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Surya Kanta Sau
Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Amit Shit
Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Kherwal Raj Kisku
Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Corresponding Author:
Surya Kanta Sau
Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Natural carotenoids and its function for ornamental fish colouration: A review

Surya Kanta Sau, Amit Shit and Kherwal Raj Kisku

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Abstract
Keeping ornamental fish is the second hobby globally. The vibrant and lucrative colour variation of any ornamental fish fetch more money or profit to the breeders and culturists. The prominent and long-lasting colour depends on the feeding to fish with natural carotenoids rich feed ingredients. The deposition or accumulation of colour pigment on skin, fin and flesh of ornamental fish is species specific. But the colour development is influenced by water quality parameters, temperature, photoperiod, and light intensity/quality, balanced feed and carotenoids sources. The majority of carotenoids are mainly C40 tetraterpenoid pigments and derived from two C20 geranylgeranyl pyrophosphate (GGPP) molecules. There are more than 600 known carotenoids that fall into two categories: i) Xanthophylls which include astaxanthin, lutein, and zeaxanthin and contain oxygen as a functional group, and ii) Carotenes which include lycopene and β-carotene and contain a hydrocarbon chain and no functional group. The carotenoids are also important for growth, reproduction, and disease resistance in fish. Different natural ingredients like marigold, papaya, rose petal, carrots etc. are used for colour enhancement of different fishes.

Keywords: Ornamental fish, Colour enhancement, Natural carotenoids, Xanthophylls, Carotenes, Fish nutrition, Pigment deposition

Introduction

A popular and stress-relieving hobby with global appeal is raising ornamental fish. Ornamental fish with extremely bright colours and morphological attributes are important in evaluating customer's interest and worldwide market demand. The capacity to quickly create attractive, healthy fish is the basis for the ornamental fish trade's worth. The commercial value of these fish reflects this necessity; consequently, ornamental fish farmers are continually seeking techniques to improve skin colouration. Fish and many other animals have pigment-containing, light-reflecting cells called chromatophores (Fujii *et al*., 2000) [18]. Chromatophores are often conveyed by light absorption or interference phenomena involving light-reflecting compounds and are present in the dermis, epidermis, and around the neurovascular system in response to various stimuli (Kelsh *et al*., 2009) [24]. Fish have six distinct types of chromatophores, such as melanophores, xanthophores, erythrophores, iridophores, leucophores, and cyanophores, which contain different pigments (Das, 2016) [14]. Melanins, pterins, and carotenoids are the three main pigments that give all vertebrates their flesh color (Grether *et al*., 2001; Steffen and McGraw, 2007) [20]. Melanins and pterins are the only pigments that animals can synthesize, whereas carotenoid pigments cannot. As a result, dietary carotenoids, whether natural or synthetic, must be supplied regularly with feed to produce their color pigmentations. (Alonso-Alvarez *et al.* 2008; Vinkler and Albrecht, 2010) [22]. Under high culture conditions, naturally colored fish frequently lose their color. Maintenance of the bright colors appears attainable with the incorporation of pigments like astaxanthin and canthaxanthin into the varied diets continuously. Several studies have demonstrated that fish can be colored by adding natural carotenoids to their diets (Kurnia *et al*., 2019; Paul *et al*., 2024) [26, 30]. Competing physiological characteristics, such as pigments, may be transferred from mother to child in fish. A rise in yolk carotene accumulation increases offspring growth and survival, increases the immunity of offspring, and reduces oxidative damage (Blount *et al*., 2002; McGraw and Ardia, 2003; Bazyar Lakeh *et al*., 2010) [7, 5].

Factors Influencing the Color of Ornamental Fish

Many internal and external factors regulate the process of fish pigmentation. Internal factors such as body size, life cycle, and genetic traits.

1. **Body size:** Larger body sizes frequently connect with enhanced or expanded color patterns.
2. **Life cycle:** Color shifts over life stages: juveniles may exhibit muted tones that become brighter as they mature, such as in cardinals, where red intensifies throughout the body in adults as opposed to just the back in neons. Hormonal control of pigment dispersion causes age-related changes, with breeding adults displaying maximal saturation for mating displays. (Lall and Tibbetts 2009)
3. **Genetic traits**: Variations such as sexual dichromatism, polychromatism, or strain-specific patterns in species like guppies and cichlids are caused by genes that regulate chromatophore types (melanophores, xanthophores, and iridophores) and pigment production. Selective breeding amplifies desired features, and several genes (such as mc1r and sox10) combine to generate colors ranging from yellow-red carotenoids to black-brown melanins. (Silverstein, 2002) [34]

External factors, such as environmental factors, diet, and carotenoid sources regulate the process of fish pigmentation

1. **Environmental factors:** Color expression is influenced by water quality (pH, oxygenation), temperature, photoperiod, and light intensity/quality; stress-induced pigment constriction and dullness are caused by unfavorable conditions. In ornamental species, high levels of stress from crowding or inadequate sunlight upset hormonal balance, which lowers carotenoid stability and vibrancy.
2. **Dietary Influences:** Balanced nutrition directly influences pigmentation, as fish rely on external sources for carotenoids unable to synthesize, with deficits leading to faded hues.
3. **Carotenoid sources:** Natural or synthetic carotenoids raise yellow-red pigments in xanthophores, with ideal levels enhancing intensity and market value under controlled upbringing. Antioxidants in these sources protect pigments from oxidative loss, boosting deposition efficiency (Dong *et al*., 2014) [16].

Fish in the wild mostly consume planktonic copepod crustaceans, which are tiny zooplanktonic creatures that float and swim in the water column, to get the majority of their color pigments. These zooplanktonic creatures are also dependent on microscopic algae and plants (phytoplankton) for their pigments. Pigments are directly consumed by wild herbivorous fish from autotrophs (Choat and Clements, 1998) [10].

Overview of Carotenoids

The most common class of isoprenoid pigments is carotenoids, which are produced by some non-photosynthetic bacteria, fungi, and photosynthetic organisms like plants. While the majority of carotenoids are indeed C40 tetraterpenoid pigments, they are derived from the condensation of two C20 geranylgeranyl pyrophosphate (GGPP) molecules to form phytoene. Carotenoids are due to the existence of a lengthy system of conjugated double bonds, and carotenoids are strongly colored and substantially absorbed in the visible area, between 400 and 500 nm (Zechmeister *et al*.,1960) [38]

There are more than 600 known carotenoids that fall into two categories: i) Xanthophylls (formerly called phylloxanthins), which include astaxanthin, lutein, and zeaxanthin and contain oxygen as a functional group, and ii) Carotenes, which only contain a hydrocarbon chain and have no functional group, such as lycopene and β-carotene (Olson and Krinsky, 1995) [28]. For example, high-value ornamental fish are naturally colored with dietary astaxanthin (Ambati, Phang, Ravi, & Aswathanarayana, 2014) [3]. Fish chromatophore cells can either deposit carotenoid pigment directly or through cellular metabolism, giving the skin and other tissues a variety of colors (Chapman and Miles 2018) [9]. Carotenoids are also important for growth, reproduction, and disease resistance in fish. (Carvalho and Caramujo 2017) [15]

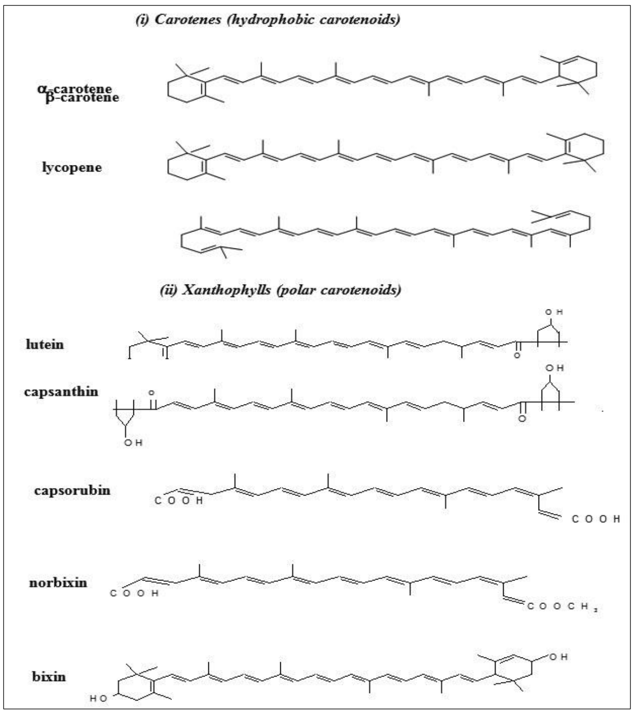

Fig 1: Shows the chemical structure of several common carotenoids.

Carotenoids in Ornamental Fishes

Fish have a variety of carotenoids, the predominant of which is unique to the species in question. The carotenoids that give fish colors are tunaxanthin (yellow), lutein (greenish-yellow), β-carotene (orange), β-doradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red), and taraxanthin (yellow).

Cyprinidae fish can generate (3S, 3´S)-astaxanthin from zeaxanthin by oxidative metabolic conversion. However, astaxanthin cannot be produced from other carotenoids by Perciformes and Salmonidae fish. Even some writers state that controversy persists over the biological roles of carotenoids in fish (Choubert *et al*., 2005) [11]. Others think of these substances as crucial micronutrients that fish cannot produce and must be incorporated into the diet (Baker *et al*., 2002) [4].

Natural Carotenoid Sources

Carotenoids are widely distributed in nature and, generally speaking, all colored fruits and vegetables are excellent sources of these substances.

There are two types of carotenoids: those from plants and those from animals. Microalgal pigments are the primary source of plant-based carotenoids; additional aqua feed formulations include marigold, yellow corn, corn gluten, and alfalfa. Aquaculture uses algae like *Chlorella vulgaris*, *Dunaliella salina*, *Arthospira maxima*, and *Haematococcus pluvialis* as a source of carotenoids. Astaxanthin in marigolds gives them a reddish-yellow hue; canthaxanthin in yeast provides them an orange hue; lutein and zeaxanthine in alfalfa give them a yellow-orange hue; and β-carotene and α-carotene in algae give them a greenish-yellow hue. Seaweeds are also thought to be an excellent source of plant-based carotenoids, such as fucoxanthin and beta carotene, which provide a greenish-yellow hue. Foods that contain carotenoids are frequently red, yellow, or orange, though this isn't always the case. Carotenoids can be found in a variety of fruits and vegetables, including carrots, yams, potatoes, papaya, watermelon, cantaloupe, mangos, spinach, tomatoes, bell peppers, and oranges (Britton *et al*.,2008; Nakano, 2003) [8].

Potential animal-based sources of carotenoids include shellfish like shrimp, krill, crabs, lobsters, etc. They are also high in proteins (25-50%), chitin (25-35%), and mineral salts (15-35%). The two most popular plant sources of carotenoids are yeast (*Phaffia rhodozyma)* and the fermentation product of Xanthophyllomyces dendrorhous, which are the primary sources of astaxanthin. These are a high source of astaxanthin, a carotenoid that is used as an ingredient in aqua feed formulations to improve fish color (Britton *et al*.,2008; Maoka, 2020; Schiedt, 1998) [8, 27, 31].

Function of Carotenoids

- **Vitamin A precursors:** In ornamental fish species, carotenoids such as beta-carotene, astaxanthin, canthaxanthin, and zeaxanthin function as precursors to vitamins A. These substances are transformed by enzymes into retinol, or vitamin A, which is necessary for growth, reproduction, eyesight, and the preservation of epithelial tissue (Schiedt *et al.*, 1985; Guillou *et al.*, 1992; Christiasen *et al.*, 1994; White *et al.*, 2003.) [32, 22, 37] Astaxanthin, canthaxanthin, and isozeaxanthin, together with carotene, were found to be precursors for vitamin A in both platies (*Xiphophorus variatus*) and guppies (*Lebistes reticulatus*) (Gross and Budowski, 1966) [21]. In rainbow trout (*Oncorhynchus mykiss*), astaxanthin, canthaxanthin, and zeaxanthin were precursors of both A1 and A2, although the rate of incorporation was strongly influenced by fish size and age.
- **Communication:** Females frequently like brighter, carotenoid-rich males because their color indicates a healthy diet, robust immune, and good genes. Carotenoid pigmentation is therefore a crucial characteristic in sexual selection. Carotenoids build up in the insides of fish. Integumentary carotenoids may play a role in signaling, camouflage, and photoprotection, including breeding color. Fish exhibit color reactions during excitement and courting, as well as color changes in response to background colors (Fujii,1969) [19].
- **Growth:** The importance of carotenoids for fish growth is debatable; some studies showed no effect, while others found a positive one. Adding papaya pulp to the diet of banded chichlids improves both color and growth (Paul *et al*., 2024) [30]. However, Biswas *et al.* (2024) [6] could not discover any notable alterations in dwarf chameleon fish (*Badis badis*).
- **Reproduction and fertility:** Aquatic organisms also accumulate carotenoids in their gonads. Although it is believed that carotenoids are essential for aquatic life to procreate, Tveranger (1986) discovered no link between the administration of astaxanthin and rainbow trout fertility**.**
- **Colour enhancement:** According to Jain *et al.* (2019) [23], different types of natural carotenoid sources (plant and animal) are used in the ornamental fish diet for colour enhancement. Gold fish (*Carassius auratus*) were used in a sixty-day feeding experiment. The results showed that the optimum effects could be achieved by adding up to 6% of carrot meal to their meals that included 30% crude protein (Tiewsoh *et al.*, 2019) [35]. Koi carp (*Cyprinus carpio*) fries were tested for 120 days using 1%, 3%, 5%, 7% and a control with 0% carrot meal incorporated diets. Results showed the highest colour intensity at 5% level of carrot powder incorporation in comparison to 1%, 3% and 7% carrot meal (Jain *et al*., 2019) [23]. Rosy barb (*Puntius conchonius*) showed the highest pigmentation with rose petals at 4% in the diet over 45 days (Pailan *et al.*, 2012) [29]. The diet supplemented with 3 g/100 g of papaya pulp powder showed the best pigmentation in banded cichlid fish (*Heros severus*) (Paul *et al.*, 2024) [30].

Stability of fish colouration: The term "fish color stabilization" describes the long-term maintenance of body pigmentation's brightness, intensity, and consistency. Stabilization guarantees that pigments stay visible and do not fade after reaching ideal coloration (via food, genetics, or environmental control).

After 90 days of upbringing, Dananjaya *et al.* (2017) [13] found that goldfish skin coloration was unstable. It could be caused by environmental stress, nutritional deficiencies, or other physiological or biological factors.

Conclusion

The coloration, general health, and visual appeal of ornamental fish are all dependent on essential carotenoids. Fish require a constant nutritional supply of carotenoids from natural or supplemented sources to maintain brilliant coloration because they are unable to synthesize them from scratch. Carotenoid-rich diets have been shown in numerous studies to improve skin and fin pigmentation, improve color stability in captivity, and considerably raise the market value of attractive species. In ornamental aquaculture, carotenoids are multipurpose bioactive substances that enhance immunity, antioxidant defense, reproductive efficiency, and stress tolerance in addition to their color. For the purpose of creating commercial aqua feed, a great deal of research must be done on the natural sources of carotenoids derived from plants and animals.

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