

Pathogenicity of bacterial isolate GM 01 in gourami (Osphronemus goramy)

by Slamet B. Prayitno

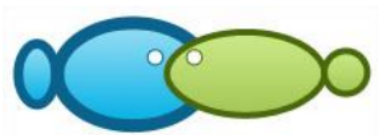
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Pathogenicity of bacterial isolate GM 01 in gourami (*Osphronemus goramy*)

¹Sarjito, ¹Reka K. Wati, ¹Alfabetian H. C. Haditomo, ¹Desrina, ²Aninditia Sabdaningsih, ¹Slamet B. Prayitno

¹ Aquaculture Department, Faculty of Fisheries and Marine Science, Diponegoro University, Tembalang, Semarang, Indonesia; ² Aquatic Resources Department, Faculty of Fisheries and Marine Science Faculty, Diponegoro University, Tembalang, Semarang, Indonesia. Corresponding author: Sarjito, sarjito@live.undip.ac.id

Abstract. Fish farming is inseparable from pests and diseases, one of which is bacteria. Several studies have been carried out to determine the causative agent of bacterial diseases in gourami (*Osphronemus goramy*). However, the studies only reached the Koch postulate test. The purpose of this study was to determine the pathogenicity of GM 01 bacterial isolates in *O. goramy* from Magelang, Central Java, Indonesia. The study was conducted on clinical symptoms, mortality, LD50 value, and blood profile. The present study used an experimental method, which consisted of 4 treatments with three replications. Experimental animals were 270 *O. goramy* with a length of 8-10 cm. The concentration of GM 01 bacterial isolate used in this study was treatment A (10^0 CFU mL⁻¹), B (10^4 CFU mL⁻¹), C (10^5 CFU mL⁻¹), D (10^6 CFU mL⁻¹), E (10^7 CFU mL⁻¹), and F (10^8 CFU mL⁻¹). Clinical symptoms of *O. goramy* infected by GM 01 bacterial isolates swimming abnormally, have low appetite, presents necrosis, reddened mouth, flaking, and bleeding at the fins, blackened body, and exophthalmia. The highest percentage of fish mortality occurred in treatments E and F, with 100% mortality at the 90th hour post-infection. Treatment D, C, and B were followed at 120, 132, and 144 hours post-infection, also with 100% mortality. LD50 value showed that the concentration of bacteria that could kill *O. goramy* populations by 50% in 96 hours was 4.2209×10^5 CFU mL⁻¹. Based on these values, GM 01 bacteria are included in the category of pathogens. The percentage of post-infection lymphocyte, neutrophil, and monocyte cells were 72-79%, 12-21%, and 3-8% respectively.

Key Words: blood profile, fish diseases, LD₅₀, mortality, *Yersinia intermedia*.

Introduction. Gourami (*Osphronemus goramy*) is one of the main freshwater commodities that is widely cultivated in Indonesia (Kusumawaty et al 2017; Rozi et al 2017; Nuryanto et al 2018). *O. goramy* also has a high value in fisheries commodities, as it is evidenced by the price reaching 3.2-3.9 USD kg⁻¹. Consumption of *O. goramy* has increased in recent years. Based on general data of the Ministry of Marine and Fisheries (2014), *O. goramy* production also appears to be increasing every year. In 2010 the production of *O. goramy* only reached 56,889 tons. In 2011 production increased to 64,252 tons, in 2012 increased to 84,681 tons, and in 2013 production reached 86,773 tons. Based on these data, the average increase of *O. goramy* production from 2010 to 2013 was 15.74%.

The emergence of the disease often hampers *O. goramy* culture. Disease arises when the aquaculture environment is not suitable for the needs of the species requirements. Intensive *O. goramy* culture causes environmental changes as a result of high pollution and wrong decision making, one of which is access to the amount of stocking density and feed that results in the emergence of disease problems. The emergence of the disease can cause deflation in production. Incoming disease agents can be contagious or non-communicable. Some infectious disease agents in *O. goramy* are parasites, bacteria, and viruses (Havixbeck et al 2016; Mishra et al 2017; Sen & Mandal 2018). Intensive is the culture, higher is the prevalence of bacteria. Based on previous reports, the bacteria that attack *O. goramy* are *Aeromonas hydrophila*, *Staphylococcus*

saprophyticus, *Aeromonas caviae*, and *Flavobacterium* sp. (Kusumawaty et al 2017; Rozi et al 2017; Rozi et al 2018).

Fish farming activities cannot be separated from fish pests and diseases. The disease can arise through an unbalanced interaction between three factors, namely the host, pathogen, and the environment, which cause stress and lower the immunity and leaves the fish vulnerable to disease (Makrinos & Bowden 2016; Li et al 2017; Yengkhom et al 2019). A bacterial disease that attacks *O. goramy* is a contagious disease (Hidayatullah et al 2018; Sen & Mandal 2018; Kousar et al 2019). Disease agents are one of the important factors that must be investigated to get the certainty of the causes and to find a cure. After that, the Koch challenge test or postulate is tested to ascertain whether the bacterium is a pathogen or not. Koch's postulates are guidelines for testing diseases caused by certain microorganisms or for determining causative agents (Ross & Woodward 2016; Wang & Lai 2018).

Fish stress conditions can facilitate the entry of disease agent. Disease agents in fish can be caused by parasites, fungi, bacteria, and viruses (Idowu et al 2017; Hidayatullah et al 2018). The bacterial disease is one of the obstacles in the culture of *O. goramy* because it can cause death and economic losses. Bacteria that often attack *O. goramy* are *Chryseobacterium aquifrigidense*, *Echerichia fergusonii*, *Staphylococcus saprophyticus*, and *Bacillus pumilus*. This kind of bacterial invasion can cause death in *O. goramy*; therefore, it can cause economic losses to fish farmers (Xue et al 2017; Luis et al 2019; Kossack et al 2020).

Due to the disease outbreak, which resulted high mortality in *O. goramy* in Magelang District, the isolation of the causative agent of the disease was performed. The preliminary study indicates that isolate GM 01 causes 80% mortality in fish. The purpose of this study was to determine the pathogenicity of GM 01 bacterial isolates in *O. goramy* from Magelang District, Central Java, based on clinical symptoms, mortality, LD₅₀ value, and blood profile. Previous experiments only reached the Koch postulate test. Therefore, further research needs to be done regarding the pathogenicity test on bacterial agents that cause disease in *O. goramy*, in order to determine the level of pathogenicity when bacteria infect fish. The results of this study are expected to provide information about the effect of bacterial density with GM 01 codes with different dosage on the blood profile as a representation of the immune response of *O. goramy* originating from Magelang District, Central Java.

Material and Method. The fishes used in this study were 270 healthy *O. goramy* specimens with a length of 8-10 cm. The experimental fishes were stored in 18 aquariums with dimensions of 30 x 40 x 50 cm filled with 15 L freshwater and aerated during the experiment. The fishes were cultivated for six days, and acclimatization was carried out three days before the examination.

This study used an experimental method with a Completely Randomized Design (CRD) consisting of 6 treatments and three repetitions, and then data was analyzed descriptively. The pathogenicity test was done by injecting GM 01 isolates intramuscularly or known by injecting bacteria just below the dorsal fin at an angle of 65°. The bacterial concentrations used were A (10⁰ CFU mL⁻¹), B (10⁴ mL⁻¹), C (10⁵ mL⁻¹), D (10⁶ mL⁻¹), E (10⁷ mL⁻¹), F (10⁸ mL⁻¹). All treatments were performed through intramuscular injection (Sarjito 2010).

The bacterial isolate used in this study was GM 01, isolated from contaminated *O. goramy*, from Magelang District, Central Java. The GM 01 bacteria were cultured in Trypticase Soy Broth medium, then incubated at 22°C for 48 hours. Bacteria were harvested and washed using Phosphate-Buffered Saline solution. After harvest, the bacteria density was measured using the McFarland 0.5 method as a standard procedure (Maftuch et al 2016; Mohammed & Omer 2019). The pathogenicity test was carried out to find bacteria that have infected fish which have been previously isolated, while the blood profile preparations process was performed following the steps presented in Figure 1.

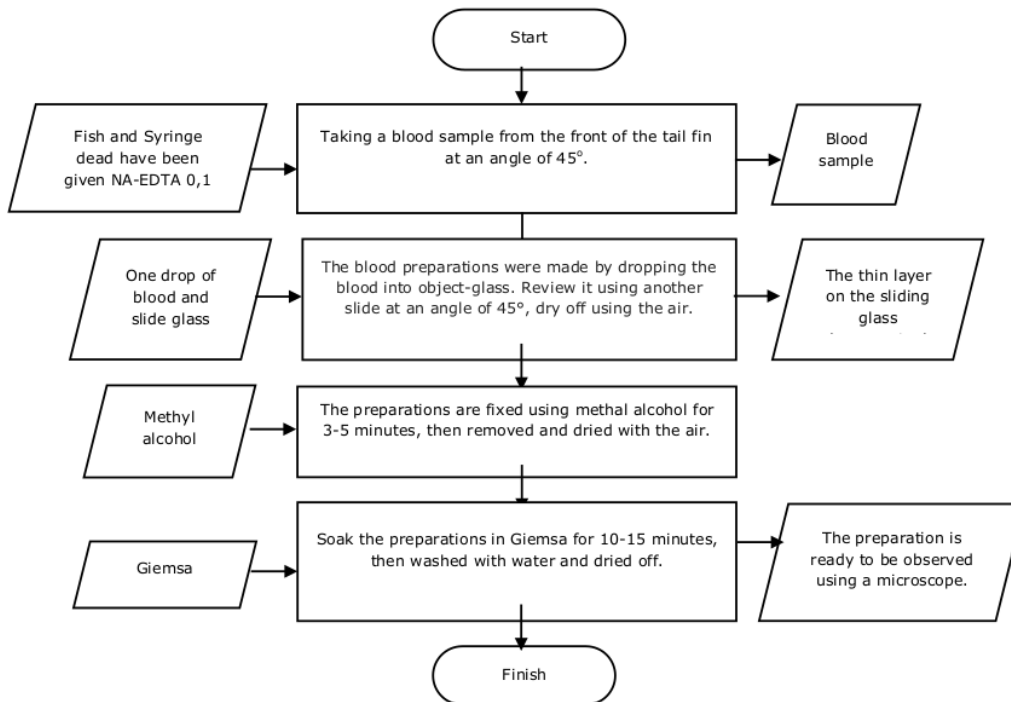


Figure 1. Flowchart of the of the blood sample preparations.

Data analysis

Fish mortality calculation. Total fish mortality was counted using Svobodova & Vykusova (1991) method, with the formula:

$$Z = \frac{N_0 - N_t}{N_0} \times 100\%$$

Where:

Z - mortality rate

No - total fish at the beginning of the experiment

Nt - total fish at the end of the experiment

LD₅₀ scores. The LD₅₀ scores were determined using the method used in the previous research of Sarjito (2010), which using the formula:

$$m = Xi + d \frac{50 - \%Xi}{\%xi + 1 - \%xi}$$

Where:

M - log LD50

Xi - log bacterial dosage below LD50 and above LD50

%xi - accumulation of mortality percentage on bacterial dosage below LD50

Xi+1 - accumulation of mortality percentage on bacterial dosage above LD50

Blood Profile

Lymphocytes. Total lymphocytes were counted using Shabirah et al (2019) method, with the formula:

$$\text{Percentage Limfosit (\%)} = \frac{L}{100} \times 100\%$$

Neutrophil. Total neutrophils were counted using a method proposed by Shabirah et al (2019), with the formula:

$$\text{Percentage Neutrofil (\%)} = \frac{N}{100} \times 100\%$$

Monocytes. Total monocytes were counted using the method of Shabirah et al (2019), with the formula:

$$\text{Percentage Monosit (\%)} = \frac{M}{100} \times 100\%$$

Water quality. Some water parameters in the experimental media were measured concerning temperature, dissolved oxygen (DO), and acidity (pH). Water quality was observed at the beginning and end of the experiment (Puspaningsih et al 2019).

Results

Clinical symptoms. Based on the result observations, the clinical symptoms found in *O. goramy* infected by GM 01 isolate bacteria were exhibited erratic swimming, low appetite, necrosis, reddened mouth, flaking and bleeding at the fins, blackened body, and *exophthalmia* (Table 1 and Figure 2).

Table 1
The clinical symptoms of *Osphronemus goramy* infected with GM 01 bacteria isolate from total sample (n = 45)

No.	Treatment	Percentage of fish	Behavior/ Clinical sign
1	A	100	Swim actively
		100	Swim normally
		100	High appetite
2	B	62.2	Swim normally
		46.67	Low appetite
		31.1	Hemorrhage on the fin base
3	C	51.1	Swim normally
		57.78	Low appetite
		55.56	Reddened mouth
4	D	48.89	Exophthalmia
		68.89	Low appetite
		60	Erratic swimming
5	E	64.4	Reddened mouth
		60	Exophthalmia
		82.2	Low appetite
6	F	75.56	Erratic swimming
		71.1	Reddened mouth
		75.56	Necrosis
6	F	40	Fins flakiness
		91.1	Erratic swimming
		75.56	Reddened mouth
6	F	82.2	Exfoliating scales, necrosis,
		66.67	exophthalmia
		73.3	Color changes, blackened
6	F	48.89	Haemorrhage on the fin

Based on the observations of the clinical symptoms found in *O. goramy* infected with GM 01 bacterial isolates, it appeared that clinical symptoms in all treatments after infection are still normal: fish can still swim actively and has a normal appetite. Clinical symptoms begin to appear at the sixth hour after infection but are only seen on F treatment, where necrosis occurred on the *O. goramy* scale. Clinical symptoms in *O. goramy* infected with GM 01 bacterial isolates, in the form of changes in behavior and morphology of the body, can be clearly seen in Figure 2.

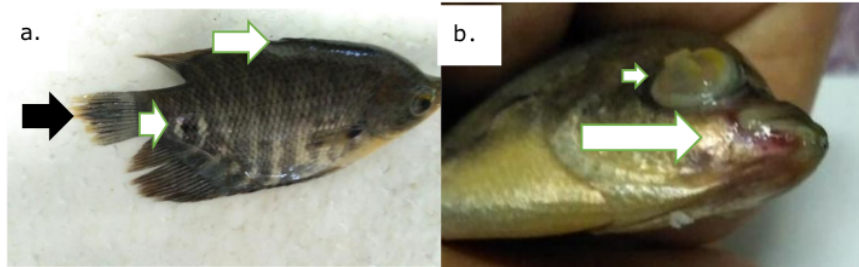


Figure 2. The clinical symptoms arising from fish infected with GM 01 bacteria.

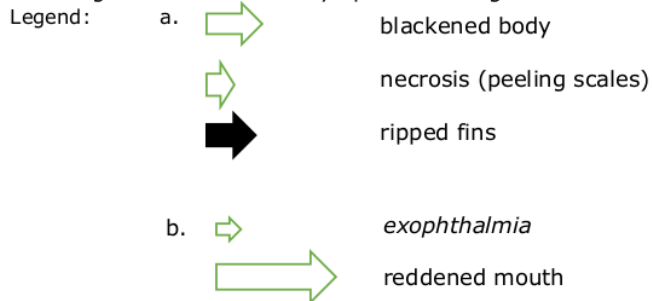


Figure 2 shows that the clinical symptoms found in *O. goramy* infected with GM 01 isolates are characterized by changes in behavior and body morphology. The changes that can be seen from the behavior of fish, among others, are decreased appetite, swim weakly on the bottom of the water and abnormal, such as swim vertically or sloping, while morphological changes consisted of peeling scales, changing body color which becomes more blackened, fins flakiness, reddened mouth, and *exophthalmia* or protruding eyes.

Mortality. The observation of *O. goramy* death time during the pathogenicity test was done every 6 hours for 96 hours and observation 12 hours after that until the end of the study. As for the cumulative mortality pattern of *O. goramy* after being infected with GM 01 bacteria, isolate code is presented in Figure 3.

Based on Figure 3, mortality of infected specimens by GM 01 bacteria isolate, in E and F treatment occurred on the sixth day post-infection, then death of *O. goramy* within the D treatment started at 12 hours after infection, while in treatment B and C *O. goramy* mortality occurred 24 hours after infection. Treatment E and F could kill all individuals within 90 hours after infection, followed by treatment D which has the same results in 120 hours after infection, then treatment C in 132 hours after infection, then all deaths the last fish sample occurred in treatment B, which was within 144 hours after infection. The results of the fastest percentage of fish deaths occurred in treatments E and F, namely as many as 100% of fish died in 90 hours after infection, then followed by treatments D, C, and B at 120 hours, 132 hours, and 144 hours after infection occurred also a 100% mortality.

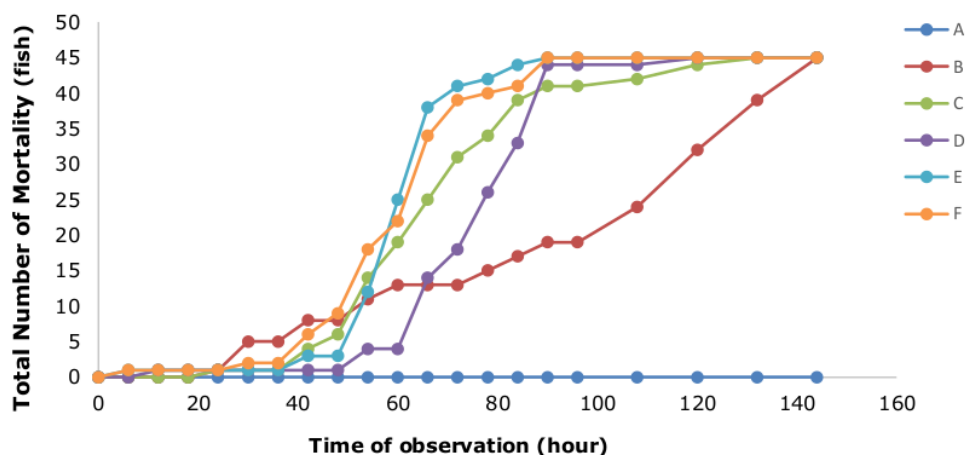


Figure 3. Mortality quantification according time.

Legend: Treatment A (PBS); B (density of bacteria 10^4 CFU mL⁻¹); C (density of bacteria 10^5 CFU mL⁻¹); D (density of bacteria 10^6 CFU mL⁻¹); E (density of bacteria 10^7 CFU mL⁻¹); and F (density of bacteria 10^8 CFU mL⁻¹).

LD₅₀ value. LD₅₀ test was conducted to determine the level of concentration of bacterial isolates that cause 50% mortality in *O. goramy* in 96 hours. The results of LD₅₀ calculations of GM 01 isolates in *O. goramy* can be seen in Table 2.

LD₅₀ of GM 01 bacteria isolate in *Osphronemus goramy* Table 2

Variables	Treatment					
	A	B	C	D	E	F
Samples (n)	45	45	45	45	45	45
Mortality (n)	0	19	41	44	45	45
Alive (n)	45	26	4	1	0	0
Mortality (%)	0	42.22	91.11	97.78	100	100
LD ₅₀ (CFU mL ⁻¹)			4.2209 x 10 ⁵			

Treatment A (PBS); B (density of bacteria 10^4 CFU mL⁻¹); C (density of bacteria 10^5 CFU mL⁻¹); D (density of bacteria 10^6 CFU mL⁻¹); E (density of bacteria 10^7 CFU mL⁻¹); and F (density of bacteria 10^8 CFU mL⁻¹).

Based on Table 2, the LD₅₀ test results with GM 01 isolate which produced 50% mortality of the *O. goramy* population within 96 hours is a bacterium with a density of 4.22×10^5 CFU mL⁻¹, so the bacteria are included in the virulent category.

Percentage of lymphocyte cells. The percentage of lymphocytes was obtained from a comparison between the number of lymphocyte cells with the total number of leukocyte cell samples that are counted as 100 cells, then multiplied with 100. Examination of D-1 blood profile was done before infection, D+1, and D+4 after infection. Examination results of lymphocyte cell percentage in *O. goramy* infected with GM 01 isolates with different bacterial densities can be seen in Table 3.

Based on Table 3, it can be seen that the average percentage of *O. goramy* lymphocyte cells infected by GM 01 isolates has decreased from day to day. The percentage of lymphocyte cells after infection, especially on the 4th day showed that lymphocytes in *O. goramy* blood is abnormal, because the average percentage of lymphocyte counts was less than 80%, which ranged between 72 and 79% while the normal lymphocyte cells for health fish is more than 80%.

Table 3

Average percentage of lymphocyte cell per treatment

Treatment	D-1	D+1	D+4	Normal
A	82.00±1.00	82.00±2.00	80.00±1.00	
B	84.00±1.00	81.00±1.73	79.00±2.00	
C	82.00±2.00	79.00±3.00	76.00±1.00	More than 80%
D	80.00±4.00	79.00±2.00	75.00±2.00	(Roberts 2012)
E	80.00±2.00	77.00±2.65	74.00±2.00	
F	71.00±2.00	76.00±3.00	72.00±1.73	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL⁻¹); C (density of bacteria 10^5 CFU mL⁻¹); D (density of bacteria 10^6 CFU mL⁻¹); E (density of bacteria 10^7 CFU mL⁻¹); and F (density of bacteria 10^8 CFU mL⁻¹).

Percentage of neutrophil cells. The percentage of the neutrophils is obtained from the ratio between the number of neutrophil cells and the total number of leukocyte cell samples which were counted as many as 100 cells, then multiplied by 100. Examination of blood profile was done D-1 before infection, D+1, and D+4 after infection. The results of an examination of the average percentage of neutrophil cells in *O. goramy* infected by GM 01 isolates with different bacterial densities can be seen in Table 4.

Table 4

Average percentage of neutrophils cells per treatment

Treatment	D-1	D+1	D+4	Normal
A	13.00±2.00	13.00±2.00	12.00±2.65	
B	13.00±2.65	16.00±2.00	17.00±4.58	
C	15.00±1.73	16.00±2.65	18.00±4.36	6-8%
D	15.00±3.00	17.00±4.36	18.00±2.65	(Roberts 2012)
E	16.00±1.73	17.00±3.61	20.00±2.65	
F	11.00±3.00	19.00±1.73	21.00±3.00	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL⁻¹); C (density of bacteria 10^5 CFU mL⁻¹); D (density of bacteria 10^6 CFU mL⁻¹); E (density of bacteria 10^7 CFU mL⁻¹); and F (density of bacteria 10^8 CFU mL⁻¹).

Based on Table 4, it appears that the average percentage of neutrophil cells in *O. goramy* after the infection has increased. The percentage of the neutrophil cells after infection, i.e., on the first day and day 4 showed that the number of neutrophils in *O. goramy* blood is higher than normal, because the average percentage of neutrophil in the treatments was around 12-21%, whereas, according to Roberts (2012) the amount of neutrophil cells for normal fish is approximately 6-8%.

Percentage of monocyte cells. The percentage of monocytes was obtained from the ratio between the number of monocyte cells with the total number of leukocyte cell samples counted, which were 100 cells, then multiplied by 100. Examination of blood profile was done D-1 before infection, D+1, and D+4 after infection. The results of the examination concerning the average percentage of monocyte cells in *O. goramy* infected by GM 01 isolates with various bacterial densities can be seen in Table 5.

Table 5

Average percentage of monocyte cells per treatment

Treatment	D-1	D+1	D+4	Normal
A	5.00±2.65	5.00±2.65	8.00±2.00	
B	3.00±2.65	3.00±1.00	4.00±2.00	
C	3.00±1.73	5.00±1.73	6.00±1.73	1-2%
D	4.00±3.00	4.00±1.00	7.00±2.00	(Roberts 2012)
E	4.00±1.00	6.00±2.00	7.00±4.00	
F	1.00±2.00	5.00±2.00	7.00±2.00	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL⁻¹); C (density of bacteria 10^5 CFU mL⁻¹); D (density of bacteria 10^6 CFU mL⁻¹); E (density of bacteria 10^7 CFU mL⁻¹); and F (density of bacteria 10^8 CFU mL⁻¹).

Based on Table 5, it can be seen that the average percentage of monocyte cells in *O. goramy* after the infection begins to increase from day to day at each treatment. The percentage of monocyte cells from before infection to post-infection showed that the number of monocytes in *O. goramy* blood was above normal conditions because the average percentage of monocyte counts in the above treatments was in the range of 3-8%, while according to Roberts (1978) the amount of normal monocyte cells of fish is between 1 and 2%.

Discussion

Clinical symptoms. Based on observations of clinical symptoms found in *O. goramy* infected by GM 01 bacterial isolates, normal parameters could be found in all treatments after infection: fish could swim actively and had normal appetite. Clinical symptoms began to appear at the sixth hour after infection but were only seen in F treatment, where presented necrosis on the fish scale. Clinical symptoms appeared in treatment B, visible at the 12th hour after infection; namely, the appetite of the fish begins to decrease. At the 30th hour, fish begins to move passively, and bleeding at the base of the fin after 48 hours after infection. Then in treatment C, clinical symptoms that appeared were reddish mouth that started appearing at 36 hours after infection and exophthalmia at 42 hours. Not very different from treatment C, the clinical symptoms that occurred in treatment D were passive movements, abnormal swimming, or at the bottom and tilt, reddened mouth, and exophthalmia. It is just that clinical symptoms appeared faster than in previous treatment. The E and F treatments showed almost the same symptoms: in treatment E, the fish were swimming abnormally, presented reddish mouth, dorsal, anal and caudal fins flaked, and necrosis occurred at the 30th hour after infection. While in the treatment F clinical symptoms such as necrosis began to appear after the 6th hour post-infection, followed by redness of the mouth at the 18th hour, besides that the fish were also seen swimming abnormal at the bottom, body-color blacks, and followed by the occurrence of exophthalmia and bleeding at the base of the fins at the 30th-hour post-infection. In contrast to treatment E, in this treatment, were no visible flakes of fish fin.

Based on the results of the clinical symptoms that could be seen *O. goramy* infected by GM 01 in all treatments, the clinical picture indicated a close relationship with species *Yersinia intermedia*. This could be seen from the most prominent clinical symptoms of fish, namely flaky scales, reddened mouth, *exophthalmia*, and blackened body-color (Kumar et al 2015; Yamasaki et al 2017; Acar et al 2018; Kazamikova et al 2019; Tkachenko et al 2019; Wrobel et al 2019). Morphological changes such as peeling scales cause fish to be more susceptible to skin injury. Clinical symptoms observed in the present study are in accordance with the report of Muziasari (2016), that among the clinical symptoms that arise from infection by *Yersinia* sp. bacteria are behavioral (slow movements) and morphological changes, where the body color becomes darker, and the mouth turns red. *Yersinia* sp. bacteria that have been reported by Hiko et al (2018) have attacked several freshwater fish, such as *Oreochromis niloticus*, *O. goramy*, *Clarias* sp. Rozi et al (2018) also reported *O. goramy* infestation by *Yersinia* sp. where the most prominent clinical symptoms were peeling scales and darker body color.

According to our observation, considering all treatments, fish samples infected by GM 01 bacterial isolates did not showed the same clinical symptoms, but the higher was the concentration of the injected bacteria, faster were the clinical symptoms manifestations. These aspects were more evident in fish samples from treatments E and F, against clinical symptoms of treatment B, C, and D.

Pathogenicity test. Pathogenicity tests included mortality patterns and LD50. During the pathogenicity test, the time of death was observed every 6 hours for 96 hours, and after that observation every 12 hours until the end of the experiment. Death occurred on the first day after infection, in the treatment D, E, and F. According to Table 2, *Y. intermedia* infected *O. goramy* within E and F treatment, were found death starting at 6 hours after infection, followed by D treatment where mortality started at 12 hours after

infection, while specimens from treatment B and C faced mortality at 24 hours after infection. Treatment E and F produced 100% mortality within 90 hours after infection, followed by treatment D, where the 100% mortality occurred within 120 hours after infection. Treatment C produced 100% mortality within 132 hours after infection, and finally, treatment B within 144 hours after infection. The fastest mortality was obtained within treatment E and F, with 100% in 90 hours after infection, followed by treatments D, E, and B, which with 100% mortality within 120 hours, 132 hours, and 144 hours after infection respectively. High levels of bacteria pathogenicity can cause mortality to reach 100% (Rozi et al 2017; Hardi et al 2018; Safinska 2018). It can be concluded that *O. goramy* died due to differences in bacterial concentrations; the higher the concentration of infection in fish, the higher the mortality that occurs.

The alleged cause of occurred mortality was the morphological changes such as flaked scales so that bacterial agents could more easily and quickly infect *O. goramy*. Scales are one of the external protections of fish. Therefore if scales exfoliate, fish will be more susceptible to bacterial infections (Maftuch et al 2016; Putra et al 2016; Yengkhom et al 2019).

LD50 results showed that the concentration of bacteria that could cause 50% mortality of *O. goramy* population in 90 hours was 4.2209×10^5 CFU mL⁻¹. Hamed et al (2018) reported that bacteria that had LD50 scores of 103-105.5 CFU mL⁻¹ are categorized as deadly infectious bacteria, whereas LD50 scores above 10^7 CFU mL⁻¹ denotes low-level virulence. Thus *Y. intermedia* used in the present experiment are included in the virulent category.

Blood profile

Lymphocyte cells. The percentage of lymphocyte cells in *O. goramy* after infection with *Y. intermedia* was below the normal range of 80%, namely (72-79%). The percentage of lymphocytes was obtained by comparing the number of lymphocyte cells with the total number of leukocyte cell samples that are counted as 100 cells, then multiplied with 100. Examination of blood profile was done H-1 before infection, H+1, and H+4 after infection.

A decrease in the percentage of lymphocytes from *O. goramy* samples is caused by stress due to poor environment in aquaculture and fishing, whereas larval transportation and aquaculture are stress factors in Teleostei or Elasmobranch fish. Stress and a poor environment condition cause an increase in the hormone cortisol, causing a decrease in lymphocyte cells in the bloodstream. A decrease in lymphocyte cells occurs in immunopathy cases or due to certain stress factors. In the present experiment, *O. goramy* might experience stress, which caused a decrease in lymphocyte cells (Makrinos & Bowden 2016; Fatimah et al 2017; Aceves et al 2019; Lee et al 2019). Under stress conditions, cortisol levels in the blood increase. Cortisol can cause lymphopenia by reducing mitosis or producing lymphocytes. Cortisol also plays a role in reducing lymphocyte cells in the blood circulation due to the redistribution of lymphocytes to the bone marrow and other target locations. Reduced lymphocyte cells can also be caused by *Y. intermedia* infection. Disease attacks can reduce the concentration of antibodies so that fish have low lymphocytes in response to antigens. In other words, lymphocytes functioned to develop cellular responses to immunity (not specific) as triggering response to fish immunity system (Sahan et al 2017; Aliko et al 2018; Shabirah et al 2019; Kossack et al 2020).

Neutrophil cells. Neutrophil cells are the first cells produced by the body to fight antigens. Monocytes and neutrophils have a role of strong phagocytes. One neutrophil cell can phagocyte 20 antigens, while one monocyte can phagocyte up to 100 antigens before the cell becomes inactive. The percentage of monocytes and neutrophils is less than lymphocytes because monocytes and neutrophils are short-lived cells, so the total cells in the blood are less than others (Nahendorf & Swirski 2015; Havixbeck et al 2016; Kordon et al 2018).

The percentage of neutrophyl cells in *O. goramy* after *Y. intermedia* bacterial infection was above normal, which is between 12-19%, during the normal percentage in

blood. The total percentage of neutrophil is obtained by comparing the total of neutrophil cells with the number of leukocyte cell samples counted, 100 cells, and multiplied with 100. The blood profile was examined D-1 before infection, D+1, and D+4 after infection. In the present experiment, the percentage of neutrophils was less than of lymphocyte cells. The increase in neutrophil cells above normal is caused by stress and bacterial infections. Total neutrophil cells in the blood will increase when bacterial infections occur. Under stress conditions, cortisol can affect the production of neutrophils from bone marrow (Douxflis et al 2017; Maryani et al 2018; Herlina et al 2019). This, results in an increase in neutrophil cells. Physical stress caused by handling and treatment can cause damage to fish skin mucus, which affects the increasing frequency of microorganism infections. Low levels of neutrophils are thought to occur because neutrophils concentrate in the area of bleeding or injury. In addition, the total amount of neutrophil is less than the total blood population.

The increase in neutrophil cells is stimulated by the appearance of foreign matter, which in the present experiment was *Y. intermedia*. An increase in neutrophil cells shows an increase in macrophage collection in the area of infection so that macrophages can easier damage foreign bodies. The function of neutrophils is the destruction of foreign matter through the phagocytosis process is chemical when cells will migrate to particles, cell adhesion, digestive cell particles, and destruction of particles by the enzyme lysozyme in phagolysosomes. Therefore an increase in neutrophil cells occurs due to foreign body reactions, which can be bacterial, viral, or pathogenic neutrophil (Mahasri 2016; Dias et al 2019; Zhang et al 2019).

Monocytes cells. The percentage of monocytes in *O. goramy* after infection with *Y. intermedia* was above normal, which is between 3 and 10%. The normal percentage of monocytes in the blood is between 1 and 2% of the total leukocyte population (Subramani et al 2016; Deboutteville et al 2019; Lulijiwa et al 2019). The percentage of total monocytes is calculated by comparing the total monocyte cells to the total monocyte cells with the total number of leukocyte cell samples counted, which were 100 cells, then multiplied with 100. The blood profile was examined D-1 before infection, D+1, and D+4 after infection. The increase in monocyte cells occurs due to bacterial infections that cause certain diseases. Monocytes can occur due to chronic diseases, especially if many cell impurities need to be cleaned, for example, fungal infections, granulomatous inflammation, and certain other diseases (Chen et al 2016; Ding et al 2019; Niu et al 2019).

Monocytes are large leukocytes, commonly called macrophages. An increase in the number of monocyte cells is related to monocytes themselves as macrophages, which are needed for phagocytes, which are caused by massive infections in the body that stimulate monocyte production so that the number of monocyte cells becomes higher (Fazio 2019; Haghghi et al 2018; Huang et al 2020; Nhu et al 2019). In other words, monocytes can be used as markers of pathogens in T cells so that these pathogens can be identified and killed, or can be used to produce antibodies. Close contact between the lymphocyte and monocyte surfaces is needed for maximum immunological response (Shen et al 2018; Dang et al 2019; Sanchez et al 2019).

Conclusions. Results of mortality and lethal dosage (LD₅₀) in the present research showed that GM 01 bacteria were classified into pathogenic bacteria. The clinical symptoms of *O. goramy* infected with GM 01 isolates showed that the result of a pathogenicity test is similar to the symptoms of a disease caused by *Y. intermedia*.

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References

- Acar U., Parrino V., Kesbic O. S., Paro G. L., Saoca C., Abbate F., Yilmaz S., Fazio F., 2018 Effects of different levels of pomegranate seed oil on some blood parameters and disease resistance against *Yersinia ruckeri* in rainbow trout. *Frontiers in Physiology* 9:1-7.
- Aceves M. A. B., Lionetti L., Faggio C., 2019 Multidisciplinary hematology as prognostic device in environmental and xenobiotic stress-induced response in fish. *Science of the Total Environment* 670:1170-1183.
- Aliko V., Qirjo M., Sula E., Morina V., Faggio C., 2018 Antioxidant defense system, immune response, and erythron profile modulation in gold fish, *Carasius auratus*, after acute manganese treatment. *Fish & Shellfish Immunology* 76:101-109.
- Chen J., Chen Q., Lu X. J., Chen J., 2016 The protection effect of LEAP-2 on the mudskipper (*Boleophthalmus pectinirostris*) against *Edwardsiella tarda* infection is associated with its immunomodulatory activity on monocytes/macrophages. *Fish & Shellfish Immunology* 59:66-76.
- Dang Y., Meng X., Jianfei L., Liu L., Jiale L., 2019 Role of mannose-binding lectin in regulating monocytes/macrophages functions during in grass carp, *Ctenopharyngodon idella*. *Developmental and Comparative Immunology* 99:28-36.
- Deboutteville J. D., Kawasaki M., Zoccola E., Heath C. M., Bowater R. O., Barnes A. C., 2019 Interactions of head-kidney leucocytes from giant grouper, *Epinephelus lanceolatus* with pathogenic *Streptococcus agalactiae* strains from marine and terrestrial origins. *Fish & Shellfish Immunology* 90:250-263.
- Dias M. K. R., Yoshioka E. T. O., Rodriguez A. F. R., Ribeiro R. A., Faria F. S. E. D. V., Ozoriod R. O. A., Dias M. T., 2019 Growth, physiological, and immune responses of *Arapaima gigas* (Arapaimidae) to *Aeromonas hydrophila* challenge and handling stress following feeding with immunostimulant supplemented diets. *Fish & Shellfish Immunology* 84:843-847.
- Ding F. F., Li C. H., Chen J., 2019 Molecular characterization of the NK-Lysin in a teleost fish, *Boleophthalmus pectinirostris*: antimicrobial activity and immunomodulatory activity on monocytes/macrophages. *Fish & Shellfish Immunology* 82:256-264.
- Douxflis J., Castro C. F., Mandiki S. N. M., Emile W., Tort L., Kestemont P., 2017 Dietary β -glucans differentially modulate immune and stress-related gene expression in lymphoid organs from healthy and *Aeromonas hydrophila*-infected rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* 63:285-296.
- Fatimah F., Rachmawati F. N., Wibowo E. S., 2017 Leukocyte differential of Anguillid Eel, *Anguilla Bicolor* McClelland, exposed to varied salinities. *Scripta Biologica* 4(2):79-83.
- Fazio F., 2019 Fish hematology analysis as an important tool of aquaculture: A review. *Journal of Aquaculture* 500:237-242.
- Haghighi M., Pourmoghim H., Rohani M. S., 2018 Effect of *Origanum vulgare* extract on immune responses and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Oceanography & Fisheries* 6(3):1-6.
- Hamed S. B., Paiva M. J. T. R., Tachibana L., Dias D. D. C., Ishikawa C. M., Esteban M. A., 2018 Fish pathogen bacteria: adhesion, parameters influencing virulence, and interaction with host cells. *Fish & Shell Immunology* 80:550-562.
- Hardi E., Saptiani G., Kusuma I. W., Suwinarti W., Nugroho R. A., 2018 Evaluation of traditional plant extracts for innate immune mechanisms and disease resistance against fish bacterial *Aeromonas hydrophila* and *Pseudomonas* sp. *IOP Conference Series Earth and Environmental Science* 139:1-10.
- Havixbeck J. J., Rieger A. M., Wong M. E., Hodgkinson J. W., Barreda D. R., 2016 Neutrophil contributions to the induction and regulation of the acute inflammatory response in teleost fish. *Journal of Leukocyte Biology* 99:241-252.
- Herlina C., Yanuhar U., Maftuch, 2019 Effect of extracellular proteins (ECPS) *Edwardsiella tarda* on the innate immune response in Nile tilapia (*Oreochromis niloticus*). *International Journal of Scientific & Technology Research* 8(7):38-42.

- Hidayatullah A., Rosidah, Subhan U., Suryadi I. B. B., 2018 Hematology parameters of Nile tilapia strain (*Osteochilus* sp.) infected by *Aeromonas hydrophila*. *Global Scientific Journal* 6(7):322-329.
- Hiko A., Tasisa K., Agga G. E., 2018 Helminthiasis and gram-negative enteric bacteria in freshwater fish from selected lakes of haramaya district, Ethiopia. *Fisheries and Aquaculture Journal* 9:2, DOI: 10.4172/2150-3508.1000242.
- Huang M., Mu P., Li X., Ren Q., Zhang X. Y., Mu Y., Chen X., 2020 Functions of TNF- α 1 and TNF- α 2 in large yellow croaker (*Larimichthys crocea*) in monocyte/macrophage activation. *Developmental and Comparative Immunology* 105:103576. doi: 10.1016/j.dci.2019.103576..
- Idowu T. A., Adedeji H. A., Sogbesan O. A., 2017 Fish disease and health management in aquaculture production. *International Journal of Environmental & Agricultural Science* 1: 002.
- Kazarnikova A., Trishina A., Galeotti M., Manzano M., Abrosimova N., Ermakov A., 2019 Detecting *Yersinia ruckeri* on the death of carp (*Cyprinus carpio*) farmed in southern Russia. *IOP Conference Series Earth and Environmental Science* 403:012039.
- Kordon A. O., Karsi A., Pinchuk L., 2018 Innate immune responses in fish: antigen-presenting cells and professional phagocytes. *Turkish Journal of Fisheries and Aquatic Sciences* 18:1123-1139.
- Kossack C. F., Montero R., Kollner B., Maisey K., 2020 Chilean aquaculture and the new challenges: pathogens, immune response, vaccination, and fish diversification. *Fish & Shellfish Immunology* 98:52-67.
- Kousar R., Shafia N., Andleeb S., Alic N. M., Akhtara T., Khalida S., 2019 Assessment and incidence of fish associated bacterial pathogens at hatcheries of Azad Kashmir, Pakistan. *Brazilian Journal of Biology* <https://doi.org/10.1590/1519-6984.217435>.
- Kumar G., Ledouble S. M., Saleh M., El-Matbouli M., 2015 *Yersinia ruckeri*, the causative agent of enteric redmouth disease in fish. *Veterinary Research* 46, 103. <https://doi.org/10.1186/s13567-015-0238-4>.
- Kusumawaty D., Suhandono S., Pancoro A., Aryantha I. N. P., 2017 Induction of toll-like receptor (TLR) 2 and myeloid88-dependent TLR-signaling response to *Aeromonas hydrophila* infections in Gourami (*Osphronemus gourami*). *IOP Conference Series: Materials Science and Engineering* 180(1), 012270.
- Lee J. W., Chiu H., Hwang U. K., Kang J. C., Kang Y. J., Kim K. I., Kim J. H., 2019 Toxic effect of lead exposure on bioaccumulation, oxidative neurotoxicity, and immune responses in fish: a review. *Environmental Toxicology and Pharmacology* 68:101-108.
- Li T., Li H., Gatesoupe F. J., She R., Lin Q., Yan X., Li J., Li X., 2017 Bacterial signatures of "Red-Operculum" disease in the gut of crucian carp (*Carassius auratus*). *Microbiology of Aquatic System* 74:510-521.
- Luis A. I. S., Campos E. V. R., Oliveira J. L. D., Fraceto L. F., 2019 Trends in aquaculture sciences: from now to use of nanotechnology for disease control. *Reviews in Aquaculture* 11:119-132.
- Lulijiwa R., Alfaro A. C., Merien F., Meyer J., Young T., 2019 Advances in Salmond fish immunology fish immunology: a review of methods and techniques for lymphoid tissue and peripheral blood leucocyte isolation and application. *Fish & Shellfish Immunology* 95:44-50.
- Maftuch, Kuniawati I., Adam A., Zamzani I., 2016 Antibacterial effect of *Gracilaria verrucosa* bioactive on fish pathogenic bacteria. *Egyptian Journal of Aquaculture Research* 42:405-410.
- Mahasri G., 2016 Development of spore protein of *Myxobolus koi* as an immunostimulant for prevent of Myxobolus on Gold fish (*Cyprinus carpio* Linn) by oral immunization. *IOP Conference Series Earth and Environmental Science* 55: 012009.
- Makrinos D. L., Bowden T. J., 2016 Natural environmental impacts on teleost immune function. *Fish and Shellfish Immunology* 53:50-57.
- Maryani, Monalisa S. S., Rosita, Rozik M., Pratasik S. B., 2018 Effectivity of *Arcangelisia flava* as immunostimulant to prevent streptococcosis on Nile tilapia, *Oreochromis niloticus*. *AACL Bioflux* 11(6):1834-1843.

- Mishra S. S., Dhiman R. D., Choudhary P., Debbarma J., Sahoo S. N., Barua A., Giri B. S., Ramesh R., Ananda K., Mishra C. K., Swain P., 2017 Present status of fish disease management in freshwater aquaculture in India: state-of-the-art-review. HSOA Journal of Aquaculture & Fisheries 1:003, DOI: 10.24966/AAF-5523/1000.
- Mohammed S. A., Omer A. F., 2019 Assessment of antimicrobial activity of methanolic extract of lemon peels against clinical bacterial isolates. African Journal of Medical Sciences 4(11):1-5.
- Muziasari W. I., 2016 Impact of fish farming on antibiotic resistome and mobile elements in Baltic Sea sediment. Doctoral School Dissertation, Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, Faculty of Agriculture and Forestry, Doctoral Programme in Microbiology and Biotechnology, University of Helsinki, Finland, 44 p.
- Nahendorf M., Swirski F. K., 2015 Immunology. Neutrophil-macrophage communication in inflammation and atherosclerosis. Science 349 (6245):237-238.
- Nhu T. Q., Hang B. T. B., Bach L. T., Hue B. T. B., Leclercq J. Q., Scippo M. L., Phuong N. T., Kestemont P., 2019 Plant extract- based differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & Shellfish Immunology 92:913-924.
- Niu J., Huang Y., Liu X., Luo G., Tang J., Wang B., Lu Y., Cai J., Jian J., 2019 Functional characterization of galectin-3 from Nile tilapia (*Oreochromis niloticus*) and its regulatory role on monocytes/macrophages. Fish & Shellfish Immunology 95:268-276.
- Nuryanto A., Amalia G., Khairani D., Pramono H., Bhagawati D., 2018 Molecular characterization of four giant gourami strains for java and Sumatra. Biodiversitas 192:578-584.
- Puspaningsih D., Supriyono E., Nirmala K., Rusmana L., Kusmana C., Widiyati A., 2019 Water quality, hematological parameters, and biological performances of snakehead fish (*Channa striata*) reared in different stocking densities in a recirculating aquaculture system. AACL Bioflux 12(5):1546-1558.
- Putra R. A., Syahrul S., Buchari D., 2016 Flour characteristic from fish waste in form of Gourami (*Osphronemus gourami*) scales, skins, and catfish (*Pangasius hypophthalmus*) bones as industry main ingredients. Jumfaperika 2(4):1-11.
- Roberts R. J., 2012 Fish pathology. 4th edition, Wiley-Blackwell: New Jersey, USA, 590 p.
- Ross L. N., Woodward J. F., 2016 Koch's postulates: An interventionist perspective. Studies in history and philosophy of biological. Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences 59:35-46.
- Rozi, Rahayu K., Daruti D. N., Stella M. S. P., 2017 Study on characterization, pathogenicity, and histopathology of disease caused by *Aeromonas hydrophila* in gourami (*Osphronemus gouramy*). IOP Conference Series Earth and Environmental Science 137(1):012003.
- Rozi K., Rahayu, Daruti D. N., 2018 Detection and analysis of hemolysin genes in *Aeromonas hydrophila* isolated from Gourami (*Osphronemus gourami*) by polymerase chain reaction (PCR). IOP Conference Series Earth and Environmental Science 137, 012003, doi:10.1088/1755-1315/137/1/012003.
- Safinska A. P., 2018 Contemporary threats of bacterial infections in freshwater fish. Journal of Veterinary Research 62(3):261-267.
- Sahan A., Yilmaz H. A., Eroldogan O. T., 2017 Determination of hematological status as health indicator of European sea bass (*Dicentrarchus labrax* L.) fed different dietary fatty acids. Turkish Journal of Agriculture-Food Science and Technology 5(4):445-450.
- Sanchez A. P., Estensoro I., Pozo R. D., Piazzon M. C., Palazuela O., Bobadilla A. S., 2019 acquired protective immune response in a fish-myxozoan model encompasses specific antibodies and inflammation resolution. Fish & Shellfish Immunology 90:349-362.
- Sarjito, 2010 Application of bio-molecular investigation to detect causative agent causing Vibriosis of Kerapu fish and potency of sponge-associated bacteria as anti vibriosis.

- Dissertation, Doctoral Program of Coastal Resources Management. Diponegoro University, Semarang, Indonesia.
- Sen K., Mandal R., 2018 Fresh-water fish diseases in West Bengal, India. *International Journal of Fisheries and Aquatic Studies* 6(5):356-362.
- Shabirah A., Rosidah, Mulyani Y., Lili W., 2019 Effect of types isolated lactic acid bacteria on hematocrit and differential leukocytes fingerling common carp (*Cyprinus carpio* L.) infected with *Aeromonas hydrophila* bacteria. *World News of Natural Sciences* 24:22-35.
- Shen Y., Wang D., Zhao J., Chen X., 2018 Fish red bloods cells express immune genes and responses. *Aquaculture and Fisheries* 3:14-21.
- Subramani P. A., Narasimha R. V., Balasubramanian R., Narala V. R., Ganesh M. R., Michael R. D., 2016 Cytotoxic effects of *Aeromonas hydrophila* culture supernatant on peripheral blood leukocytes of Nile tilapia (*Oreochromis niloticus*): possible presence of a secreted cytotoxic lectin. *Fish & Shellfish Immunology* 58:604-611.
- Tkachenko H., Buyun L., Majewska E. T., Honcharenko V., Prokopiv A., Osadowski Z., 2019 Preliminary *in vitro* screening of the antibacterial activity of leaf extracts from various *Ficus* species (Moraceae) against *Yersinia ruckeri*. *Fisheries & Aquatic Life* 27:15-26.
- Svobodova Z., Vykusova B., 1991 Diagnostics, prevention and therapy of fish disease and intoxications: manual for International Training Course on Fresh-Water Fish Diseases and Intoxications: Diagnostics, Prophylaxis and Therapy. Vodnany, Czechoslovakia: Research Institute of Fish Hydrobiology, 270 p.
- Wang E., Lai W., 2018 preparation, characterization, and evaluation of the immune effect of alginate/chitosan composite microspheres encapsulating recombinant protein *Streptococcus iniae* designed for fish oral vaccination. *Fish & Shell Immunology* 73:262-271.
- Wrobel A., Leo J. C., Linke D., 2019 Overcoming fish defences: The virulence factors of *Yersinia ruckeri*. *Genes (Basel)* 10(9). pii: E700. doi: 10.3390/genes10090700.
- Xue S., Xu W., Wei J., Sun J., 2017 Impact of environmental bacterial communities on fish health in marine recirculating aquaculture system. *Veterinary Microbiology* 203:34-39.
- Yamasaki M., Sakai T., Ito T., Mori K., 2017 Bactericidal effects of disinfectants on *Yersinia ruckeri*. *Fish Pathology* 52(4):198-201.
- Yengkhom O., Shalini K. S., Subramani P. A., Michael R. D., 2019 Stimulation of non-specific immunity, gene expression, and disease resistance in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), by the methanolic extract of the marine macroalga, *Caulerpa scalpelliformis*. *Veterinary World* 12:271-276.
- Zhang L., Wang C., Liu H., Fu P., 2019 The important role of phagocytosis and interleukins for Nile tilapia (*Oreochromis niloticus*) to defense infection of *Aeromonas hydrophila* based on transcriptome analysis. *Fish & Shellfish Immunology* 82:54-63.
- *** Ministry of Marine and Fisheries, 2014 Laporan Tahunan 2013. <https://www.djpb.kkp.go.id/public/upload/download/Pustaka/06PUSTAKA/LAPTAH%20PRODUKSI%20%202013.pdf>.

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Authors:

Sarjito, Diponegoro University, Faculty of Fisheries and Marine Science, Aquaculture Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: sarjito@live.undip.ac.id

Reka Kurnia Wati, Diponegoro University, Faculty of Fisheries and Marine Science, Aquaculture Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: rekakurniawati772@gmail.com

Alfabetian Herjuno Condro Haditomo, Diponegoro University, Faculty of Fisheries and Marine Science, Aquaculture Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: condrohaditomo@gmail.com

Desrina, Aquaculture Department, Diponegoro University, Faculty of Fisheries and Marine Science, Aquaculture Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: rinadesrina@yahoo.com

Aninditia Sabdaningsih, Diponegoro University, Faculty of Fisheries and Marine Sciences, Aquatic Resources Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: aninditiasabdaningsih@live.undip.ac.id

Slamet Budi Prayitno, Diponegoro University, Faculty of Fisheries and Marine Science, Aquaculture Department, Indonesia, Semarang – 50275, Tembalang, Jl. Prof. Soedarto SH, e-mail: sbudiprayitno@gmail.com

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