Iranian Journal of Fisheries Sciences 19(6) 2770-2789 2020
DOI: 10.22092/ijfs.2020.122739

Research Article

Effects of different dietary supplementation of plant carotenoids on growth, coloration and behaviour of giant gourami, *Trichogaster fasciata* (Bloch and Schneider, 1801)

Bano F.[1]; Kashyap A.[1]; Serajuddin M.[1]*

Received: March 2018 Accepted: April 2019

Abstract

Effects of different dietary supplements of plant carotenoid sources on growth, coloration and behavioural activities of *Trichogaster fasciata* were carried out. Three groups of fish were fed with petal powder of rose (*Rosa chinensis*), China rose (*Hibiscus rosasinensis*) and marigold (*Tagetes erecta*) separately as carotenoid source along with the same basic ingredients for 90 days. The mean final weight of marigold petal powder fed group was significantly different (p<0.05) from other groups after 90 days of the experiment. The absolute growth rate, relative growth rate, weight gain percent and specific growth rate were highest in the group of fish fed with marigold petal powder while feed conversion ratio was lowest in this group. Carotenoid content in the tissue of different groups of the fish was found to be significantly different (p<0.0001). Marigold petal powder was most effective to enhance the pigmentation and coloration in the different color zones (color of eye and opercular ocellus, jugular darkening, anal, ventral and dorsal fin color etc.) of the skin of *T. fasciata*. Pigmentation affected the behavioural activities of the fish where male aggression and selection of males by females was high in the group fed with marigold petal powder. No significant difference was observed in the social interactions and habitat preference.

Keywords: Growth indices, Female selection, Habitat preference, *Trichogaster fasciata*, Social interaction

1- Fish Biology Research Lab, Department of Zoology, University of Lucknow, Lucknow, Uttar Pradesh, India

*Corresponding author's Email: lu.fisheries@gmail.com

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

Introduction

Ornamental fish are characterized by wide diversity of colors and their different patterns that act as signaling tool to attract a mate for courtship and other types of social behavior. Fish cannot synthesize their own coloring pigments *de novo* and need to be incorporated into their diet (Chatzifotis *et al*., 2005; Jorjani *et al*., 2019a, b). Carotenoids are synthesized from geranylgeranyl diphosphate (Giuliano *et al*., 2000). They are essential for the normal diet of the fish (Simpson and Chichester, 1981; Goodwin, 1986; Hamrang Omshi *et al*., 2019) because they play an important role in the color enhancement phenomenon (Olson, 1989; Sueki, 1991; Gupta *et al*., 2007). Besides these, carotenoids have many functions such as: Vitamin A precursors (Simpson et al., 1981), produce marked effect on reproductive performance (James *et al*., 2009; Vasudhevan and James, 2011; Karga and Sagar, 2016), a potent antioxidant (Burton, 1989; Naguib, 2000), predominant role in immune response (James *et al*., 2006; Abdel-Tawwab and Ahmad, 2008; Vasudhevan, 2007; Mustafa *et al*., 2013; Vasudhevan *et al*., 2013; Reddy *et al*., 2014), acting on sense organs (Nandeesha *et al.*, 1998), liver structure (Segner *et al*., 1989). Halten *et al*. (1997) reported a direct relationship between dietary carotenoids and pigmentation in fish.

Behavioural studies in fish are important for welfare research where growth is an indicator of health status and physiology of the fish (Huntingford *et al*., 2006). Carotenoid pigments are extensively used for the coloration of the integument of the fish which boost their immune system and also advertise their health leading to preferential selection by the sexual partner, heighten aggression activity (Baron *et al*., 2008). Intraspecific aggression is recognized as a problem in aquaculture which generates stress (Ejike and Schreck, 1980), impaired immune function (Pottinger and Pickering, 1992), caused unequal growth (Jobling and Reinsnes, 1986; Jobling *et al*., 1995). Costs of aggressive defence might include injury (Haller, 1994) and can indirectly affect the welfare of fish (Oldfield, 2011).

Wild fish obtain a balanced diet from the aquatic water bodies in the form of phytoplankton and zooplankton which produces the optimum coloration. But in case of the aquarium fish the coloration may fade with time particularly in those which are kept for longer period of time; so supplementary carotenoids are needed to maintain mainly yellow, red and pink color in skin and flesh of fish (Awasthi *et al*., 2014). Fish farmer and hobbyist of all over the world wish to enhance the color of ornamental fish for their good health and demand. A variety of carotenoids are being developed from synthetically or naturally occurring products for the enhancement of color in ornamental fish (Duncan and Lovell, 1993). Carotenoids derived from natural sources are alpha, beta carotenes, zeaxanthin, astaxanthin, lutein, cryptoxanthin etc. Beta-carotene is the synthetic carotenoid which is considered to be very expensive and harmful as compared to natural

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

carotenoids. But the high doses of synthetic carotenoids (β -carotene) may increase the risk of lung and prostate cancer, intracerebral haemorrhage and cardiovascular diseases particularly in people who smoke cigarettes or have a history of high-level exposure to asbestos (Stahl *et al*., 1998).

Gouramis are a group of medium to large sized freshwater fish that are comprised under the family, Osphronemidae and order Perciformes, particularly known for their ornamental worth. A number of researchers have supplemented the natural carotenoid sources to enhance the growth and pigmentation in gouramis and notable among them are *Trichogaster leeri* (Fey and Myers, 1980), *Colisa labiosa* (Segner *et al*., 1989), *Trichogaster lalia* (Baron *et al*., 2008; Awasthi *et al*., 2014; Baksi *et al*., 2017) *Trichogaster trichopterus* (Frankel, 1992, Alagappan *et al.,* 2004; Hamlin *et al*., 2013; Priyanka *et al*., 2015; Khanzadeh *et al*., 2016) and Kissing gourami (Kopecký, 2013). But the only study includes restoration of pigmentation in *T. fasciata* was carried out by Dey and Goswami (2016) using plants and animals carotenoid sources. The utilization of fairy shrimp as a live food can improve the skin color of ornamental fish and also enhance the color and reproductive performance of freshwater ornamental prawns (Seidgar 2015; Seidgar *et al.,* 2016). Hence, the present study was proposed to investigate the efficiency of different plants carotenoid sources to develop coloration on the skin, their role in the establishment of social interaction, habitat preference and evaluation of growth in the giant gourami, *Trichogaster fasciata*.

Materials and Methods

Rearing conditions

The rectangular glass tanks (length× width×depth: 75×30×36 cm) were used in the study for a period of 90 days. The trial was conducted in 12 glass tanks with four tanks per treatment. Tanks were brushed every 2 days to minimize algal growth and to ensure exchange of water for appropriate aeration and to improve dissolved oxygen levels. Various physicochemical parameters such as temperature (23-26 ºC), pH (8.5-8.6), dissolved oxygen (6.5-7.0 mg L^{-1}) and total ammonium nitrogen (0.30-0.51 mg L^{-1}) were recorded as per standard methods (APHA, 2012) daily in the morning before their feeding. After feeding, the experimental tanks were siphoned off to remove uneaten feed and faeces residues. The 50% of water in each experimental tank was changed every day using fresh and clean tap water.

Experimental diets

The 100g feed was prepared containing 85g basic ingredients [soyabean (15.22g), fishmeal (15.22g), groundnut oil cake (11.28g), wheat flour (13.04g), tapioca flour (13.04), rice bran (12.2), vitamins (5.0)] and 15g carotenoid supplements as used by Ramamoorthy *et al.* (2010). The control diet (D1) was prepared using the basic ingredients only without any carotenoid supplements. Three experimental diets such as D2, D3 and D4 contained basic

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

ingredients and additional carotenoid supplements such as rose (*Rosa chinensis*), China rose (*Hibiscus rosasinensis*) and marigold (*Tagetes erecta*) respectively. For the feed preparations, the petals of rose, China rose and marigold flower were collected from the garden, air dried in the dark room to prevent their denaturation, powdered, sieved (particle size of 0.5 mm) and mixed to a unanimous homogeneity with the basic feed by the use of water. The resulting pellets (1.2 mm) were air dried and stored at -20ºC to avoid oxidation of the carotenoids.

Experimental fish and feeding trial

A total of 120 fish specimens of *T. fasciata* (weight: 1.2 to 1.7 g) were collected using cast and drag nets from river Gomti at Lucknow region (26º 56" N 80º 43" E) and acclimatized to laboratory conditions for a month prior to the experiment. The individuals of giant gourami were randomly distributed into 12 tanks (10 fish each) as the experiment was carried out in triplicates. Prior to the experiment, fish were conditioned by the treatment of control diet without supplement of carotenoid sources for 4 weeks. At the beginning of the feeding trial, fish were kept on fast for 24 hour and their weights were taken individually using an electronic weighing machine sensitive to 0.001mg (Kerro, BL3002). Each diet was assigned to four tanks and fish were hand-fed twice a day at 10:00 and 17:00 hour with their respective feed at the rate of 5% of their body weight to achieve their apparent satiation. The palletisation was carried out at the rate of 15g $100g^{-1}$ feed. The weight of fish was recorded before feeding at the intervals of 15 days at 9:00 hour and the daily ration of feed was adjusted accordingly.

Mean body weight and growth parameters

The weight of the individuals of different groups of fish exposed to the different trials was taken using an electronic balance sensitive up to 0.001g. Growth parameters such as specific growth rate (SGR), relative growth rate (RGR), absolute growth rate (AGR), weight gain percent and feed conversion ratio (FCR) were calculated as per Degani *et al*. (1989):

Percentage of SGR=[(Final mean weight (g)–Initial mean weight (g))/ Rearing period (days)]×100

Percentage of RGR=[(Final mean weight (g)–Initial mean weight (g))/ Initial mean weight]×100

Percentage of AGR=Final mean weight (g)–Initial mean weight(g)

Weight Gain Percent=[(Final mean weight (g)–Initial mean weight (g))/ Initial mean weight (g)/Rearing period (days)]×100

FCR=Weight of dry feed used/Wet weight of fish (g)

Evaluation of enhancement of color

The images of the fish were taken using a digital camera (Cannon Digital PC-1585, China) under the standardized light condition. The specific zones on the body of the fish were selected to evaluate the enhancement of coloration (both control and treated) as carried out

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

by Resear (1967). The remarkable specific zones were: (1) eye color, (2) opercular ocellus, (3) longitudinal bar, (4) general body, (5) jugular, (6) anal, (7) dorsal, (8) ventral and (9) horizontal chin bar (Fig. 1).

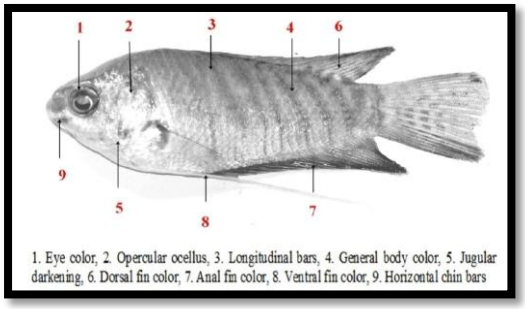

Figure 1*:* Color zones on the body of *T. fasciata* to compare color enhancement under different carotenoid sources.

Analysis of carotenoid content

Carotenoid content of the tissue was analysed as per Awasthi *et al.* (2014). Spectrophotometer was used to evaluate the optical density of the samples at 450 nm and total carotenoid content was calculated using the following formula:

Carotenoid content=Optical density/0.25×Sample weights (g)×10

Where, 0.25 is extinction coefficient and 10 is the dilution factor

Behavioural study

After the feeding trial of 90 days, the males and females of *T. fasciata* were segregated to carry out the behavioural study as done by Baron *et al*. (2008). The basis of sexual dimorphism includes the external morphology such as upper lip, fin morphology (Swarup *et al*., 1972), body size and color (Das and Kalita, 2006) and color patterns, fin color and abdominal morphology (Dehadrai *et al*., 1973; Das and Kalita, 2006). The behavioural tanks were provided with the artificial floating plants and stones (hiding places).

(A) Female selection

The male and female individuals of *T. fasciata* were selected from D2, D3, and D4 while only females of the same size were selected from D1 (control) to study the female preference for male. The selected male and female fish were maintained for the acclimatization of 30 minutes separately in the same tank by erecting an opaque partition in the centre (Fig. 2A). The central opaque partition was perforated to allow sufficient movement of water. The number of approach and time spent by the female of particular group for the male of specific group was observed and counted to study the female preference for male after removing the centrally placed opaque partition of the tank as carried out by Baron *et al.* (2008). The observational study was made for 1 hour at an interval of five minutes to assess the female choice between D1 vs D2, D3 and D4 individuals of *T. fasciata* in the experimental tank, separately.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

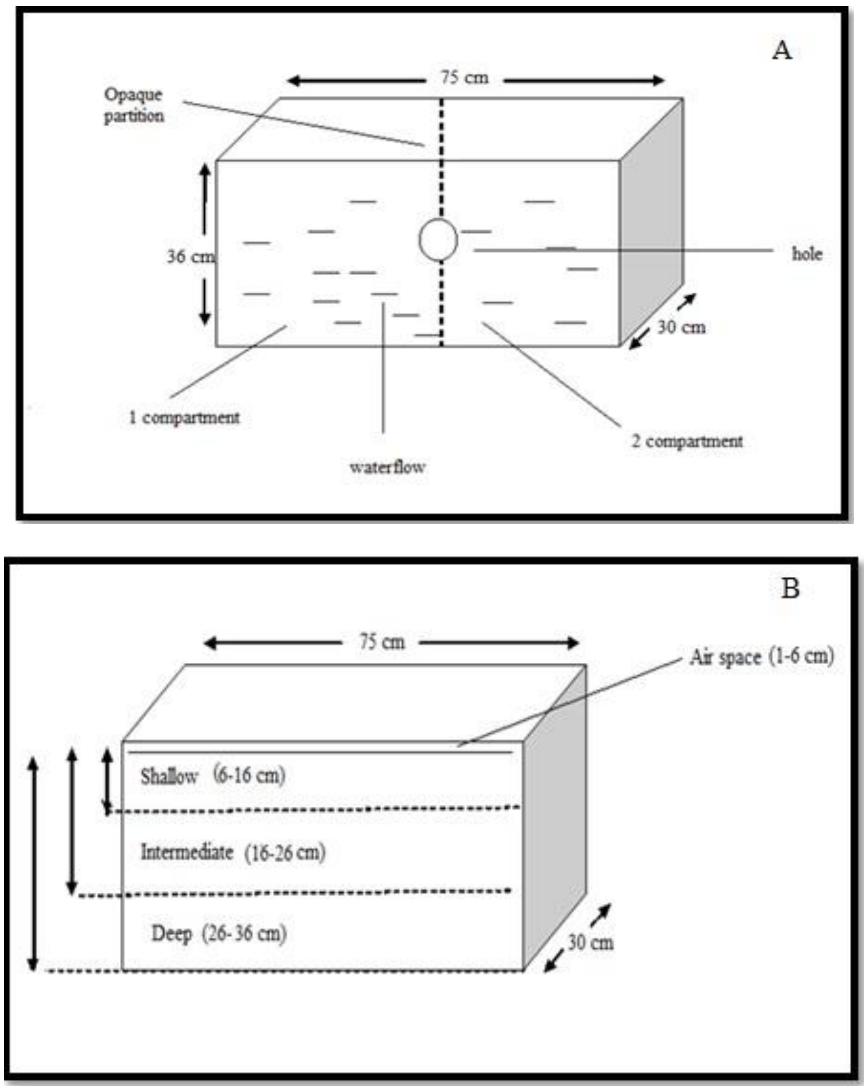

Figure 2: Test apparatus used for behavioural study: (A) for female selection, aggression and social interaction (B) habitat preference.

(B) Aggressive behaviour

The 10 male individuals of D1 group were kept separately with the 10 males of D2, D3 and D4 groups in the same tank and separated by erecting an opaque partition in the centre for acclimatization of 30 minutes duration. The aggressive behaviour was observed for 1hour duration at an interval of five minutes in the experimental tank.

The tank employed was similar as used for female selection but was covered with black plastic to minimize the external disturbances. Thereafter by lifting the partition the males were allowed for aggressive interactions and behavioural patterns were recorded as per Cooper *et al*. (1988):

Tail beating (TB): The fish stay parallel while they beat each other with their tails.

Ramming (R): The attacking fish swims quickly towards the partner, ramming it at head or side.

Biting (B): The attacking fish hits the partner with an open mouth trying to bite it.

The individuals of different group were marked as winner or loser.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

(C) Social interaction

Social interaction between males and females of the same group was carried out as done by Baron *et al.* (2008). The experimental tank was similar as used for aggression. The number of physical contacts given and received by male and female of respective group were counted and repeat trials were performed for all the groups separately.

(D) Habitat preference

The experimental tanks were divided into three sections based on different depth from the bottom: shallow (06-16 cm), intermediate (16-26 cm) and deep (26-36 cm) (Fig. 2B), to compare the habitat preference of *T. fasciata* as per Keller and Brown (2008). Initially 10 fish of the control group (D1) were introduced into the tank and left to stay for 10 minutes and inspected for habitat preferences. After the duration of 10 minutes the different carotenoid supplemented grouped fish (10 fish in each group) were introduced separately (D1 vs D2, D1 vs D3 and D1 vs D4). The influence of carotenoid supplemented fish on the habitat selection of control group (D1) was recorded by observing each individual after every 5 minutes for 1hour duration.

Statistical analyses

One-way ANOVA was designated to find out the statistical differences of mean carotenoids content and the growth parameters. Data from feeding and time of trials were analysed using two-way ANOVA and post hoc Bonferroni test. Also paired-sample 't' tests (two tailed) were used to test the statistical differences for female selection and the time spent in it. Statistical analysis for the various aggressive patterns between the different groups was calculated by one-way ANOVA: Post hoc Tukey's HSD. Social interaction (contacts given and contacts received) and the habitat preference were calculated using one-way ANOVA (Non-Parametric). Data are presented as Mean±standard error of mean and a probability level of 5% was used as the minimal criteria of significance. All statistical analysis was carried out through Graph Pad Prism software (version 5.01) and Paleontological software (PAST, version 3.12).

Results

The same diet with different carotenoid sources were accepted equally well by all the groups of *T. fasciata* and they grew normally without any sign of disease. Two-way ANOVA and the post hoc Bonferroni test showed the significant difference in the mean body weight (p=0.0013; F=8.013) and the time period (p=0.0001; F=24.13) in the group where marigold as carotenoid source was used after 90 days of the experiment. One-way ANOVA revealed that the mean carotenoid content for different groups of the fish were significantly different (p=0.0001; Table 1). The gouramis fed with marigold (D4) as carotenoid source showed the higher values in all the growth parameters except FCR. The details are given in Table 1.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

Table 1: Mean body weight (g) and growth parameters after 90 days under the influence of different plant carotenoid sources.

Growth parameters	D1	D2	D3	D4
Initial Body Weight	2.50±0.453	2.51±0.038	2.51±0.312	2.52±0.198
Final Body Weight	2.89±0.058	3.110±0.169	3.358±0.286	3.501±0.183*
Carotenoid content	3.093±1.014	5.667±1.034	7.103±0.306	8.901±0.034**
FCR	1.093±1.358	0.667±1.016	0.451±0.286	0.352±0.171*
SGR	0.412±0.394	0.498±0.798	0.615±0.530	0.745±0.561*
RGR	2.338±0.218	3.104±0.745	4.491±3.077	5.481±3.057*
AGR	0.061±0.859	0.083±0.075	0.125±0.087	0.156±0.097*
Weight gain Percent	32.110±06.65	46.562±05.183	67.375±4.159	82.225±4.857**

Mean±SE, values with different subscript in the same row are significantly different (p<0.05).
*less than 0.05 and ** less than 0.01. D1=Control, D2=Rose, D3=China rose and D4=Marigold.

Evaluation of enhancement of color

The male and female fish treated with marigold as carotenoid source was leading in the development and color enhancement process. The remarkable zones consist of the dark eye color with deep orange iris, intensified blue color of the opercular ocellus, dark brown longitudinal bars, blue to grey body color, iridescent blue-black dorsal, anal and ventral fins, dark blue jugular and deep orange in the distal edge of the fin tips. The individuals of the control group were found to be least colored. The details are well explained in Table 2 and Fig. 3.

Table 2: Description of the color zones in *T. fasciata* under the influence of different plant carotenoid sources.

S.N.	Parameters	D1	D2	D3	D4
1.	Eye Color (around the eyes)	Light bronze	Light silvery and the iris a pale orange	Uniformally dark with orange iris	dark with deep orange iris
2.	Opercular Ocellus	Faint blue color	Blue color	Intensified blue color	Highly intensified blue color
3.	Longitudinal Bars	Light blue	Deep blue, with almost silvery background	Dark brown in color	Extremely dark brown and nearly blend in with the darkened body and fins
4.	General body color	Faint blue	Deep Blue	Blue to grey	Blue to grey with dorsum appears somewhat darker gray
5.	Jugular Darkening	Absent/Silvery	Silvery to blue	Dark blue often approaching black	Dark blue often approaching black
6.	Dorsal Fin Color	turtuoise blue, the distal edge of the fin tipped in white	Deep turtuoise blue, the distal edge of the fin tipped in intense orange	Iridescent blue, the distal edge of the fin tipped in deep orange. The soft dorsal has black spots on an orange background	Deep Iridescent blue-black in color, the distal edge of the fin tipped in deep orange. The soft dorsal has black spots on an orange background
7.	Anal Fin Color	Blue with diffuse black spots in the faint orange area along the posterior margin of the fin	Blue with diffuse black spots in the orange area along the posterior margin of the fin	Turquoise blue with diffuse black spots in the orange area along the posterior margin of the fin	Turquoise blue to a deep, iridescent blue-black with intensified orange color along its exterior margin
8.	Ventral Fin Color	Completely white in color	Light orange	Light orange followed by deep orange area along the posterior margin of the fin	intensified orange color along its exterior margin
9.	Horizontal Chin Bars	Faint brown	Faint brown	Brown to Grey	Nearly Black

D1=Control, D2=Rose, D3=China rose and D4=Marigold.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

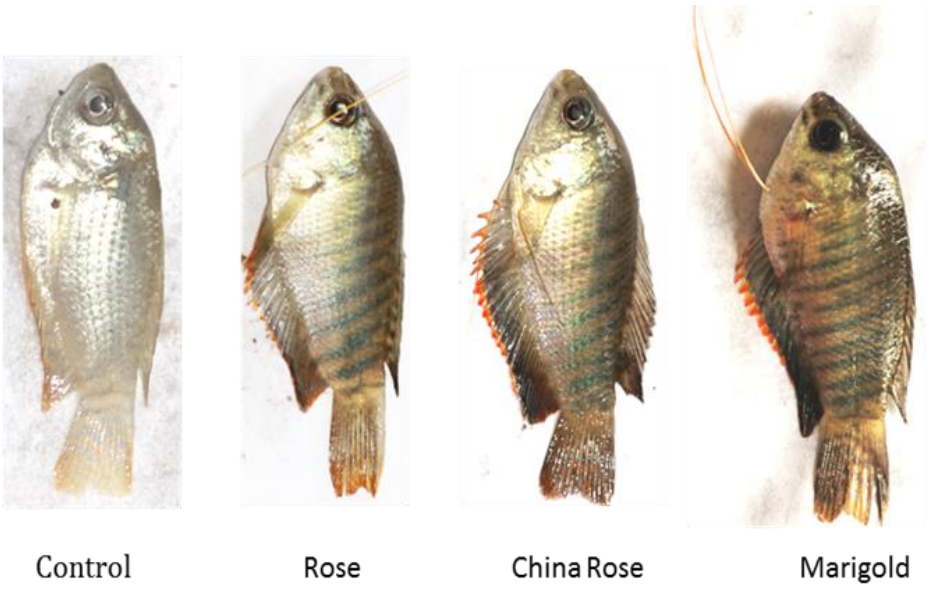

Figure 3: *T. fasciata* under the influence of different plant carotenoids sources.

(A) Female selection

On keeping the males and females of carotenoid treated groups (D2 or D3 or D4) together with the females of control group of the fish, the territory was established by both the males and females of the carotenoid treated groups. Although the initial attack was started by males (supplemented) over the females of control group. The female selection for the courtship behaviour were found to be significantly different (p=0.0055; Fig. 4A) for the 'D4' group. Also, the time spent by the males and females of 'D4' group was maximum and significantly different (p=0.0383; Fig. 4B) from the other groups. The "non-selected" females from control group were found to be hidden along with the plants, gravels and pebbles.

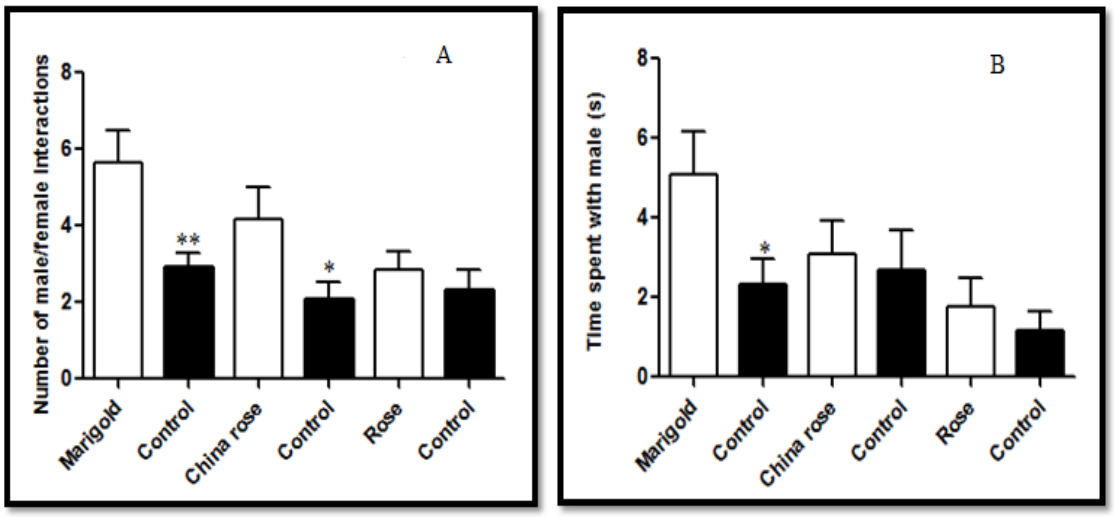

Figure 4: Influence of diet on female preference between control and pigmented males, (values are mean±SE). (A) male-female interactions and (B) time spent (s) with each male. White bars depict the carotenoid supplemented groups and black bars depict the control groups.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

(B) Aggression

After 15 minutes of the experiment, the giant gourami showed aggressive interactions. Carotenoid induced males (D2, D3 and D4) display stronger territorial and aggressive behaviour, dominated and performed the first attack biting the males of control group. Dominant fish (colored) were aggressive and reinforce the high aggressiveness toward their partners (subordinates/losers) threatening and attacking them by tail beating (p=0.0001; F=7.004), ramming (p=0.0001; F=7.004), biting (p=0.0001; F=7.004). The details are given in Fig. 5. After the fight, the subordinate fish showed passive behaviour consisting of attempts to escape by searching for refuge behind the stone or around the aquatic plants. Again, Tukey's HSD showed that the individuals of 'D4' showed significantly higher bites (p=0.00027).

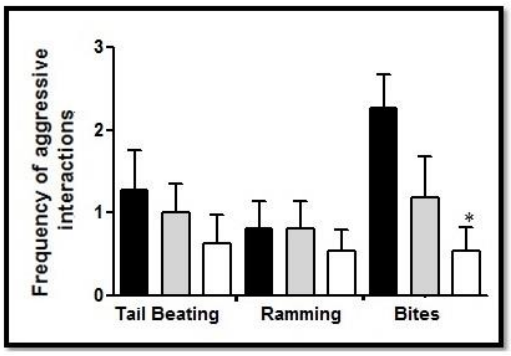

Figure 5: Influence of diet on male aggression between control and pigmented males, (values are mean±SE). Interactions included: D1 vs D4 (Black bars), D1 vs D3 (Grey bars) and D1vs D2 (white bars).

(C) Social interaction

No significant difference was noted for the social interaction in different groups of the fish where total contacts given (p=0.5401; F=0.6787) and received (p=0.2567; F=2.801) by the males and females of the same group (Fig. 6).

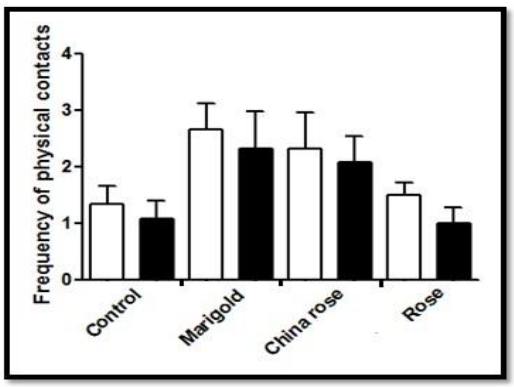

Figure 6: Influence of diet on social interaction in giant gouramis, (values are Mean±SE). White bars represent: total contacts given and black bars represent: Total contacts received respectively.

(B) Habitat preference

The majority of the individuals of control group (D1) preferred the shallow and intermediate zone of the experimental tank as their ideal habitat and was found to be significantly different (*p*=0.0173; F=4.597; Fig. 7A). But after the introduction of the individuals of carotenoid groups (D1 vs D2, D1 vs D3 and D1 vs D4), no significant difference was observed (*p*=0.2969; F=1.260; Figure 7B). The fish of the control group changed their ideal habitat and randomly distributed to all the three zones but most of them were forced to the deep zone of the experimental tank.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

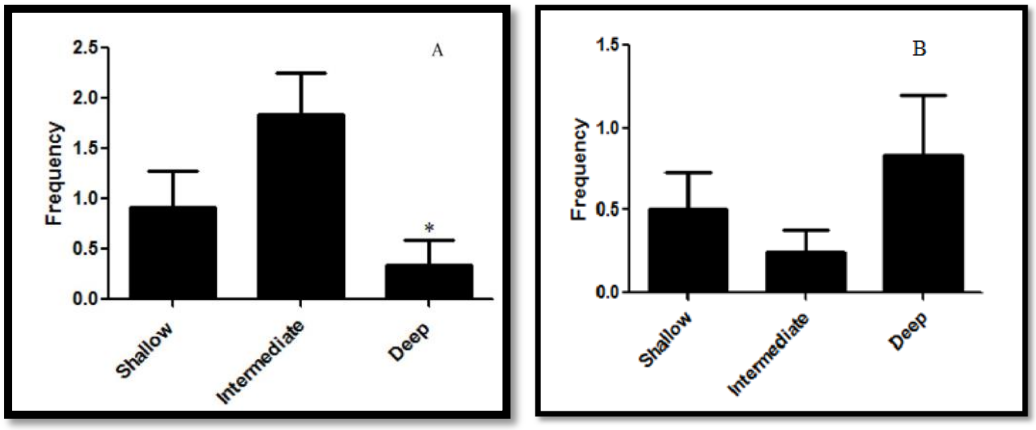

Figure 7: Habitat preference of control group fish: 'A' before and 'B' after the exposure of the carotenoid supplemented giant gouramis, (values are Mean±SE).

Discussion

The supplemented carotenoids sources (rose, China rose and marigold) used in present study were effective and produced varying degree of pigmentation in skin of giant gourami but the marigold was found to be most effective. Carotenoid accumulation on fish is affected by their kinds and dietary concentration (Kalinowski *et al*., 2004). Boonyaratpalin and Unprasert (1989) pointed out that the rate of development of color in the skin of fish depend on the amount and nature of carotenoid pigment used as source, while the carotenoid source and its effectiveness on fish body is species specific (Ha *et al*., 1993). Carotenoids are taken by fish in their diet as precursor pigments and absorb through the intestinal mucosa after digestion (Furr and Clark 1997) and metabolically oxidize to other forms (Katayama *et al*., 1973) before deposition into chromatophores in skin of the fish (Katayama *et al*., 1973; Chatzifotis *et al*., 2005).

Mean body weight was found to be significantly different in the group 'D4' during 75-90 days of the experiment. A number of workers such as, Sinha and Asimi (2007) and Pan *et al*. (2010) reported the correlation between the supplemented carotenoids and growth enhancement in *Carassius auratus* and *Hyphessobrycon callistus* respectively, while contrary to these other workers such as Kalinowski *et al*. (2004), Wang *et al*. (2006) and Kop *et al*. (2010) reported not any significant growth on the supplementation of carotenoids in the diet in *Pagrus pagrus*, *Hyphessobrycon callistus* and *Cichlasoma severum* respectively.

The survival rate was 100% in all the groups of the fish in the present study but the growth parameters such as weight gain percent, absolute growth rate, specific growth rate, relative growth rate and feed conversion ratio were significantly improved in the individuals of the fish fed with marigold as a carotenoid source. The similar results were reported on the supplement of coriander with diet in *Carassius auratus* by Ahilan *et al.* (2008). The increase of carotenoid content in the exoskeleton and abdomen

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

of the pacific white shrimp (*Litopenaeus vannamei*) was reported by Ponce-Palafox *et al*. (2006). Jagadeesh *et al*. (2015) also reported increased in weight gain, relative growth rate, specific growth rate and low feed conversion ratio on the supplemented diet of marigold oleoresin fed with 120 ppm.

Picciolo (1964) reported that visual cues are imperative in intraspecific sex discrimination in the genus *Colisa*. Male *T. fasciata* attained more carotenoids and become more attractive with alternating red and blue bands whereas females are pale and more silvery in color (Dey and Goswami, 2016). The present investigation indicated that female giant gouramis exhibited strong preferences for males with high body coloration as reported and justified by many workers in other species where pigmentation was developed using carotenoid sources (McLennan and McPhail, 1990; Milinski and Bakker, 1990; Evans *et al*., 2004). Baron *et al*. (2008) reported that the skin pigmentation of male red flame dwarf gouramis can be increased with the inclusion of a synthetic astaxanthin (Lucantin Pink) and reported that the sexually active females of red flame dwarf gourami associated more with males with greater red skin coloration than with less-pigmented males. All the males selected by females in the present study were bright in color from groups (D2, D3 and D4) over control indicated that color patterns have pronounced function in the context of sexual communications for mate choice in *T. fasciata.*

Aggression is a flexible behaviour modified on the costs and benefits of fighting over resources (Huntingford and Turner, 1987). The aggressive interactions in *T. fasciata* corroborated that the male individuals of carotenoid groups were aggressive, strong and dominated as depicted by greater bites and chases over the males of control. The bright red color of male has dual purpose, as a signal to the female that the male is ready to spawn and of aggression and territorial defence to other males (Frischknecht, 1993). In dominant-subordinate relationship, aggression has been shown to depend on social status with dominant individuals displaying higher levels of aggression indicated by greater bites and chases than sub-ordinates individuals (Borg *et al*., 2012; Ricci *et al*., 2013). The consequence of such relationships the dominant becomes more pronounced in the context of mating when the dominant individuals, by virtue of higher aggressiveness can monopolize territories as well as potential mates while the subordinates are deprived (Kodric Brown, 1992). Dominant males are successful in siring more offspring than the subordinates, thereby accounting for higher reproductive success (Paull *et al*., 2010; Watt *et al*., 2011).

The level of social interaction between males and females of same groups (D2, D3 and D4) did not differ from the control group and as a conclusion, the social interaction was found to be no longer under the influenced of carotenoid sources. The occurrence of social interaction within

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

the same diet and size-matched groups on dwarf gouramis by Baron *et al.* (2008), and the research work pointed out, it is dubious that color played a role in social interaction. The social interaction and competitive abilities are possible, due to the variation in physiology such as innate cardiorespiratory capacities, enzyme systems (Sloman and Armstrong, 2002) and stress parameters (plasma cortisol concentrations: Ejike and Schreck, 1980).

The changes in preferred habitat of non-pigmented group (D1) after the introduction of carotenoid treated/pigmented groups (D2, D3 and D4) indicated that normal body fitness is essential for the determination of true habitat preference in fish. The non-pigmented individuals of *T. fasciata* were considered to be unfit and forced to use a less preferred habitats in the present study. The result was supported by the hypothesis which was anticipated to explain the survival differences with reference to its consequences and application in fish feeding ecology (Sogard, 1994; Sogard and Olla, 1996).

Plant sources such as marigold, China rose and rose petals have been proved to be effective carotenoid sources for coloration in fish. The marigold petal meal as a natural carotenoid source is considered to be the best to obtain the appropriate growth, coloration and other behavioural activities in *T. fasciata*.

Acknowledgements

The authors thank the Head, Department of Zoology, University of Lucknow for providing facility and administrative support. F.B. is thankful to the University Grants Commission (UGC), New Delhi for the SRF [Ref No-19/06/2016(i) EU-V], which has been given to undertake the advanced research.

References

Abdel-Tawwab, M. and Ahmad, M.H., 2009. Live Spirulina (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic *Aeromonas hydrophila*. *Aquaculture Research*, 40, 1037-1046. DOI:/10.1111/j.1365-2109.2009.02195.x

Ahilan, B., Jegan, K., Felix, N. and Ravaneswaran, K., 2008. Influence of botanical additives on the growth and colouration of adult goldfish, *Carassius auratus* (Linnaeus). *Tamil Nadu Journal of Veterinary Animal Science*, 4, 129-134.

Alagappan, M., Vijila, K. and Archana, S., 2004. Utilization of spirulina algae as a source of carotenoid pigment for blue gouramis (*Trichogaster trichopterus* Pallas). *J Aquaricult Aquatic Sci*, 10, 1-11.

APHA (American Public Health Association, American Water Works Association, Water Environment Federation) 2012. Standard Methods for the Examination of Water and

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

Wastewater, 22nd ed. Washington: American Public Health Association, New York, USA. 1360 P.

Awasti, M., Kashyap, A. and Serajuddin, M., 2014. Effect of Plant Meal as a Carotenoid Source on the Development of Pigmentation in Dwarf Gourami, *Colisa lalia* (Hamilton, 1822). *Proceedings of National Academy of Sciences, India: Section B, Biological Sciences,* 84, 1031-1034. DOI:10.1007/s40011-013-0274-7.

Baksi, S., Behera, S., Bhakta, D., Kumar, S., Jomang, O., Saha, S. and Mondal, A., 2017. Effects of spirulina powder in colouration and growth enhancement of an indigenous ornamental fish *Trichogaster lalius*. *International Journal of Advance Biological Research,* 7, 263-267.

Baron, M., Davies, S., Alexander, L., Snellgrovet, D. and Sloman, K.A., 2008. The effect of dietary pigments on the colouration and behaviour of flame-red dwarf gourami, *Colisa lalia*. *Animal Behaviour,* 75, 1041-1051. DOI:10.1016/ j.anbehav.2007.08.014.

Boonyaratpalin, M. and Unprasert, N., 1989. Effects of pigments from different sources on colour changes and growth of red *Oreochromis niloticus*. *Aquaculture,* 79, 375-380. DOI:10.1016/0044-8486(89)90479-1.

Borg, A.A., Asmul, T., Bolstad, G.H., Viken, A., Berglund, A. and Rosenqvist, G., 2012. Interactions among female guppies (*Poecilia reticulata*) affects growth and reproduction. *Ethology,* 118, 752–765. DOI:10.1111/j.1439-0310.2012.02065.x.

Burton, G.W., 1989. Antioxidant action of carotenoids. *J Nutr,* 119, 109-111.

Chatzifotis, S., Pavlidis, M., Donate, J. C., Vardanis, G., Sterioti, A. and Divanach, P., 2005. The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). *Aquaculture Research,* 36, 1517-1525. DOI:10.1111/j.1365-2109.2005.01374.x.

Cooper, E.L., Peters, G., Ahmed, I.I., Faisal, M. and Ghoneum, M., 1988. Aggression in Tilapia affects immunocompetent leucocytes. *Aggressive Behavior* 15, 13-22. DOI:10.1002/1098-2337(1989)15:1<13::AID-AB2480150104>3.0.CO;2-C

Das, S.K. and Kalita, N., 2006. Seed production technology of ornamental gouramis *Colisa fasciata* and *C. lalia* under captive conditions-an experience in Assam, India. *Aquaculture Asia Magazine,* 11, 13-14.

Degani, G., Ben-Zvi, Y. and Levanon, D., 1989. The effect of different protein levels and temperatures on feed utilization, growth and body composition of *Claris gariepinus* (Burchell, 1822). *Aquaculture,* 76, 293-301. DOI:10.1016/0044-8486(89)90082-3

Dehadrai, P.V., Banerji, S.R., Thakur, N.K. and Das, N.K., 1973. Sexual dimorphism in certain air-

breathing teleosts. *Journal of Inland Fisheries Society of India*, 5, 71-77.

Duncan, P.L. and Lovell, R.T., 1993. Natural and synthetic carotenoids enhance pigmentation of ornamental fish. *Highlights of agricultural research-Alabama Agricultural Experiment Station*, 40, 8.

Dey, S. and Goswami, U.C., 2016. Restoration of Pigmentation in *Trichogaster fasciata*. *Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 11, 52-58. DOI: 10.9790/3008-11215358

Ejike, C. and Schreck, C.B., 1980. Stress and social hierarchy rank in coho salmon. *Transactions of the American Fisheries Society*, 109, 423-426.

Evans, J.P., Bisazza, A. and Pilastro, A., 2004. Female mating preferences for colourful males in a population of guppies subject to high predation. *Journal of Fish Biology*, 65, 1154-1159. DOI:10.1111/j.0022-1112.2004.00502.x

Fey, M. and Meyers, S.P., 1980. Evaluation of Carotenold-Fortified Flake Diets with the Pearl gourami *Trichogaster leeri*. *J Aquaricult*, 1, 16-19.

Frankel, J.S., 1992. Inheritance of Trunk Coloration in the Three-Spot Gourami, *Trichogaster trichopterus* (Pallas). *Journal of Fish Biology*, 41, 663-665. DOI:10.1111/j.1095-8649.1992.tb02693.x

Frischknecht, M., 1993. The breeding colouration of male three-spined stricklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. *Evolutionary Ecology*, 7, 439-450. DOI:10.1007/BF01237640.

Furr, H.C. and Clark, R.M., 1997. Intestinal absorption and tissue distribution of carotenoids. The *Journal of Nutritional Biochemistry*, 8, 364-377. DOI:10.1016/S0955-2863 (97)00060-0.

Giuliano, G., Aquilani, R. and Dharmapuri, S., 2000. Metabolic engineering of plant carotenoids. *Trends in Plant Science*, 5, 406-409. DOI:10.1016/S0014-5793 (02)02699-6.

Goodwin, T.W., 1986. Metabolism, nutrition, and function of carotenoids. *Annu Rev Nutr*, 6, 274-297. DOI:10.1146/annurev.nu.06.070186.001421.

Gupta, S.K., Jha, A.K., Pal, A.K. and Venkateshwarlu, G., 2007. Use of natural carotenoids for pigmentation in fishes. *Natural Product Radiance*, 6, 46-49.

Ha, B.S., Kang, D.S., Kim, J.H., Choi, O.S. and Ryu, H.Y., 1993. Metabolism of dietary carotenoids and effects to improve the body colour of cultured flounder and sea bream. *Bull. Korean Fish Soc*, 26, 91-101.

Halten, B., Arnmesan, A., Jobling, M. and Bjerkeng, B., 1997. Carotenoid pigmentation in relation to feed intake, growth and social integration in Arctic char, *Salvelinus aipinus* (L), from two anadromous strains. *Aquaculture Nutrition*, 3, 189-199. DOI:10.1046/j.1365-2095.1997.00087.x.

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

Hamrang, Omshi A., Bahri. A., Khara, H., Mohammadizadeh, F., 2019. The effects of lucantin red, yellow and astaxanthin on growth, hematological, immunological parameters and coloration in the Tiger Oscar (Astronotus ocellatus Agassiz,1831). *Iranian Journal of Fisheries Sciences***.** 18 (4), 798-811**.** DOI: 10.22092/ijfs.2019.118801

Hamlin, R.S., Jansi, M. and Vasudhevan, I., 2013. Effect of dietary *Spirulina plantensis* on feeding parameters of blue gourami, *Trichogaster trichopterus. International Journal of Research in Fisheries and Aquaculture*, 3, 103-106.

Huntingford, F.A. and Turner, A.K., 1987. Animal conflict. Chapman and Hall, London. 448 P.

Huntingford, F.A, Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P. and Turnbull, J.F., 2006. Current issues in fish welfare. *Journal of Fish Biology*, 68, 332-372. DOI:10.1111/j.0022-1112.2006.001046.x

Jagadeesh, T.D., Murthy, H.S., Surendranath, S.V., Panikkar, P., Manjappa, N. and Mahesh, V. 2015. Effect of supplementation of Marigold (*Tagetus erecta*) oleoresin on growth, survival and pigmentation of Rosy Barb, *Puntius conchonius* (Hamilton). *The Bioscan: An International Quarterly Journal of Life Sciences*, 10, 1431-1435.

James, R., Sampath, K., Thangarathinam, R. and Vasudevan, I., 2006. Effect of dietary spirulina level on growth, fertility, coloration and leucocyte count in red Swordtail, *Xiphophorus helleri*. *Israeli Journal of Aquaculture-Bamidgeh*, 58, 97-104. DOI:10524/19166.

James, R., Vasudhevan, I. and Sampath, K., 2009. Interaction of spirulina with different levels of Vitamin E on growth, reproduction, and colouration in goldfish (*Carassius auratus*). *Israel Journal of Aquaculture-Bamidgeh*, 61, 330-338. DOI:10524/19299.

Jobling, M. and Reinsnes, T.G., 1986. Physiological and social constraints on the growth of Arctic charr. *Salvelinus alpinus*: An investigation of factors leading to stunting. *Journal of Fish Biology*, 28, 379-384. DOI:10.1111/j.1095-8649.1986.tb05174.x

Jobling, M., Arnesen, A.M., Baardvik, B.M., Christiansen, J.S. and Jorgensen, E.H., **1995.** Monitoring voluntary feed intake under practical conditions, methods and applications. *Journal of Applied Ichthyology*, 11, 248–262. DOI:10.1111/j.1439-0426.1995.tb00025.x

Jorjani M, Sharifrohani M, Mirhashemi Rostami A, Tan S., 2019a. Effects of *Hibiscus rosasiensis* as a natural carotenoid on growth performance, body composition, pigmentation and carotenoid in blood plasma of blue gourami, Trichogaster trichopterus at different stocking densities. *Iranian Journal of Fisheries Sciences*. 18

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

(4), 619-634. DOI: 10.22092/ijfs.2018.119315

Jorjani, M., Sharifroohani, M., Mirhashemi Rostami, A., Enayat Gholampoor, T., Mirzaei, M., Tan Shau Hwai, A., 2019b. The effects of Marigold as natural carotenoids on scale chromatophores' variations in blue gourami under different stocking densities. Survey in Fisheries Sciences. 5(2), 29-36. DOI: 10.18331/SFS2019.5.2.4

Kalinowski, C.T., Robaina, L.E., Fernadez-Palacios, H., Schuchardt, D. and Izquierdo, M.S., 2004. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus Pagrus*) growth and skin colour. *Aquaculture*, 244, 223-231. DOI:10.1016/j.aquaculture.2004.11.001

Karga, J. and Sagar, C.M., 2016. Effect of different feeds on growth, survival and reproductive performance of zebrafish, *Danio rerio* (Hamilton, 1822). *Aquaculture Nutrition*, 23, 406-413. DOI:10.1111/anu.12407

Katayama, T., Shintani, K. and Chichester, C.O., 1973. The biosynthesis of astaxanthin. *Comparative Biochemistry and Physiology*, 448, 253-257.

Keller, K. and Brown, C., 2008. Behavioural interactions between the introduced plague minnow *Gambusia holbrooki* and the vulnerable native Australian ornate rainbowfish *Rhadinocentrus ornatus*, under experimental conditions. *Journal of Fish Biology*, 73, 1714-1729. DOI: 10.1111/j.1095-8649.2008.02045.x

Khanzadeh, M., Fereidouni, A.E. and Berenjestanaki, S.S., 2016. Effects of partial replacement of fish meal with *Spirulina platensis* meal in practical diets on growth, survival, body composition, and reproductive performance of three-spot gourami (*Trichopodus trichopterus*) (Pallas, 1770) *Aquaculture International*, 24, 69-84. DOI:10.1007/s10499-015-9909-4

Kodric-Brown, A., 1992. Male dominance can enhance mating success in guppies. *Animal Behaviour*, 44, 165-167. DOI:10.1016/S0003-3472 (05)80766-3.

Kop, A., Durmaz, Y. and Hekimoglu, M., 2010. Effect of natural pigment sources on the colouration of cichlid (*Cichlasoma severum* sp. Heckel, 1840). *Journal of Animal and Veterinary Advances*, 9, 566-569.

Kopecký, J., 2013. The Effect of Astaxanthin and β-carotene on the Colour of the Kissing Gourami (*Helostoma temminckii*). *Acta Fytotechnica et Zootechnica*, 16, 65-68.

McLennan, D.A. and McPhail, J.D. 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial colouration in *Gasterosteus aculeatus*: the relationship between male colour and female behaviour. *Canadian Journal of Zoology*, 68, 482-492. DOI:10.1139/z90-071.

Milinski, M. and Bakker, T.C.M. 1990. Female sticklebacks use mate coloration in mate choice and hence avoid parasitized males. *Nature*, 344, 330-333. DOI:10.1038/344330a0.

Mustafa, A., Randolph, L. and Dhawale, S., 2013. Effects of nutritional supplements phosphatidylcholine and beta-carotene on growth and selected stress and immune parameters in Nile tilapia, *Oreochromis niloticus* (L.) *Advances in Zoology and Botany*, 1, 57-61. DOI:10.13189/azb.2013.010302.

Naguib, Y., 2000. Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agriculture Chemistry*, 48, 1150-1154. DOI:10.1021/jf991106k.

Nandeesha, M.C., Gangadhara, B., Varghese, T.J. and Keshavanath, P., 1998. Effect of feeding Spirulina platensis on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio*. *Aquaculture Research*, 29, 305-312. DOI:10.1046/j.1365-2109.1998.00163.x

Oldfield, R.G., 2011. Aggression and Welfare in a Common Aquarium Fish, the Midas Cichlid. *Journal of Applied Animal Welfare Science*, 14, 340-360. DOI:10.1080/10888705.2011.600664.

Olson, J.A., 1989. Biological actions of carotenoids. *The Journal of Nutrition*, 119, 94-95. DOI:10.1093/jn/119.1.94.

Pailan, G.H., Archana, S. and Mrinal, K., 2012. Rose petals meal as natural carotenoid source in pigmentation and growth of rosy Barb (*Puntius Conchonius*). *Indian Journal of Animal Nutrition*, 29, 291-296.

Pan, C.H., Chien, Y.H. and Wang, Y.J., 2010. The antioxidant capacity response to hypoxia stress during transportation of characins (*Hyphessobrycon callistus* Boulenger) fed diets supplemented with carotenoids. *Aquaculture Research*, 41, 973-981. http://doi.org/10.1111/j.1365-2109.2009.02380.x

Paull, G.C., Filby, A.L., Giddins, H.G., Coe, T.S., Hamilton, P.B. and Tyler, C.R., 2010. Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. *Zebrafish*, 7, 109-117. DOI:10.1089/zeb.2009.0618.

Picciolo, A.R., 1964. Sexual and nest discrimination by species of *Colisa* and *Trichogaster Ecological Monographs*, 34, 53-77. DOI:10.2307/1948463.

Pottinger, T.G. and Pickering, A.D., 1992. The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. *Journal of Fish Biology*, 41, 35-447. DOI:10.1111/j.1095-8649.1992.tb02672.x

Ponce-Palafox, J.T., Arredondo-Figueroa, J.L. and Vernon-Carter, E.J., 2006. Carotenoids from plants used In diets for the culture of the Pacific white shrimp (*Litopenaeus*

[DOR: 20.1001.1.15622916.2020.19.6.16.8]

vannamei). *Revista Mexicana de Ingenieria Quimica,* 5, 157-165

Priyanka, M., Camellia, N., Nilofer, K. and Ruma, P., 2015. Mixed algal diet for skin colour enhancement of ornamental fishes. *Journal of Algal Biomass Utilization,* 6, 35- 46

Ramamoorthy, K., Bhuvaneswari, S., Sankar, G. and Sakkaravarthi, K., 2010. Proximate composition and carotenoid content of natural carotenoid sources and its color enhancement on marine ornamental fish *Amphiprion ocellaris* (Cuveir 1880). *World Journal of Fisheries and Marine Sciences,* 2, 545-550.

Reddy, D.C., Delhi, B.S. and Kalarani, V., 2014. Influence of spirulina on growth and Immunity of the fingerlings common carp, (*Cyprinus carpio,* l.1758), challenged with pathogenic Aeromonas hydrophila. *International Journal of Pharmacy and Life Sciences,* 5, 3590-3602.

Resear, J.A., 1967. Courtship and Reproductive behaviour in the Giant Gourami, *Colisa fasciata* (Bloch and Schneider). Thesis. pp. 1-44

Ricci, L., Summers, C.H., Larson, E.T., O'Malley, D. and Melloni, Jr. R.H., 2013. Development of aggressive phenotypes in zebrafish: Iinteractions of age, experience and social status. *Animal Behaviour,* 86, 245-252. DOI:10.1016/j.anbehav.2013.04.011

Segner, H., Arend, P., Poeppinghaussen, K.V. and Schmidt, H., 1989. The effect of feeding astaxanthin to *Oreochromis niloticus* and *Colisa labiosa* on the histology of the liver. *Aquaculture,* 79, 381-390. DOI:10.1016/0044-8486(89)90480-8

Seidgar, M., 2015. The effects of fairy shrimp *Phallocryptus spinosa* (Branchiopoda: Anostraca) as live food on reproduction performances and color of freshwater ornamental fish prawns. *International Journal of Aquatic Science,* 6(**1**), 37-44.

Seidgar, M., Nekuie Fard A., Jafarpour M., 2016. Fairy shrimps as a source of carotenoids for fish feeding. *Poeciliid Research,* 6(**1**), 1-3.

Simpson, K.L. and Chichester, C.O., 1981. Metabolism and nutritional significance of carotenoids. *Annual Review of Nutrition,* 1, 351-374. DOI: 10.1146/annurev.nu.01.070181.002031.

Simpson, K.L., Katayama, T. and Chichester, C.O., 1981. Carotenoids in fish feeds: Carotenoids as colorants and vitamin A precursors. *Academic Press New York,* 4, 463-538.

Sinha, A. and Asimi, O.A., 2007. China rose (Hibiscus petals: a potent natural carotenoid source for goldfish (*Carassius auratus* L.). *Aquaculture Research,* 38, 1123-1128. DOI:10.1111/j.1365-2109.2007.01767.x

Sloman, K.A. and Armstrong, J.D., 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? *Journal of Fish Biology,* 61, 1-23. DOI:10.1111/j.1095-8649.2002.tb01733.x

Sogard, S.M., 1994. Use of suboptimal foraging habitats by fishes: consequences to growth and survival. In: Stouder DJ, Fresh KL, and Feller RJ (Eds.) Theory and Application of Fish Feeding Ecology. The Belle W. Baruch Press, Columbia, SC. 18, 103–131.

Sogard, S.M. and Olla, B.L., 1996. Food deprivation affects vertical distribution and activity of a marine fish in a thermal gradient: Potential energy-conserving mechanisms. *Marine Ecology Progress Series*, 133, 43–55.

Stahl, W., Heinrich, U. and Jungmann, H., 1998. Increased dermal carotenoid levels assessed by noninvasive reflection spectrophotometry correlate with serum levels in women ingesting betacarotene. *The Journal of Nutrition*, 128, 903–907. DOI:10.1093/jn/128.5.903.

Sueki, K., 1991. Carotenoids: Metabolism of carotenoids and new physiological function of β carotene. *Journal of Japan Oil Chemists' Society*, 40, 893-903.

Swarup, K., Kumar, A. and Srivastava, S., 1972. Sexual dimorphism in the Giant Gourami, *Colisa fasciata* (Anabantidae). *Proceedings of the National Academy of Science*, 42, 93-94.

Vasundhevan, I., 2007. Effect of selected growth promoting immunostimulants on growth, reproduction, coloration and leucocytes count in goldfish, *Carassius auratus*. Ph.D. Thesis submitted to Manonmanian Sundaranar Univ., Tirunelveli, TN, India.

Vasudhevan, I. and James, R., 2011. Effect of optimum Spirulina with different levels of vitamin C on growth, reproduction and coloration in goldfish *Carassius auratus* (Linnaeus, 1758). *Indian Journal of Fisheries*, 58, 101-106.

Vasudhevan, I., James, R., Pushparaj, A. and Asokan, K., 2013. Effect of Azolla filiculoides on growth, coloration and leucocytes count in goldfish, *Carassius auratus*. *International Journal of Plant, Animal and Environmental Sciences*, 3, 211-219.

Wang, Y.J., Chien, Y.H. and Pan, C.H., 2006. Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. *Aquaculture*, 261, 641-648. DOI:10.1016/j.aquaculture.2006.08.040.

Watt, P.J., Skinner, A., Hale, M., Nakagawa, S. and Burke, T., 2011. Small subordinate male advantage in the zebrafish. *Ethology*, 117, 1003–1008. DOI:10.1111/j.1439-0310.2011.01953.x

[DOR: 20.1001.1.15622916.2020.19.6.16.8]