



The Effect of Giving Various Natural Sources of β -Carotene to Increased Color Intensity on the Oranda Goldfish (*Carassius auratus*)

**Muhamad Afif Adhwa Kautsar^{a*}, Walim Lili^a, Subiyanto^a
and Roffi Grandiosa Herman^a**

^a *Department of Fisheries, Department of Marine Science, Faculty of Fisheries and Marine Science, Padjadjaran University, Jl. Raya Bandung-Sumedang, Hegarmanah Jatinangor, Sumedang 45363, West Java, Indonesia.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2022/v18i230437

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/88625>

Original Research Article

Received 30 April 2022
Accepted 07 July 2022
Published 11 July 2022

ABSTRACT

This research aims to examine the effect of adding various sources of natural β -Carotene obtained from sweet potato flour, carrot flour and pumpkin flour to the color brightness of Goldfish. The research was carried out from December 2021 to February 2022 at the Aquatic Animal Physiology Laboratory Building 2, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The experimental design used in this study was a completely randomized design (CRD) with five treatments and three replications. The treatments were negative control (A), positive control (B), 10% sweet potato flour (C), 10% carrot flour (D) and 10% pumpkin flour (E). Maintenance with treatment on the test fish was carried out for 60 days. Parameters observed included color brightness observed using Toca Color Finder (TCF), growth, life, and air quality (Temperature, pH, Dissolved Oxygen). The results showed that the addition of various sources of natural carotene in the feed had an effect on increasing the brightness of the color of the Oranda Goldfish (*Carassius auratus*). The highest value of increasing fish color brightness was found in treatment E with pumpkin flour as a natural source of β -Carotene, namely 3.2 in the head, 3.3 in the body and 2.5 in the tail. Survival rate in this study is 100%. The water quality parameter values are in good

condition for the growth and survival rate of goldfish. Based on observations of water quality the results of temperature ranged from 24.2°C - 28°C, pH ranged from 6.88-7.62 and Dissolved Oxygen ranged from 5.8-8.0 mg/L.

Keywords: Goldfish; color brightness; toca color finder; β -carotene; sweet potato flour; carrot flour; pumpkin flour.

1. INTRODUCTION

The development of ornamental fish farming fisheries in Indonesia is increasing along with population growth and the development of human mindset, fish can be used as a means of recreation in the form of ornamental fish. Indonesia with a tropical climate has the potential of ornamental fish reaching 300 million heads in a year and consists of 240 species of marine ornamental fish and 226 types of freshwater ornamental fish [1].

Goldfish is a dominating commodity in increasing ornamental fish production in Indonesia after koi and betta fish. The increase in goldfish production is known to have amounted to 66.823.000 heads in 2010 to 72.997.000 in 2014 [2]. According to the DJPB performance report, goldfish production in 2015 amounted to 28.731 heads in 2016, which doubled, which was 61.768 heads but in 2017 there was a decrease in production of 39.409 heads and again rose in 2018 by 81.284 heads with an average increase of 31.1% [3].

Color is one of the reasons ornamental fish are in demand by the community, so cultivators need to maintain the color of ornamental fish by giving feed containing color pigments [4]. These additives are the main source in the pigmentation process in tropical ornamental fish that are yellow, red and other colors, better known as carotenoids [5]. Carotenoids can come from chemicals or natural materials whether they come from plants or animals. Carotenoids derived from chemicals are relatively less safe for both fish and aquatic environments whereas carotenoids from natural ingredients are safer [6].

Natural sources of carotenoids are found in plants and fruits [7]. There are three natural ingredients that can be used as a source of natural β -Carotene, including carrots, sweet potatoes and yellow pumpkin. The content of β -Carotene at 100 grams of carrot powder is 20.550 μg [8]. The content of β -Carotene in 100 grams of purplish red sweet potatoes is 9.900 μg [9]. While the content of β -Carotene levels in

yellow pumpkin fruit is 1.000-1.300 μg [10]. Because the content of β -Carotene can be obtained from these three ingredients, carrot flour, sweet potato flour and yellow pumpkin flour can be used as an alternative as an additional ingredient in making fish feed.

2. MATERIALS AND METHODS

This research was conducted from December 2021 to February 2022 at the Water Animal Physiology Laboratory Building 2 of the Faculty of Fisheries and Marine Sciences, Padjadjaran University. Oranda Goldfish are 3 months old with an average length of 5-8 cm as many as 60 heads obtained from Parung Ornamental Fish Market, Bogor West Java. Fifteen (15) aquariums with a size of 39.8 cm x 25.4 cm x 28 cm are used as maintenance containers. The study lasted for 60 days. During the feeding frequency study 3 times a day at 08.00 WIB, 12.00 WIB and 16.00 WIB. The amount of feed will be adjusted every 10 days based on the increase in fish weight after sampling. The color brightness observation was done visually with three non-colorblind panelists using the standard value of the Toca Colour Finder (Fig. 1). The assessment starts from the smallest score of 1 to the largest score of 7 with color gradations from orange to dark red. Observations of color, fish weight and water quality were made once every 10 days during the research.

The research method used is an experimental method and the experimental design used in this study is a Complete Random Design (CRD). The study used five treatments with three repetitions. The treatment tested was the difference in mixing natural β -Carotene from sweet potato flour, carrot flour and yellow pumpkin flour in commercial feed.

The treatments tested in this study include:

- Treatment A = Negative Control (without mixture)
- Treatment B = Positive Control (commercial ornamental fish feed brand Hikari Oranda Gold)

- Treatment C = Commercial feed added 10% Sweet Potato Flour
- Treatment D = Commercial feed added 10% Carrot Flour
- Treatment E = Commercial feed added 10% Yellow Pumpkin Flour

2.1 Research Procedures

2.1.1 Test fish feed preparation

The feed used during the study was in the form of commercial fish feed mixed with yam flour, carrot flour and yellow pumpkin flour in accordance with the treatment. The three mixtures used are flour in dry form. The feed used for control does not contain sweet potato flour, carrot flour or yellow pumpkin flour. The three added flours are adjusted to the treatment dose. Mixing is done by adding Carboxymethyl cellulose (CMC), which is a synthetic adhesive or binder added to the feed. According to Tarmizi et al. [11] the dose of CMC commonly used as a binder in fish and shrimp feed is 2% - 3%.

The stages of mixing sweet potato flour, carrot flour and yellow pumpkin flour in this feed refer to Barus [12] namely each flour such as sweet potato flour, carrot flour and yellow pumpkin flour with a concentration of 10% put in a tray and mixed with commercial feed as much as 100 grams at each concentration of treatment and stirred evenly, feed added with CMC as an adhesive by spraying on the feed and stirred until it sticks and evenly, then continued with the drying process. After drying the feed is wrapped in a zip lock according to the treatment and marked with label paper.

2.1.2 Preparation of test fish maintenance media

The preparation is to prepare an aquarium with a size of 39.8 cm x 25.4 cm x 28 cm as many as Fifteen (15) pieces, then the aquarium is cleaned and rinsed with clean water and then dried for 24 hours. After drying the aquarium in the contents of the water up to a height of 16 cm with a volume of Fifteen (15) liters of water and equipped with an aeration installation.

2.1.3 Maintenance of test fish

Every day the test fish will remove dirt or leftover feed that causes the water to become dirty, therefore it is carried out daily. Water reception is done after the aerator is turned off, it is done so that dirt and fish food waste go down to the bottom of the maintenance container so that the

call is easier to do. The amount of water volume that will be responded to $\pm 10\%$ of the initial volume. Then, the wasted water will be replaced again with clean water as much as the amount of water volume wasted during the call-off process.

2.2 Data Analysis

Survival and growth data are analyzed with analysis of variance (ANOVA) test F with a confidence level of 95%, then if there is a difference between treatments followed by duncan double distance test. The color improvement data was analyzed using the Kruskal-Wallis analysis, then if there was a difference between treatments, the Z test was conducted with a confidence level of 95%. Water quality data is analyzed descriptively.

2.2.1 Color brightness

How to calculate the intensity of color in fish is to compare the increase in color in each treatment and repeat by observing the color changes in sampling fish and seeing the increase in weight in M-TCF (Fig. 1). Observations were made on the 0th, 10th, 20th, 30th, 40th, 50th and 60th days. Visual observations were made by three panelists who were not colorblind by comparing the original color of the fish on color measuring paper that had been given a weighting value. Ratings start from the smallest 1 to the largest score of 7 with color gradations from orange to dark red.

2.2.2 Heavy growth

The weight growth formula used based on Effendie [13] is as follows :

$$W = W_t - W_0$$

Information :

- W : Average weight growth of fish (grams)
- W_t : Final fish weight growth (grams)
- W_0 : Early fish weight growth (grams)

2.2.3 Long growth

The long growth formula used based on Effendie [14] is as follows :

$$W = P_t - P_0$$

Information :

- P_m : Average length growth (cm)
- P_t : Final length growth (cm)
- P_0 : Initial length growth (cm)








No.	Toca Colour Finder TCF	Description
1.		Score 1 TCF Code 0614
2.		Score 2 TCF Code 0814
3.		Score 3 TCF Code 0615
4.		Score 4 TCF Code 0815
5.		Score 5 TCF Code 0616
6.		Score 6 TCF Code 0816
7.		Score 7 TCF Code 0916

Fig. 1. Toca Colour Finder (TCF)

2.2.4 Survival rate (SR)

The survival rate formula used based on the Effendie formula [13] is as follows:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Information :

- SR : Survival Rate (%)
- N_t : Number of fish that live at the end time (tail)
- N_0 : Number of fish that lived at the beginning (tail)

2.2.5 Water quality

The water quality observed at the time of the study for supporting materials was pH,

Temperature and Dissolved Oxygen. Observations were made on the 0th, 10th, 20th, 30th, 40th, 50th and 60th days.

3. RESULTS AND DISCUSSION

3.1 The Brightness Level of the Goldfish Color

Research on discoloration in goldfish was observed in three different parts of the body, namely the color on the fish head, fish body and goldfish tail. Color enhancement research on goldfish was done visually by comparing the original color in fish with the Toca Color Finder (TCF) which was rated with a value range of 1 to 7. The observations were made by three panelists with criteria not colorblind.

Table 1. Average Increase in goldfish TCF score

Treatment	Color TCF Score Improvement (60th-0th)		
	Head	Body	Tail
A (Negative Control)	2,4 ^a	2,6 ^a	1,9 ^a
B (Positive Control)	2,6 ^{abc}	2,7 ^{ab}	2,0 ^{ab}
C (Sweet Potato Flour)	2,9 ^{abc}	2,9 ^{ab}	2,1 ^{ab}
D (Carrot Flour)	2,9 ^{bc}	3,3 ^{ab}	2,1 ^{ab}
E (Pumpkin Flour)	3,2 ^c	3,3 ^b	2,5 ^b

Description : Different notations show a noticeable influence $P < 0.05$ (Double distance test Z)

Based on observations made during 60 days of research with 5 treatments, the average color change was obtained in The Oranda Goldfish. The results of the count showed that all test fish experienced an increase in color brightness. Based on Table 1, it was found that the highest increased brightness value of fish color was found in treatment E giving yellow pumpkin flour as a natural source of β -Carotene, namely 3.2 on the head, 3.3 on the body and 2.5 on the tail while for the lowest value found in treatment A or negative control, namely 2.4 on the head, 2.6 on the body and 1.9 on the tail.

Based on the results of research shows that the addition of sweet potato flour, carrot flour and yellow pumpkin flour can affect the orange color on the head, body and tail of the goldfish. The increase in color score indicates the influence of the treatment of yam flour, carrot flour and yellow pumpkin flour on the orange color of goldfish. The development of the color of goldfish based on TCF from the beginning of the study to the end of the study can be seen in the following graph.

Based on the graph above about the increase in color on the head of the oranda goldfish found that there was an increase in color scores. At the beginning of the research got a score with a range of 2.5 - 2.7 and at the end of the research got a score of 5.1 - 5.7 with the best treatment on the E treatment with the addition of pumpkin flour.

Based on the graph above about the increase in color on the body of the oranda goldfish, it was found that there was an increase in color scores. At the beginning of the research got a score with a range of 1.9 - 2.0 and at the end of the research got a score of 4.5 - 5.3 with the best treatment on treatment E with the addition of pumpkin flour.

Based on the graph above about the increase in color on the tail of the oranda goldfish found that there was an increase in color scores. At the beginning of the research got a score with a range of 2.3 - 2.6 and at the end of the research got a score of 4.3 - 5.1 with the best treatment on treatment E with the addition of pumpkin flour.

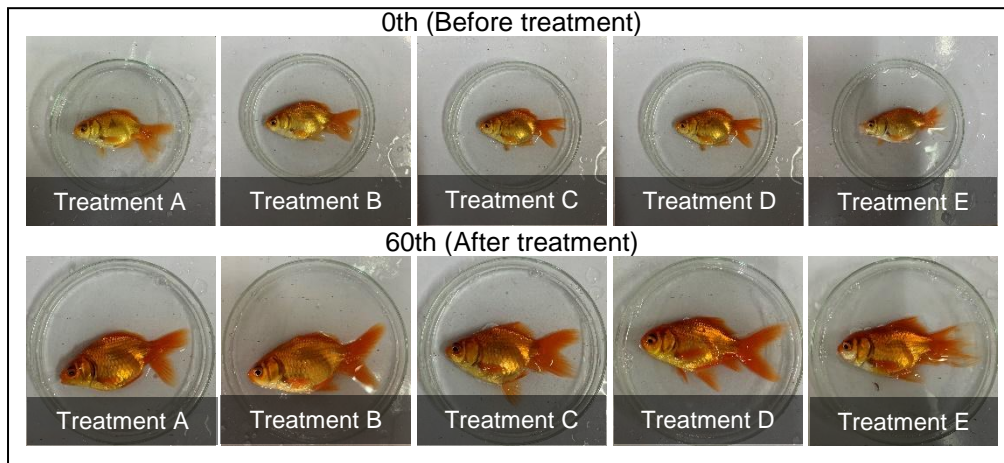


Fig. 2. Observation of the brightness level of goldfish color

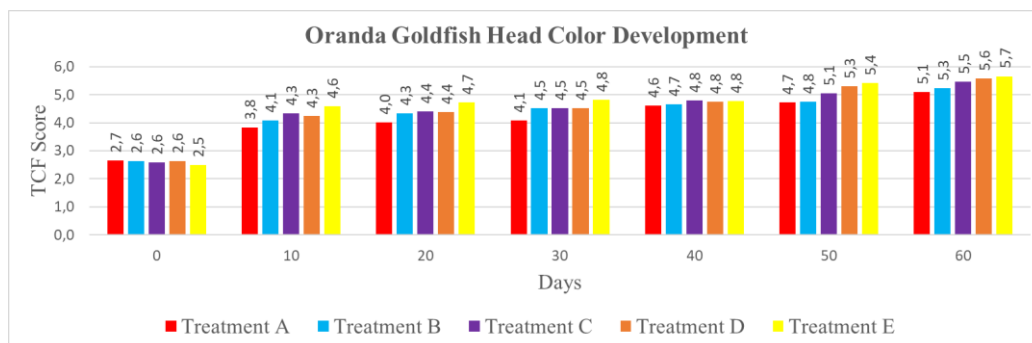


Fig. 3. Oranda goldfish head color

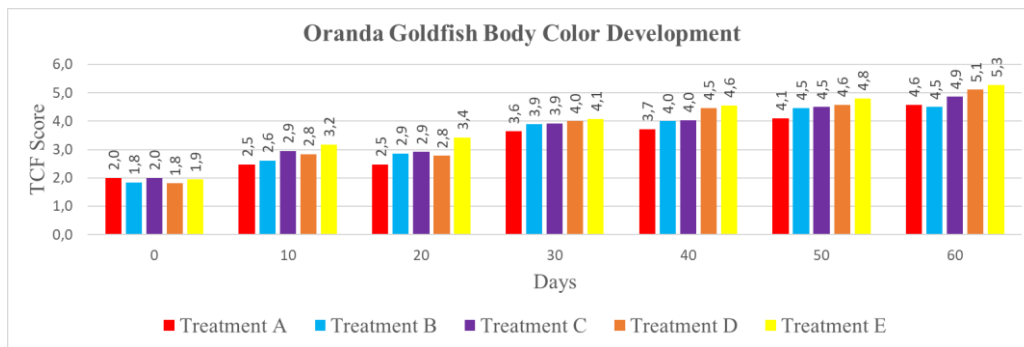


Fig. 4. Oranda goldfish body color development

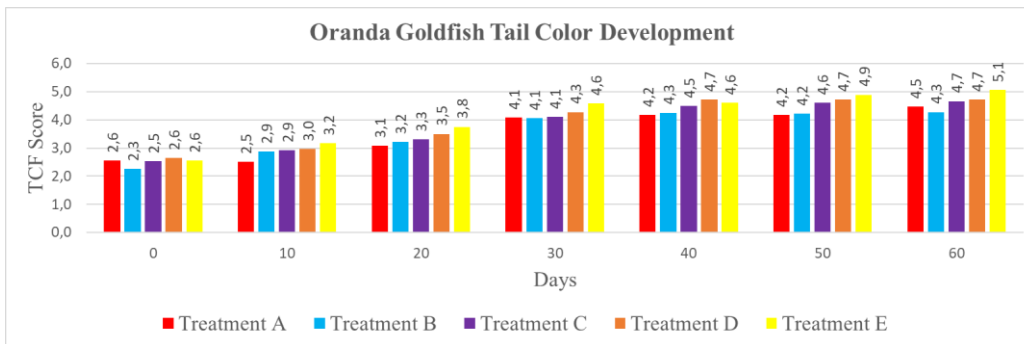


Fig. 5. Oranda Goldfish Tail Color Development

The highest color increase based on graph is occurring in Treatment E with the addition of pumpkin flour. This is suspected because the absorption ability of goldfish and the amount of β -Carotene content contained in yellow pumpkin has been sufficient in the pigment absorption process so that the pigment absorption process takes place well and produces better color appearance [10]. This is supported by the statement [15] that fish take longer to break down carotenoid ingredients into color pigments if the amount of carotenoid content contained in the feed is more and more, and vice versa.

Lesmana and Sugito [4] stated that the discoloration in fish depends on the amount of feed, especially the component of color ingredients in the composition of the feed which indicates that the greater the dose of carotenoids in the feed, the greater the value of chroma. Nasution [5] stated that the greater the content of carotenoids in the body of fish, the brighter the color in fish.

The process of overhauling carotenoids contained in feed ingredients into β -Carotene enters the bloodstream after 24 hours, even in Brown Trout Fish carotenoids are still found in the blood after 72 hours [16]. Carotenoids that

enter the body will be synthesized into certain color pigments. Like other food substances, pigments will be carried along with the bloodstream and then the pigment is deposited in chromatophores contained in the dermis. According to Storebakken and Hong [16] carotenoids that have been synthesized into pigments will be placed on xanthophores (yellow color) and eritophores (red or orange).

This is also reinforced by the statement of Kurniawati et al. [15] that hormones have limits in the ability to work. Giving excessive sources of pigment can decrease the work of hormones, added by (Satyani, 1997 Kurniawati et al. 2012) [17] that if the administration of excess carotenoids, at some point will not give a better color change and may even lower the color value.

3.2 Goldfish Growth

Growth in living things can be defined as an increase in weight and length in a given time. As for one of the factors that affect the growth of fish is good feed. Good feeding can increase the growth rate of fish. The observations showed that the administration of various flours as a natural

source of β -Carotene in feed showed varying results.

The results of the fingerprint analysis of the variety of the Analysis of Variance (ANOVA) test, showed that the treatment of natural β -Carotene sources for 60 days did not have a real influence ($P>0.05$) on the growth of absolute length and absolute weight of oranda goldfish as stated in Table 2.

Table 2. Average measurement results of the length and weight of goldfish

Treatment	Absolute weight growth (g)	Absolute long growth (cm)
A	2,3 ^a	1,5 ^a
B	3,2 ^a	1,5 ^a
C	3,6 ^a	1,7 ^a
D	3,7 ^a	1,4 ^a
E	3,7 ^a	1,7 ^a

Growth data on the yellow pumpkin flour feeding treatment received the highest value including absolute weight growth of 3.7 g and absolute length growth of 1.7 cm. The low growth value of oranda goldfish on the treatment of the addition of various natural sources of β -Carotene can be caused by several factors. According to Soleha et al. [18] the crude fiber content in feed is increasing in line with the addition of the concentration of a mixture of sweet potato flour, carrot flour and yellow pumpkin flour as a natural source of β -Carotene. This is in line with the

statement of Solihah et al. 2015 that the content of carotenoids in feed has no effect in the process of fish growth. According to Azuri [19] that carotenoids that have been added in the feed do not affect the growth process of goldfish. Carotenoids given to ornamental fish will be more widely used for the process of improving the quality of their color.

3.3 Survival Rate (SR)

Survival is a comparison between the number of organisms living at the end of the study with the number of initial organisms at the time of distribution expressed in the form of percent, the greater the percentage value indicates the more organisms that live during maintenance [14]. Survival can be used as a benchmark to determine the tolerance and ability of cultivated organisms to live. The survival rate of goldfish from observations for 60 days in each treatment can be seen in the following graph (Fig. 6).

3.4 Water Quality

Water quality is one of the things that need to be considered in aquaculture activities because it greatly affects the growth and development of fish. Good water sources in aquaculture activities should meet the criteria for water quality standards so that fish can grow and develop properly. The results of water quality measurements in maintenance containers during the study include Temperature, pH and Dissolved Oxygen presented in Table 3.

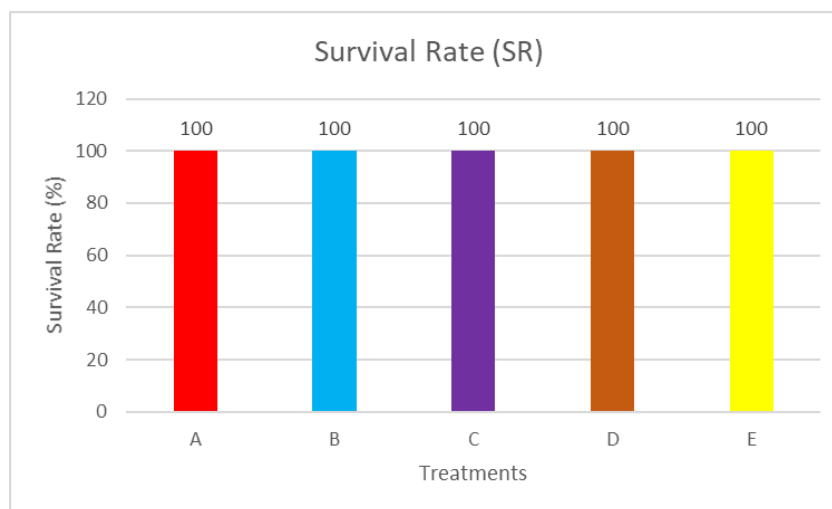


Fig. 6. Goldfish Survival rate

Table 3. Water quality

Treatment	Water quality parameter range		
	Temperature (°C)	pH	Dissolved oxygen (mg/l)
A	24,4 – 27,5	6,89 – 7,62	5,8 – 7,2
B	24,3 – 28	6,89 – 7,44	5,8 – 8,0
C	24,4 – 27,3	6,88 – 7,62	5,8 – 8,0
D	24,4 – 27,3	6,92 – 7,62	5,8 – 7,3
E	24,2 – 27,5	6,91 – 7,61	5,9 – 7,9

Water quality observations in this study were conducted every ten days. Research on water quality in this study is still within the limits of eligibility. The results of temperature observations during the study ranged from 24.2°C – 28°C with optimum temperature values according to Manurung and Basuki [20] ranging from 22°C – 30°C, the results of pH observations during the study ranged from 6.88 – 7.62 with the optimum value of pH according to Diansyah et al. [21] which is 6–9 while for do observations during the study ranged from 5.8 – 8.0 mg / L with the optimum value of Dissolved Oxygen according to Solihah et al. [22] which is >3 mg / L.

4. CONCLUSION

The results showed that giving yellow pumpkin flour as a natural source of β -Carotene added to the feed can increase the brightness of the color of the goldfish with an increased score on the head of 3.2, on the body 3.3 and on the tail 3.5 but does not support optimal growth, namely with an absolute length growth of 1.7 cm and an absolute weight growth of 3.7 grams. The results of water quality observations during the study were temperatures ranging from 24.2°C – 28°C, pH ranging from 6.88 – 7.62 and Dissolved Oxygen ranging from 5.8 – 8.0 mg / L.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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