Journal of Aquaculture Science
DOI: https://doi.org/10.20473/joas.v8i2.48705

Secondary Stress Response of Tilapia (*Oreochromis Niloticus*) in Different Doses of Hydrogen Peroxide (H_2O_2) During the Transport Period

Viola Nur Maghribfa[1], Putri Desi Wulan Sari[2*], Lailatul Lutfiyah[3]

[1]Program Study of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115, East Java, Indonesia
[3]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115, East Java, Indonesia

*Corresponding author: putri.dw@fpk.unair.ac.id
Submitted: 9 May 2023 Revised: 22 July 2023 Accepted: 31 July 2023 Publish: 28 October 2023

Abstract

Fish transportation is a crucial step in maintaining fish quality during the shipping process from one region to another. The closed-wet system, which is commonly used for fish transportation, has several obstacles in the form of fish stress and mortality. Stressed fish generate primary, secondary, and tertiary stress responses. The purpose of this study is to assess the secondary stress response of tilapia during transportation by giving different doses of hydrogen peroxide. 3% hydrogen peroxide applied to water resulting in three different doses i.e. 0.3 ml.L^{-1}, 0.1 ml.L^{-1}, 0.08 ml.L^{-1} and without hydrogen peroxide used as control groups. Data was obtained by measuring blood glucose levels and the ventilation rate of tilapia. The results showed an alteration in blood glucose levels and ventilation rate of tilapia during transportation. Treatment with 0.1 ml.L^{-1} hydrogen peroxide gave the lowest glucose levels during 10 hours of transportation. Changes in ventilation rate of tilapia were observed in the treatment with 0.1 ml.L^{-1} of hydrogen peroxide which indicated miled stress conditions. It can be concluded that the optimal dose of hydrogen peroxide in the transportation process is 0.1 ml.L^{-1} of hydrogen peroxide.
Key word: fish stress, blood glucose, hydrogen peroxide, ram ventilation

INTRODUCTION

The transportation of live fish has the risk of causing stress and leading to mortality. Stress in fish is a physiological change caused by environmental disturbances. During the stress condition, fish will produce a response such as primary, secondary, and tertiary stress responses known as hemostatic defense (Roy et al., 2022; Widiastuti et al., 2022). The primary stress response is a response from neuroendocrine cells that triggers the secretion of catecholamine hormones and cortisol hormones from chromaffin cells and internal cells that lead to secondary responses (Aliza et al., 2013). Secondary stress activates a number of metabolic pathways, which cause changes in blood and hematology, especially blood glucose levels. Consequently, blood glucose levels can be utilized to predict fish stress (Lestari and Syukriah, 2020).

Fish blood glucose levels in normal conditions are 40 – 90 mg.dL^{-1}. Various factors cause an alteration in blood glucose levels, such as temperature, pH, ammonia and dissolved oxygen (Cahyanti and Irsha, 2022). The secretion of catecholamine and cortisol hormones causes the sympathetic nervous system to increase heartbeat, respiratory rate, and energy storage to increase blood glucose (Galhardo and Rui, 2009).

Respiration is the way organisms exchange gasses with their environment. Fish respiration is recognized by the opening and closing of the mouth or the operculum movement (Syahidah et al., 2019). The acceleration movement of the operculum in fish occurs when they are stressed. They often swim to the surface to get air (Putra et al., 2014). Fish mortality during transportation can be reduced by providing additional ingredients such as hydrogen peroxide (H_2O_2). Hydrogen peroxide will decompose in water into environmentally friendly products, namely water and oxygen molecules (Bogner et al., 2021). The utilization of hydrogen peroxide in aquaculture has been approved by the United States environmental regulatory agency from the Food and Drug Administration (FDA, 2020).

Several previous studies have shown the success of hydrogen peroxide in maintaining survival rates during fish transportation (Nurjanah et al., 2006; Manullang, 2019). However, different species of fish have diverse physiological and metabolic system. Therefore, this research purposed to analyze the effect of hydrogen peroxide administration on tilapia, a popularly consumed fish. Tilapia has been widely cultured and potentially cultivated because it is easy to adapt, rapidly grow, and easy to breed (Mulyadi et al., 2014). Based on statistical data from the Indonesian Ministry of Maritime Affairs and Fisheries in 2022, the highest yield from aquaculture is tilapia, with a total production of 358,094 tons (KKP, 2022).

MATERIALS AND METHODS

Study site

This research was carried out in December 2022 - January 2023 at the Fish Cultivation and Anatomy Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga.

Tools and materials

The equipment used in this research was 60 cm x 40 cm x 0.3 mm plastic, 1 ml syringe, easy touch brand glucometer, hand counter, Hanna™ water quality control, ammonia test kit Merck™ 1.11117. The materials used were 100 of tilapia with a size of 7 – 9 cm, 3% of hydrogen peroxide, and pure oxygen.

Research design

The research was carried out by an experimental design with the dosage of hydrogen peroxide referring to Manullang (2019) with modifications through preliminary research, which took the results from a fish survival value of 100%. The treatments were without hydrogen peroxide (P0), hydrogen peroxide dose 0.3 $ml.L^{-1}$ (P1), hydrogen peroxide dose 0.1 $ml.L^{-1}$ (P2), and hydrogen peroxide dose 0.08 $ml.L^{-1}$ (P3).

Fish Transport Process

Fish are fasted for two days before the transportation process to provide sufficient oxygen content during the transportation process and reduce feces production (Rudiansyah and Wahidin, 2021). Tilapia transportation is performed using a closed-wet system method. Fish are transported using four-wheeled vehicles with a time duration of 10 hours. Water quality parameters such as temperature, pH, dissolved oxygen, ammonia are measured before and after transportation.

Examination of Blood Glucose Levels

Examination of tilapia blood glucose levels was carried out referring to the method performed by Maryani et al., 2021 and Irdalisa et al., 2015 with modifications and adjustments according to research needs.

Calculation of operculum

The calculation of ventilation rate is based on the method of Prariska et al.,

DOI: https://doi.org/10.20473/joas.v8i2.48705

(2017), with modifications performed during the transport process with a calculation interval of once per hour.

Data analysis

All data obtained during the research were analyzed using the Completely Randomized Design (CRD) ANOVA test with a confidence interval of 95% and continued with the homogeneity test using the Duncan test. Data were analyzed using the SPSS version 26 application.

RESULTS AND DISCUSSIONS

Blood Glucose Levels

The blood glucose levels of tilapia before and after transportation are displayed in Table 1. The utilization of hydrogen peroxide in different dosages does not affect the blood glucose levels of tilapia before transportation ($p > 0.05$). However, the blood glucose levels after transportation revealed a significant difference compared to control group P0 ($p < 0.05$). The blood glucose level of P0 has a significant difference with P1, P2 and P3. Blood glucose levels are increasing, indicating high stress in fish (Odhiambo et al., 2020; Pratama et al., 2022; Renitasari et al., 2021). The blood glucose levels of the fish before transportation were within the normal range from 40 - 90 $mg.dL^{-1}$ (Cahyanti and Irsha, 2022). One of the factors affecting high blood glucose levels before transportation is fish fasting, which affects the glycogen content in the liver (Yustiati et al, 2017).

After the transport process, the blood glucose levels in the control treatment experienced a higher increase compared to P1, P2, and P3 (Table 1). Increasing the blood glucose levels due to the transportation process and the long transportation time indicated a stress response in fish (Suwandi et al., 2012: Sreani et al., 2023). The provision of hydrogen peroxide in the P1, P2, and P3 treatments increases dissolved oxygen in the water, which reduces the metabolic rate and keeps blood glucose levels from rising too high (Taylor and Ross 1988). The high blood glucose levels in stressed fish come from the glucose consumption by muscle and other tissues from excess glycogen stored in the liver (Odhiambo et al., 2020). Stressed fish will release the cortisol hormone from interrenal cells as a primary stress response. Then, it will stimulate a secondary stress response, for example, increasing blood glucose levels (Sadoul, B., & Geffroy, B. 2019). Besides the increasing blood glucose levels, stress in fish can be seen from the rapid movements of the fish's operculum (Slavik et al., 2022). The release of catecholamine hormones from chromaffin cells causes a response from the sympathetic nervous system that activates movements heart rate and respiratory rate characterized by accelerated opening and closing of the fish operculum (Galhardo and Rui 2009).

Fish that experienced severe stress were found in the control treatment group (P0) due to the closed and prolonged transportation process. The rapid movement of operculum indicated increased metabolic activity (Ismi et al., 2016). The stressed fish is characterized by producing a secondary response due to releasing catecholamine hormones, which stimulate an increase in the opening and closing of the fish's operculum. Then, over a long period fish begins to have difficulty breathing until it experiences a decrease in ventilation rate before completely dying (Urakov, 2017).

DOI: https://doi.org/10.20473/joas.v8i2.48705

Table 1. ANOVA test results for blood glucose levels of tilapia during the transportation period

Time period	Treatment	Mean ± SD ($mg.dL^{-1}$)	F-count	*p*
Before Transportation	P0	$92.8^a \pm 10.71$	0.207	0.890
	P1	$87.4^a \pm 14.046$		
	P2	$90.2^a \pm 7.759$		
	P3	$90^a \pm 9.874$		
After Transportation	P0	$354^a \pm 144.07$	6.435	0.005
	P1	$169,2^b \pm 46.78$		
	P2	$141^b \pm 40.67$		
	P3	$190.8^b \pm 62.17$		

*note=P0: control/ without hydrogen peroxide; P1: 0.3 $ml.L^{-1}$; P2: 0.1 $ml.L^{-1}$; P3: 0.08 $ml.L^{-1}$.

Ventilation rate

The secondary stress response is observed by calculating the respiration rate or opening and closing of the operculum during the transportation period. When the respiration rate increases, the opening and closing of the operculum also become faster. Different doses of hydrogen peroxide affected the movement of the tilapia operculum during the transportation period ($p < 0.05$) (Table 2). The motion of the operculum decreased since the 1st hour of transport process. The frequency of opening and closing the operculum fluctuates due to stress in the fish. The acceleration movements of the fish operculum in P1, P2, and P3 (Table 2) due to the introduction of foreign material (hydrogen peroxide), which made the fish have to adapt to the new environment and marked by the high intensity of the operculum motion. The rate of oxygen consumption in the first minutes of treatment is higher because the fish adapt to the new environment, then the fish tend to be restless until they move actively (Syamdidi et al., 2006; Rama and Vinod, 2018; Simon et al., 2021). Adding hydrogen peroxide to water during transportation activities increases the dissolved oxygen content in water. It is an effort to protect the fish from excessive stress during transportation. Hydrogen peroxide wil decompose into water and oxygen as this following equation: $2H_2O_2 \rightarrow 2 H_2O + O_2$ (Khopsoh et al., 2022). Hydrogen peroxide is recommended in aquaculture because it can increase the dissolved oxygen in water and help fish from the stress due to the lack of oxygen during transportation (Urakov, 2017).

Table 2. Ventilation rate of tilapia after 10 hours of transportation

Treatment	Average± SD ($time.minute^{-1}$)	Stress category	F-value	*p*
P0	$113.73^a \pm 3.62$	Heavy	42.8	0.000
P1	$147.10^b \pm 11.94$	Light		
P2	$162.65^c \pm 5.73$	Light		
P3	$159.91^c \pm 6.88$	Light		

* Note=P0: without hydrogen peroxide; P1: 0.3 $ml.L^{-1}$; P2: 0.1 $ml.L^{-1}$; P3: 0.08 $ml.L^{-1}$.

Water quality

The results of water quality parameters before transportation between treatments were not much different. However, changes in water quality occurred after transport process (Table 3). Factors affecting water quality during transport include fish activity that

DOI: https://doi.org/10.20473/joas.v8i2.48705

limits dissolved oxygen content, thereby increasing the metabolic rate of the fish. The water temperature decreases because the transportation process is performed in the afternoon until evening. A decrease in temperature during transportation occurs when no light enters the plastic (Suwandi et al., 2011).

The low pH value during transport can be due to the influence of respiration products (carbon dioxide), which causes the decreasing pH in the water (Suwandi et al., 2011). Dissolved oxygen in the treatment using hydrogen peroxide showed a drastic decrease compared to before transportation. Due to the increased fish metabolism and activity throughout the transportation time, there is a drop in dissolved oxygen, which causes an increase in ammonia (Yanto, 2009). Decreasing dissolved oxygen in water is also caused by the respiration activity of fish during the transportation period (Ismi et al., 2016). In contrast to the control treatment, the high dissolved oxygen was due to decreased oxygen consumption in the fish due to damage to the gills caused by severe stress during transport and high ammonia. Thus, making fish difficult to breath (Arini et al., 2018).

Extreme water quality conditions at P0 due to high ammonia in the water can cause toxicity to fish, which causes physiological and metabolic disorders (Fajriyani and Bayu, 2021). High ammonia content in water weakens the opening and closing of the fish's operculum during transportation. Increased ammonia in the water causes ammonia excretion in the gills to be inhibited, which can reduce fish respiration rate, gill damage, and mass death of fish (Ismi et al., 2016; Agung et al., 2022; Wahyu et al., 2015). The ventilation rate on fish weakens during transport, causing dissolved oxygen in the media to be ineffectively used /by the fish.

Table 3. The water quality measurement before and after transportation

Treatment	Before transportation				After transportation			
	Temp	pH	DO	Ammonia	Temp	pH	DO	Ammonia
P0	29,8	7,8	10,1	-	27,2	6,8	9,7	10
P1	28,4	7,7	8,1	-	27,3	7	2,1	4,6
P2	28,1	7,8	7,2	-	27,4	6,9	2,1	3,4
P3	28,5	7,8	8,1	-	27,8	7,1	1,9	4,6

Ammonia is a chemical compound found in aquatic environments that causes stress in fish. High ammonia content also causes an increase in blood glucose levels. Thus, fish under stress will try to maintain their body's homeostasis (Shabrina et al., 2018). The effect of increasing ammonia is also caused by decreasing pH values, which form more hydrogen ions, and ammonia becomes more dominant (Suwandi et al., 2011). The increase in ammonia is caused by high fish activity and metabolic waste that appears because the fish are under stress during transportation (Farida et al., 2015).

Increased blood glucose during the stress phase can be caused by catecholamine reactions in the glycogen center in the liver and body tissues. Furthermore, high blood glucose will stimulate the thyroid gland to produce thyroxine, which results in lymphocytopenia in the blood. This condition causes the nervous system to contract, resulting in increasing respiratory rate and blood pressure (Renitasari et al., 2021). The increase in

DOI: https://doi.org/10.20473/joas.v8i2.48705

ammonia that occurs when treated with hydrogen peroxide is lower. This is because hydrogen peroxide can suppress the metabolic rate of fish and can reduce the ammonia content in the water. Hydrogen peroxide can inhibit the ammonia content in water, but increases the dissolved oxygen content in the water (Arini et al., 2018).

CONCLUSION

Based on the results, it can be concluded that there are changes in blood glucose levels and gill opening of tilapia fish with different doses of hydrogen peroxide during the transportation period. This study recommends the addition of hydrogen peroxide at 0.1 ml/L (P2) to suppress the increase in blood glucose levels and open the lid of the tilapia operculum during the transportation period. Hematology profile and cortisol levels need to be evaluated for further study to find more details about hormone calculations.

REFERENCE

Adji, M. 2018. Analisis gula darah ikan Nila (*Oreochromis Niloticus*) dari sungai Jagir Kota Surabaya Jawa Timur (Doctoral dissertation, Universitas Brawijaya).

Aliza, D., & Sipahutar, L. W. 2013. Efek peningkatan suhu air terhadap perubahan perilaku, patologi anatomi, dan histopatologi insang ikan nila (*Oreochromis niloticus*). *Jurnal Medika Veterinaria*, 7(2):142-145

Arini, P. D., Muhammad, F., Baskoro, K., & Fahris, N. 2018. Pengaruh pemberian hidrogen peroksida (H_2O_2) dalam pengendalian ektoparasit, dan kelangsungan hidup benih ikan Nila Salin (*Oreochromis niloticus*) Di Balai Besar Perikanan Budidaya Air Payau, Jepara. Bioma: *Berkala Ilmiah Biologi*, 20(1): 59-65.

Bögner, D., Bögner, M., Schmachtl, F., Bill, N., Halfer, J., & Slater, M. J. 2021. Hydrogen peroxide oxygenation and disinfection capacity in recirculating aquaculture systems. *Aquacultural Engineering*, 92, 102140.

Cahyanti, Y., & Awalina, I. 2022. Studi Literatur: Pengaruh suhu terhadap ikan Nila (*Oreochromis Niloticus*). Panthera: *Jurnal Ilmiah Pendidikan Sains dan Terapan*, 2(4):224-235.

Fajriyani, F., & Bayu, B. 2021. Analisis kadar amonia pada media pemijahan ikan tiger (*Datnioides microlepis*). *Buletin Teknik Litkayasa Akuakultur*, 19(1): 39-42.

Farida., Rachini., & J. Ramadhan. 2015. Imotilisasi benih ikan jelawat (*Leptobarbus Hoevenii*) menggunakan konsentrasi larutan daun bandotan (*Ageratum Conyzoides*) yang berbeda pada transportasi tertutup. *Jurnal Ruaya*, 5 (1): 22-28

FDA, U. 2020. Approved Aquaculture Drugs. US Food and Drug Administration.

Galhardo, L., & Oliveira, R. F. 2009. Psychological stress and welfare in fish. *Annual Review of Biomedical Sciences*, 1-20.

Irdalisa, I., Safrida, S., Khairil, K., Abdullah, A., & Sabri, M. 2015. Profil kadar glukosa darah pada tikus setelah penyuntikan aloksan sebagai hewan model hiperglikemik. *Jurnal EduBio Tropika*, 3(1).

Ismi, S., Kusumawati, D., & Asih, Y. N. 2016. The effects of fasting duration and different densities of grouper seed transported in closed system. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 8(2): 625-632.

Kementrian dan Kelautan Perikanan Indonesia. 2022. Produksi Perikanan Budidaya. https://kkp.go.id. Diakses pada tanggal 01/02/2023.

Khopsoh, B., Diyaningsih, M. V., & Haryuni, N. 2022. Penggunaan H_2O_2 (Hidrogen Peroksida) untuk mengurangi kadar coliform air pada peternakan ayam petelur di Kabupaten Blitar. Brilliant: *Jurnal Riset dan Konseptual*, 7(1):187-196

Lestari, D. F., & Syukriah, S. 2020. Manajemen stres pada ikan untuk akuakultur berkelanjutan. *Jurnal Ahli Muda Indonesia*, 1(1): 96-105.

Manullang, H. M. 2019. Dosis hidrogen peroksida (hp) terhadap derajat kelulusan hidup ikan mas (*Cyprinus Carpio Linn*). *Jurnal Edu Science*, 6(1): 1-7.

Maryani, M., Rozik, M., Nursiah, N., & Pudjirahaju, A. 2021. Gambaran aktivasi sistem imun ikan nila (*Oreochromis niloticus*) terhadap pemberian daun sangkareho (*Callicarpa longifolia Lam.*) melalui pakan. *Jurnal Akuakultur Sungai dan Danau*, 6(2): 74-81.

Tang, U., & Yani, E. S. 2014. Sistem resirkulasi dengan menggunakan filter yang berbeda terhadap pertumbuhan benih ikan nila (*Oreochromis niloticus*). *Jurnal Akuakultur Rawa Indonesia*, 2(2): 117-124.

Nasichah, Z., Widjanarko, P., Kurniawan, A., & Arfiati, D. 2016. Analisis kadar glukosa darah ikan tawes (*Barbonymus gonionotus*) dari bendungan rolak songo hilir sungai brantas. In Prosiding seminar nasional kelautan. Universitas Trunojoyo. Madura.

Nurjanah, Komari., & Susanto, E. 2010. Penambahan hidrogen peroksida (H_2O_2) dalam mempertahankan waktu hidup ikan kerapu lumpur. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 9(2):

Odhiambo, E., Angienda, P. O., Okoth, P., & Onyango, D. 2020. Stocking density induced stress on plasma cortisol and whole blood glucose concentration in Nile tilapia fish (*Oreochromis niloticus*) of Lake Victoria, Kenya. *International Journal of Zoology*, 2020: 1-8.

Prariska, D., Tanbiyaskur, T., & Azhar, M. H. 2017. Uji toksisitas ekstrak akar tuba (*Derris elliptica*) pada ikan nila merah (*Oreochromis sp*). *Jurnal Ilmu-ilmu Perikanan dan Budidaya Perairan*, 12(1):41-47

Pratama, A. R., & Sopyan, A. 2022. Pengaruh eugenol terhadap glukosa darah dan sintasan benih ikan sepat mutiara (*Trichogaster Leeri*) selama dan pasca transportasi. Jendela *ASWAJA*, 3(2): 1-9.

Putra, D. A., & Pribadi, T. A. 2014. Ram jet ventilation, perubahan struktur morfologi dan gambaran mikroanatomi insang ikan lele akibat paparan limbah cair pewarna batik. *Life Science*, 3(1):53-58

Rama Rao, K.. and Kumar, V. 2018. Effect of temperature on oxygen consumption and metabolic rate in puntius sophore (Hamilton-Buchanan, 1822). *International Journal of Life Sciences*, 6(2): 415-419.

Kurniaji, A. 2021. Blood glucose of tilapia fish Oreochromis Mossambicus as a water bio indicator in the downstream of Brantas waters, east java. *AACL Bioflux*, 14(4):2040 -2049

Roy, T., Ghosh, S., & Bhattacharya, S. 2022. A new growth curve model portraying the stress response regulation of fish: Illustration through particle motion and real data. Ecological Modeling, 470, 109999.

Rudiansyah, R. 2021. Kemampuan puasa dan tingkat konsumsi oksigen benih ikan lele (*Clarias Batrachus*). *Jurnal Perikanan Darat dan Pesisir*, 2(2): 1-6.

Shabrina, D. A., Hastuti, S., & Subandiyono, S. 2018. Pengaruh probiotik dalam pakan terhadap performa darah, kelulushidupan, dan pertumbuhan ikan tawes (*Puntius javanicus*). Sains Akuakultur Tropis: *Indonesian Journal of Tropical Aquaculture*, 2(2): 26-35.

Das, S. K., De, M., Ghaffar, M. A., Noor, N. M., Mazumder, S. K., & Bakar, Y. 2021. Effects of temperature on the oxygen consumption rate and gill fine structure of hybrid grouper, Epinephelus fuscoguttatus♀× E. Lanceolatus♂. *Journal of King Saud University-Science*, 33(2): 101358.

Slavík, O., Horký, P., Valchářová, T., Pfauserová, N., & Velíšek, J. 2022. Comparative study of stress responses, laterality and familiarity recognition between albino and pigmented fish. *Zoology*, 150, 125982.

Suwandi, R., Jacoeb, A. M., & Muhammad, V. 2011. Pengaruh cahaya terhadap aktivitas metabolisme ikan lele dumbo (Clarias gariepinus) pada simulasi transportasi sistem tertutup. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 14(2):92-97

Suwandi, R., Nugraha, R., & Novila, W. 2012. Penurunan metabolisme ikan nila (Oreochromis niloticus) pada proses transportasi menggunakan ekstrak daun jambu biji (Psidium guajava var. pyrifera). *Jurnal Pengolahan Hasil Perikanan Indonesia*, 15(3): 252-260.

Syahidah, D., Mastuti, I., Mudeng, C. C., & Mahardika, K. 2019. Respon Tingkah Laku Ikan Cantang (Ephinephelus Fuscoguttatus-Lanceolatus) Terhadap Anesthesia. Prosiding: Konferensi Nasional Matematika dan IPA Universitas PGRI Banyuwangi, 1(1), 115-121.

Taylor, N. I., & Ross, L. G. 1988. The use of hydrogen peroxide as a source of oxygen for the transportation of live fish. Aquaculture, 70(1-2): 183-192.

Urakov, A. L. 2017. Hydrogen peroxide can replace gaseous oxygen to keep fish alive in hypoxia. *International Scientific Research Journal*, (5-2 (59), 106-108.

Supriyono, E., Nirmala, K., & Harris, E. 2015. The effect of fish density during transportation on hematological parameters, blood pH value and survival rate of juvenile snakeheads Channa Striata (Bloch, 1793). *Jurnal Iktiologi Indonesia*, 15(2): 165-177.

Wibowo, A. A. 2019. Lama waktu transportasi menggunakan sistem tertutup terhadap kelangsungan hidup benih Ikan Tengadak (*Barbonymus schwanenfeldii*) (Doctoral dissertation, Fakultas Perikanan dan Ilmu Kelautan).

Widiastuti, R., & Widodo, M. S. 2022. Respon hormon stress dan glukosa darah benih Ikan Maru (*Channa Marulioides*) terhadap suhu berbeda. *Syntax Idea*, 4(5), 843-851.

Yanto, H. 2009. Penggunaan MS-222 dan larutan garam pada transportasi ikan jelawat (*Leptobarbus hoevenii Blkr*.) ukuran sejari. *Jumal Ilmu-ilmu Perairan dan Perikanan Indonesia*, 16(1), 47-54.

Yatiningsih, R., Besono, H., & Sardiyatmo, S. 2017. Analisis perubahan salinitas terhadap tingkat kematian dan tingkah laku Ikan Nila Merah (*Oreochromis Niloticus*) sebagai pengganti umpan hidup pada penangkapan cakalang. *Journal of Fisheries Resources Utilization Management and Technology*, 7(1): 01-10.

Yustiati, A., Pribadi, S. S., Rizal, A., & Lili, W. 2017. Pengaruh kepadatan pada pengangkutan dengan suhu rendah terhadap kadar glukosa dan darah kelulusan hidup ikan nila (Oreochromis niloticus). *Akuatika Indonesia*, 2(2):138-146