gây bệnh và độc lực của vi khuẩn *E. ictaluri* trên cá tra (*Pangasianodon hypophthalmus*) hương giai đoạn 45 ngày tuổi. 1170 cá tra tương đương được cảm nhiễm *E. ictaluri* ở các nồng độ 0, 10³, 10⁴, 10⁵ và 10⁶ CFU/mL bằng phương pháp ngâm. Sau 9 ngày cảm nhiễm, kết quả cho thấy tỉ lệ chết tích lũy của cá tra dao động từ 3,08 - 100%. Liều gây chết (LD₅₀) của vi khuẩn *E. ictaluri* trên cá tra hương giai đoạn 45 ngày tuổi là 7.59 × 10⁴ CFU/mL. Ở tất cả các nồng độ, các chỉ tiêu sinh trưởng của nghiệm thức cá tra cảm nhiễm *E. ictaluri* đều giảm so với nghiệm thức đối chứng.

Từ khóa: *Edwardsiella ictaluri*; giai đoạn cá hương; LD₅₀; *Pangasianodon hypophthalmus*; cá tra