# Identification of Causative Agent of Dewa Fish (*Tor douronensis*) Mortality in Kuningan Regency, West Java

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## ABSTRACT

Dewa Fish (Tor douronensis) is become an icon of Kuningan Regency. The names that are carried by this freshwater fish are indeed diverse. In Central and East Java, it is known as Tombro fish, in southern Sumatra it is called Semah fish, in West Java it is called Kancra fish. This fish is a source of regional economy through ecotourism activities. In addition, the living habitat in the highlands causes the existence of this fish to be increasingly limited so that it needs to be preserved. However, based on information from the community, there were quite a lot of mortality in Manis Kidul Village area, so it is necessary to identify the cause of Dewa fish mortality. The method consists of field observation (on-site) and laboratory examination. Identification was also carried out on the characteristics and physical parameters of fish, the possibility of pathogen infection and water quality. The causative agent of Dewa Fish mortality was the emerge of pathogen from bacteria namely Aeromonas salmonicida. This pathogen had been defined in regards to clinical symptoms (characteristics and physically) and laboratory test result. Aeromonas salmonicida belongs to the quarantine fish disease group II. The recommendations to overcome the problem were providing medical treatment for fish and performing quarantine measures by separating fish from healthy fish, cleaning ponds regularly to minimise pathogen vectors from outside the pond, seeking water pumps and/or blowers to overcome low oxygen levels at night and conduct periodic water quality tests.

Keywords: Dewa fish; Tor douronensis; Fish disease; Bacteria; Fish mortality, Aeromonas

### **INTRODUCTION**

Dewa Fish (Tor douronensis) is become an icon of Kuningan Regency (9). In Central Java and East Java, it is known as Tombro fish, in southern Sumatra it is called Semah fish, in West Java it is called Kancra fish. In other areas, there are also mentions of Batak fish, Curong fish, Lempon fish, Ihan fish, Sepan fish, Kelah fish, Masheer fish, and Torsoro fish. Meanwhile, in West Java, especially Kuningan, Dewa Fish is a fish that is sacred by the community. So that, it becomes a tourist attraction and has a positive effect on the economy of community.

Previous study explained that Dewa fish are generally endangered species due to forest destruction and overfishing (9). The existence of uncontrolled logging causes a decrease in the flow of springs so that there is a decrease in the water level in natural ponds. Dewa Fish live in groups (schooling) and have location-specific characteristics, namely developing in certain habitats such as swift, clear, and rocky river waters and are included in omnivore category.

In other words, Dewa fish has only lived in a source of clear, clean, and flowing water continuously (8). Meanwhile, Dewa fish also has a smooth and three dorsal fin, a half rows of scales, swims normally around the bottom of the water and the head is not conical (11,15).

Though many information about physical condition of Dewa Fish, currently, there is lack of information about the disease on Dewa fish. There is only mortality case that a few times occur in



place. Also, at this moment, the mortality of Dewa fish already occured then became concerns from various parties regarding the cause of the mortality of the fish. These mortalities came from Cibulan waters tourist attraction. Sequentially, these fish suddenly found floating on the surface. Moreover, water during observation, there were seen fish with a pale white body condition, fins resembling "pineapple skin" (reddish and slightly raised). Such attributes are fish which were infected by certain pathogen that later on causing a fish disease.

Fish disease is one of the inhibiting factors in fish cultivation and the balance of ecosystems in freshwaters. As an example, outbreaks of furunculosis and Motile Aeromonas Septicemia (MAS) have occurred in Java, Sumatra, and Kalimantan in 2013 (1). This experienced could be a reminder that the presence of disease needs to be cautious so that it does not spread to other areas. Especially for fish diseases that are included in the quarantine fish disease category.

As Dewa fish is included in Cyprinidae, so there are some usual pathogen that infect Cyprinidae cultivation. In previous study, some types bacterial infection on cultured fish are from *Aeromonas salmonicida*. This bacteria known infect to carp, gouramy and catfish. Besides, another pathogen infiltration also occured. For example, the infection of Edwardsiella group usually experienced in carp, gouramy, tilapia, and catfish (1;10;13; 18).

Therefore, to identify the causative agent or a certain pathogen of Dewa fish mortality and in line with the potential of spreading fish diseases, the team is urged to actively control measures through observations and laboratory examination.

## MATERIAL AND METHODS Fish Material

Dewa Fish (*Tor douronensis*) reared in conventional water column. Fish sample was alive and taken from population in pond by targeted sampling. Targeted sample is chosen due to clinical symptoms such as unstable swimming, hemorrhage, necrosis, and lession on skin. The length of sample is 40 cm. At the laboratory, the fish was being necropsy and isolated to the agar medium.

## **Location and Time**

This Research had been conducted in Cibulan waters, Manis Kidul Village, Jalaksana District, Kuningan Regency, West Java Province and Fish Quarantine and Inspection Agency Cirebon. The time of research was August 21<sup>st</sup> until September 10<sup>th</sup>, 2022.

## **Bacteria Inoculation**

At laboratory, bacteria isolation was carried out on the kidneys. Organ samples were taken by cutting the belly of the fish and then the kidneys were taken using tweezers. Then sample was placed in a petri dish to be isolated using a sterile ose needle. Tests were executed on bacterial parameters using conventional Biochemical test methods.

Identification and purification of bacterial colonies done by looking at the morphology of Gram properties, motility, and other cultural/cultural characteristics such as fermentation ability, Indole test, Methyl Red, Voges-Proskauer, and Citric (IMViC) and Biochemical tests. Biochemical testing carried out on all isolates separately conventional against motility, catalase, test triple sugar iron (TSIA) media. agar oxidase. oxidative/fermentative (O/F), GSP, KIA, gelatin, growth at 28°C-37°C, glucose, lactose, sucrose, raffinose, sorbitol, maltose, arabinose, dulcitol, lysin decarboxylase, urea, citrat, ornithin, indol, mannitol, sorbitol, rhamnose, maltose, trehalose, xylose, and mio innositol.



#### **Analysis Method**

The methods used in this research were in-situ observations and laboratory testing on fish samples. In-situ observations were carried out by observing on the condition of fish and water ponds. In addition, the inlets and outlets of the Cibulan waters were also observed. Then, sample was being necropsy and Biochemical test.

A descriptive comparative method was conducted by comparing the result of Biochemical characteristic from the samples with the bacteria characteristic on identification book or literature. To minimise the bias, Biochemical test were carried out in duplicate, namely two isolates. The comparative of Biochemical tests using two literatures (2,3).

### RESULT

#### **Field Observation**

During observation in Cibulan waters founded that Dewa fish shown pale white bodies, fins resembling "pineapple skin" (reddish and slightly raised) (Figure 1). This characteristics in line with the characteristics of fish that are altered by pathogens of the Aeromonas group.



Figure 1. Dewa Fish with hemorrhage all over the body

Meanwhile, internal organ of Dewa fish has the clinical symptom as the sign of microbe infiltration. At the laboratory testing, kidney organ was suffered and having an alteration or damage.



In addition, the behaviour of fish swimming towards the water, unstable and tends to stay around inlets. This is a characteristic that the physiological condition of the Dewa fish has a certain tendency in terms of maintaining its body metabolism to survive. With the condition of such body parts, there are various possible causes, possibly the confrontation of fish body with microbes, or poor water quality thus the body's response changes. For example, if a freshwater fish is in a condition with a high enough nitrite content, the body will become "limp" and in some fish it will look "a bit red".

### Laboratory Examination

The results of the Dewa fish testing in the laboratory showed that the isolates contained *Aeromonas salmonicida* bacteria (Table 1). Of the total Biochemical test component, it determines that there is *A. salmonicida* bacteria in isolate 1 and isolate 2. The Biochemical test components used are a combination of two literatures (28 components), thus it is much more than in other similar studies.

#### Table 1. Biochemical Test Result

	Parameter	Isolate 1	Isolate 2	A. salmonicida
1	Gram KOH 3%	-	-	-
2	Catalase	+	+	+



3	Oxidase	+	+	+
4	Motility	-	-	-
5	H2S	-	-	-
6	Glucose	+	+	+
7	OF	F	F	F
8	GSP	yellow	yellow	yellow
9	Gelatin	+	+	+
10	TSIA (B/S), Gas, H <sub>2</sub> S	K/A. H2S	K/A, H2S	K/A
11	Lysin Decarboxylase	-, g	-,g	v
12	Urea	-	-	-
13	Citrat	+	-	V
14	Ornithin	-	-	-
15	Indol	+	-	V
16	MR	+	-	-
17	VP	+	-	+
18	Arabinose	-	-	V
19	Sucrose	+	+	v
20	Mannitol	+	+	v
21	Sorbitol	-	-	v
22	Rhamnose	-	-	-
23	Maltose	+	+	v
24	Manose	+	+	+
25	Trehalose	+	-	v
26	Lactose	+	+	v
27	Xylose	-	-	-
28	Mio Inositol	-	-	-
		A. salmonicida	A. salmonicida	

Description : (+) positif; (-) negative; (v) variable; (g) gas; A/K: acid/Alkali; c/m: coloni/media; B/S : Butt/Slant; ND : tidak ada data; H2S: Hidrogen sulfida, MR: Methyl red; VP: VogesProskauer

The water quality and the character of Dewa Fish can be equated with the standard requirements like other Cyprinidae, such as goldfish water quality standards (with some adjustments). For example, Dewa fish live in waters with a temperature of 18°C - 26°C. As for goldfish (for example) the standard water temperature is 25-30°C. For other parameters, it is relatively similar as freshwater fish in general. The results of water quality measurements are in table 2.

Table 2. Water Quality

No	Parameter	Main tandon (inlet)	Pond I (outlet)	The standards (Ref: 5; 12)
1	Temperature	25	24.6	18-26 °C
2	pН	7.5	8	7-8.5 C
3	DO	4.4	5.3	> 5 mg/L
4	Nitrite	0.1	0	< 0.06 mg/L

## DISCUSSION

#### **Field Observation**

The clinical symptoms has a characteristics which in line with the characteristics of fish that are altered by pathogens of the Aeromonas group. This group of pathogens could cause bleeding from tissue wounds at the base of the pectoral and pelvic fins (2; 14). The fish will become weak, dark in color, ulcerated, exopthalmus and can be followed by a secondary infection by fungi or other bacteria.

For fish swimming in unstable conditions, this occurs in the majority of fish whose body conditions are abnormal. Also, the suffered kidney on Dewa fish indicated as an impact of pathogen infection. As mentioned in literature, those clinical symptoms are identical to previous studies that the disease caused by



Aeromonas sp. Showing symptoms likewise loss of appetite, wounds on the body surface, bleeding in the gills, enlarged stomach filled with fluid, loose scales and tail fin, if surgery is performed, swelling and damage to liver, kidney and spleen tissue will be seen (16;17). Meanwhile, in regards to fish gather or tend to be "stayed" at a certain point, presumably it were because the dissolved oxygen content did not meet the standards.

Members of the genus Aeromonas are gram-negative, straight rod-shaped with rounded cell ends approaching a spherical shape, 0.3-1.0 m in diameter and 1.0-3.5 m in length (6). Cells are unicellular or grouped. Members of this genus are facultative anaerobes, having both respiratory and fermentative metabolic types. Members of the genus Aeromonas grow at optimal temperatures ranging from  $22 - 28^{\circ}$ C and many members of the species grow well at  $37^{\circ}$ C.

In specific, Bacteria *A. salmonicida* live in water, mud and detritus media, host fish, other aquatic animals and especially fish carriers. There are also studies that provide an overview of the presence and survival of *A. salmonicida* in these habitats. Based on the literature (2), the recovery of water from the presence of *A. salmonicida* bacteria as soon as 24 hours and at the latest it can reach 19 days. Meanwhile, in brackish waters and unfiltered marine waters, it reaches 16-25 days and 24 hours-8 days respectively.

The disease caused by A salmonicida called Furunculosis. is Furunculosis causes inflammation of the skin of chronically infected fish (17). Subacute or chronic form of furunculosis. characterised by weak fish, slight exophthalmia, bleeding fins, bleeding from the nostrils, and multiple bleeding in muscles and other tissues. Internally, bleeding in the liver, swelling of the spleen and necrosis of the kidney may occur most commonly especially in growing and adult fish. Another thing is that fish usually die within 2-3 days, and cause high mortality (17).

## **Biochemical test**

In general, the result of Biochemical test from two isolates indicated to Aeromonas salmonicida. In the fifth column. is the standard characteristic of the Biochemical test results of A. salmonicida. While two columns (columns 3<sup>rd</sup> and 4<sup>th</sup>) are the results of Biochemical tests of two isolates. In this test, morphologically all bacterial isolates had circular colonies, convex colony elevations, and cream coloured bacterial colonies on TSA media, while on GSP agar the inhibition zone was visible, yellow (10). According to literature, bacteria are able to ferment carbohydrates contained in GSP agar media, resulting in a change in colony colour (13). The nature of this fermentation can be used as a distinguish between the criterion to Aeromonas and Pseudomonas groups.

Based on the basic Biochemical test according to table 2, the two bacterial isolates indicated that the isolates were *A. salmonicida*, namely gram negative, catalase positive, oxidase positive, motility negative, and glucose positive. This is in line with previous research which stated that *A. salmonicida* is a Gram-negative, non-motile bacteria, produces a positive reaction in the oxidase test, positive glucose, and some isolates produce brown pigment (1).

The next Biochemical test parameters, namely TSIA, H2S, O/F, GSP and citrate from the test results are dominantly similar to previous studies conducted by (16), namely TSIA K/A, H2S positive, Indole positive, and facultative anaerobes. Indole and Citrate which have different results, namely isolate 1 is positive and isolate 2 is negative. This is because the indole and



citrate parameters can appear positive or negative (they are variable parameters). Likewise with several other sugar test parameters that are included in the "variable" type, there are differences in results between isolate 1 and isolate 2, namely trehalose. However, the majority of the test parameters in both isolates had the same results as the standard characteristics of A. salmonicida bacteria, both having definitive and variable characteristics. This shows that the results of Biochemical tests was an Α. salmonicida bacteria in fish samples from Cibulan waters.

## Water quality test

Regarding on Table 2, Cibulan waters are still within the standard range. Therefore, the water quality should not considerred as the factor effects on the fish condition.

To summarise, according to the results of in-situ observations and laboratory examination, it is clear that the causative agent of Dewa fish mortality was Aeromonas salmonicida. The recommendations of the Fish Ouarantine team are providing medical treatment for fish and performing quarantine measures by separating fish from healthy fish, cleaning ponds regularly to minimise pathogen vectors from outside the pond, seeking water pumps and/or blowers to overcome low oxygen levels at night and conduct periodic water quality tests. In systematic management of addition. Cibulan waters, both in terms of water quality, conservation, and nutrition of Dewa fish could be implemented to reduce the risk of disease exposure.

## CONCLUSION

The causative agent of Dewa Fish mortality was the emerge of pathogen from bacteria namely *Aeromonas salmonicida*. This pathogen had been defined in regards to clinical symptoms (characteristics and physically parameters of fish) and laboratory test result. Aeromonas salmonicida is categorised as quarantine fish disease group II. Besides, the management of water columns is need to be developed. Cibulan waters are an economic source for the surrounding community, so it needs to be sustained. Therefore, cultivation good and biosecurity management should be implemented daily. As a result, the mortality or negative impact of pathogens in the waters or even on a fish can be prevented.

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