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*
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 (Nandeesh 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...
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 °C), 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
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 -20°C 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 (26° 56” N 80° 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 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=**\[
\frac{\text{Final mean weight} \ (g) - \text{Initial mean weight} \ (g)}{\text{Rearing period} \ (\text{days})} \times 100
\]

**Percentage of RGR=**\[
\frac{\text{Final mean weight} \ (g) - \text{Initial mean weight} \ (g)}{\text{Initial mean weight}} \times 100
\]

**Percentage of AGR=**\[
\text{Final mean weight} \ (g) - \text{Initial mean weight} \ (g)
\]

**Weight Gain Percent=**\[
\frac{\text{Final mean weight} \ (g) - \text{Initial mean weight} \ (g)}{\text{Initial mean weight} \ (g)/\text{Rearing period} \ (\text{days})} \times 100
\]

**FCR=**\[
\frac{\text{Weight of dry feed used}}{\text{Wet weight of fish} \ (g)}
\]

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
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.](image)

**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:

\[
\text{Carotenoid content} = \frac{\text{Optical density}}{0.25 \times \text{Sample weights (g)} \times 10}
\]

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

<table>
<thead>
<tr>
<th>Growth parameters</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body Weight</td>
<td>2.50±0.453</td>
<td>2.51±0.038</td>
<td>2.51±0.312</td>
<td>2.52±0.198</td>
</tr>
<tr>
<td>Final Body Weight</td>
<td>2.89±0.058</td>
<td>3.110±0.169</td>
<td>3.358±0.286</td>
<td>3.501±0.183*</td>
</tr>
<tr>
<td>Carotenoid content</td>
<td>3.093±1.014</td>
<td>5.667±1.034</td>
<td>7.103±0.306</td>
<td>8.901±0.034**</td>
</tr>
<tr>
<td>FCR</td>
<td>1.093±1.358</td>
<td>0.667±1.016</td>
<td>0.451±0.286</td>
<td>0.352±0.171*</td>
</tr>
<tr>
<td>SGR</td>
<td>0.412±0.394</td>
<td>0.498±0.798</td>
<td>0.615±0.530</td>
<td>0.745±0.561*</td>
</tr>
<tr>
<td>RGR</td>
<td>2.338±0.218</td>
<td>3.104±0.745</td>
<td>4.491±3.077</td>
<td>5.481±3.057**</td>
</tr>
<tr>
<td>AGF</td>
<td>0.061±0.859</td>
<td>0.083±0.075</td>
<td>0.125±0.087</td>
<td>0.156±0.097**</td>
</tr>
<tr>
<td>Weight gain Percent</td>
<td>32.110±6.65</td>
<td>46.562±0.158</td>
<td>67.375±4.159</td>
<td>82.225±4.857**</td>
</tr>
</tbody>
</table>

Means±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.

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

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

(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).

(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.
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
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 subordinates 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
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.

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