

# TIK-79 Effect of Coal Particles on Embryonic Development, Egg Hatching Rate and Larvae Survival Rate of Climbing Perch Fish *Anabas testudineus* BLOCH

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# Effect of Coal Particles on Embryonic Development, Egg Hatching Rate and Larvae Survival Rate of Climbing Perch Fish *Anabas testudineus* BLOCH

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## Abstract

This study aimed to analyze the impact of coal particles on the embryo development, egg hatching rate and larvae survival rate of climbing perch fish *Anabas testudineus* Bloch. The research was done in the wet laboratory of the Faculty of Fisheries and Marine, Lambung Mangkurat University for six months. The treatment in this study was a dose of coal particles exposure to eggs and larvae. The coal particles measuring 150 µm can penetrate the eggshell and interfere with embryo development at the stage of organ formation (organogenesis) and larval development. The eggs were exposed to coal particles 15 mg/litre of water. It turns hatching rate was 87% lower than without exposure to 98%. Similarly, the larvae survival rate (up to day 4) were exposed to coal dust 80% and without exposure to 93%.

**Keywords:** coal particles, embryonic development, egg hatching rate, climbing perch fish

## Introduction

Kalimantan Selatan is one of the areas with extensive mining land, such as coal. This area is known for its abundance of mining material reserves, especially coal. Until nowadays, its production can achieve 10% of total coal production nationally. In 2011, the production reached 101.1 million tons. In general mining in Kalimantan Selatan is carried out with an open-pit technique, which is by the clearing of land, stripping of topsoil, stripping of overburden, and cleaning and mining the coal. The transport from land mines is completed through special routes using dump trucks to a special port, which located on beaches or riverbanks. Upon arrival in the special port, the transfer from the trucks to barges is performed, and then the coal will be shipped to Java islands or overseas destinations.

Facts in the field show that almost all of the dump trucks are not covered when transporting coals, as well as the barges when they drift in the waters (rivers, swamps, estuaries or seas). This condition causes the coal particles to be airborne, and some of them fall to the waters. If the dump trucks mostly drop the coal particles to the swamps, the barges to the rivers, swamps, estuaries, or seas. Fisheries resource management problems are not only a matter of population decline or fish resource stocks due to overfishing but also due to habitat destruction due to pollution, sedimentation and decrease of conservation areas.<sup>1,2</sup>

One of the wetland types is a swamp in South Kalimantan, in its swamp, live the climbing perch fish *Anabas testudineus* BLOCH that make the swamp as its natural habitat.<sup>3</sup> Climbing perch fish is an Anabatidae family and ordo labyrinthichi, which means it has a breathing aid in the form of a labyrinth that is able to take oxygen directly from the air.<sup>4</sup> In the biolimmology study of climbing perch fish, turns out that this fish has native habitat in marsh waters with various types,

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namely monotonous swamp and tidal swamp. This fish is an economically important fish in South Kalimantan.<sup>5</sup> The production data of climbing perch caught in South Kalimantan's public waters in 2017 was 7,511.4 tons.<sup>6</sup> While in 2017 the production decreased significantly to only 3,361.2 tons.<sup>7</sup> This shows that the population of climbing perch fish is very large in the last ten years. This is allegedly there are two main factors causing the decline, namely; destruction of climbing perch fish habitat and overfishing.

The data shows that the population of climbing perch fish is declining annually. The cause of the decrease is suspected from the destruction or pollution that impacts the swamps, thus endangering the habitat of swamp fishes and biotas, including the climbing perch fish. In its reproduction, the eggs of climbing perch fish tend to be not moving and need ideal temperature and sufficient dissolved oxygen. The pollution of the swamp, which is caused by coal particles, is suspected to be able to increase the temperature of the water, decrease the supply of oxygen and probably closed the pores of the eggs that is used to breathe and delays the development of the climbing perch fish embryos even reducing the capability of the eggs to hatch. In order to answer the mentioned presumption and phenomenon, a research that analyse the impact of coal particles on eggs and embryonic development of climbing perch fish is very essential to be conducted.

### Materials and Methods

The research site is in the Wet Laboratory of Fisheries and Marine Faculty of Lambung Mangkurat University, with duration six months. Materials and equipment for the research consist of climbing perch fish, plastic pond, ovaprim hormone, fish feed, aquarium and its accessories, microscope, plankton net, basin, pail, scoop net and other supporting equipment. The research is experimental; where there are 12 aquariums each filled with 100 eggs of climbing perch fish that are

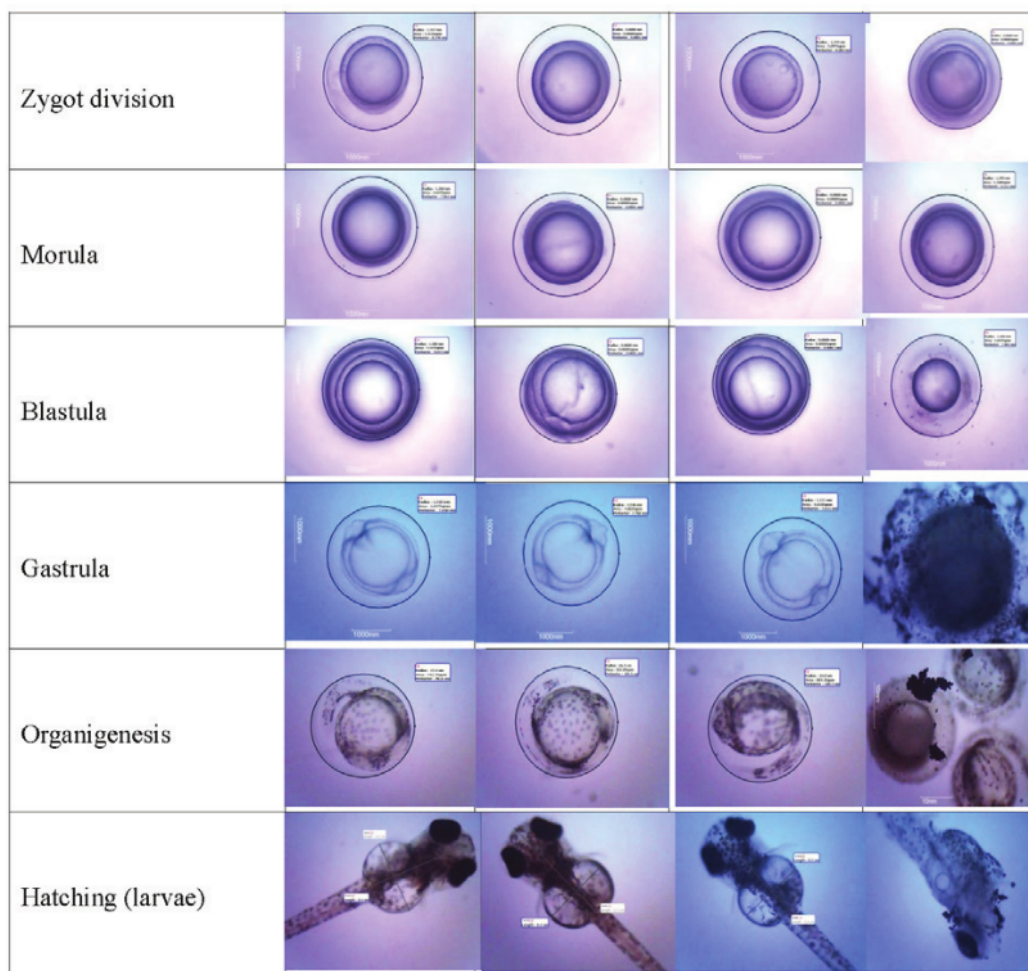
results of an ovaprim hormone-stimulated breeding. There several treatments: treatment O (control), which is without coal particles (0 mg/l water), treatment A with 5 mg/l water of coal particles, treatment B with 10 mg/l water of coal particles, and treatment C with 15 mg/l water of coal particles.

The main parameters in this study are the embryo development, egg hatching rate and survival rate. The observation of climbing perch fish's embryogenesis is performed on a microscope where the results will tell whether the development is normal or abnormal due to the effects of coal particles that penetrated through the egg pores. The observation of the embryogenesis is performed in the third, sixth, ninth, twelfth, fifteenth, thirteenth, sixteenth, and nineteenth hours after fertilization. Embryogenesis process that will be observed includes morula, blastusa, gastrula, eyes, pharynx, tail, veins, and yolk sac.<sup>8</sup> Afterwards, every one hour the eggs are taken its sample and observed its embryo development on a digital LCD microscope with 160X magnification and will be documented. Then the hatching rate and survival rate of the climbing perch fish will be calculated until the fourth day with the assumption that the yolk has been separated. Calculation of the percentage of hatching eggs is done by counting the eggs that hatch and eggs that do not hatch on the sample eggs that are in the experimental aquarium units, amounting to 50 eggs.

### Findings and Discussion

#### Embryo Development

The development of climbing perch fish's embryo occurs after the sperm fertilizes the egg, which is followed by several phases: cell division, morula, blastula, and gastrula and stadia organogenesis. The observation results time phases of embryo development stadia of climbing perch fish in a different exposure of coal particles that are shown in Table 1.

**Table 1. Phases of embryo development of climbing perch fish**

The observation result shows that in treatment O (control), the process of embryo development is relatively normal, from zygote division stadia until hatching into larva. Treatment A (exposure to 5 mg/l water coal particles), turns out doesn't affect the climbing perch fish's embryo development, as well as treatment B (exposure to 10 mg/l water coal particles) Meanwhile, treatment C (exposure to 15 mg/l water coal particles), it is evident that the coal dusts affects embryo

development, where coal particles enters eggshell—which is assumed by diffusion—that can disrupt and damage and even kill the embryo within the egg.

#### **Hatching Rate**

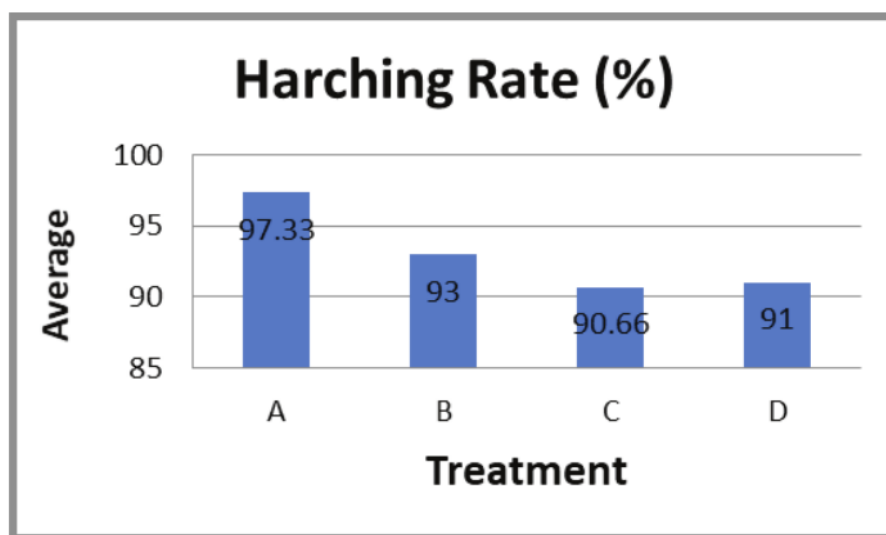
The hatching rate data of climbing perch fish's eggs in every treatment can be seen in Table 2.

**Table 2. Hatching rate average of larvae of climbing perch fish**

Treatment	1	Repetition 2	3	Hatching Rate (%)
O (0 mg/l)	98	96	98	97,3
A (5 mg/l)	93	92	94	93,0
B (10 mg/l)	92	84	96	90,7
C (15 mg/l)	87	95	91	90,0

Based on Table 2, the hatching rate of climbing perch fish's eggs ranges between 90.7% - 97.3%, where highest hatching rate is produced from treatment O that is 97.3%, trailed by treatment A as much as 93.0%, 91% from treatment C, and the lowest from treatment A which is 90.7%. This result indicates that the greater the exposure of coal particles, the lower the eggs' hatching rate. When the eggs hatch, the larva will try to get out of its shell, if the eggs exposed to the coal dust even

covered by the coal dust then the eggs will not be able to hatch. However, the result of variety analysis shows that it does not significantly impact the hatching rate of climbing perch fish's eggs based on a calculation:  $F_{count}(2,03) < F_{table}$  5% (4,07) and 1% (7,59). Therefore, coal dusts exposure up to 15 mg/l water is not significant in making the difference of the hatching rate. An average chart of the hatching rate of climbing perch fish's eggs during the research period can be seen in Figure 1.



**Figure1. Chart of average the hatching rate of larvae of climbing perch fish**

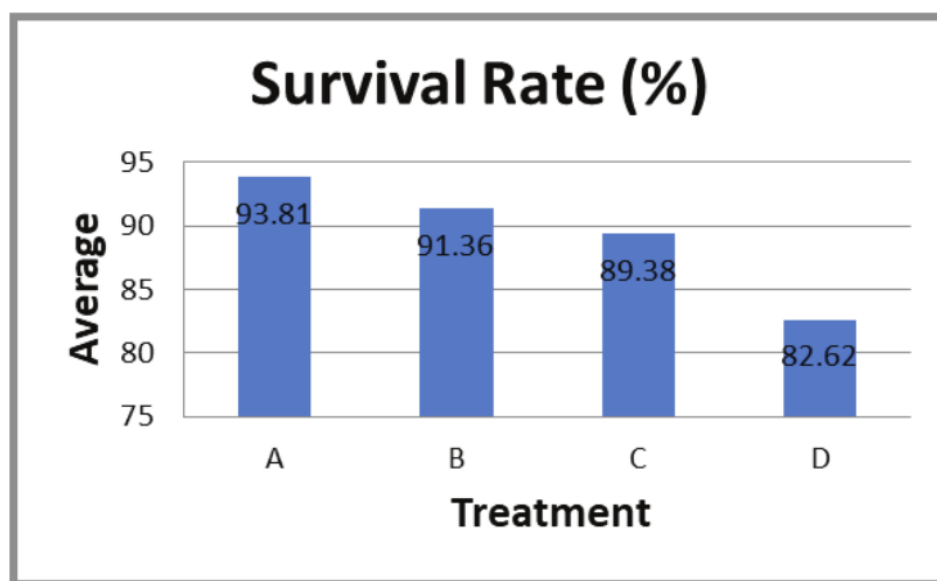
### Survival Rate

The data of the survival rate of climbing perch fish *Anabas testudineus Bloch* in every treatment is shown in Table 3.

**Table 3. Survival rate average of the larvae of climbing perch fish**

Treatment	1	Repetition 2	3	Survival Rate (%)
O (0 mg/l)	94,89	91,66	94,89	93,81
A (5 mg/l)	93,54	89,13	90,42	91,36
B (10 mg/l)	85,86	91,66	90,62	89,38
C (15 mg/l)	91,95	77,89	78,02	82,62

Based on Table 3, the survival rate of climbing perch fish ranges from 82.62% to 93.81%, where the highest survival rate is resulted by the treatment that is 93.81%, followed by treatment A 91.36%, 89.38% from treatment B, and the lowest 82.62% from treatment C. The average of survival rate during the research is shown in Figure 2.



**Figure 2. Chart of average the survival rate of larvae of climbing perch fish**

The result of variety analysis (Anova), shows that there is no significant difference between treatments based on the survival rate of the larvae of climbing perch fish. Therefore, it is assumed that the exposure of coal particles of to 15 mg/l does not cause larva mortality, but if observed visually, it can disturb damage, or cause abnormality in growth and the development of larva of climbing perch fish.

Embryo development is an early part of the life cycle that deals with aspects of evolution, heredity,

developmental mechanisms and environmental influences on the shape and structure of organisms.<sup>9</sup> The eggs of climbing perch fish that is exposed to coal particles with different dosage undergo an embryo development that is started with the formation of blastodisk. Blastodisk formation is a creative process of one blastomere cell in animal poles with a lump as a result. Morula stadia occur when cell division continuously happens, so the cell numbers increase in large amounts with a smaller size.

These cells will eventually form a lump and undergo a thickening at the animal poles. This is in accordance with the statement of Rahayu (2013), morula stadia is a stadia where the number of blastomere is so thick it is very small and very difficult to be counted. Blastula stadia are started with the clear formation of two layers that form a sac.<sup>10</sup>

Organogenesis process is marked with the emergence of a segment in the dorsal body of the embryo. The formation of the spine of embryo's body is marked with the appearance four segments in the median body of the embryo that consists of two layers where each of them four parts have, and the segments of embryo's body will increase along with the growth of embryo's body until hatching. In the organogenesis process, the disturbance due to the exposure of coal particles started, especially with greater dosage which is 15 mg/l water. At the time when the hatching occurs, embryo will make movements to keep off from the yolk inside the chorion so the chorion layer will become flaccid and eventually cracked.<sup>11</sup> Embryo will keep moving in circle faster so the chorion layer grows flaccid due to the movements or due to the chorionase enzyme that causes the chorion layer to be cracked.<sup>8</sup>

The observation of the newly-hatched climbing perch fish's larva shows that the larva body has eyes pigments and transparent brown-black spots in every parts of its body with straight and located on the surface of the water body. The newly-hatch larva of climbing perch fish has been equipped with an imperfectly formed fin with a big size of jaw. The newly-hatched larva of climbing perch fish has black eyes pigments and brown-black spots in parts of its body that are called melanophore.<sup>10</sup> Then, the fertilized egg will develop an embryo and will hatch immediately after 14-20 hours,

depending on the temperature and light obtained. From climbing perch fish egg hatchability data, it can be seen that the range is between 70.2 - 87.1%, with mortality ranging from 2.4 - 47.4%.<sup>12</sup> Furthermore, an important factor that must be considered when incubating an egg (embryo) is water temperature and pH water. Temperature and pH water strongly influences embryo metabolism and to embryonic development, hatching rate and egg hatching rate.<sup>13</sup> The embryogenesis process will take place when the temperature incubation starts from the stage of cleavage, morula, blastula, gastrula, and continued with organogenesis, which then hatches.<sup>14</sup>

By observing the condition of egg, embryo, and larva above, it is proven that coal particles with a size less or equal to 150 µm (pass through the plankton net) and its exposure is about 15 mg/l water can penetrate the egg and disturb or damage the embryo and larva phases (after hatching). It is suspected that diffusion is the main route of alien material enters the climbing perch fish eggs, especially through passive diffusion through semipermeable membrane of the egg skin that is covered with fat. The coal particles are a toxicant that is able to affect or give negative response toward an ecological system, which can seriously damage its structure and function or even cause mortality. The bad impacts here is meant as a depiction and measurement results or quantification that exceeds a normal range that is found in a healthy organism. The toxicant is able to enter the water ecosystem and significantly change the water quality and make the environment to be unsuitable for the organism. The impacts of exposure of the pollutant in an organism are various, from instant death, sub-lethal effect to unobservable effects. The impacts are called ecologically significant when the pressure of the pollutant affects the survival rate, productivity or population structure.<sup>15</sup>

The hatching rate of climbing perch fish's eggs, in the normal state, can achieve 95%.<sup>16</sup> The hatching rate for eggs that have been injected with ovaprim hormone ranges from 85.87% to 87.03%.<sup>17</sup> The hatching rate with different spreading thickness ranges from 96.50% to 98.58%.<sup>18</sup> The cause of the low level of hatching rates is poor qualified sperms that fail to bring spermatozoa to fertilize the eggs, so the eggs don't divide in blastosis phase after the fertilization and the embryo died before hatching.<sup>17</sup>

The highest degree of hatching was at incubation temperature of 31°C, pH 6.7 - 7.6 and dissolved oxygen from 3.3 to 3.8 mg/l.<sup>19</sup> Furthermore, incubation of eggs with different temperatures will have an impact on the morphological development of the prolarva and post larvae of climbing perch fish, where the optimum incubation temperature is obtained at  $30 \pm 1^{\circ}\text{C}$ .<sup>20</sup> One of the environmental parameters that significantly influence the hatchability and development of fish larvae is water temperature. Media temperature has an important effect on larvae organ development, hatching rate and larvae behavior.<sup>21</sup>

Climbing perch larvae are very sensitive, so high mortality often occurs in the larval period. Especially when the larvae change their food from the yolk they contain (endogenous food) to food that comes from outside (exogenous food).<sup>22</sup> Climbing perch fish hatched by induced breeding obtained 100% spawning data, number of eggs 8,978 - 39,868 eggs, hatching degree 69.40 - 98.14%, and degree of survival of tillers to four months age 0, 17 - 0.54%.<sup>23</sup> Survival rates of climbing perch that exceed 70% have shown good results. Even if seen from the monthly period graph, the fry experienced high mortality at the time of initial stocking, which shows that they are still in the adaptation phase.<sup>18</sup>

### Conclusion

1. The exposure of coal particles as much as 15 mg/l water can give damaging impacts toward the development of embryo of climbing perch fish, while exposure of 10 mg/l and 5 mg/l has not give significant impacts.

2. The exposure of coal particles as much as 5 mg/l, 10 mg/l and 15 mg/l lessened both the hatching rate and survival rate of the larva of the climbing perch fish compared to without exposure to coal dusts, but has not give significant impact.

**Ethical Clearance:** This research has gone ethical feasibility testing by the Ethical Research Commission of the *Natural Resource and Environment Management Study Program, Lambung Mangkurat University*.

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**Conflict of Interest:** The authors declare that they

have no conflict interests.

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