The effects of the antibiotic erythromycin on Oryzias javanicus in gender

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The effects of the antibiotic erythromycin on O*ryzias javanicus* in gender perspective

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Abstract. Erythromycin (ERY) is an antibiotic that is widely prescribed in human and veterinary medicine to treat several bacterial infections. This antibiotic was reported to have various effects on aquatic environments. This study aimed to evaluate whether there is a difference in sensitivity to ERY between male and female *Oryzias javanicus*. The experimental fish were exposed to ERY (2 mg/L) for 96 hours. In this experiment, each experimental unit used five fish with five replications. Male and female animals were kept in different containers during the experiment The parameters measured were oxygen consumption and survival rate. The oxygen consumption of male and female of *O. javanicus* before the experiment began showed a statistically significant difference ($p < 0.05$). The average oxygen consumption of female *O. javanicus* was 0.752 mgO_2/g body weight/hour, while the average oxygen consumption of males was 0.474 mgO_2/g body weight/hour. After exposure to ERY, the oxygen consumption of male *O. javanicus* was reduced to 0.353 mgO_2/g body weight/hour. In contrast, there was 100% mortality of the female *O. javanicus* by the end of the experiment. After exposure to ERY, the percent mortality of male and female *O. javanicus* were statistically no different ($p < 0.05$). Males were not more resistant to ERY than females. This study concluded that there was no difference in sensitivity to ERY between male and female *O. javanicus*.

1. Introduction

Antibiotics are antimicrobial drugs that function to kill or inhibit bacterial growth. Worldwide, the use of antibiotics reaches 100,000 tonnes per year [1]. The use of antibiotics in large quantities has an impact on the environment. Antibiotics are drugs that are found as pollutants in water [2]. Antibiotic concentrations have been detected in both freshwaters and marine habitats. Antibiotics used to treat human and animal diseases are not fully absorbed by the body but almost 90% will be excreted together with urine and feces which will then enter the aquatic environment [3].

The introduction of antibiotics into the waters can pose serious threats to the environment such as the spread of bacteria and genes that are resistant to antibiotics. This can lead to an increase in the number of infections that are increasingly difficult to treat as the antibiotics used are less effective [4]. Although antibiotics are specifically used to treat pathogenic infections [1], the introduction of antibiotics in water will have an impact on non-target organisms even at ng/l concentrations [5]. The presence of antibiotics continuously in the aquatic environment causes the organisms to be chronically exposed. In addition, active antibiotics even in low concentrations can cause toxic effects either because of their active ingredients or additives used in their formulations [6].

One of the non-target organisms that can be affected by exposure to antibiotics is fish. In general, fish is considered the most likely organisms for environmental monitoring because they occupy the upper trophic level in the aquatic ecosystem as a carrier of low to high energy flow and absorption of drugs through the surface of the skin and gills carried by water or sediment [2].

One of the most commonly used antibiotics is Erythromycin (ERY). ERY is a macrolide antibiotic that has been used since the 1950s to treat diseases in humans and is also used in treating bacterial infections in fish farms. ERY is of particular concern in the aquatic field when compared to other antibiotics due to its use, release and persistence, and toxicity [2]. Research shows that ERY exposure can accumulate in the body of Carassius auratus fish and be maximally concentrated in muscle tissue [7]. ERY can cause oxidative stress in fish, *Labeo rohita* [8]. Li & Zhang (2020) explained that a concentration of 2 mg/l ERYs can reduce the swimming speed of *Oryzias latipes* [9]. Several studies have shown that ERY exposure can affect fish metabolism.

Metabolism is all the organized chemical activities carried out by cells [10]. Metabolic rate can be estimated by measuring the amount of oxygen consumed by living things per unit of time [11]. Increased oxygen consumption indicates an increased metabolic rate in fish and indicates that the fish is experiencing stress so that it takes a lot of energy to adjust [12]. Oxygen consumption is an important physiological parameter for assessing the toxicity of toxins because it is an important indicator of energy expenditure during metabolism [13]. Fish that are exposed to toxic materials require large amounts of oxygen to detoxify these toxins [9].

It is important to consider sex as crucial in toxicological tests. This is because the distribution of contaminants in the fish body can differ between sexes in both behavior and physiology of fish [14]. In such cases, the mercury concentration in muscle tissue and liver of *Petromyzon marinus* L showed that the mercury concentration in male fish muscle tissue was about 10% higher than that of female fish while the mercury concentration in the liver of female fish was about 60% greater than that of male fish [15]. The difference in accumulation will result in a difference in toxicity [14].

Based on this, ERY exposure can have an effect on the metabolic rate of fish which can be measured from the rate of oxygen consumption. Although there have been many studies on the effects of ERY exposure in fish, there is still less research on differences in fish gender sensitivity as measured by the level of oxygen consumption of male and female fish after exposure to pollutants, so this research needs to be carried out. The test fish used in this study was *Oryzias javanicus*, which widespread in Indonesia that has the potential to be a sentinel organism and is one of the test animals that are often used in ecotoxicology, but the information is still lacking regarding differences in gender sensitivity to pollutants such as antibiotics.

2. Materials and methods

2.1. Chemical
Erythromycin 500 mg was purchased at a local pharmacy.

2.2. Fish sampling
The sampling was carried out at the location of the Salarang River flow, Maros Baru District, Maros Regency, South Sulawesi. The fish were caught using a fishing net with a mesh size of 3 mm. The fish samples were put into a container that has been given an aerator and transferred to the laboratory.

Figure 1. Oryzias javanicus sampling location.

Figure 2. *Oryzias javanicus* from the Salarang River.

2.3. The exposure experiment

The collected *Oryzias javanicus* consisted of 50 fish with a length of 3 -3.5 cm consisting of 25 male fish and 25 female fish which were acclimatized in the laboratory. The fish were separated into two aquariums based on gender. Each aquarium was given aeration for maintaining oxygen supplies. This acclimatization aims to adapt to the physiological conditions of fish in the laboratory and was carried out for a minimum of 10 days. During the acclimatization process, the fish were fed two times a day with the amount of feed 5% of the total body weight of the fish. The media were changed once a day to maintain water quality.

The ERY concentration used in the study refers to the research of Li & Zhang (2020), which was 2 mg/l [9]. Five fish were used in each experimental unit in one liter of experimental media with five replications. The exposure was carried out for 96 hours. During the exposure process, the fish are fed once a day before changing the water. Observations were made every 6 hours. During the observation, the number of dead fish and water quality such as pH and temperature are recorded.

Measurement of oxygen consumption was carried out twice for male and female test animals before and after ERY exposure, using a respiration bottle. Oxygen consumption measurements after 96 hours of ERY exposure were carried out on fish that were still alive at the end of the study. After 96 hours of exposure, the fish were transferred to a glass jar without ERY exposure to measure their oxygen consumption. The dissolved oxygen measurement procedure in this study was carried out using the iodometric titration method.

Figure 3. The exposure experiment.

2.4. Research variables

2.4.1. Oxygen consumption of fish. Oxygen consumption of fish (mgO_2/g body weght/hour) is estimated using the following formula:

$$\mathbf{OC = ((DO_0 – DO_t)/ W \ x \ t) \ x \ V}$$

OC = oxygen consumption (mgO_2/g body weight/hour), DO_0 = dissolved oxygen at the beginning of observation (mg/l) DO_t = dissolved oxygen at the end of observation (mg/l), W = weight of test fish (g), V = volume of water (l), t = time of observation (hours)

2.4.2. Survival rate. The survival rate is the percentage of the number of live fish and the number of fish at the end of the study. The survival rate (SR) calculation formula is: SR = Nt / No x 100%. SR = survival (%) Nt = the number of fish at the end of the study (individual) No = the number of fish at the beginning of the study (individual).

2.5. Data analysis

The student's t-test was used to determine differences in oxygen consumption of male and female fish. To analyze the relationship between length and weight on oxygen consumption, Pearson correlation analysis was carried out. Before the tests were performed, the data were first tested for normality and homogeneity. Data analysis was performed using Ms. application. Excel, GraphPad Prism 8, and SPSS 25.

3. Results and discussion

The results of measuring the oxygen consumption of male and female *Oryzias javanicus* before exposure to ERY 2 mg/l found that there was a statistical difference in oxygen consumption between male and female *Oryzias javanicus* and female *Oryzias javanicus* ($p < 0.005$).

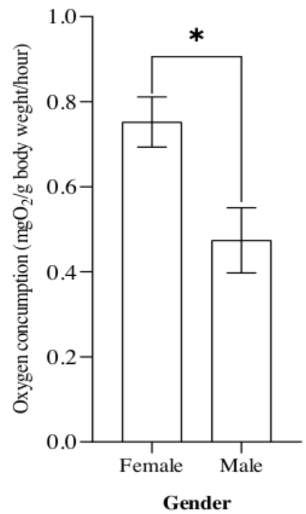

Figure 4. Comparison of oxygen consumption of male and female *Oryzias javanicus* before exposure to ERY. * Asterix showed a statistically significant difference (P <0.05).

The results of statistical tests using Student's t-test analysis showed that oxygen consumption between male and female *O. javanicus* was significantly different (P <0.05). This shows that the oxygen demand for female fish is more than that of male fish. This is thought to be related to the higher energy requirements of female *Oryzias javanicus* compared to male *O. javanicus*, because female *O. javanicus* fish are preparing to produce prime quality eggs with thick chorions as a consequence of the K strategy.

The relationship between length, weight, and oxygen consumption of *Oryzias javanicus* fish can be seen in Figure 5. From the results of the correlation analysis using the Pearson correlation analysis, it was known that the correlation between length and oxygen consumption was 0.425, and the correlation between weight and oxygen consumption was -0.541. From the results of statistical analysis, it was found that there was no correlation between oxygen consumption and fish length (p=0.2210 and weight (p=0.107). This means that oxygen consumption for adult fish is not affected by weight and total length.

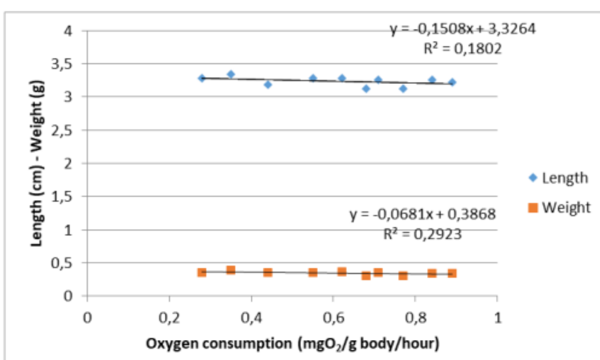

Figure 5. The relationship between length and weight of fish and oxygen consumption.

After exposure to ERY 2 mg/l for 96 hours, it was found that the oxygen consumption of male fish was 0.353 mgO_2/g body/hour. This value indicates that the oxygen consumption of male fish after exposure is lower than before exposure, namely 0.474 mgO_2/g body/hour. Meanwhile, all-female fish experienced total death at the end of the period. There was a decrease in oxygen consumption in male *Oryzias javanicus* and total mortality in female *Oryzias javanicus* after exposure to ERY antibiotics. These were presumably because ERY enters the fish's body more through the gills than other pathways such as skin so that it can interfere with the performance of the gills which were the respiratory organs. This disturbance ultimately resulted in death through an increase in the amount of mucus in the gills and a decrease in the rate of oxygen consumption [2,16,17].

Based on the results of the study, it was found that male *Oryzias javanicus* fish experienced a decrease in the survival rate to 92% in 6 hours. Meanwhile, the female experienced the same decrease in survival rate as the male fish in the 12 hours. However, after entering the 48 hours it was found that the survival rate of female fish was lower than that of male fish and continued to decline, resulting in total mortality in the 72 hours. Male fish can survive until the end of the 96 hours with a survival rate of 8%. The same decrease in survival rate at different periods between these fish indicates that male fish were more sensitive in the early period to ERY contaminants than female fish. However, at longer periods, female fish become more sensitive than male fish.

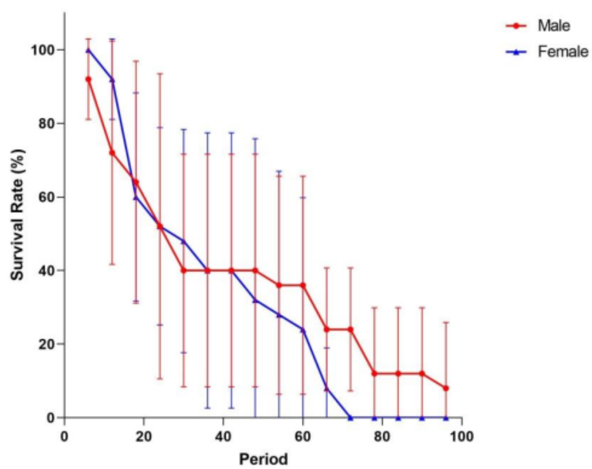

Figure 6. The survival rate of male and female *Oryzias javanicus*.

The results of the current study were consistent with research by Amal and co-workers (2018) that revealed *Oryzias javanicus* experienced mortality in the fourth period after being exposed to *Streptococcus agalactiae* while female fish only experienced mortality in the sixth period [18]. Liu co-workers (2014) who showed differences in sensitivity in *Oryzias latipes* that male fish were more sensitive than female fish to exposure to 2,4,6-trichlorophenol [7]. This was in contrast to the current study which showed no significant difference between the percent mortality of male and female animals.

ERY is a macrolide antibiotic. Macrolide antibiotics can cause DNA damage through the induction of oxidative stress in fish. ERY also causes significant changes in gill with acute or chronic exposure [19]. This research is also supported by Zhao (2016) research that the activity of antioxidant enzymes in the liver, namely Superoxide Dismutase (SOD) and catalase (Cat), increases in females. This mechanism can reduce the chemical content to maintain homeostasis. As the main defense system against exterior compounds, COD and CAT activity play an important role in antioxidant defense. Inhibition of SOD and lack of CAT response indicates some susceptibility to the antioxidant system and oxidative stress in organisms caused by pollutants. SOD activity was significantly induced in the female

Oryzias melastigma group during the initial period of sulfamethazine exposure. Meanwhile, male Oryzias melastigma showed no significant increase in SOD induction at all concentrations of sulfamethazine exposure. Male *Oryzias melastigma* at a period of two hours showed a slower response

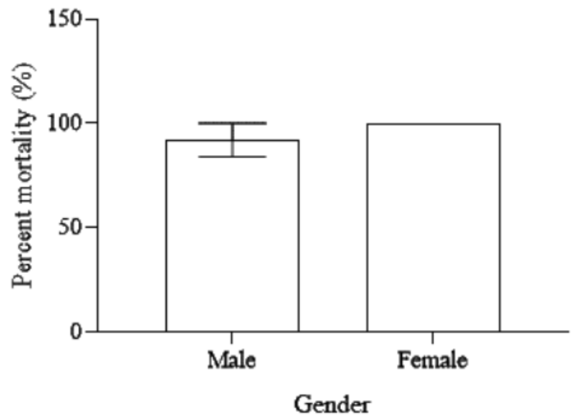

Figure 7. The percent mortality of male and female *Oryzias javanicus*.

than female fish. Hence, male fish are more susceptible than female fish to chemical exposure in the early period. However, for CAT activity, there was a decrease within 12 hours in female fish. The activity of this antioxidant enzyme also affects the time and concentration of exposure. SOD activity in males was induced at high doses. It can be found that at a concentration of 40μg/l male fish had the highest metabolites while female fish at a concentration of 40μg/l had liver damage and decreased activity of CAT in 24 hours and CAT inhibition in the next section. This shows that the liver of female fish is more susceptible than male fish. This is because the excessive accumulation of sulfamethazine in the fish liver causes inhibition of CAT function [20].

4. Conclusion

The results of this study concluded that:

1. There was a difference in oxygen consumption between male and female *Oryzias javanicus*. The average oxygen consumption of female *Oryzias javanicus* was 0.752 mgO_2/g body/hour, while the average consumption of male Oryzias javanicus was 0.474 mgO_2/gr. The results depicted that the oxygen consumption of females was greater than that of male fish.
2. The correlation value between length and oxygen consumption is -0.425 and the correlation between weight and oxygen consumption is -0.541. The results of statistical analysis showed that there was no correlation between length and weight and oxygen consumptions.
3. There is no difference in the sensitivity of male and female Oryzias javanicus to erythromycin.

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References

[1] Danner M C, Robertson A, Behrends V and Reiss J 2019 Antibiotic pollution in surface fresh waters: Occurrence and effects *Sci. Total Environ.* **664** 793–804
[2] Rodrigues S, Antunes S C, Correia A T and Nunes B 2018 Toxicity of erythromycin to Oncorhynchus mykiss at different biochemical levels: detoxification metabolism, energetic

balance, and neurological impairment *Environ. Sci. Pollut. Res.* **26** 227–39

[3] Liu K, Zhang D, Xiao X, Cui L and Zhang H 2020 Occurrence of quinotone antibiotics and their impacts on aquatic environment in typical river-estuary system of Jiaozhou Bay, China *Ecotoxicol. Environ. Saf.* **190** 109993

[4] Felis E, Kalka J, Sochacki A, Kowalska K, Bajkacz S, Harnisz M and Korzeniewska E 2020 Antimicrobial pharmaceuticals in the aquatic environment - occurrence and environmental implications *Eur. J. Pharmacol.* **866** 172813

[5] Pereira A, Silva L, Laranjeiro C, Lino C and Pena A 2020 Selected pharmaceuticals in different aquatic compartments: Part II-Toxicity and environmental risk assessment *Molecules* **25**

[6] Grenni P, Ancona V and Barra Caracciolo A 2018 Ecological effects of antibiotics on natural ecosystems: A review *Microchem. J.* **136** 25–39

[7] Liu J, Lu G, Ding J, Zhang Z and Wang Y 2014 Tissue distribution, bioconcentration, metabolism, and effects of erythromycin in crucian carp (*Carassius auratus*) *Sci. Total Environ.* **490** 914–20

[8] Renuka S, Umamaheswari S, Shobana C, Ramesh M and Poopal R K 2019 Response of antioxidants to semisynthetic bacteriostatic antibiotic (erythromycin) concentrations: A study on freshwater fish *Acta Ecol. Sin.* **39** 166–72

[9] Li Y and Zhang J 2020 The effect of acute erythromycin exposure on the swimming ability of Zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) *Int. J. Environ. Res. Public Health* **17**

[10] Sanjana M C 2019 Carbohydrate Metabolism- A Constant Supply of Energy *Epra Int. J. Res. Dev.* **4** 151–6

[11] Suharsono S 2018 Perbedaan Jumlah Konsumsi Oksigen (O2) Pada Respirasi Berbagai Hewan Invertebrata Kelas Insekta *J. Kesehat. Bakti Tunas Husada J. Ilmu-ilmu Keperawatan, Anal. Kesehat. dan Farm.* **18** 212–20

[12] Putra A N 2015 Jurusan Perikanan, Fakultas Pertanian Universitas Sultan Ageng Tirtayasa, Jl. Raya Jakarta Km. 4 Pakupatan, Serang Banten *J. Perikan. dan Kelaut.* **5** 13–8

[13] Tilak K S and Swarna Kumari R 2009 Acute toxicity of Nuvan®, an organophosphate to freshwater fish *Ctenopharyngodon idella* and its effect on oxygen consumption *J. Environ. Biol.* **30** 1031–3

[14] Madenjian C P, Rediske R R, Krabbenhoft D P, Stapanian M A, Chernyak S M and O'Keefe J P 2016 Sex differences in contaminant concentrations of fish: A synthesis *Biol. Sex Differ.* **7** 1–16

[15] Pedro S, Caçador I, Quintella B R, Lança M J and Almeida P R 2014 Trace element accumulation in anadromous sea lamprey spawners *Ecol. Freshw. Fish* **23** 193–207

[16] Puspitasari R, Purbonegoro T and Putri D I 2018 Short time effect of cadmium and copper on java medaka (*Oryzias javanicus*) as bioindicator for ecotoxicological studies *AIP Conf. Proc.* **2026**

[17] Juanda S J and Edo S I 2018 Histopatologi insang, hati dan usus ikan lele (*Clarias gariepinus*) di kota kupang, nusa tenggara timur (Gill, Liver and Gut's Histopathology of Catfish (Clarias gariepinus) in Kota Kupang, East West Nusa) *SAINTEK Perikan. Indones. J. Fish. Sci. Technol.* **14** 23

[18] Amal M N A, Zarif S T, Suhaiba M S, Aidil M R M, Shaqinah N N, Zamri-Saad M and Ismail A 2018 The effects of fish gender on susceptibility to acute Streptococcus agalactiae infection in Javanese medaka *Oryzias javanicus Microb. Pathog.* **114** 251–4

[19] Yang C, Song G and Lim W 2020 A review of the toxicity in fish exposed to antibiotics *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* **237** 108840

[20] Zhao S, Wang X, Li Y and Lin J 2016 Bioconcentration, metabolism, and biomarker responses in marine medaka (*Oryzias melastigma*) exposed to sulfamethazine *Aquat. Toxicol.* **181** 29–36

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