Physiological condition of fish living in Linow Lake, Indonesia

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Abstract. The environment greatly influences the physiological state of an organism. Linow Lake is a volcanic lake that has geothermal activity. The organisms that can live in it are able to adapt to the environmental conditions. The current research aimed to determine the types of fish that can live in Linow Lake and study their physiological conditions. The research showed that two species of fish dominated the catches, namely *Anabas testudineus* and *Channa striata*. The study also determined the values of physiological parameters (antioxidants and also hematological and biochemical blood marker concentrations) of the experimental specimens.
Key Words: *Anabas testudineus*, antioxidant, blood biochemistry, *Channa striata*, hematology.

Introduction. Linow Lake is located in a geothermal area near the Tomohon City, North Sulawesi, Indonesia. Geologically, the Linow Lake Basin is considered a natural crater resulting from an eruption that occurred 0.5 million years ago (Bujung & Wenas 2022), consisting of a hot water lake that has a high sulfur content. The area of the Lake Linow is only about 34 km^2. Lake Linow is famous for changing its color, from noon to evening, due to the reflection of radiation by its waters, which contain sulfur and other chemicals. There are several hot springs, both large and small, located on the edge of the lake. Its water supply also consists of the river which flows in from the north. The temperature in the area around the lake has high values; the mud pool is 72.3–92.1˚C, the hot spring is 100˚C and the stream out 94.5-100˚C. The pH value in mud pools and hot springs ranges from 3 to 4 (Silangen 2017).

The uniqueness of a habitat will influence the condition of the living organisms that live in it (Jawad et al 2004). Adaptation is needed so the organisms can optimize their survival. Adaptation can cause physiological changes to occur. The physiological response is an indicator that can show the condition and ability of the fish to adapt to the environment. In the natural environment of the Lake Linow, the presence of high sulfur content can be a limiting factor for the survival of fish.

Sulfur in the aquatic environment can be found in the form of H_2S compounds. H_2S is a strong inhibitor of aerobic respiration. Exposure to H_2S causes oxidative stress in fish and results in lipid peroxidation (Sreejai & Jaya 2010). Lipid peroxidation occurs due to the high production of free radicals, which exceeds the availability of endogenous antioxidants (Rahman 2007). Malondialdehyde (MDA) is the end product of lipid peroxidation and is used as an indicator of tissue damage (Sewerynek et al 1996). Superoxide dismutase (SOD) is an antioxidant that captures the superoxide anion radicals which contribute to an excessive hydrogen peroxide production (Ho et al 1998). The novelty of this research consists of using the blood chemistry and hematologic characteristics to describe the physiological condition of the fish living in the Linow Lake.

Material and Method

Research sites. The research location is at Linow Lake, Tomohon City, North Sulawesi, Indonesia (Figure 1).

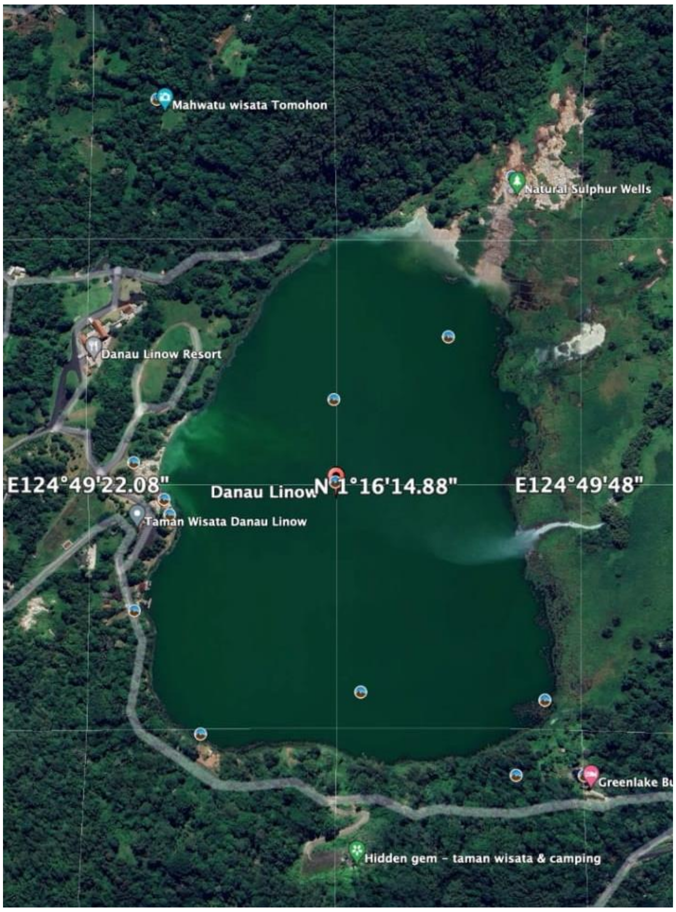

Figure 1. Linow Lake.

Experimental procedure. The fish was collected by using gill nets and pole and line. The fish obtained were then identified. Analysis of physiological condition was carried out by collecting blood and liver organ samples. The blood was taken using a 3 mL syringe from the caudal vein. The blood obtained was used for hematological analysis, and a part of the blood sample was centrifuged at 3,000 rpm for 10 minutes to obtain serum. The serum obtained was used for the analysis of glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), and blood biochemistry. Then fish was dissected to remove its liver organs. The liver was used for the analysis of Malondialdehyde (MDA), Superoxide dismutase (SOD), and Glutathione peroxidase (GPx).

Parameters measurements. Malondialdehyde (MDA) assay of the liver to determine the peroxidation activity of liver cell membranes was measured by using the tribarbituric acid (TBA) method (Singh et al 2002). SGPT and SGOT assays were measured by using the kit Reagents BTS-350 (BioSystem, Spain). The determination of sample SOD activity was carried out according to Misra & Fridovich (1972). The concentrations of glucose, protein, cholesterol, and albumin were measured by using the kit Reagents BTS-350 (BioSystem, Spain) and using a spectrophotometer to read it. The red blood cells (RBC) and white blood cells (WBC) measurement was performed according to Blaxhall & Daisley (1973), the hemoglobin measurement was performed according to Wedemeyer & Yasutake (1977), and the hematocrit measurement was performed according to Anderson & Siwicki (1995). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated indirectly with reference to RBC, hematocrit and hemoglobin. The water analysis was conducted in the Water Laboratory Nusantara (WLN) Indonesia, by measuring the temperature, pH, H_2S, ammonia, and dissolved oxygen.

Statistical analysis. Data obtained were tested for normality using the Kolmogorov-Smirnov test. $P<0.05$ was considered statistically significant. One-way analysis of variance (ANOVA) was used to determine the significant differences in all parameters measured among the species. Statistical analyses were performed using MINITAB 19 software.

Results and Discussion

Collected fish from Linow Lake. The fish obtained from this sampling are *Anabas testudineus, Channa striata*, and *Clarias sp*. The fish that dominated the catches were *A. testudineus* (75%) and *C. striata* (24%) (Figure 2).

Anabas testudineus *Channa striata*

Figure 2. The fish that dominated the catches in Linow Lake.

Physical and chemical analysis of Linow Lake surface water. Appetite and metabolism will increase with the water temperature increase. However, if the temperature is too high, the fish will become stressed, sick, or susceptible to pathogens (disease and even death). For this reason, an optimum temperature is needed for an optimal fish life and growth. In this study, the temperature obtained was 26.7°C (Table 1). Temperature greatly influences chemical and biological processes (Suparno et al 2023). The results show that although there are several hot springs in the Linow Lake, they do not really affect the surface water temperature, due to the water inputs from the river. Therefore, the Lake Linow waters temperature is still tolerated by fish.

Table 1

Physical and chemical analysis of Linow Lake surface water

Test description	*UoM*	*Result*	*Method references*
Temperature in situ (Water)	°C	26.7	SNI-06-6989-23-2005
pH in situ	n/a	6.25	APHA-4500H^+.B(2017)
Dissolved oxygen in situ	mg L^{-1}	3.34	APHA-4500O-G(2017)
Ammonia (N-NH_3)	mg L^{-1}	0.54	WI-(ID)-[ehs]-LA-064(FIA)
Unionized sulfide (H_2S)	mg L^{-1}	2.47	APHA-4500s^2-H(2017)

According to Yunita et al (2014), *A. testudineus* can tolerate pH values up to 5.18. Research conducted by Puspaningsih et al (2019) shows that *C. striata* can survive in the pH range of 6.5–9. The pH range that *C. striata* can tolerate in cultivation media is 4–9. If the pH is low or acidic, the O_2 in the water will decrease. pH conditions can affect fish appetite. In this study, the data obtained showed that the pH of the waters of the Lake Linow, at the location where the biota was found, was around 6.25 (Table 1). The pH suitable for life in the waters is around 6.3-9.0, while the tolerance limits of fish ranged from 4 to 11 (Pillay 1992).

The O_2 content can be interpreted as dissolved oxygen (DO), in the water column. Dissolved oxygen in water comes from atmospheric oxygen diffusion, currents or water flows, and photosynthetic activity by aquatic plants and phytoplankton (Novonty & Olem 1994). Although the studied waters have low concentrations of O_2, *A. testudineus* can live in these conditions (Yunita et al 2014). The waters dissolved oxygen minimum concentration required for fish growth is around 3 mg L^{-1} (Boyd & Lichkoppler 1991), and in the waters of the Linow Lake it complies to the standard for living organisms (Table 1).

NH_3 and its salts dissolve in water. Free NH_3 cannot be ionized, but ammonium (NH_4) can be ionized. Free NH_3 which cannot be ionized tends to be toxic to the aquatic organisms (Yunita et al 2014). Ammonia entering the aquatic environment can come from the waste of organisms that live in these waters (Ip & Chew 2010). The form of ammonia is affected by the temperature and pH of the water. Ammonia can affect fish either directly or indirectly depending on its concentration in the water. At low concentrations, of around 0.05 mg L^{-1}, unionized ammonia is dangerous for fish, which can result in growth inhibition, decreased fecundity, and increased stress and susceptibility to disease. At concentrations exceeding 2.0 mg L^{-1}, ammonia causes potentially lethal gill and tissue damage (Hargreaves & Tucker 2004). Tolerance to ammonia toxicity will be different in different species (Boyd 2013). In this study, it appears that *A. testudineus* and *C. striata* can tolerate an exposure to 0.54 mg L^{-1} ammonia (Table 1).

H_2S is a compound that has toxic effects on various organs and tissues (Chen et al 2019; Liu et al 2022). The H_2S Lc50 (lethal concentration 50%) value for freshwater fish ranges from 20 to 50 μg L^{-1}, while for seawater fish species it ranges from 50-500 μg L^{-1} (Boyd 2014). The H_2S value detected in Linow Lake in this study was relatively high, 2.47 mg L^{-1} (Table 1), because the Linow Lake is a geothermal area that can produce H_2S gas.

Physiological responses to the oxidative stress. MDA concentration in the liver tissue of *A. testudineus* and *C. striata* (Table 2) showed no significant differences. The increase in MDA values is strongly influenced by exposure to oxidizing substances, which damage the bonds in cell membranes, thereby releasing MDA compounds (Rawung et al 2021). This is why the MDA concentration can be a reference for the state of cell damage caused by cell oxidation. Reactive oxygen species (ROS) are not only a by-product of normal oxidative metabolism in eukaryotes, but can also be caused by the presence of compounds originating from the environment in which the organism lives (Soorya et al 2013). Exposure to H_2S can cause oxidative stress (Liu et al 2022) and result in lipid peroxidation in fish Sreejai & Jaya (2010). Lipid peroxidase causes damage to the biological membranes and generates lipid peroxides. MDA is formed from the breakdown of polyunsaturated fatty acids and serves as an appropriate index to determine the level of damage caused by lipid peroxidation. Sreejai & Jaya (2010) showed that there was an increase in MDA concentration in the liver, gills, kidneys, and brain in fish exposed to H_2S. Meanwhile, the SOD concentration in *C. striata* tends to be higher than in *A. testudineus* (Table 2). The mechanism of action of SOD as an antioxidant is by changing superoxide anions into less reactive molecules, such as oxygen and H_2O_2 (Norberg & Arner 2001). The decrease in SOD activity occurs due to its inactivation through the interaction with O_2 radicals or to an excessive H_2O_2 production, because SOD is deactivated by its own reaction products. Research conducted by Liu et al (2022) shows that H_2S exposure can cause a decrease in SOD activity in zebrafish larvae. Increased SOD activity indicates that more protein is needed to protect the cells against superoxide radicals (Soorya et al 2013). Glutathione peroxidase (GPx) is an antioxidant enzyme that prevents molecules from being damaged by the oxidative effects of increasing H_2O_2 concentrations during the oxidative stress (Niki 2012). The GPx concentration in the liver of the two types of fish did not show any differences (Table 2).

There are significant differences in the SGPT and SGOT concentrations for these fish (Table 2). *A. testudineus* showed the highest SGPT and SGOT concentrations. In conditions of hepatic impairment, the SGPT and SGOT concentrations increase in plasma (Li et al 2014). SGPT is found in the kidneys, and muscles and has the greatest concentration in the liver. Meanwhile, SGOT was observed in all body tissues (Gowda et al 2009). Therefore, an increase in the SGPT concentration is more specific for liver damage compared to SGOT (Kulkarni 2017). However, in this study, the increase in both SGPT and SGOT concentrations in *A. testudineus* was estimated, due to the relatively high physical activity which was recorded. This is shown by the behavior of *A. testudineus* which often jumps on the surface of the water during the observation. According to Pettersson et al (2008), physical activity can increase levels of SGPT and SGOT in the blood.

Table 2

Liver concentrations of MDA, SOD and GPx, and serum concentrations of SGPT and SGOT (mean±standard error) of the dominant species in the Linow Lake fish catches

Parameter	*Anabas testudineus*	*Channa striata*
Liver MDA (μmol g^{-1})	2.19±0.7	2.43±0.2
Liver SOD (%)	89.35±17.27	116.61±46.09
Liver GPx (nmol)	44.66±21.28	50.95±7.15
SGPT (U L^{-1})	28.67±7.23*	10.67±4.51
SGOT (U L^{-1})	44.00±11.5*	24.33±5.03

* Significantly different, at $p<0.05$.

Blood chemistry of fish from Linow Lake. Glucose, protein, cholesterol, and albumin concentrations can be indicators of the physiological condition of fish. Blood glucose levels of *A. testudineus* and *C. striata* showed no difference (Table 3). Glucose is an important monosaccharide that can be absorbed by the body to produce energy (Blanco 2017). Blood glucose enters the body through foods containing carbohydrates. The type of food consumed by fish will affect the fish's blood glucose levels (Serdiati et al 2023). According to Fazio et al (2013), the blood glucose levels of carnivorous fish are higher than those of herbivorous fish. Apart from the type of food consumed, increased blood sugar levels can also occur due to a response to stress (Barton 2002).

Table 3

The blood chemistry parameters (mean±standard error) of fish from the Linow Lake

Parameters	*Anabas testudineus*	*Channa striata*
Glucose (mg dL^{-1})	146±50.3	93.5±0.58
Protein (g L^{-1})	232.5±114.2*	32.5±0.58
Cholesterol (mg dL^{-1})	384.75±27.4*	203.5±29.4
Albumin (g L^{-1})	6.32±4.14	4.48±1.9

* Significantly different, at $p<0.05$.

Proteins in blood serum play an important role in the transport of various types of chemicals, in the defense of organisms against infections, parasites, xenobiotics, and they support various other functions. The concentration of protein serum levels depends on fish species, age, life cycle and gonadal maturity, feed, health, and environmental factors (Kovyrshina & Rudneva 2012). This study showed that the protein levels of *A. testudineus* were significantly different, compared to *C. striata* (Table 3), probably due to physiological differences between fish species, but also by the ongoing gonad maturation of *A. testudineus* fish: the fish samples obtained for *A. testudineus* were dominated by female fish which were undergoing gonadal maturity, whereas in *C. striata* no gonadal ongoing maturation had been detected (no discriminant between males and females). An increase in serum protein during gonadal maturation can occur due to the mobilization of the vitellogenin protein which is quite high in the circulatory system (Rawung et al 2021). The vitellogenin itself is a precursor of egg yolk protein.

The significant difference between *A. testiduneus* and *C. striata* was not only observed in serum protein levels but also in serum cholesterol levels (Table 3). According to Chatzifotis et al (2004), during the reproductive period, nutrients are mobilized from several organs which are transported through the circulatory system to the developing oocyte, resulting in an increase in the concentration of lipids, triglycerides, protein, and cholesterol in the blood. During the reproductive period, cholesterol in the female parent is needed in the formation of cell membranes and the endogenous structure of eggs (Svobada et al 2001).

Albumin protein has an important role in maintaining homeostasis in the fish body (Dwijayanti et al 2016). According to Wada et al (2017), amino acids play a close role in albumin synthesis in tissues. The main factor that influences the synthesis of albumin is the type of amino acids that enter the body through food (Wei et al 2021). The results of this study showed that albumin levels in the serum of *A. testudineus* and *C. striata* were not different (Table 3).

Hematology analysis of fish collected from Linow Lake. Blood parameters such as the number of red blood cells, white blood cells, hemoglobin, and hematocrit can indicate an individual's response to changes in the environment. Complete blood cell count is very important in monitoring the health status of fish in response to changes in nutrition, water quality, and the presence of disease (Fazio 2019). Changes in hematological profiles (hematocrit, leukocytes, hemoglobin) are one of the secondary stress responses in fish (Barton 2002). This study showed that the hematologic values of *A. testudineus* and *C. striata* were not different (Table 4). The hematologic values of C. striata in this study are different from the results of the research conducted by Puspaningsih et al (2019). This is due to the influence of the environment (Jawad et al 2004). According to Roberts (1978), the normal number of red blood cells in fish ranges from 1.05 to 3.00 x 106 mm^{-3}. In this study, the red blood cell counts of *A. testudineus* (3.4 x 10^6 mm^{-3}) and *C. striata* (3.58 x 10^6 mm^{-3}) (Table 4) tended to be slightly higher than the normal number of red blood cells in fish. According to Puspaningsih et al (2019), this can be caused by fish activities, which always take oxygen directly from the air. A research study, conducted by Fazio et al (2013), on the red blood cells of several types of wild fish showed a range of values between 1.45 and 3.73 x 10^6 mm^{-3}. White blood cells are the body's defense cells. According to Douglas & Jane (2010), white blood cell levels have implications for the immune response and the animal's ability to fight infection. Species with higher levels of white blood cells will be able to fight infection more effectively than other species.

Table 4

The hematology parameters (mean±standard error) of fish from Linow Lake

Parameters	*Anabas testudineus*	*Channa striata*
RBC (10^6 mm^{-3})	3.4±0.48	3.58±0.30
WBC (10^3 mm^{-3})	18.63±5.05	15.35±2.78
Hemoglobin (g dL^{-1})	12.75±2.01	11.93±1.19
Hematocrit (%)	42.75±5.12	41.5±7.00
MCV	127.90±24.67	116.12±18.08
MCH	37.58±3.71	33.45±3.51
MCHC	29.91±3.98	29.22±4.70

This study shows that the hemoglobin values in *A. testudineus* and *C. striata* are not different (Table 4). The average hemoglobin value for *A. testudineus* in this study was 12.75 g dL^{-1} and for *C. striata* it was 11.93 g dL^{-1}. A research conducted by Kumar et al (2018) showed that the average hemoglobin level in *A. testudineus* which was not treated was 14.36 g dL^{-1}. Podeti & Benarjee (2015) showed that hemoglobin levels in C. striata were 11.3 g dL^{-1}. This proves that the hemoglobin values of both fish are still within the normal range. Hemoglobin levels in fish blood are closely related to the number of erythrocytes. Hemoglobin has a physiological function as a transporter of oxygen in the blood (Rummer & Brauner 2015).

Hematocrit is a calculation of the volume of erythrocytes in 100 ml of blood or the ratio of erythrocytes and blood plasma in percent (%). The hematocrit value in this study is shown in Table 4. The hematocrit value of *A. testudineus* obtained was 42.75%, and the same value was also shown in a study conducted by Kumar et al (2018), 42.85%. Meanwhile, the *C. striata* hematocrit value in this study was 41.5%, higher than in the research conducted by Podeti & Benarjee (2015), which was 26.3%, and in the study of Puspanigsih et al (2019), which was 31.20%. An increase in hematocrit values can be associated with an increase in temperature (Bozorgnia et al 2011). MCV, MCH, and MCHC are calculated indirectly with reference to RBC, hematocrit, and hemoglobin. Therefore, the changes will be directly related to these blood parameters. This study shows that the hematologic conditions of *A. testudineus* and *C. striata* were not significantly different. Hematologic studies contribute to the understanding of the relationship between blood and habitat characteristics and of the adaptability of species to their environment.

Conclusions. Linow Lake can offer a suitable habitat for certain species. The conditions of the aquatic environment in the Linow Lake still shows appropriate values for the growth and development of fish species which can tolerate a high H_2S content. The physiological condition of the fish in Linow Lake shows their ability to survive. The types of fish that dominate the aquatic environment of Linow Lake are *A. testudineus* and *C. striata*.

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Conflict of interest. The authors declare no conflicts of interest.

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