



World Scientific News

An International Scientific Journal

WSN 133 (2019) 45-55

EISSN 2392-2192

Effect of Using Low Temperature in the Beginning of Transportation with Closed System of Goldfish juvenile (*Carassius auratus* L.)

Walim Lili, Nurmuklis Rubiansyah*, Zuzy Anna and Kiki Haetami

Faculty of Fisheries and Marine Sciences, Padjadjaran University, Bandung, Indonesia

*E-mail address: nurmuklisrubiansyah@gmail.com

ABSTRACT

This research aims at analyzing the optimum of low temperatures (12 °C, 16 °C, 20 °C and temperature control (24 °C)) used at the beginning of transport on the survival rates of goldfish juvenile (*Carassius auratus* L.), with the size of about 3 cm. Each time 20 goldfish is placed in 2 litres of water in transportation with closed systems for 8, 10 and 12 hours where the goldfish is transported at 21:00 pm. This research used an experimental method, Factorial Randomized Group Design, which consists of two factors that is, a four-level temperature factor and a three-level time factor and repeated three times. The parameters observed were the survival rate, water quality, duration of the process anesthesia, duration of the process recovery after transportation and pisciculture after transportation. The results showed the low temperature and time of transportation had a significant effect on the survival rate of goldfish (*Carassius auratus* L.) in transportation with closed systems. The optimum temperature and duration of time for transportation of goldfish (*Carassius auratus* L.) were: temperature of 16 °C and 12 hours of transportation time, with 98.33% survival obtained. This species of fish comes from the regions of Japan, South Korea, South-East China, Malaysia, Thailand, Vietnam, Indonesia, and Philippines. Currently, this fish is found worldwide as a breeding copy.

Keywords: Closed system transportation, low temperature, duration of transportation, goldfish juvenile, *Carassius auratus*

1. INTRODUCTION

Goldfish (*Carassius auratus* L.) is an ornamental fish commodity that has a very unique shape and has a variety of colors. Goldfish in the domestic market have a stable position showing even an increase. For example, goldfish production in 2016 in the Tulungagung area of East Java in Indonesia reached a total production in one year of 4,416,166,660 goldfish.

The high market demand from the domestic market cannot be maximally utilized by farmers because of the limitations in the technique of handling live fish transportation. In Indonesia, the transportation using water media is one of the simplest and most popular transportation techniques compared to transportation using dry media. Dry system transportation does not use water media so it is easier to do but has a high risk of death if transported for a long time, while the use of wet systems is usually done to ensure all activities, such as metabolism and respiration to continue running normally in long-distance transportation [1].

The problem faced in the transportation of live fish is the mortality rate of fish because of the results of metabolism and relatively high temperatures during carrying which increases oxygen consumption during transportation. The method of lowering the metabolic system can be done by using low temperatures to get the conditions of the fish to be unconscious. Temperature is a factor that affects metabolism [2-20].

Temperature is one of the physical parameters that affects the life process of fish, because fish are poikilothermal animals whose body temperature is influenced by the temperature of their habitat so that metabolism is highly dependent on the surrounding environment [14]. Increasing the environmental temperature results in the decrease of dissolved oxygen concentration in water, whereas the oxygen consumption by fish increases. In addition, increasing temperature will increase the process of respiration. In this case, the energy for respiration is included in the metabolic value so that it can be concluded that the increase in temperature will cause an increase in metabolism. Increased metabolic activity of these fish will increase CO₂ concentration in water and reduce the dissolved oxygen to a lethal level of fish because of relatively high temperatures. The fish acclimatized at 20 °C has a dead point at a temperature of 31-34 °C.

Low temperature is one of the keys in the transportation of live fish. At these conditions metabolic and respiration rates are so low that fish or crustaceans can be transported for a long time with high survival rates. The purpose of this research is to analyze the optimum of low temperatures used at the beginning of handling on the survival rate of goldfish juvenile during the transportation of live fish with closed water media systems.

2. MATERIALS AND METHODS

The material used in this research is goldfish juvenile (*Carrasius auratus* L.) from Cimalaka fish farmers with a size of about 3 cm in length, ice cubes as a material to reduce the temperature in the water, and oxygen tubes for providing oxygen. The research was conducted with an experimental model using a Factorial Randomized Group Design, which consisted of two factors, namely a four-level temperature factor and a three-level time factor and repeated three times. In this research, the goldfish juvenile with a density of 20 fish/ 2 litres of water were transported, in each treatment using low temperatures at the beginning of transportation

in closed systems (temperature control (24° C), 20 °C, 16 °C, and 12 °C), where the goldfish juvenile was transported for 8, 10 and 12 hours at night at 21:00 pm. The parameters observed were the survival rate, water quality, duration of the anesthesia process, duration of the process recovery after transportation, and pisciculture after transportation.

2. 1. Survival of Fish

The survival of the goldfish was observed after being transported and 7 days pisciculture after transportation. The survival rate of fish can be calculated from the comparison of the number of fish that live at the end of the period with those living at the beginning of the period, using the formula:

$$SR (\%) = \frac{Nt}{No} \times 100\%$$

where:

SR – Survival rate of fish during the trial

Nt – Number of fish at the end of the experiment

No – Number of fish at the beginning of the experiment.

2. 2. Water Quality Parameters

Water is the main factor for the fish life. Temperature, dissolved oxygen, pH, and ammonia are the parts of observable water quality parameters.

2. 3. Data analysis

Effect of using the low temperature in the beginning of transportation with closed system of goldfish juvenile (*Carassius Auratus* L.) was analyzed using the F test. If the treatment has a significant effect, then further testing is done with Duncan's multiple range test ($\alpha = 0.05\%$). Survival rate pisciculture after transportation and water quality data were analyzed descriptively.

3. RESULTS AND DISCUSSION

3. 1. Effects of Temperatures on the Activity of Goldfish juvenile

At the control temperature (24 °C), the activity and condition of fish are relatively normal. The position of the fish is upright, active swimming, movement is very agile, and very responsive to external stimuli. Movements of limbs such as gills, dorsal fin, pectoral fin and caudal fins look to be active, and the operculum moves normally.

At a temperature of 20 °C, the activity and condition of the fish are relatively reduced. This is indicated by most of the fish which in an upright position have begun to calm down. Fish are less active in swimming, but still responsive to physical stimuli from the outside. In addition, the operculum movement has begun to slow down. At the temperature of 20 °C the goldfish juvenile experience the fainted condition, and at the fifth minute on average after the temperature drop process. At a temperature of 16 °C, the activity and condition of the fish begin to weaken. This is indicated by the majority of the fish being upright and weak. Fish are less

active swimming (slow), and less responsive to physical stimuli from the outside. At this temperature range, the fish begins to be easily caught when removed from the water, the fish still struggles and the operculum moves slowly. At a temperature of 16 °C, the goldfish juvenile experience the fainted condition at the third minute on average after the temperature drop process. At a temperature of 12 °C, the activity and condition of the fish weakens. Some fish begin to enter the collapsing phase, fish swim very slowly, do not swim actively, and are less responsive to physical stimulation from the outside. At this temperature range, fish are easily caught. When removed, the fish struggles weakly and the operculum moves slowly. At 12 °C the goldfish juvenile experience the fainted condition at the first minute after the temperature drop process.

Anaesthetic process before transportation has an effect on the metabolic rate when the fish is transported. The use of low temperatures will make the metabolic process of fish to decrease down to basal metabolic rate. Basal metabolism is defined as a minimum energy level to maintain the structure and function of body tissues (to stay alive). Basal metabolism includes energy requirements for blood circulation, replacing damaged cells, respiration and intestinal peristaltic movements. Basal metabolism makes fish to be passive, so the respiration process and the metabolites released by the body become low. The decrease in temperature causes the activity of the respiration rate and metabolism of gouramy to tend decrease, along with a decrease in the water temperature of fish living media. Thus the efforts to improve fish survival in transportation can be done by decreasing the rate of fish metabolism.

3. 2. Duration of Recovery

Analysis of recovery time is presented in diagrams in Figure 1.

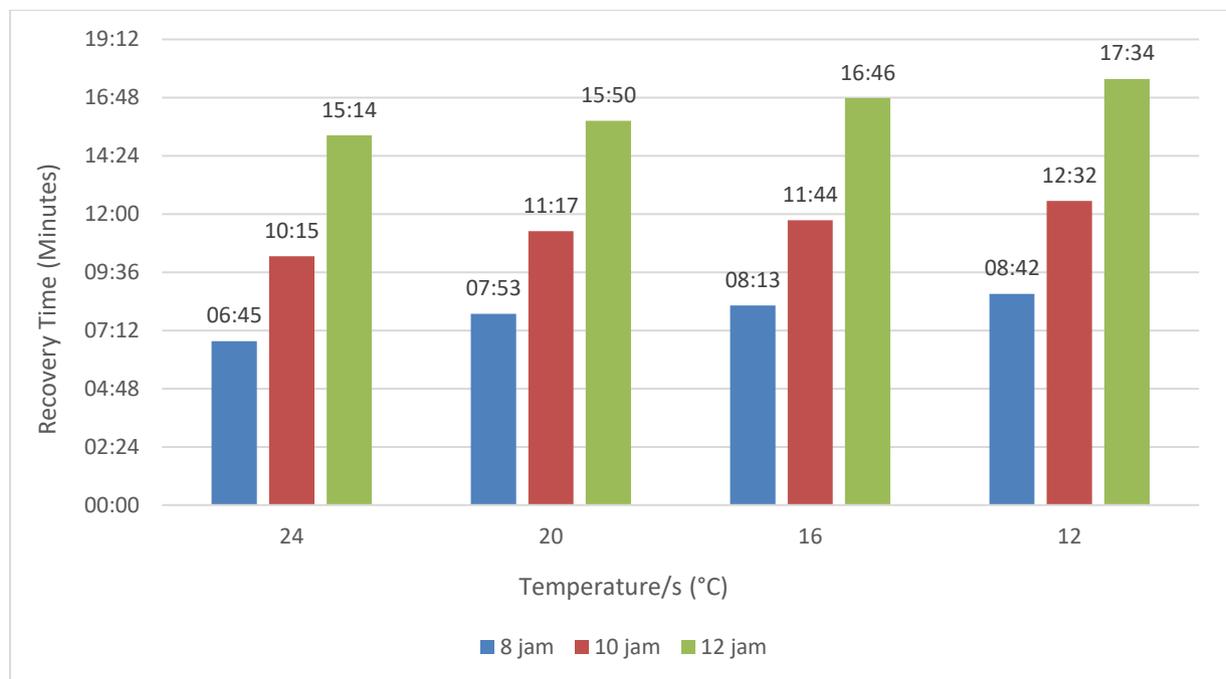


Figure 1. The Average Diagram of the Duration of Time for Goldfish Recovery.

Note: Treatment S_0 : Water temperature 24 °C ±1 °C, S_1 : 20 °C ±1 °C, S_2 : 16 °C ±1 °C, S_3 : 12 °C ±1 °C, Treatment W_1 : for 8 hours, W_2 : 10 hours, W_3 : 12 hours.

In transportation of live fish, after the fish are transported, a process of recovery is carried out to determine the survival rate. The recovery process aims to restore the condition of the fish.

The temperature of recovery media is adjusted to fish habitat. Figure 1 shows that the average recovery of goldfish ranges from 6:45-17:34 minutes. The longest time when the process of recovery is on the transport treatment 12 hours and the temperature is 12 °C (W₃S₃) where the average duration of time for recovery is 17:34 minutes. In addition, the fastest time when the recovery process is on the transport treatment 8 hours and the temperature is 24 °C (W₁S₀) where the average duration of time for recovery is 6:45 minutes.

Figure 1 can be analyzed that the longer the time of transportation (8, 10 and 12 hours) and the lower the temperature (24 °C, 20 °C, 16 °C and 12 °C) will affect the process of recovery the goldfish. The longer the transportation time will cause the longer the process of recovery, because the fish are in a weak condition and lose more energy during transportation, so the goldfish requires a longer time to regain consciousness. In addition, the lower the temperature of transportation will cause the longer the process of recovery because the fish need time to adjust to the conditions of their habitat when doing the recovery. The recovery process is carried out by entering fish that are already unconscious in normal temperature water (about 27 °C). In fact, the normal temperature of 28 °C is ample to support the success of the tests carried out. During the process of fish recovery, the condition of goldfish in general shows the same activity. First, the condition of goldfish begins with a very slow swimming movement, then gradually becomes normal. After that, the condition of the limbs returning to normal, such as the movement of the fins and operculum which is gradually normal, even though it is still in a weak condition. During the process of recovery, fish that did not show signs of movement of the limbs after 10 minutes were dead.

3. 3. Survival Rate of Fish

Analysis of survival rate of goldfish transportation can be seen in diagrams (Figure 2).

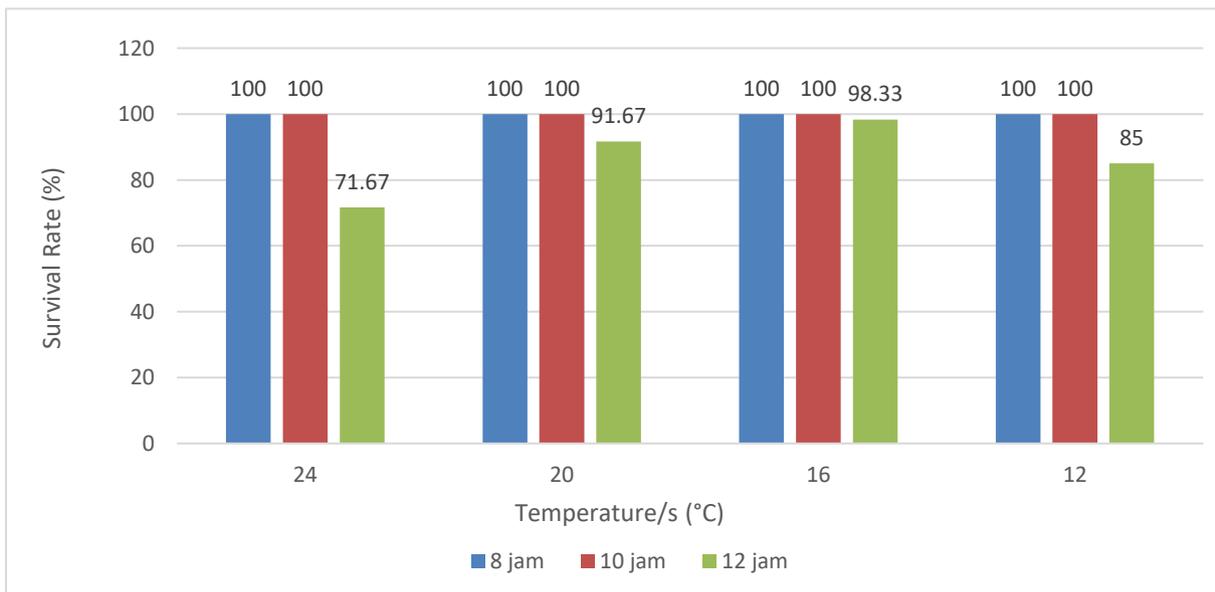


Figure 2. Survival Rate of Goldfish juvenile.

The following survival rates of goldfish juvenile after 8, 10 and 12 hours, which are transported using low temperatures (temperature control 24 °C), 20 °C, 16 °C and 12 °C) with closed systems can be seen in Table 1.

Table 1. Survival rate of Goldfish juvenile

Time (W)	Temperature/s (°C)	Average (%)	Notation
8 hours	24	100	d
	20	100	d
	16	100	d
	12	100	d
10 hours	24	100	d
	20	100	d
	16	100	d
	12	100	d
12 hours	24	71.67	a
	20	91.67	b
	16	98.33	d
	12	85	c

Note: The value followed by the same letter is not significantly different, Treatment S₀: Water temperature 24 °C ±1 °C, S₁: 20 °C ±1 °C, S₂: 16 °C ±1 °C, S₃: 12 °C ±1 °C, Treatment W₁: for 8 hours, W₂: 10 hours, W₃: 12 hours

Table 1 shows that the effect of used low temperatures at the start of the transport of goldfish juvenile that are transported at a density of 20 fish/2 litres of water for various duration of transport results in the survival rates of diverse goldfish juvenile. Following the statistical analysis between the treatment of low temperature and the duration of transport one may notice significantly different results in some of these interactions.

Figure 2 shows the average survival rate of goldfish juvenile ranging from 71.67 to 100%. All treatments of low temperatures of 24 °C, 20 °C, 16 °C and 12 °C (S₀, S₁, S₂, and S₃) with a duration of transport time of 8 hours and 10 hours (W₁ and W₂) revealed the results were not significantly different from the survival rate of 100% (Table 1). This is due to transportation time. The goldfish juvenile in this research are transported at night. After 8 hours and 10 hours (W₁ and W₂), the fish transportation is finished in the morning.

Transport time greatly affects the water temperature of the media during transport. At night and in the morning the ambient temperature is relatively lower so that affecting relatively lower water temperature. The low temperature of this water can last in the plastic for longer time so that the metabolism of fish remains low and the availability of dissolved oxygen is adequate. Low water temperature can reduce the activity and level of oxygen consumption of fish. In general, live fish transportation, good for consumption or juvenile size, is carried out at night until the morning in order to get successful achievement in view of keeping dissolved oxygen high and avoid fish stress during transport.

At the time of transportation for 12 hours the survival rate of goldfish juvenile began to decline and the results were significantly worse (Table 1). In transportation with a treatment temperature of 16 °C and a duration of transport time of 12 hours (S₂W₃), the highest survival rate was 98.33%. on the other hand, in transportation with a treatment temperature of 24 °C and a duration of transport time of 12 hours (S₀W₃), the lowest survival rate was 71.67%. In addition, at a treatment temperature of 20 °C and a duration of transport time of 12 hours (S₁W₃) and at a treatment temperature of 12 °C and a duration of transport time of 12 hours (S₃W₃), the success rates obtained were 91.67% and 85%, respectively.

At the time of the treatment of transporting for 12 hours (W₃) goldfish juvenile experienced death or mortality. The death or mortality resulted from the duration of transportation time and oxygen dissolved in water. In the transportation with closed system the factors that need to be considered include dissolved oxygen in water, density (here 20 fish/2 litres), and the time of transport. The prolongation of time of the transport causes the temperature to rise due to environmental influences, the rising temperature causes a decrease in dissolved oxygen and increased carbon dioxide (CO₂); they result in increased activity and high levels of stress so that the fish suffocates during transportation.

Hyperactive fish will consume a lot of O₂ and release CO₂ a lot so that O₂ dissolves in water and the transport media become rapidly reduced and CO₂ increases too quickly. Fish transported at relatively high temperatures will need more oxygen. Every increase in water temperature above 10 °C results in oxygen consumption by fish, by increasing 3-5 times. Fish acclimatized at 20 °C have dead spots at temperatures of 31-34 °C.

Based on the data it can be concluded that the longer the transport time is carried out, the lower temperatures should be used to keep or increase the survival rate. The fainted goldfish and the transport temperature of 16 °C are the best method to be applied for duration time goldfish transportation with 12-hour transport time. To decrease the temperature used, the expected ratio of water and ice should be: 1 litre of water and: 0.2 kg of ice.

Pisciculture of goldfish juvenile is intended to determine the effect of using low temperatures at the beginning of transportation. This pisciculture has been carried out for 7 days. Based on the observations of survival values, in Figure 3 all treatments with received SR values of 100% are presented.

The effects after transport that occur do not influence the physiological processes of fish which could reduce the health and endurance of fish, so handling with low temperatures does not affect the life of post-transport goldfish. In handling with low temperatures it is declared safe because there are no chemicals introduced that could harm fish.

Thus, it was found that handling using low temperatures for goldfish transport does not have an impact on the lives of goldfish and pisciculture after transportation. At the time of pisciculture there are no goldfish died after transportation is done.

The average survival of post-transport goldfish juvenile is presented in Figure 3.

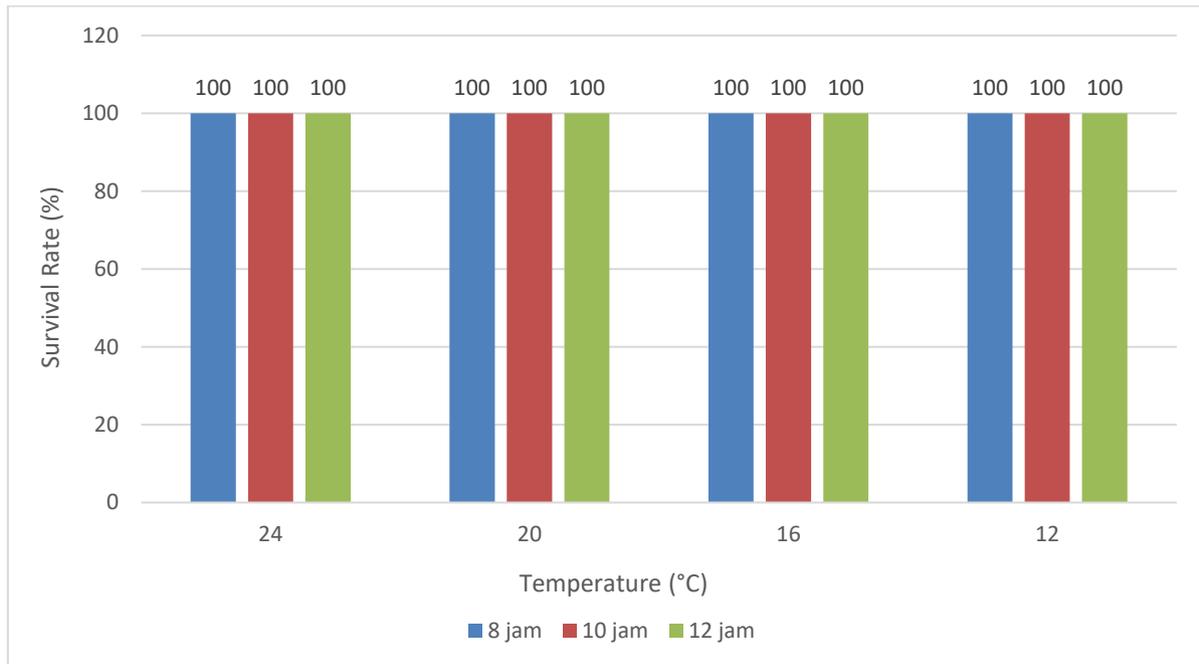


Figure 3. Diagram of Survival Rate in Pisciculture Goldfish

Based on the observations of survival values shown (Figure 3), all treatments received SR values of 100%. The effects after transport that occur do not affect the physiological processes of fish which could reduce the health and endurance of fish, so handling with low temperatures does not have an influence on the life of post-transport goldfish. In handling with low temperatures it is declared safe because it does not contain chemicals that can harm fish. Thus, this shows that handling using low temperatures on goldfish transport does not have an impact on the lives of goldfish when pisciculture after transportation. It appears, at the time of pisciculture there are no goldfish died after fish transportation is done.

3. 4. Water Quality

Temperature conditions at the start of the transport of goldfish juvenile using low temperature treatments were: 24 °C, 20 °C, 16 °C, and 12 °C. Then, post-transportation observations were carried out, changes in temperature occurred where post-transportation average temperatures ranged from 21-28 °C. Temperature is very influential on transportation closed system, because the rising water temperature will make the fish's metabolic rate will change so with its energy needs. Increasing temperatures will increase the process of respiration. In this case, energy for respiration is the energy that is included in the metabolic value. Thus it can be concluded that the increase in temperature will cause an increase in metabolism. Every increase in water temperature of 10 ° C will increase the oxygen consumption by fish for 3-5 times. Fish acclimatized at 20 ° C have dead spots at temperatures of 31-34 °C. Pisciculture temperature conditions after transportation at Laboratory Ciparanje Padjadjaran University Indonesia at the beginning of pisciculture ranged from an average of

24.4 - 25.1 °C and observations at the end of pisciculture ranged from 25 to 25.6 °C. These conditions are still ideal for the life of the goldfish juvenile. The ideal temperature for tropical ornamental fish is around 25-32 °C.

Dissolved oxygen (DO) conditions at the beginning of the transportation of goldfish juvenile ranged from an average of 3.9-4.8 mg/L; the condition is not good for the life of the goldfish during transportation. Then, pure oxygen is added to the water before the fish is transported. After oxygen is added, transportation is performed, then post-transport DO observations are carried out. Changes occur at the end of transportation, where DO values range on average of 4.9-7.4 mg/L. The dissolved oxygen conditions at the initial pisciculture of post-transport goldfish ranged from an average of 6.6 to 8 mg/L, then the DO observation at the end of pisciculture ranged from 6.1 to 6.6 mg/L. Water for fish maintenance must have oxygen content of 5 mg/L.

The pH conditions at the start of the goldfish transportation ranged from an average of 6.5 to 6.74. Then on the expression of pH, at the end of transportation that pH value obtained on average of 6.38-6.59, whereas the condition of pH when pisciculture the goldfish of post-transport was equal ranging on average of 7.5 - 7.4. This condition has been recognized feasible for the life of the goldfish juvenile during the research. Optimal pH in the maintenance of ornamental fish ranges from 5.5 to 9.

4. CONCLUSION

The effects of low temperature and duration of transportation time have been studied. It was found that at transportation in closed system these parameters significantly influence the survival of goldfish juvenile (*Carassius auratus* L.) of size of about 3 cm. The most optimum temperature and duration of time for the transport of goldfish juvenile (*Carassius auratus* L.) is a temperature of 16 °C and a duration of transport time of 12 hours. The obtained survival of 98.33% was noted when the fish was carried out at night, where temperature decreases using temperature comparisons of water and ice: 1 L of water and 0.2 kg of ice.

References

- [1] Nani, M. Zaenal, A. Bagus, D. H. S. 2015. Effectiveness of Tilapia (*Oreochromis* sp) Transport System for Consumption Using Wet, Semi-Wet and Dry Systems. *Indonesian Swamp Aquaculture Journal*, 3 (2): 84-90.
- [2] Duncan, J.A. and Storey, K.B. Role of enzyme binding in muscle metabolism of the goldfish. *Can. J. Zool.*, 69: 1571–1576, 1991.
- [3] Holopainen, I.J., Hyvarinen, H., and Piironen, J. Anaerobicwintering of crucian carp (*Carassius carassius* L.). II. Metabolic products. *Comp. Biochem. Physiol. A Physiol.*, 183: 239–242, 1986.
- [4] Zikic, R.V., Stajn, A., Saicic, Z.S., Spasic, M.B., Ziemnicki, K., and Petrovic, V.M. The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. *Physiol. Res.*, 45: 479–481, 1996.

- [5] Zhu, Z., Li, G., He, L., Chen, S. Novel gene transfer into the fertilized eggs of gold fish (*Carassius auratus* L. 1758). *Journal of Applied Ichthyology*, Volume1, Issue 1, May 1985, pp. 31-34. <https://doi.org/10.1111/j.1439-0426.1985.tb00408.x>.
- [6] Jinlin Chen, Zheng Fan, Dejie Tan, Dongneng Jiang and Deshou Wang, A Review of Genetic Advances Related to Sex Control and Manipulation in Tilapia. *Journal of the World Aquaculture Society*, 49, 2, 277-291, (2017).
- [7] Minghui Li and Deshou Wang. Gene editing nuclease and its application in tilapia. *Science Bulletin*, 62, 3, (165), (2017).
- [8] Kang Xu, Wei Duan, Jun Xiao, Min Tao, Chun Zhang, Yun Liu, and ShaoJun Liu. Development and application of biological technologies in fish genetic breeding. *Science China Life Sciences*, 58, 2, (187), (2015).
- [9] Yoji Yamamoto, Naoki Kabeya, Yutaka Takeuchi, Kentaro Higuchi, Takashi Yatabe, Kazunobu Tsunemoto, Ryosuke Yazawa, Takaichi Kawamura and Goro Yoshizaki. Establishment of a stable transgenic strain in a pelagic egg spawning marine teleost, Nibe croaker *Nibea mitsukurii*. *Aquaculture*, 313, 1-4, (42), (2011).
- [10] Duan, M., Zhang, T., Hu, W., Guan, B., Wang, Y., Li, Z., and Zhu, Z. Increased mortality of growth-enhanced transgenic common carp (*Cyprinus carpio* L.) under short-term predation risk. *Journal of Applied Ichthyology*, 26, 6, (908-912), (2010).
- [11] Duan, M., Zhang, T., Hu, W., Sundström, L.F., Wang, Y., Li, Z., and Zhu, Z. Elevated ability to compete for limited food resources by 'all-fish' growth hormone transgenic common carp *Cyprinus carpio*. *Journal of Fish Biology*, 75, 6, (1459-1472), (2009).
- [12] Li, D., Hu, W., Wang, Y., Zhu, Z., and Fu, C. Reduced swimming abilities in fast-growing transgenic common carp *Cyprinus carpio* associated with their morphological variations. *Journal of Fish Biology*, 74, 1, (186-197), (2009).
- [13] Volodymyr I. Lushchak, Ludmyla P. Lushchak, Alice A. Mota, and Marcelo Hermes-Lima. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, Volume 280, Issue 1, January 2001, pp. R100-R107, <https://doi.org/10.1152/ajpregu.2001.280.1.R100>.
- [14] Peter, R.E., Gill, V.E. A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carassius auratus*. *The Journal of Comparative Neurology*, Volume159, Issue1, 1 January 1975, pp. 69-101.
- [15] Ayelén Melisa Blanco, Lakshminarasimhan Sundarrajan, Juan Ignacio Bertucci, and Suraj Unniappan. Why goldfish? Merits and challenges in employing goldfish as a model organism in comparative endocrinology research. *General and Comparative Endocrinology*, 10.1016/j.ygcen.2017.02.001, 257, (13-28), (2018).
- [16] Jan A. Mennigen, Hélène Volkoff, John P. Chang, and Vance L. Trudeau. The nonapeptide isotocin in goldfish: Evidence for serotonergic regulation and functional roles in the control of food intake and pituitary hormone release. *General and Comparative Endocrinology*, 10.1016/j.ygcen.2017.09.008, 254, (38-49), (2017).

- [17] Dillon F. Da Fonte, Lei Xing, Myy Mikwar, and Vance L. Trudeau. Secretoneurin-A inhibits aromatase B (cyp19a1b) expression in female goldfish (*Carassius auratus*) radial glial cells. *General and Comparative Endocrinology*, 10.1016/j.ygcen.2017.04.014, (2017).
- [18] Elsie Tachie Mensah, Ayelén Melisa Blanco, Andrew Donini, and Suraj Unniappan. Brain and intestinal expression of galanin-like peptide (GALP), galanin receptor R1 and galanin receptor R2, and GALP regulation of food intake in goldfish (*Carassius auratus*). *Neuroscience Letters*, 10.1016/j.neulet.2016.11.037, 637, (126-135), (2017).
- [19] Ratanak Ou, and Naoyuki Yamamoto. Forebrain atlas of Japanese jack mackerel *Trachurus japonicus*. *Ichthyological Research*, 10.1007/s10228-016-0509-8, 63, 3, (405-426), (2016).
- [20] Nikhil V. Palande, Rahul C. Bhoyar, Saikat P. Biswas and Arun G. Jadhao. Short-term exposure to L-type calcium channel blocker, verapamil, alters the expression pattern of calcium-binding proteins in the brain of goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 10.1016/j.cbpc.2015.07.006, 176-177, (31-43), (2015).