Adaptation strategy for Jambal Catfish (*Pangasius djambal*)
to stress the aquatic environment

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Abstract. Oxygen is a major element in the metabolic process of cells in an aquatic animal’s body. Water oxygen solubility fluctuations will affect the metabolic process. Acute oxygen deficiency conditions can cause physiological changes in the body of fish. The research aims to analyze morphometric characters and genetic characters as environmental pressure. Test fish used in this research are 24.21 ± 0.96 cm jambal fish (*P.djambal*) with 223.42 ± 31.23 g. The treatment provided is an environment with different oxygen solubility, i.e., low oxygen (hypoxia), high oxygen (normoxia), and high fluctuating conditions naturally as control. The observed variables include the main parameters: the extent of gills filaments, the hemoglobin levels in the blood, and the expression of Lactate Dehydrogenase (LDH) genes. Supporter parameters are growth and mortality as well as water quality parameters. LDH gene expression analysis on jambal catfish (*P.djambal*) using Reverse Transcriptase Chain Reaction (RT-PCR) method. An analysis shows the morphometric characters of jambal catfish living in a hypoxia environment (*P < 0.05*). The genetic character of jambal catfish (*P.djambal*) shows that the hypoxic environment of gene expression LDH is better than fish living in a normoxia and natural environment. Gene LDH indicates the strategy of jambal catfish (*P.djambal*) adaptation used in the selection program to produce jambal catfish (*P.djambal*) resistant to the hypoxic environment.

1. Introduction

Jambal catfish (*P.djambal*) is one of the 14 species of catfish of Indonesian waters, especially in Sumatra and Borneo. Jambal catfish (*P.djambal*), with its meat characteristic white or better known white meat jambal catfish, is likely an export commodity and resembles the type of *Pangasius bocourti*, which is a type of export potential fish in Vietnam ¹. Jambal catfish is a type of fish that requires an environment with high oxygen content normoxia and tends not to be resistant to low oxygen [2;3;4]. The oxygen content for jambal catfish (*P.djambal*) reached more than 3 mg.l⁻¹. Normoxia refers to relatively high, and natural oxygen conditions are water oxygen solubility or undersaturation ⁵. Hypoxia is also a condition in which oxygen is insufficient for the purpose of cell, tissue, or organ. Hypoxic pressure on a species population creates biological reactions in the adaptation that causes morphometric changes to the species. The mechanism is a form of phenotypic plasticity in a species to maintain its life [6]. Research results [7] suggest that fish living in water conditions with high levels of turmoil caused the fish’s diameter to become more mature. The hypoxic environment's fish population has a wider gill surface or longer filaments than fish in the environment with high oxygen ⁸; ⁹; ¹⁰; ¹⁰; ¹¹; ¹². Jambal
catfish (P. djambal) character requiring high oxygen requires optimal aquatic ecosystems. In general, the aquatic ecosystem tends to be relatively low quality. Such conditions lead to low levels of oxygen or hypoxic conditions. Jambal catfish (P. djambal) response in the face of environmental pressure in the form of hypoxia is adaptable to survive. This condition can lead to morphometric and physiological character changes as a strategy to survive on jambal catfish [13]. The mechanism of adaptation in jambal catfish (P. djambal) to hypoxia is the increasing glycolysis, anaerobic, which is awakened by increasing lactate dehydrogenase enzymes. The understanding of the adaptation mechanism to hypoxia at the molecular level has grown far. Research results [14] show that there are five LDH isozymes induced liver network systemically. There has not been much research on the relationship between LDH that plays hypoxic conditions. Jambal catfish (P. djambal) is a relatively newly domesticated fish derived from lowland river waters with relatively high oxygen water characteristics (≥ 28 °C) [15]. The characteristic location of cultivation activities in the lowlands is generally aquaculture stagnant water ponds with small discharge, so it has low dissolved oxygen content. Related to this condition, it is necessary to obtain a jambal catfish (P. djambal) with low oxygen resistant characters starting with the morphometric approach and molecular markers expected to provide necessary information related to low oxygen resistance. The research aims to analyze morphometric characters and genetic characters as a strategy for jambal catfish adaptation in aquatic environmental pressure.

2. Material and method

Test fish used in this research are 24.21 ± 0.96 cm jambal catfish (P. djambal) with 223.42 ± 31.23 g. Maintenance is performed in a concrete pond of 50 m² with a water depth of 70 cm by nine. The stocking density of fish was two individual m⁻² to support optimum fish growth and feed 28% protein levels of 5%-6% fish weight day⁻¹. The design used is Random Complete Block Design, with three treatments and three replications, and fish maintenance for 10 months at Sukamandi, West Java. Grouping is based on low oxygen (hypoxia) environment and oxygen oversaturation (normoxia). The treatment was determined based on the oxygen requirement of catfish is A. Oxygen levels ≤ 1.5 mg.L⁻¹ (hypoxic condition); B. Oxygen levels ≥ 3 mg.L⁻¹; C. Natural environment with fluctuating oxygen levels (as control). The treatment was based on hypoxia conditions set without aeration and photosynthesis by closing maintenance containers, so hypoxic conditions can be maintained during research. The normoxia condition is regulated using aeration during the research. Morphometric observation of test fish is done morphometrically and molecular at the end of the research. Meristic characters have measured the height of hemoglobin in the blood of jambal catfish. The observed supporter parameters are the growth of standard length and body weight of fish, survival rate, and ammonia (NH3) levels performed every once a week. Soluble oxygen levels are observed daily as long as research takes place. A DO meter was used to measure dissolved oxygen in the morning. Water quality parameters are observed using reagent kits. Water samples from each maintenance vessel are entered into the comparator kits, then the water sample is given a chemical as an indicator, then compared with the standard value.

Molecular observation of the jambal catfish (P. djambal) pituitary organ is conducted to evaluate the expression of LDH genes catfish at the end of the research. LDH gene expression analysis on jambal catfish using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method. The molecular test stage includes RNA extraction, reverse transcriptase-polymerase chain reaction (RT-PCR), and semi-quantitative PhGH gene expression analysis of RT-PCR products. RNA Extraction Total RNA extracted from jambal catfish pituitary organ. According to its usage instructions, RNA extraction uses Tri Reagent Kit (Molecular Research Center, Inc., Cincinnati, OH, USA). A total of 10 mg organs are extracted, then added 1 mL next sample smooth with a pastel pallet. Samples are incubated at space temperature for 5 minutes, then added 100 μL bromochloropropane and rotated for 15 seconds. Samples incubated at space temperature for 15 minutes then centrifuged at 12,000 x g speed for 15 minutes at 4 °C. The top layer of liquid 800 mL (supernatual) is transferred into a new 1.5 mL microcentrifuge tube, next added 200 mL isopropanol. Samples centrifuged at 8000 x g speed for 15 minutes at 4 °C temperature. Isopropanol solution isopropanol titan, RNA that settles at the base of a washed tube with 1 mL 75% ethanol. RNA is dried up at space temperature until ethanol is gone, then RNA added 200
mL RNase-free water and 1mL DNAse. The level of purity and quantity is measured using Gene Quant (Qiagen). The quantity of RNS is highly reckoned for the CDNA synthesis process. CDNA synthesis uses Ready-To-Go CDNA TM RT-PCR Beads (GE Healthcare). Material composition for CDNA synthesis uses 30 ml RNA and 1.6 mL Oligo d(T) 16 primary (Roche, Germany) and 1.3 mL RNase-free water. Sample incubated at 37°C for 1 hour. Obtained CDNA is stored at temperature 20°C before use for the next step.

3. Result and discussion

The metabolic rate in living creatures' cells is influenced by the availability of oxygen from media that can be absorbed into the body of fish through breathing. Gills filament on fish serve as the site of oxygen transfer from media to the body. Therefore, the size or extent of the gills filament's surface has an effect on the provision of oxygen in the body. LDH’s lactate gene expression is an intracellular enzyme in almost all metabolic cells, with the highest concentration found in the heart, framework muscle, liver, kidney, brain, and red blood cells. LDH gene expression analysis uses the RT-PCR method and uses beta-actin as a mineral control of expression. The most highly expressed LDH genes are found in hypoxia treatment [4;14;18]. This condition is proven to be a thicker amplification of 400 bp and higher intensity in this treatment than other treatments (Figure 1).

![Figure 1. LDH gene expression in fish with hypoxia treatment 1,2,3,4,7,8,10, normoxia treatment 5,6,9,13, 16, 11, 12, 14, 15, and natural treatment sample number 17 to 21.](image)

![Figure 2. Expression of LDH genes in the pituitary measured by the ratio of LDH mRNA to β-actin mRNA.](image)
Figure 2 shows the ratio between mRNA-LDH and mRNA-actin that does not appear in oxygen conditions above saturation. The ratio between LDH mRNA and actin-mRNA will be influenced by oxygen conditions in the media and increase hypoxia conditions [25].

Table 1. The range of water quality during the experiment.

| Treatment  | Temperature (°C) | DO (mg.L⁻¹) | pH | ANT (mg.L⁻¹) | Nitrite (mg.L⁻¹) | Nitrate (mg.L⁻¹) |
|------------|------------------|--------------|----|--------------|-----------------|-----------------|
| Hypoxia    | 29.7-30.8        | 0.2-1.19     | 7.28-7.56 | 0.579-1.337  | 0.007-0.015     | 0.0-0.177       |
| Natural    | 28.5-29.0        | 1.7-6.0      | 6.99-8.55 | 0.279-0.280  | 0.014-0.032     | 0.17-0.177      |
| Normoxia   | 28.8-28.9        | 3.1-7.1      | 6.9-10.4  | 0.187-0.565  | 0.088-0.021     | 0.0-0.177       |

DO = dissolved oxygen
ANT = total of ammonia-nitrogen

The research factor is the media environment's water quality, mainly dissolved oxygen in water, as seen in Table 1. Based on water quality data on Tables 1, it looks that the dissolved oxygen content for hypoxic treatment can be conditioned below 2 mgL⁻¹ is in the range of 0.2-1.19 mgL⁻¹, for normoxia treatment is in the range of 3.1-7.1 mgL⁻¹, while for natural treatment of oxygen content is more fluctuated 1.7-6.0 mgL⁻¹. In the treatment of hypoxia, total nitrogen ammonia (TAN) higher than the other treatments range 0.5788-1.337 mgL⁻¹. Nitrite content in all treatments is still under 0.1 mg or still in the safe range for the fish's life. Oxygen solubility in water during research shows that hypoxic conditions and normoxia are well maintained. The growth of fish during the research involves total length, standard length, and weight and hemoglobin levels measured at the end of the research can be seen in Table 2.

Table 2. The growth of jambal catfish (P. djambal) and hemoglobin (Hb) content in the final experiment.

| Treatment  | Standard length(cm) | Total length (cm) | Weight (g) | Hb content |
|------------|---------------------|-------------------|------------|------------|
| Hypoxia    | 25.76±1.82 a        | 31.18±1.92 a      | 217.83±28.32 a | 3.33±0.62 a |
| Normoxia   | 26.43±2.55 a        | 31.98±2.43 a      | 248.75±36.63 b | 3.23±0.59 a |
| Natural    | 26.06±2.71 a        | 31.43±2.91 a      | 222.56±39.98 a | 3.67±0.39 a |

Note: the numbers followed by the same superscript letters are not significantly different.

Survival rate

During the study, the test fish mortality occurred, especially in the hypoxia treatment (Table 3). Mortality in these treatments occurred gradually starting at the age of 18 days of the experiment period.

Table 3. Percentage of survival rate (%) for jambal catfish (P. djambal) of the experiment’s rearing period.

| Replicate | Hypoxia | Normoxia | Natural |
|-----------|---------|----------|---------|
| 1         | 77.0    | 97.0     | 100.0   |
| 2         | 75.0    | 98.0     | 100.0   |
| 3         | 90.0    | 100.0    | 100.0   |
| Average   | 80.7b   | 98.3a    | 100.0a  |

Note: the numbers followed by the same superscript letters are not significantly different.

The degree of survival of the jambal catfish (P. djambal) survives as long as the research can be seen in Table 3. Hypoxia treatment suggests the death of test fish that occurred gradually on day 18. Jambal
catfish (P. djambal) maintained in hypoxia grows slower than maintained in natural and normoxic environments (Table 3). This condition may occur due to the more dominant influence of feed on body weight than standard length. Hb levels in all three treatments do not show any real differences. This suggests that the condition of dissolved oxygen in water does not affect the Hb levels in the blood of jambal catfish (P. djambal). Fish mortality began to occur on day 18 in hypoxia environmental conditions (Table 3). The data shows that fish deaths are not solely due to low oxygen content but also a high level of total ammonia-nitrogen. This indicates that in jambal catfish (P. djambal), there is an adaptation strategy in maintaining its life in a hypoxic environment. The influence of media water quality conditions, especially dissolved oxygen levels, indicates a real difference between treatments (P< 0.05). The higher mortalities occurred in a hypoxic treatment until 19.3 %. Mortality is suspected due to low oxygen levels dissolved in the media, considering jambal catfish is a common water fish that requires high oxygen [32]. The hypoxia tolerant mechanism in the phase activates anaerobic metabolism [26], reducing ATP usage and suppressing metabolism as a whole [27]. The gills of ion leaks can be prevented by decreasing protein synthesis and holding part of the ion-exchanged flow on the gills filament [28;29]. Oxygen transfer factors increase permanently on medium-level hypoxic and decrease in acute hypoxia. LDH movement in vertebrates can be intermediate by substrates, enzymes, and concentrations of a cofactor such as a temperature, pH, and pressure [30]. Hypoxia tolerant is based on gene regulation due to a highly sensitive oxygen sensor system developed by fish and conserved through evolution on vertebrates. Neumayer’s Barb fish (Enteromius neumayeri) has a heart LDH content than fish from a normoxia environment. LDH plays an important role in anaerobic metabolism, which can then be used for glucose production. Therefore the role of LDH activity is high in the heart of fish experiencing hypoxic conditions. This is an effort to preserve the homeostasis of an organism. A theory that an adapted organism has better performance than a new organism adapted to new environments. This enables negative growth during the acclimatization process in the inappropriate environment of [31]. The distribution of LDH networks at Cichlid Amazon (Cichlasoma amazonarum), including the genus Astronotus, relates to its ability to tolerate the hypoxic environment. The goal is to accelerate, which is the expression of phenotypic phenomenon genes on heart organs. Next, this character is expressed as the basis for choosing a habitat of [23]. Controlling the flexibility of LDH-A and LDH-B expression on Amazon’s cichlid shows that the fish can affect metabolism on anaerobic glycolysis through increased expression of LDH-A during low oxygen. Therefore, the phenomenon enables to occupy the environment with low oxygen concentration stimulates the stabilization of hypoxia-inducible factor (HIF-1) and stimulates the transcription of gene hypoxia-inducible such as EPO, transfer, vascular endothelial growth factor, phosphor phosphofructokinase and LDH-A A [24]. Research results [23] show that a ten-fold increase in LDH-A expression found was in white muscles after 30 minutes of anoxia. However, there is no connection between hypoxia and increased expression levels of LDH-A mRNA in adult stadia expressing LDH-A high on normoxia, similar to anoxic animals’ expression. In adults, hypoxia does not affect the expression of LDH-A mRNA. Therefore it can be suspected that an adult developed tolerance through the mechanism and does not rely exclusively on gene regulation to remain alive on hypoxic conditions. [25] explains that oxygen pressure causes stabilization of HIF-11 (inhibiting factor hypoxia), which is very different between types and cell types. If this is true, then stabilization or decrease in LDH-A expression can be explained by the adaptation process. Seeds in hypoxia treatment show a real difference (P<0.05) with normoxia and natural treatment; this suggests that dissolved oxygen content affects the survival of the jambal catfish (P. djambal). Low oxygen levels lead to unraveling organic materials imperfect, thus toxic to fish. If the oxygen is low, there is a process of morphometric and enzymatic adaptation. Oxygen content dissolved that low in the water for a long time causes the fish to adapt to maintain its life. Fish adaptation strategies to maintain its life in extreme environmental conditions can be done in many ways as reportedly [17] that there is hyperplasia in its gills lamellae in beluga fish (Huso huso) structure as a response to the body maintaining the hypoxia environment. Strategies used by fish to survive on hypoxia conditions are anaerobic metabolic activation and suppress energy changes depending on the adjustment of key enzyme activities in a certain way and on a network-specific basis. LDH levels in the heart of the jambal catfish (P. djambal) maintained in the
hypoxia environment are much higher than the LDH content in the natural environment and normoxia (Figure 2). This suggests that in hypoxia conditions, the jambal catfish (P. djambal) conducts an adaptation strategy to maintain its life. Thus it can be stated that in jambal catfish (P. djambal) can so that the population can survive on low oxygen conditions as a strategy then be conserved to be passed to the next generation. Judging in terms of genetic potential, this result can reference the selection of jambal catfish (P. djambal) populations with the mechanism as a population that will be used as a low oxygen tolerant strain.

4. Conclusion
LDH gene expression in jambal catfish (P. djambal) under hypoxia was shown by gill filament surface, which is wider than fish that live in the normoxia environment and natural environment. This expressive genetic character condition indicates the genes that can be utilized to deal with hypoxia. Jambal catfish (P. djambal) populations with specific characteristics deserve to be candidates for a base population that has the potential to withstand the stresses of the aquatic environment.

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