

Research Article

Xanthophore Distribution of Nemo (*Amphiprion ocellaris*) Skin Based on various concentrations of carotenoid sources.

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Abstract

The decreased color intensity of Nemo is related to the amount of carotenoid concentration in the xanthophore of Nemo's skin. Therefore, efforts are needed to maintain stable carotenoid production. Tomato is one of the most abundant carotenoid producers. The utilization of carotenoids can maintain the morphological color of aquatic organisms. This study aimed to analyze the effect of carotenoid compounds on total carotenoid content, the size, and distribution of xanthophores on Nemo skin. A total of 60 Nemo with an average initial weight of 0.84 ± 0.14 g and an average length of 2.95 ± 0.08 cm. The research was conducted using a completely randomized design (CRD), consisting of four carotenoid concentrations in addition to fish pellet, 0%, 0.5%, 1%, and 1.5% with three replications. The results showed that the optimum dose of carotenoid compounds in increasing the total carotenoid content in nemo fish skin is 1.5%. Carotenoids added to the fish feed affected the distribution and maturity of xanthophore. However, it did not affect the size of the xanthophore.

1. Introduction

Amphiprion ocellaris is a type of marine ornamental fish known as clownfish or Nemo fish (Luo et al., 2021). Nemo are very popular because they have striking bright colors that are a combination of black, white, and orange. The problem with Nemo is that there is a decrease in morphological color. This is caused by a deficiency in nutrients related to carotenoids, which are responsible for fish morphological color. In the frying phase, there is a transition from natural to artificial feed. Artificial feed does not contain carotenoids. Because carotenoids cannot be synthesized by the fish body, they can only be obtained through external feed consumption.

Generally, color degradation in fish is caused by several factors such as unfavorable environmental conditions (Kumar et al., 2022; Díaz-Jiménez et al., 2021), stress (Liu et al., 2022; Vissio et al., 2021), disease (Marudhupandi et al., 2022), nutrient deficiencies related to pigmentation (Makri et al., 2021; Ebenezzar et al., 2020), and aging (Díaz-Jimenez et al., 2021). As demonstrated by Luo et al. (2021), color changes in fish are divided into two, namely physiological color changes and morphological color changes. Color changes in fish that occur rapidly in seconds to hours are referred to as physiological color changes, usually achieved by the movement of pigments or nano-sized structures in specialized cells called chromatophores (Ligon and Cartney, 2016). Variations in the number of skin pigment cells, as well as changes in density, morphology, and distribution, which are influenced by the amount and composition of feed containing carotenoid sources, are referred as morphological color changes (Fang et al., 2022). This process is slow, taking days or weeks to complete, with a long-term stimulus after comprehensive adaptation to specific conditions such as background patterns, feed nutrition, UV light, and social interactions (Cal et al., 2018). Variables such as environment and feed nutrition can be modified in captive aquaculture to ensure that fish can maintain their original characteristics for as long as possible.

The decrease in color intensity in Nemo is related to the amount of carotenoid production in the xanthophore of Nemo's skin. Carotenoids are easily oxidized and damaged due to heat, which affects their stability in the product. Due to the presence of highly conjugated double bonds in its structure, a bioactive chemical such as carotenoid is easily oxidized and susceptible to light, heat, and singlet oxygen during food processing and storage, resulting in the loss of both its color and bioactivity. Therefore, efforts are needed to protect carotenoids from damage due to heat, oxidation, light,

or isomerization by using an encapsulation system (Alfionita et al., 2022). Xanthophores are pigment cells that contain xanthosomes that are responsible for the orange, yellow, and red colors of fish. The process of xanthosomes formation on fish skin begins with the absorption of carotenoid compounds found in fish food, such as plankton, fruits and aquatic plants. These carotenoid compounds are then transported to fish skin cells. Fish skin cells contain chromatophores, which are cells that contain pigments that play a role in giving color to fish skin. Xanthophore is one type of chromatophore. Xanthosomes and xanthophores are pigment structures found in some animals, especially fish and amphibians (Djurdjevic et al., 2015). Xanthosomes are composed of carotenoid pigments, while xanthophores are pigment cells that contain xanthosomes (Hirata et al., 2003). The carotenoids stored in xanthosomes have red, orange, and yellow colors and can be altered by several factors such as food, environment, and fish health. While xanthophores function to disperse and organize carotenoid pigments in fish skin. The color found in fish is related to carotenoids (Nhan et al., 2019; Tran et al., 2022). Three main pigments are responsible for coloration in all vertebrates: melanin, pteridine, and carotenoids. Melanin and pteridine can be synthesized while carotenoids cannot (Micah et al., 2022). Carotenoids are natural pigments that can be found in animals, plants, and algae (Maoka, 2020) It has a spectrum of yellow, orange to red. Based on their structure, most carotenoids show maximum absorption at around 450 nm of canthaxanthin (Langi et al., 2018; Stahl and Sies, 2003; Riaz et al., 2021). Xanthophore also plays a role in protecting carotenoids from damage such as free radicals or excessive light. Carotenoids have important roles in animal life. As physiological functions as vitamin A precursors, antioxidants, modulators of cell development (Nakano, 2020; Meléndez-Martínez, 2019; Lim et al., 2023), and pigmentation (Merhan, 2017). Tomato is one of the most abundant sources of carotenoids. The carotenoid found in tomato is lycopene which is responsible for the red color in tomato fruit (Kumar et al., 2020; Ebenezzar et al., 2020; Bao et al., 2019).

There have been many studies on pigmentation by adding carotenoid compounds to fish feed to improve color quality. Carotenoids are stored in xanthosomes and then distributed in the form of xanthophores to the fish skin. Thus, xanthophore size is thought to be related to the color phenotype of the fish. Previous research has discussed the number, morphological structure, and distribution of melanophores in *Misgurnus anguillicaudatus* (Sheng et al., 2021). However,

research on carotenoid feeding related to the size and distribution of xanthophores as one of the Nemo fish's chromophores that produce yellow, orange, and red colors is still limited. The effect of carotenoids added to fish feed on the distribution and size of xanthophore in the skin of Nemo fish.

2. Materials and Methods

2.1 Materials

This research was conducted from July to December 2022 at the Marine Aquaculture Center, Ambon Mollucca Province, Indonesia. Nemo used during the research was obtained from the hatchery of the Ambon Marine Aquaculture Center, Maluku province. There were 60 fish and each aquarium contained five fish with an average initial weight and length of 0.84 ± 0.14 g and 2.95 ± 0.08 cm, respectively. The fish feed used pellets combined with tomato extract as a source of carotenoids. Doses of carotenoids given were 0%, 0.5%, 1%, and 1.5%. Each treatment had three replicates. Thus, this study consisted of 12 experimental units. Maintenance lasts for 50 days.

2.2 Method

Fish were put into the aquarium with a stocking density of 1 fish/L. Acclimatization was carried out for seven days for fish to adapt to their new environment. During the acclimatization process a feed with 40% protein content was given as usual. After the acclimatization process, treatment feeding was carried out by adding carotenoid extract of 0.5%, 1%, and 1.5% to pellets. Feeding was applied three times a day (08.00 a.m., 12.00 p.m., and 5.00 p.m.). Feeding was done as much as 5% of the weight of the fish. The marine water used came from Ambon waters with a water quality maintained in an optimum range of salinity (30 - 35 ppt), temperature (27-29°C), pH (6.7-8.6), DO (5.0-5.5 mg/L), nitrite (0.006-0.01 mg/L), and ammonia (0.001-0.038 mg/L). During maintenance, water was flushed twice a day to keep the water clean. Flushed using a ½ inch hose by sucking up the dirt that settles on the bottom of the aquarium.

2.3 Sampling and Parameter Analysis

2.3.1 Total carotenoids of tomato extract and fish feed

Carotenoid analysis was conducted to determine the number of carotenoids in tomato and feed by absorbance method (Zhao *et al.*, 2019). Carotenoid analysis of tomato extract and feed was conducted at the beginning of maintenance. The absorbance in the

spectrophotometer was at a wavelength of 450 nm. The carotenoid content of the Nemo feed was calculated using the following formula:

$$C = A_{450} \times EV / E^{1\%} 1 \text{ cm} \times SW \quad \dots\dots\dots(\text{Eq 1})$$

Where:

C = Total carotenoid pigment concentration (ppm)

V = Volume of extract (mL)

A = Maximum absorbance at 450 nm wavelength

E = Coefficient of extension (absorbance) of 1% standard in acetone and 1 cm cuvette tube = 2200

B = Weight of extracted sample (g wet weight)

2.3.2 Pigment distribution

Nemo skin was taken from each treatment, washed thoroughly, and placed on a slide without wrinkles, then observed using a Discovery v.12 stereo microscope. The skin was observed in the dorsal part of the orange area. Observations were made at the beginning and the end of maintenance with a total of 15 samples. This method refers to the results of research done by (Sheng *et al.*, 2021). The diameter of the xanthophore was measured using the ImageJ software.

2.3.3 Fish skin histology

To determine the histology of fish skin, samples were taken by dissecting the fish and then taking its abdomen, body, and tail. The meat attached to the skin was cleaned by scraping. The organ was then preserved with 10% formalin. The procedure of making histopathology preparations in fish is based on the method of Sheng *et al.* (2021) with several stages, namely fixation, dehydration, paraffination, deparaffination, staining, dehydration, and mounting. Histology analysis was carried out at the beginning and the end of maintenance with a total of 8 samples.

2.3.4 Water quality

During the research, water quality was checked, namely temperature, pH, DO, salinity, and ammonia. Checking the temperature, salinity, and DO of the maintenance media was done twice a day at 07.00 (a.m.) and 17.00 (p.m.) using Hanna H198192. pH measurements were taken in the morning using a pH meter. Meanwhile, a spectrophotometer took ammonia measurements at the beginning, middle, and the end of maintenance.

2.4 Data analysis

Data obtained in the study included fish skin

carotenoid content, pigment distribution, histology, and physical water chemistry. Fish carotenoid content was analyzed using analysis of variance (ANOVA). Duncan's multiple follow-up tests were conducted because there was a significant effect. To determine the closeness of the relationship as a response to the treatment, the correlation regression technique was used. Pigment distribution was analyzed using the ImageJ application and continued with Origin software. Physical parameters of water chemistry were analyzed descriptively based on the viability of Nemo fish.

3. Results and Discussion

3.1 Results

3.1.1 Total carotenoids of tomato extract and fish feed

The extract yield was calculated based on the ratio of final weight (weight of the extract produced) to initial weight multiplied by 100%. The yield value of tomato extract using acetone produced 28.12 g of extract from 500 g of simplisia or 2.81%. The total carotenoid test result in tomato extract was 11.0 µg/2gr.

The average carotenoid content in the fish feed added with carotenoids are presented in (Table 1). The results showed that there were significant differences. This indicates that Nemo fish feed contains carotenoids as a constituent of Nemo fish color. The highest average amount of carotenoids was found in the 1.5% carotenoid dose of 1.0191 ± 6.66 ppm. While the lowest average amount of carotenoids was found in the 0% carotenoid dose which was 0.0006 ± 20.20 ppm. The high number of carotenoids in fish feed is due to the high content of carotenoids in tomatoes. While the low number of carotenoids in the carotenoid dose of 0% (control) is due to the absence of carotenoids in fish feed.

3.1.2 Size and pigment distribution

At the beginning of maintenance, Nemo was given feeds without the addition of carotenoids. The skin color of Nemo fish at the beginning of maintenance was yellow (Figure 1). Nemo has three chromatophores namely yellow xanthophores, black melanophores, and white iridophores. Based on the observation the pigments distribution of xanthophores have an oval shape and are like tubes. The distribution of xanthophore in the skin of Nemo does not spread evenly and looks small. Compared to melanophore, it looks more numerous and evenly distributed. The shape of melanophore is round like a flower.

Meanwhile the iridophores look like a sheet that emits light. Based on pigment placement, xanthophore are at the top, melanophore in the middle, and iridophores at the bottom. Nemo feeding with the addition of carotenoids showed a color transition of the Nemo culture from yellow to red (Figure 2).

Based on color intensity, it is brighter than the previous maintenance. The distribution of xanthophore pigments in the 0% carotenoid dose looks small and unevenly distributed. Whereas in carotenoid doses of 0.5%, 1%, and 1.5%, the distribution of xanthophore is very abundant, almost all areas of fish skin have xanthophore, but the distribution is uneven. In contrast to melanophore and iridophores, the distribution is evenly distributed and patterned. This indicates that fish feed added with carotenoids affects the distribution of pigments and intensity of Nemo skin color based on the analysis of the average diameter of the xanthophore with various doses of carotenoids (Figure 3). Based on R-squared (R²), treatment 0%, 0.5%, 1%, and 1.5% were 0.841, 0.659, 0.837, and 0.757 respectively. Thus, the average diameter of xanthophores with various doses of carotenoids shows that the administration of carotenoids produces xanthophores with a high variable numbers and diameters at any given time. So, the regression line available does not explain all average variations in diameter distribution xanthophore. This is thought to correlate with the stages of xanthosome development. Thus, the greater amount of carotenoid deposit in the fish skin will show different variation in the production of the xanthophores' diameter produced based on xanthophore development towards mature xanthophore.

3.1.3 Histology

Based on histology results in Nemo fish skin, melanophores are distributed in the epidermis and dermis (Figure 4). Generally, xanthophores are in the upper layer followed by melanophores and iridophores (Hirata et al., 2003). However, xanthophores were not found in epidermis nor in dermis. It is thought that xanthophores contain fat-soluble carotenoids and pigments are easily dissolved in the process of dehydration and repeated washing.

3.1.4 Water quality

The results of temperature measurements of Nemo fish rearing media ranged from an average of 28.28-30°C (Table 2) which is classified as optimal. This is in line with the study of Pratiwi et al. (2022) who stated that the optimal temperature of fish rearing media ranges from 25-30°C. Meanwhile pH of the maintenance media ranges

from 7.75 - 7.1, consistent with earlier study by [Chambel et al. \(2015\)](#), Nemo can live in waters with acidity levels ranging from 6-9. The salinity of the maintenance media is 32 ppt as demonstrated by [Pietoyo et al. \(2020\)](#) who stated that the salinity of the maintenance media is still in the optimal range. The *dissolved oxygen* in the maintenance media was 5.16 mg/L. According to [Ghosh et al. \(2011\)](#), a good oxygen concentration for cultivation purposes ranges from 5-9 mg/L. Ammonia level in the maintenance media was 0.001 mg/L, which makes it very suitable for the life of Nemo.

3.2 Discussion

The advantage of maceration process is that it is easy and cheap to do with simple tools. Acetone solvent

has a relatively low yield. According to [Pataro et al. \(2019\)](#), the ideal yield value is >70%. Meanwhile, [Purnomo et al. \(2020\)](#) suggested that the effectiveness of extraction is influenced by the type of solvent used, the particle size of simplisia, the method, and the duration of extraction. Carotenoid content in feed is one of the factors that affect the morphological color of fish. Efforts to increase the brightness in fish color by adding carotenoids to fish feed are considered very effective because fish will absorb the pigment source directly from the feed. When the tomato extract is mixed with fish feed, it can be seen that the fish feed absorbs the tomato extract, indicated by the turning color of the fish feed into red. After the fish feed is crushed, the inside of the fish feed is also red.

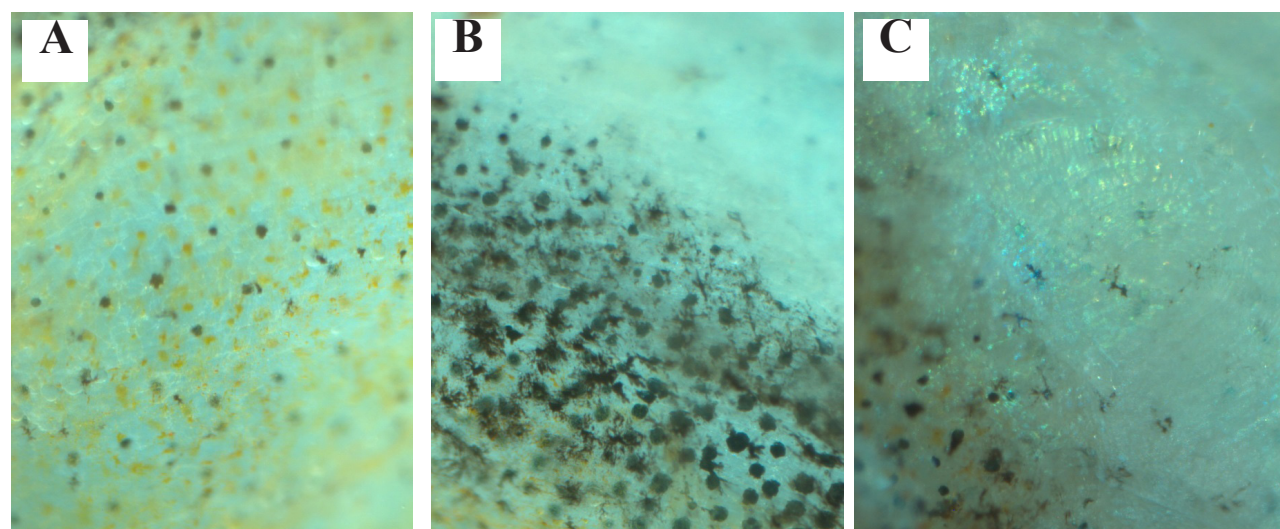


Figure 1. Pigment distribution on the surface of *A. ocellaris* body parts. (A). Xanthophore (yellow), (B). Melanophore (black), and (C). Iridophores (reflective sheet). Magnification 150

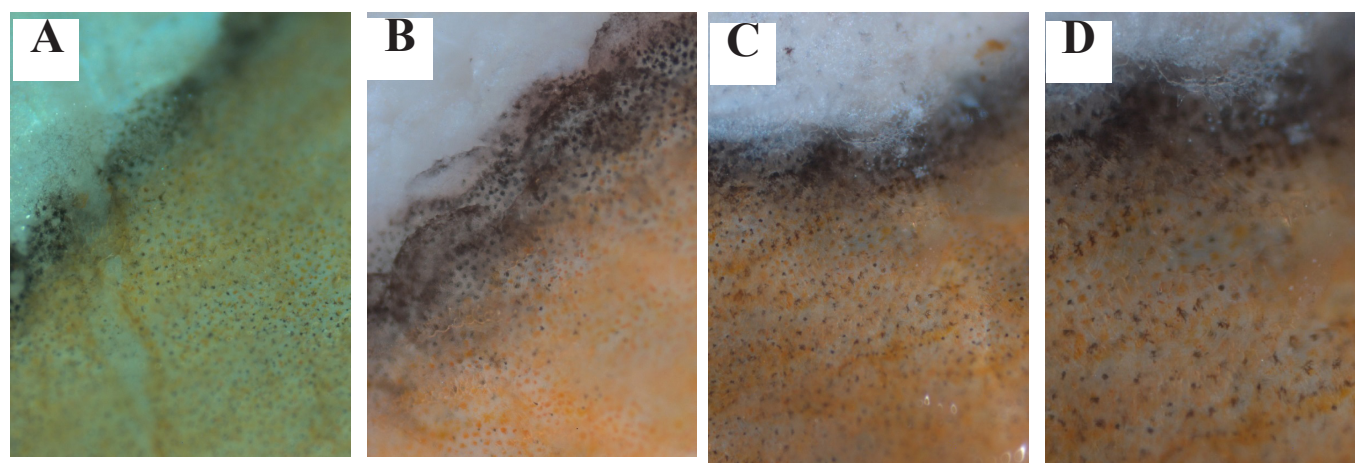


Figure 2. Pigment distribution on the skin surface of *A. ocellaris* body parts. Yellow and orange areas are xanthophore. A (control), B (0.5%), C (1%), and D (1.5%). Magnification 60

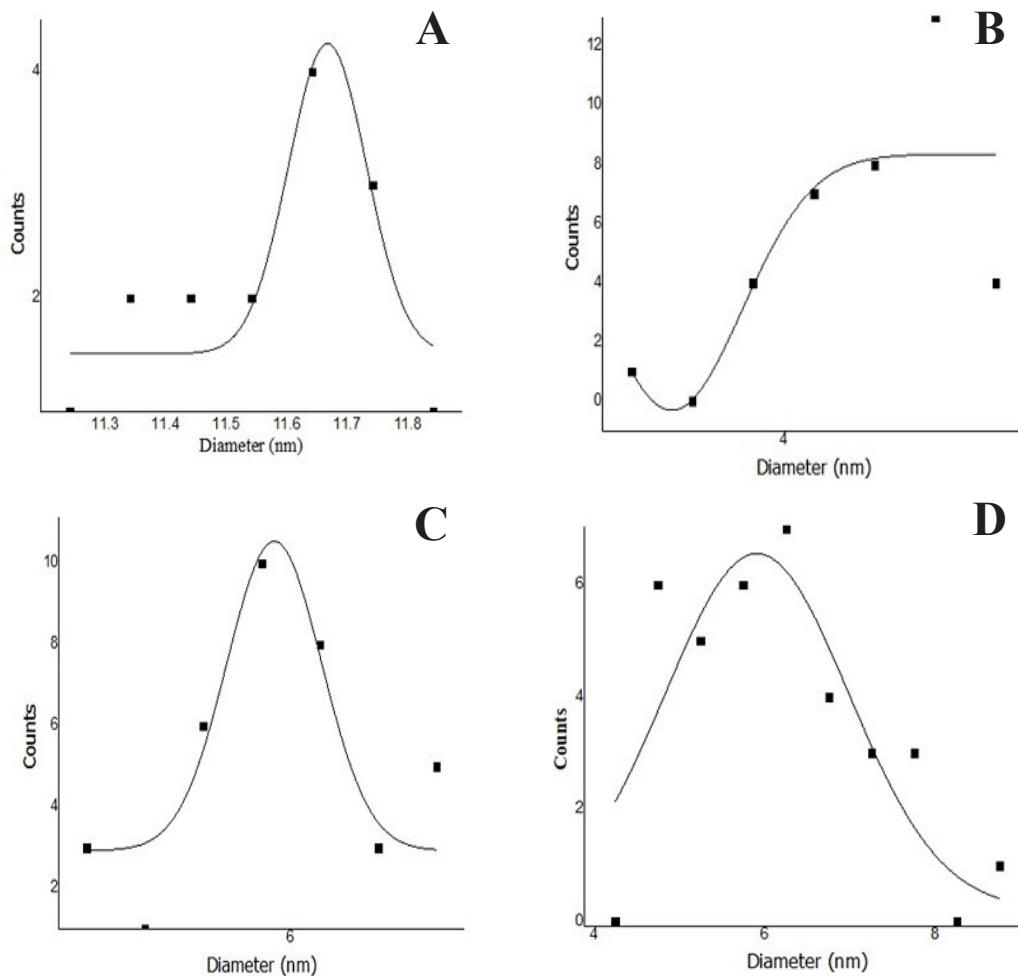
Table 1. Test results of feed that have been added with carotenoids

Carotenoid dosage (%)	Average carotenoids count (ppm)
0	0.0006 ± 20.20 ^a
0.5	1.0152 ± 18.19 ^b
1	1.0166 ± 1.15 ^{bc}
1.5	1.0191 ± 6.66 ^c

Description: Different subscript indicates significant difference between treatments at a 5% level (P<0.05).

Table 2. The results of physicochemical measurements of water in Nemo maintenance

No	Parameters	Average measurement result range	Standard Range	Reference
1	Temperature (°C)	28.28-30	27.9 - 29.6	(Pratiwi et al., 2022)
2	pH	7.75 – 7.1	33-35	(Chambel et al., 2015)
3	Salinity (ppt)	32	6.7 - 8.6	(Pietoyo et al., 2020)
4	DO (mg/L)	5.16	5.0 - 5.5	(Ghosh et al., 2011)
5	Ammoniac (mg/L)	0.01	< 0.1	(Diaz-Jimenez et al., 2021)

**Figure 3.** The number and diameter of xanthophore distribution on Nemo fish skin. A (control), B (0.5%), C (1%), D (1.5%)

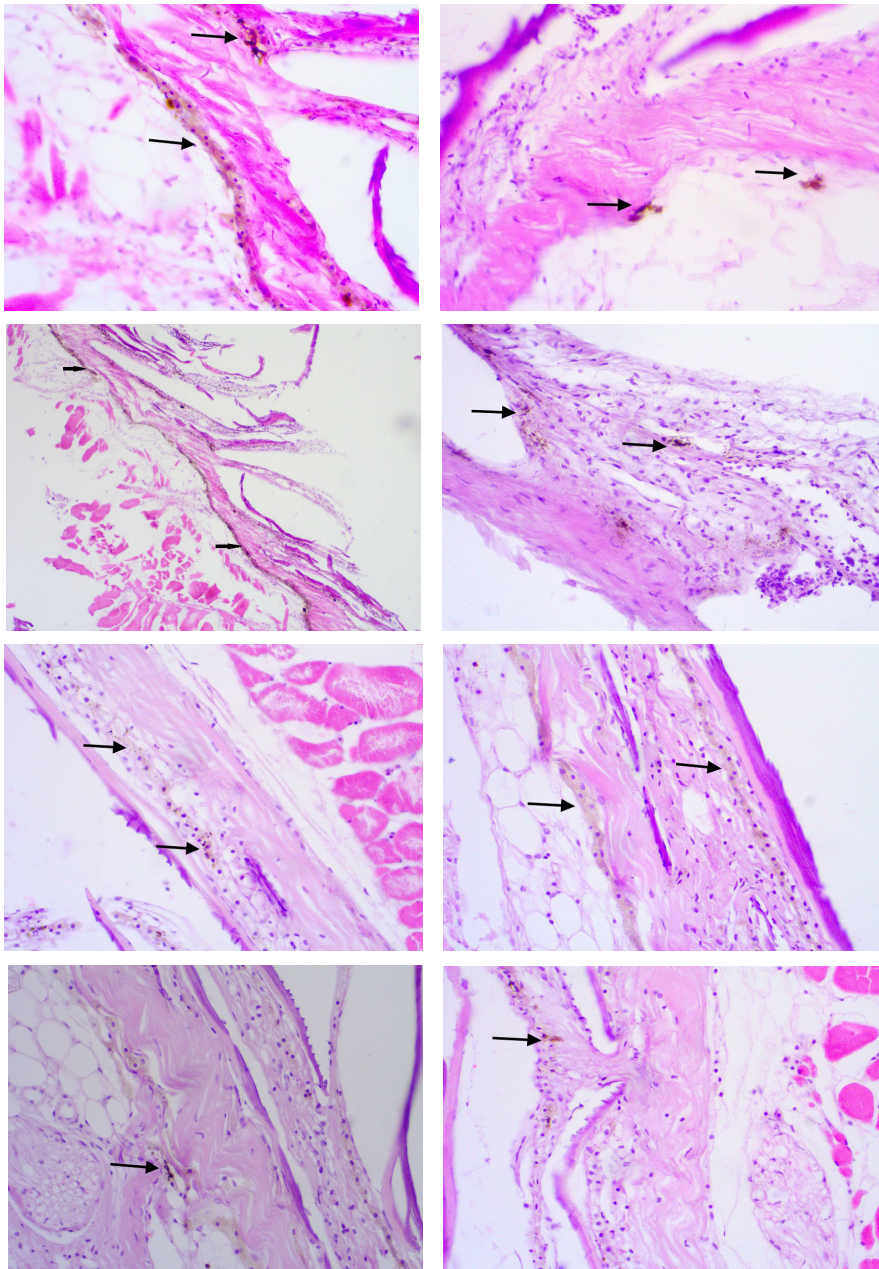


Figure 4. Distribution of melanocytes is more in the dermis than in the epidermis (first-row epidermis and second-row dermis) with 40x magnification. A (control), B (0.5%), C (1%), and D (1.5%).

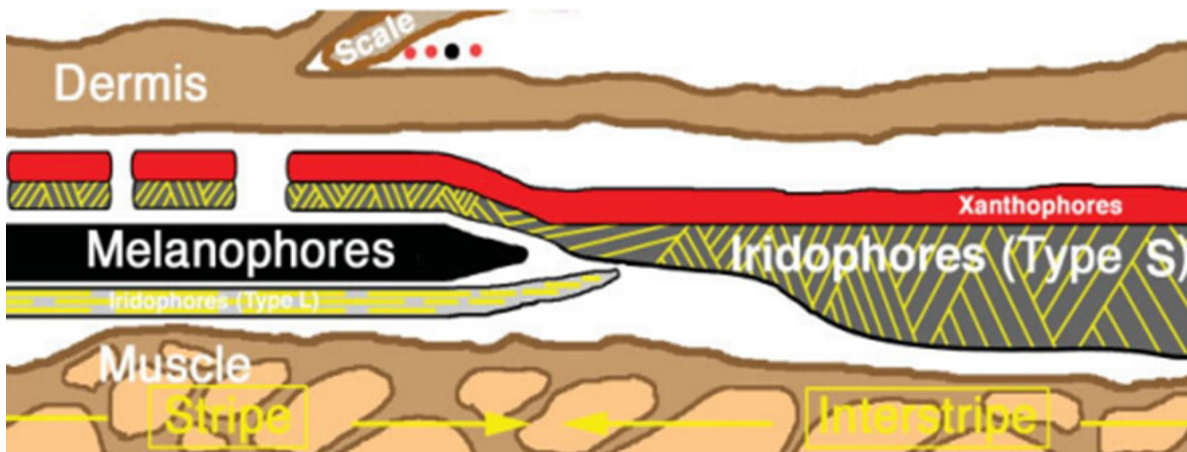


Figure 5. Pigment cell distribution in the dermis and hypodermis (Source: Hirata *et al.*, 2003)

Color patterns in fish are diverse because fish have various color types. Nemo contains three types of pigment cells, namely melanophores, xanthophores, and iridophores (Moore et al., 2023). Since Nemo is more dominant in orange/red color, xanthophores (orange) are more abundant in Nemo skin compared to melanophores (black) and iridophores (white). The process of pigment synthesis in chromatophores, transportation, and deposition plays an important role in the formation of final skin color phenotype (Tian et al., 2022). In addition, the size distribution of chromatophores is one aspect of determining the pigment pattern (Fang et al., 2022). Based on the results of microscopic analysis at the end of maintenance (50 days) after giving carotenoids to Nemo, there was a change in color from yellow to reddish-orange and the distribution of xanthophore increased, which also resulted in a brighter fish color (Figure 2). This correlates with the intake of carotenoids obtained through the Nemo feed. Different doses of carotenoids resulted in differences of fish skin color with no carotenoids. Carotenoids are organic compounds consisting of conjugated carbon skeletal bonds that give yellow to red color to various types of plants, microorganisms, and animals. This includes fish skin (Zia-Ul-Haq, 2021). Color transfer in fish is related to proteins and enzymes (Ahi et al., 2020; Zia-Ul-Haq, 2021). Carotenoid compounds are converted into simpler forms so that they are easily converted by enzymes contained in the xanthophore (Maoka, 2020; Djurdjevic et al., 2015). Such as mono galactosyl lutein or galactosyl beta-carotene (Gedi et al., 2019). Enzymes contained in the xanthophores will work to produce carotenoids in xanthosome (Djurdjevic et al., 2015). Xanthosomes are placed where carotenoids are stored or produced and distributed in the form of xanthophores in fish skin. This process involves the breakdown and incorporation of carotenoid compounds as well as the removal of certain oxygen groups to produce different xanthosome colors such as astaxanthine, or zeaxanthine, which gives fish skin its red color (Rebelo et al., 2020; Toomey et al., 2022; Nusslein-Volhard and Singh, 2017). Carotenoids are derived from tomatoes. Tomatoes have many excellent bioactive contents, especially carotenoids, namely lycopene (red) and β -carotene (orange / red) (Pinela et al., 2016). Fang et al. (2022) reported that skin color in fish is related to the type and concentration of carotenoids in the skin, based on the absorption and deposition process. Consistent with earlier study Sheng et al. (2021) also reported that the red skin of *M. anguillicaudatus* is related to carotenoid content.

Carotenoid accumulation on fish skin is uneven,

where the back to the tail looks darker orange while the other parts look bright orange. According to Hamre et al. (2021), the distribution of pigments in fish skin is affected by several factors, namely sunlight, genetics, and the environment. This is caused by carotenoid deposits as demonstrated by (Sheng et al., 2021). Meanwhile Toomey et al. (2022) stated that carotenoid deposits in fish skin are more intense in the back than in the body part of the fish. This is due to a migration of pigment cells from the dorsal midline to the tissue that eventually differentiate themselves which makes the distribution of xanthophore uneven and without pattern. One side is densely distributed but the other side has no pigment. This is related to the interactions of pigment cells that affect the concentration of pigment cells in different areas of the skin. The pigment pattern is determined mainly by the number, size, and distribution of different cell types (Bolker and Hill, 2000). Based on the pigment placement, xanthophore are at the top, melanophore in the middle, and iridophores at the bottom. The distribution of pigment cells in the hypodermis line area of zebrafish in the research of Hirata et al. (2003) showed that xanthophore is in the upper layer followed by melanophore and iridophore (Figure 5).

Xanthophore is a dendritic cell (Bajec et al., 2022). In storage, there are pigment cells, namely carotenoids that absorb light. Carotenoids are stored in their chromosomes, that is, xanthosomes (Ligon and McCartney, 2016). The results of this research indicate that the provision of carotenoids added in fish feeds affects the distribution of xanthophore in Nemo's skin. However, the variation in the average size of xanthophore was not directly related to the carotenoid supplementation, which is related to the maturity of xanthophore. Carotenoids may be more influential on the amount of pigment cell production in xanthophore. The maturity of xanthophore is assumed to have almost the same development stage as melanosomes, which have four stages. Based on the results of research by D'Alba and Shawkey (2019) that melanosomes are initially formed by the transport of various membrane proteins and soluble proteins into endosomes. At this level, tyrosinase enzyme is found but no melanin production yet. This is supported by Navarro et al. (2008) who stated that *Carassius auratus* fish stages 1 and 2 in melanosome development appear to lack pigment but contain protein fibers. Melanin synthesis begins at stage 3 and at stage 4 melanosomes fully produce pigments. Likewise, with xanthophores that the beginning of xanthophore formation is related to with proteins and enzymes (Luo et al., 2021). The first stage of xanthosome

formation in fish skin cells. Fish skin cells have special pigments namely chromatophores, one of which is xanthophore. Xanthophores produce xanthosomes. At this stage, proteins regulate and control the formation of xanthosomes (Luo *et al.*, 2021). The second stage of xanthophore has formed involves enzymes in the production of xanthosomes in the xanthophore. Stage The third stage of carotenoid compounds absorbed by fish will be stored in the xanthosomes on the xanthophore (Grether *et al.*, 2004). The fourth stage of xanthophore is distributed to the skin of the fish to provide color to the fish (Nusslein-Volhard and Singh, 2017).

At the stage when the xanthophore has been formed there is phytoene synthase (PSY) responsible for catalyzing the formation of phytoene which is the is responsible for catalyzing the formation of phytoene as the precursor for various types of carotenoids (Nakano, 2020). Phytoene undergoes successive modifications such as desaturation and isomerization to form lycopene. Through the modification of phytoene, organisms can produce carotenoids important in their biological functions. which was explained in the discussion of Rebelo *et al.* (2020) related to carotenoid synthesis engineering. Xanthophore formation in fish skin involves several enzymes and metabolic pathways that are very complicated. It depends on the nutrition and environmental conditions of the maintenance medium of the fish. The role of proteins in this process helps in regulating xanthophore formation in fish skin cells and the production of enzymes related to this process (Liang *et al.*, 2020). During formation, the xanthophore undergoes development, that is, the xanthophore undergoes a change into a mature or dendritic xanthophore. This process is influenced by nutritional, genetic, and environmental factors. Xanthophores are significantly more abundant in orange-colored skin than in black skin (Renko *et al.*, 2022). Therefore, it is suspected that there is a close relationship between phenotype, maturity, and quantity of xanthophore in Nemo fish.

The dendritic structure looks like a large flower indicating that it has entered the mature phase but immature xanthophore do not show dendritic structures (Zhang *et al.*, 2022). Without carotenoids (0%) (Figure 3) the size of xanthophores that have entered the adult phase. However, the production of pigment cells is decreasing indicated by the decrease intensity of the color in Nemo's skin, which is related to the intake of carotenoids that are not fulfilled. According to Djurdjevic *et al.* (2015), if the xanthophore loses color, the shape of the xanthophore structure remains but does not appear empty in the sense that there is no production

of pigment granules in it. Guo *et al.* (2007) stated that fish color and the formation of pigment patterns in fish are related to the number and distribution of melanophores, xanthophores, and iridophores. The treatments of 0.5%, 1%, and 1.5% showed variations in size that were not significantly different. It is suspected that xanthophore maturity occurs gradually based on the deposition of carotenoids in fish skin. As demonstrated by Sinha (2022) the amount of carotenoid deposits in the skin area is different depending on where the particles are produced and stored. Based on the research results of Zhang *et al.* (2022), the geometric structure and number of melanosomes are very diverse in controlling melanin production and metabolism. Fang *et al.* (2022) reported that the color of fish skin depends on the type and concentration of carotenoids in the skin, based on the process of deposition and absorption.

Based on the measurement results, the environment maintenance showed optimum results so that it is very supportive to the life of Nemo (Table 2). The treatment of 0%, 0.5%, 1%, and 1.5% were maintained with the same conditions so that the measurement results gave values that were not significantly different. One of the factors that can reduce morphological color in Nemo is the environment, which is temperature, pH, salinity, and DO. According to Vissio *et al.* (2021), a color decrease in fish is a response to environmental factors which can cause fish to stress in certain conditions. When the temperature of the maintenance media increases, concentration of dissolved oxygen in the maintenance media can be reduced. Low dissolved oxygen concentration can also affect the pH of maintenance media according to Pietoyo *et al.* (2020) waters that tend to be acidic or alkaline can cause fish death. This is due to low oxygen concentrations that makes respiratory activity increase and appetite decreases. Likewise, the salinity of maintenance media is interrelated with temperature, where high temperatures can increase salinity (Pratiwi *et al.*, 2022). Salinity affects the osmotic pressure of water. The higher the salinity, the higher the osmotic water, so it will affect the level of energy use (Bonanno *et al.*, 2021). During the maintenance of feeding, the reduced metabolic products and residual feed will accumulate in the aquarium.

4. Conclusion

Overall, the results of this study indicate that the yellow and orange coloration of Nemo is closely related to carotenoid content. The optimum dose of carotenoid compounds in increasing the total carotenoid content in nemo fish skin is 1.5%. Carotenoids added to fish feed

affected the distribution and maturity of the xanthophore but did not affect the size of the xanthophore. The results of this study also provide an overview of the mechanism of xanthophore formation and color change in Nemo fish. Therefore, research on the mechanisms and factors that regulate color changes or transitions in fish skin needs to be studied further.

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Authors' Contributions

The contribution from each author is as follows, Rp collected and analyzed data. Myk and Ddt, devised the main conceptual ideas and done a critical revision of the article. All authors discussed and contributed to the development of initial research ideas, the preparation, and the final manuscript.

Conflict of Interest

The authors stated and declared there is no conflict of interest in this research.

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