Blood glucose of tilapia fish *Oreochromis mossambica* as a water bioindicator in the downstream of Brantas Waters, East Java

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Abstract. Blood glucose is one biomarker that can be used as an indicator for fish which is contaminated by industry or domestic pollutant. This research was conducted in downstream Brantas (Sidoarjo and Mojokerto). The purpose of this research was to analyze tilapia fish blood glucose levels as health water indicators in the Brantas River. This research used a descriptive method. Wilcoxon – Mann – Whitney test were used to analyze the data. The result of this research showed that blood glucose in Station 1 about 118-161 mg dL^{-1} and Station 2 about 145-209 mg dL^{-1}. Blood glucose levels of tilapia fish from the two places showed that blood glucose tilapia exceeded the normal limit, and it was more than 100 mg dL^{-1}. This result indicated that tilapia was under stress because the environment was contaminated. The total blood glucose in Sidoarjo River was higher than that in the Mojokerto River. The results of water quality measurement showed that the temperature range was 29-30°C, pH was 7.7-8.7, DO was 3.3-4.0 mg L^{-1}, Ammonia was 0.035-0.052 mg L^{-1}, TSS was 110-132 mg L^{-1} and COD was 50.74-87.84 mg L^{-1} and Chlorin in Station 2 was 0.02 mg L^{-1}. It is inferred that at blood glucose in fish can be used as an effective and fast biomarker for determining the environmental health of contaminated waters.

Key words: Biomarker, environment, pollutant, water quality.

Introduction. Water pollution in the river has been an immense problem in Indonesia including East Java. Anthropogenic waste can increase contamination in rivers. The Brantas River has experienced pollution from both upstream and downstream (Roosmini et al 2018; Hayati et al 2017; Sholichin & Othman 2006). High levels of river contamination can damage to biota and river resources (Ruvinda & Pathiratne 2018). Based on Yetti (2011) reporting level of pollution in Brantas River has passed the threshold limit which negatively effects aquatic ecosystems such as fish.

Fish is the most sensitive aquatic organisms to water pollution compared to invertebrates, that recommends it as an indicator of aquatic environmental conditions (Authman et al 2015). Accumulated pollution in the fish body can affect its physiological mechanisms (El-Sappah et al 2012; Ogundiran & Fawole 2018). Tilapia fish is used as an environmental indicator because of resistance to low-quality water (Kusrini & Priyono 2000). Stress in fish caused by environmental stressors or external stimulation (Kubilay & Ulukoy 2002). Polluted environmental condition is disturbing growth, reproduction, and the immune system. One of the best indicators that can be used for knowing the stress level is blood glucose (Evans & Claiborne 2006).

Blood glucose is used to provide energy and it is an effective indicator of the aquatic environment quality (Osman et al 2018). Increasing glucose in the blood occurs as a response when fish is under stress condition (Rachmawati 2010). Blood glucose is main source of energy supply and essential substrate for cell metabolism, especially brain. Glucose is needed continuously for the sustainability of brain function (Hastuti et al 2003). Changing condition in the environment will make a high demand for blood glucose supply. Stress caused by various domestic and industrial wastes in rivers can be known by blood glucose levels in tilapia fish (*Orechromis mossambica*). Fish diagnosis by blood glucose is effectively for various stressors (Sulmartiwi et al 2013). Therefore, this study aimed to analyze blood glucose levels as a bioindicator of the health level in Brantas River water.

Material and Method. This research was conducted by survey method at two stations in the downstream Brantas River, namely station 1 in Mojokerto and station 2 in Sidoarjo. Brantas river is the longest river in East Java Province, Indonesia. This river has been polluted due to various industrial wastes, agricultural activity, and fish catching (Hayati et al 2017). Two samplings were taken from each station (Figure 1). The material in this research was the blood of tilapia fish (*O. mossambica*) and water samples. Water quality analysis was carried out at the Water Quality Laboratory, Jasa Tirta I Malang. This laboratory is accredited according to ISO 17025 standards.

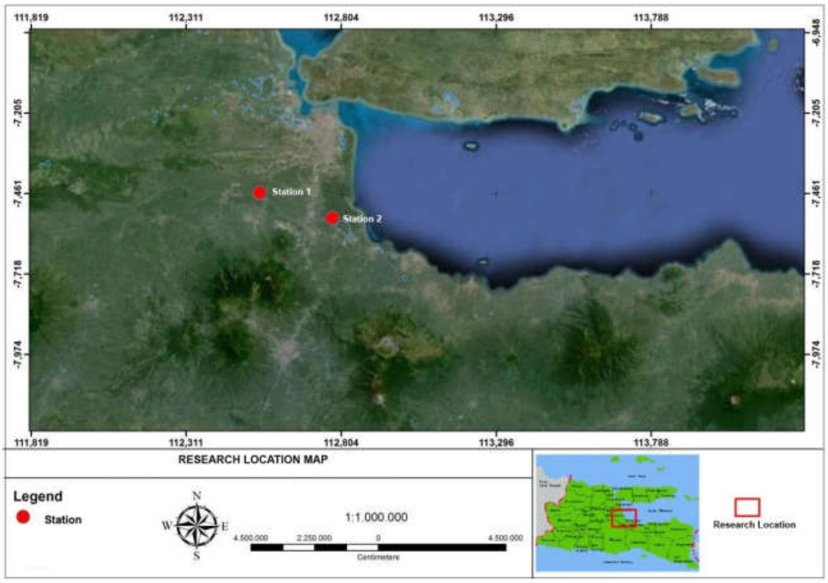

Figure 1. Sampling station map.

Fish and water quality sampling. Fish sampling was assisted by fishermen in the Brantas river. Tilapia fish sample was taken using 12 fish with a size of 11-12 cm, from all stations. The limited number of 12 fish is because of restricted fishing access and expensive analysis costs. Water sampling was using plastic bottles and stored in a cool box to maintain its quality. Water sample was analyzed for its quality in the laboratory.

Blood glucose test. Fish blood glucose was measured by Wedemeyer & Yasutake (1977) method with modifications at Clinical Pathology Laboratory, Brawijaya University, Indonesia. Fish blood was taken in caudal peduncle 3 mL with a syringe. The sample analyzed was directly done to prevent lysis. Minimum of 0.5 mL of blood was needed for glucose measurement. Examination of fish blood glucose using the Glucose Oxidase or Perioxidase method using the BioSystem BTS-350 Spectrophotometer at a wavelength of 500 nm (Sarmiento-Ortega et al 2017). This method is more accurate than using a kit method, however it is rarely used in blood glucose analysis.

Data analysis. The Man-Whitney test or U-test or Mann – Whitney – Wilcoxon (MWW), Wilcoxon rank-sum test or Wilcoxon – Mann – Whitney test were used to analyze the data according to Neuhauser (2011). The Mann – Whitney test was used to determine differences between two data sets from independent samples (independent of each other). The Man-Whitney test is a non-parametric test and an alternative to t-test (parametric test). This test was carried out on heterogeneous data for comparing two stations by different pollutant source. This method has been carried out with attention to animal welfare assessment. Fish was caught from the river using cast net fishing. Fish samples were put in a filled water container. Fish blood was sampled using a 3 mL syringe in a caudal penducle vein 0.5 mL; then, the sampled fish were directly released into the rivers.

Results. Based on the results of the analysis of Man-Whitney test, significant differences in the amount of blood glucose levels of *O. mussambica* at Station 1 Mojokerto and Station 2 Sidoarjo were found. The results showed that U value is smaller than the U table (11 U value < 33 U table) seen from the calculation of U table [11.12: 33 and U value is 11 so that it rejects H_0 with a confidence interval of 95% (α = 0.05)].

The results of *O. mossambica* research at Station 1 in sampling spot 1 showed a range of blood glucose between 146-161 mg dL^{-1} with an average of 153,167 mg dL^{-1}; while the second spot range between 118-134 mg dL^{-1}, with an average about 128.333 mg dL^{-1}. The results of research on the tilapia blood glucose (*O. mossambica*) at Station 2 in spot 1 range from 187-209 mg dL^{-1}, with an average of 198.2 mg dL^{-1}. Tilapia blood glucose levels at the second spot was about 145-172 mg dL^{-1} with an average of 160 mg dL^{-1} (Table 1).

Tabel 1

Blood glucose level of *O. mossambica* in Station Mojokerto and Sidoarjo

No	*Station 1*		*Station 2*	
	Spot 1 (mg dL^{-1})	Spot 2 (mg dL^{-1})	Spot 1 (mg dL^{-1})	Spot 2 (mg dL^{-1})
1	146	132	209	155
2	151	134	187	172
3	153	127	197	170
4	150	130	198	166
5	158	129	200	145
6	161	118	-	152
Average	153.167	128.333	198.2	160
SD	5.014	5.121	7.025	9.949

Blood glucose levels from individuals 1 to 12 revealed high blood glucose results. The average blood glucose of *O. mossambicus* was above 100 mg dL^{-1} (Figure 2) at station 1 (Mojokerto). Station 2 (Sidoarjo) of the Fish blood glucose levels was very high compared to station 1 (Figure 3). Blood glucose levels in Station 2 (Sidoarjo) were higher than those from station 1 (Mojokerto). The average blood glucose in fish showed higher levels than the normal threshold (Figure 4).

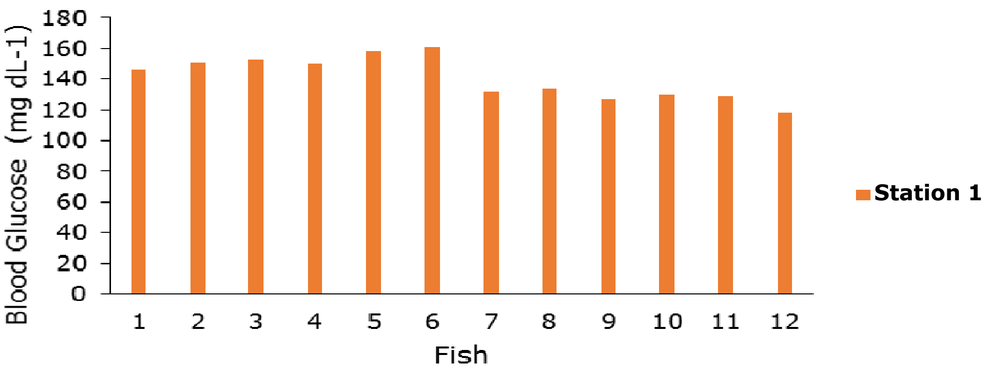

Figure 2. Blood glucose levels of *O. mosambicus* in Station 1 at Mojokerto.

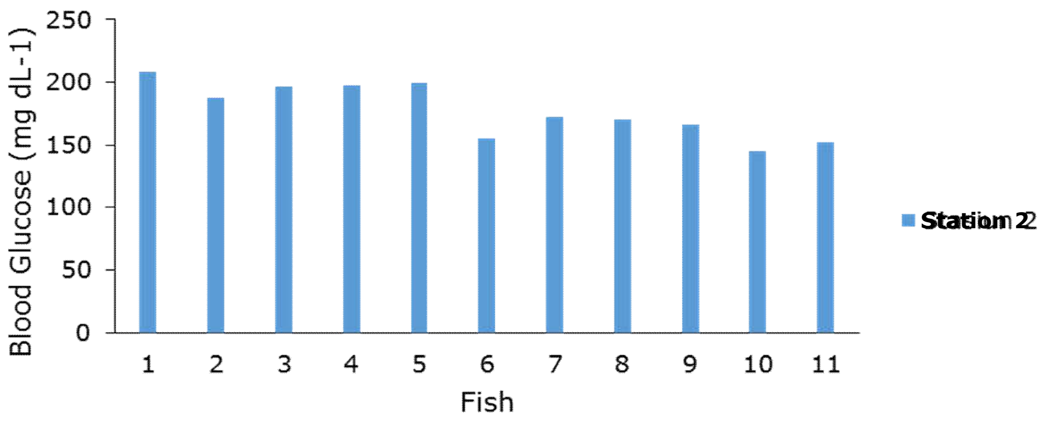

Figure 3. Blood glucose levels of *O. mosambicus* in Station 2 at Sidoarjo.

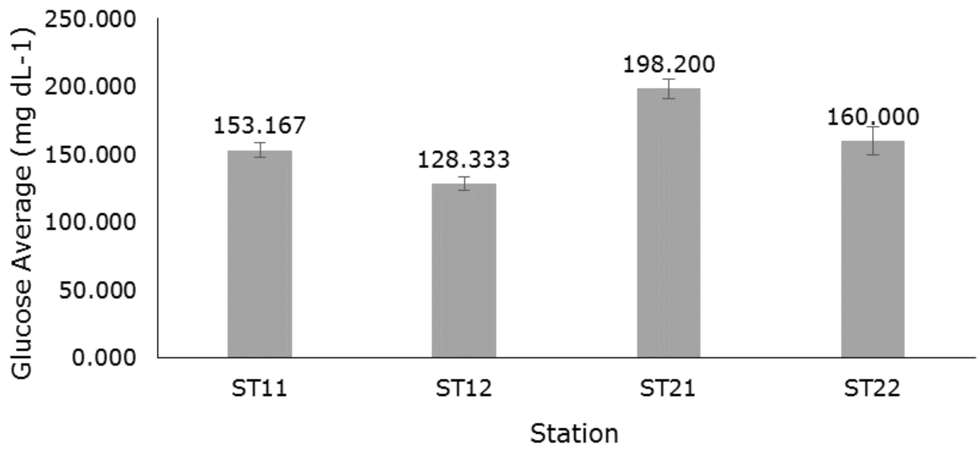

Figure 4. Average of glucose levels in both stations and spot samplings (ST11: Station 1 Spot Sampling 1, ST12: Station 1 Spot Sampling 2, ST21: Station 2 Spot Sampling 1 and ST22: Station 2 Spot Sampling 1).

There was no difference between the temperature from station 1 (Mojokerto) and station 2 (Sidoarjo); both at sampling spot 1 and sampling spot 2, the temperature ranged from 29-30^{0}C. The pH conditions at two stations were different but at the second spot, sampling was higher than the first spot. Dissolved oxygen in the downstream Brantas at Mojokerto (station 1) was showing higher than at Sidoarjo (station 2) both at first and second sampling spots. Ammonia content in station 1 was lower than station 2. Chemical Oxygen Demain (COD) in downstream Brantas River at Mojokerto (station 1) and Sidoarjo (station 2) was high. COD concentration in station 1 was lower than station 2. Data of water quality in both stations were distinguished from water quality standard of Governmental Regulation of Indonesia no. 82 of 2001 on water quality management and water pollution control. The chlorine concentration in Mojokerto (station 1) was not detected, while in Sidorjo (station 2) it was detected. The Total Suspended Solid (TSS) concentrations in Sidoarjo (station 2) was high than Mojokerto (station 1).

Table 2

Water quality data in two observation stations

No	*Parameter*	*Station 1*		*Station 2*	
		I	II	I	II
1	Temperature	30 ^{0}C	29 ^{0}C	30 ^{0}C	29 ^{0}C
2	pH	7.7	7.5	8.7	8.6
3	DO	3.9 mg L^{-1}	4 mg L^{-1}	3.3 mg L^{-1}	3.5 mg L^{-1}
4	Amonia	0.041 mg L^{-1}	0.035 mg L^{-1}	0.052 mg L^{-1}	0.048 mg L^{-1}
6	TSS	110 mg L^{-1}	117 mg L^{-1}	128 mg L^{-1}	132 mg L^{-1}
7	COD	64.86 mg L^{-1}	50.74 mg L^{-1}	87.94 mg L^{-1}	86.72 mg L^{-1}
8	Chlorine	-	-	0.018 mg L^{-1}	0.019 mg L^{-1}

Discussion. The upstream of the Brantas river is in Mount Arjuna, Malang, and the downstream at Madura Strait estuary. Downstream from Brantas river in Mojokerto and Sidoarjo then flows towards in Surabaya river. Upstream condition of Brantas is currently experiencing pollution due to human activities and will be carried downstream. Condition of Brantas river is worrying about and water quality has decreased. Downstream of Brantas in Mojokerto today is very poor because a lot of garbage that floats on the surface of water and around the sampling spot was used as a place for the disposal of waste and rubbish by residents. About 100 m from the sampling spot in Sidoarjo, there is an industry paper that discharges its waste directly into the river. The treatment of an industrial waste that does not meet the quality standards can cause pollution (Hayati et al 2017; Roosmini et al 2018).

***Blood glucose levels in tilapia fish**.* Blood glucose concentration at Station 1 and 2 up normal which was more than 100 mg dL^{-1}. It is suspected that fish are stressed due to low water quality. Fish stress was caused by the environmental conditions that imposed more energy to adapt. Based on reports from Patriche (2009), fish normally has a blood glucose content of about 40-90 mg dL^{-1}. Rahardjo et al (2011) reported that normally fish had blood glucose levels of about 40-90 mg dL^{-1}. Wedemeyer & Yasutake (1977) reported that glucose in fish blood is usually over normal at about 200 mg dL^{-1} or 100 mg dL^{-1} as an effect of relatively short-term stress.

Syawal & Ikhwan (2011) showed that environmental factors greatly influence the stress conditions of fish. Temperature affects blood glucose, urea, uric acid and, protein levels but the pattern is not consistent. Increased glucose levels in fish blood plasma during stress may be caused by the action of catecholamines at the glycogen center in the liver and tissues. Evan & Clainorne (2006) considered that stress in fish is caused by environmental changes that can increase blood glucose levels. High glucose levels stimulate thyroid gland and increase thyroxin production. High thyroxin triggers lymphocytopenia (low lymphocytes) in blood. Then the sympathetic nervous system reacts excessively, which causes lymph contractions, increases breathing and increases blood pressure. Hastuti & Subandiyono (2011) considered that the presence of blood glucose is determined by stressors with different treatments.

The Man-Wheatney Test showed that there was a difference in the blood glucose levels of tilapia fish in the two stations. The blood glucose in Sidoarjo (station 2) is higher than the blood glucose in Mojokerto (station 2). It is suspected that the sampling in Sidoarjo was adjacent to one of the waste dumps from a paper company. Wastes discharged in these waters are thought to contain free chlorine. Although the concentration of chlorine is low, it still influences the condition of fish, especially blood glucose. At a concentration of 0.02-0.03 mg L^{-1} chlorine can be toxic and even deadly to fish, while the chlorine level at the Sidoarjo sampling station is almost to 0.02 mg L^{-1}. Hastuti & Subandiyono (2011) reported that the presence of blood glucose is determined by stressors with different treatments.

The mechanism of changes in blood glucose performance during stress is caused by receptor receiving the stressor. This information is conveyed to the hypothalamic brain via the nervous system, and then the chromaffin cells receive information through the sympathetic nerve fibers to secrete the catecholamine hormone. This hormone activates the enzymes involved in the catabolism of liver and muscle glycogen stores, and suppress the secretion of the hormone insulin, so that blood glucose increases (Hastuti et al 2003).

Stressed fish due to pollution emit signs as a response to disturbances in body, that is an increase in blood glucose levels, osmoregulation balance, increased blood pressure, increase respiration and depress inflammatory response (Heath 1995). Royan et al (2014) inferred that the stress response of fish is characterized by changes in physiology and an increase in metabolism, and thus, the condition of fish can disrupt the homeostatic in their bodies (Martines-Porchas et al 2009). Stages of stress in fish: the stress response in fish is very similar to other vertebrates and can be explained in three stages (Galhardo & Oliveira 2009). The primary stage is via the two neuroendocrine axes: the hypothalamus-sympathetic-chromaffin axis which produces catecholamines, and the hypothalamus-pituitary-interrenal (HPI), which is responsible with corticosteroid production (especially cortisol). Secondary responses are physiological and behavioral adjustments during stressful conditions. This includes the activation of several metabolic pathways that cause various changes in blood chemistry and hematology, respiratory disorders, acid-base distribution with deficiency of ions in the gills, hydrothermal balance, cellular response and immune function (Galhardo & Oliveira 2009). Within tertiary responses, prolonged contact stressors affect changes in the whole organism, and have a final impact on the organism's population. Stress has an inhibiting effect on fish reproduction and inhibits growth because the metabolic effects and endocrine effects are in a one-way position. Overall due to changes and suppression of the immune system that is deep (continuously), an increased risk of disease and death appears (Galhardo & Oliveira 2009).

Fish stress conditions have an impact on the decline in the immune system, inhibit growth, produces changes in swimming behavior, reproductive disruption, and even death (Barton 2002). Besides the impact of increased glucose, which can affect changes in fish behavior in the form of rapid movement of the operculum, fish uptake air on the surface of the water, and fish become inactive (Brick & Cech 2002). Fish that are experiencing stress will continuously be exposed to death. This is caused by the high level of glucose in the blood, the signal from the nerve center indicates that the fish feels full, and the fish does not want to eat so the fish die. Hastuti et al (2003) showed that at very high stress levels, a rapid increase of blood glucose and survival at high levels of glucose will be followed by death.

Water quality – blood glucose level of O. mossambicus relationship. High Chemical Oxygen Demand (87.94 mg L^{-1}) indicates that the waters are polluted by organic matter which can interfere with fish survival. The high COD reduce the dissolved oxygen in the water especially when the dissolved oxygen content in the water is already low, around 3.3-3.5 mg L^{-1}. This causes oxygen to run out over time and cause stress on aquatic organisms, especially fish.

High TSS was about 110-133 mg L^{-1}, and low dissolved oxygen was about 3.3-4 mg L^{-1} according to Governmental Regulation of Indonesia no. 82 of 2001, a value over the standard of Class II water quality. The standard for TSS is < 50 mg/L, and dissolved oxygen is 4 ppm. Effendi (2003) considered that concentration of TSS 81-400 mg L^{-1} has an unfavorable effect on the interests of fisheries. High TSS levels result in the occlusion of gills in fish, with gill congestion making it difficult to get oxygen into the body of the fish or oxygen will not be bound by gills. This condition causes fish to experience oxygen deficiency (hypoxia) so that the fish experiences stress.

Suspended solids or TSS have a direct impact on the life of organisms through the following mechanisms: first, direct abrasion of the gills of aquatic animals; second, blockage of fish gills or other respiratory membranes; third, it inhibits the growth/ smothering of eggs or lack of oxygen intake because it is coated by solids; fourth, disruption to the feeding process, including the process of finding prey and selecting food (especially for predation and filter-feeding) and fifth, causing fish stress (Suyantri et al 2011). Beside that, high ammonia will be toxic to fish. Fish cannot tolerate too much free ammonia because it can interfere with the oxygen-binding process by the blood and can ultimately lead to suffocation. The binding process of oxygen that cannot be carried out by the blood in the body of the fish can cause the fish to experience stress and even die. It can be concluded that poor water quality causes stress in fish. Stress condition increase blood glucose levels in the body in response to maintaining homeostasis. The relationship between water quality (polluted waters) and blood glucose is the fish's rapid response to stress conditions, so that blood glucose in fish can be used as an effective and fast biomarker for determining the environmental health of contaminated waters.

Temperature. The temperatures at stations 1 and 2 are within the quality standard limits for fish life. According to Kordi (2010), the optimal temperature for tilapia fish growth is between 25-30^{o}C. Temperatures up to 22^{o}C tilapia fish can still spawn, as well as at 37^{0}C. At temperatures below 14^{o}C or more than 38^{o}C tilapia fish begin not to spawn. Deadly temperatures are at 6^{o}C and 42^{o}C. Temperature can increase the toxicity of pollutants (domestic and industrial waste) to aquatic organisms. Wastewater containing organic matter will have a higher toxicity when the water temperature rises. Yan et al (2019) considered that high water temperatures will increase the toxic power of toxic compounds such as NO_3, NH_3, and NH_3N to aquatic animals and can accelerate the metabolic activities of aquatic animals. Effendi (2003) reported that an increase in temperature can cause the solubility of gases in water such as O^2 and CO^2 gases. Furthermore, an increase in water temperature causes an increase in the speed of metabolism and respiration of aquatic organisms.

Dissolved Oxygen (DO). According to Governmental Regulation of Indonesia no. 82 of 2001 concerning water quality management and water pollution control, oxygen at Station 1 and 2 is over the quality standard limit class II. The effect of DO (Dissolved Oxygen) concentration on waste toxicity is if dissolved oxygen is low or there is no dissolved oxygen content, so that many microorganisms in water cannot survive, because dissolved oxygen is used for the degradation of organic matter (from industrial or domestic waste) in water. Furthermore, the concentration of DO will affect ammonia toxicity. If DO is low, non-ionized ammonia is toxic at lower concentrations. Ammonia at low DO cannot be broken down by decomposing bacteria, and to decompose bacterial organic matter requires oxygen. Because bacteria cannot decompose organic matter, it causes ammonia to be more toxic at low DO concentrations.

Ammonia (NH_3). Quality standard limit for ammonia content in waters is required to be at 0.02 mg NH_3 L^{-1}. Ammonia at both Mojokerto and Sidoarjo stations was over the quality standard limit for fisheries. Ammonia's toxic power is greatly influenced by temperature and water pH. According to Boyd (1979), when the temperature and pH of water are high, then the ammonia's toxicity is high, too. If the pH of the water is high, then the ammonia toxicity increases, because most of it is in NH_3, while ammonia in molecular form can penetrate parts of the cell membrane faster than NH_4^+ ions. The direct effect of high levels of ammonia that has not been deadly is the damage to the gills, in which the gill filament is swollen so that its function as a respiratory device is disrupted. As a result, in a chronic condition, the fish no longer live normally.

Total Suspended Solid (TSS). The Total Suspended Solid (TSS) values at both stations have over the quality standards normal for class II waters. The difference is almost 3 fold the quality standard, which is 50 ppm. According to Eni et al (2011), high suspended solids can create a high risk of fish live in water. Effendi (2003) showed that suspended solids into the waters can cause water turbidity. This causes a decrease in the rate of phytoplankton photosynthesis, so the primary productivity of the waters decreases, which can disrupt the food chain. High suspended solids will affect the biota in the water that is blocking and reducing the concentration of light in water bodies, thus inhibiting the process of photosynthesis by phytoplankton and other aquatic plants.

Chemical Oxygen Demand (COD). COD concentrations can be used as an indication that the waters have been polluted. The Brantas River Downstream COD concentration has exceeded the Class II of the quality standard. According to Republic of Indonesia Government Regulation No. 82 of 2001, the maximum allowable COD value is 25 ppm. Eni et al (2011) indicated that the value of COD is an indicator of pollution with industrial waste in the waters. The concentration of COD indicated the presence of organic material in Downstream Brantas. High COD causes the dissolved oxygen content in the water to be low, even completely depleted. As a result, oxygen as prerequisite of life for aquatic living organisms cannot be fulfilled, and therefore, these aquatic creatures die.

Chlorine (Cl^-). Chlorine at station 2 is higher than station 1 because sampling at station 1 is close to the waste disposal from a paper company. The chlorine value limit according to PP no 82 of 2001 free chlorite is 0.003 so that the chlorine in the Sidoarjo station is categorized as still in the threshold. However, chlorine is very dangerous for the organism if it continues to occur. High chlorine concentrations are very dangerous for fish and can even cause death in fish. At a concentration of 0.03 mg L^{-1} chlorine can cause 100% death in fish. Eni et al (2011) explained that chlorine that binds to other chemical compounds is known as bound chlorine, while free chlorine is a chloride ion that is not bound to other compounds. The speed of the chlorine reaction occurs is determined by pH (Hasan 2006).

Conclusions. The results showed that the blood glucose level of *O. mossambica* in the Downstream Brantas at Sidoarjo and Mojokerto stations is over the normal fish limit of about 70-90 mg dL^{-1}, indicating stress in fish. This result is observed from the comparison of data to data regarding healthy fish. The water in the downstream Brantas (Sidoarjo and Mojokerto) is polluted compared to the water quality standards of class II (according to Governmental Regulation of Indonesia no. 82 of 2001 on water quality management and water pollution control). Concentrations of COD, ammonia, TSS, and dissolved oxygen at both locations are not within the normal water quality standards.

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