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LIVE FEED: COLORATION AND GROWTH RATE IN ANGEL FISH (*Pterophyllum scalare)*

ALIMENTO VIVO: COLORACIÓN Y TASA DE CRECIMIENTO EN PEZ ÁNGEL (*Pterophyllum scalare)*

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ABSTRACT

Animals obtain their energy through nutrients found in their food; feeding is related to the color and growth rate in aquatic animals, both conditions are influential aspects in ornate fish market value. In this study, we used live feed -*Culex quinquefasciatus* pre-adults- to test coloration and growth in angel fish -*Pterophyllum scalare*- which is an important species for ornamental aquaculture around the world. We used 60 individuals from *P. scalare* of the black variety for a period of 90 days. During this time; fish were fed with mosquito pre-adults and commercial flakes. We hypothesize that if live feed influences the coloration and growth rate in angel fish, then there must be differences in colour intensity, colour pattern and growth rate in fish fed with mosquito pre-adults. According with this prediction, a difference between *C. quinquefasciatus* diet and commercial flakes diet was found, showing good results in growth and color intensity in fish feeding with mosquito pre-adults. *P. scalare* fish were more colorful when they fed on the *C. quinquefasciatus* diet than when they were fed with commercial flakes diet. It was concluded that live feed (*C. quinquefasciatus* pre-adults) are an excellent way to enhance growth and coloration in ornamental fish.

Keywords: Pterophyllum scalare, live feed, coloration, growth rate, Culex quinquefasciatus.

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RESUMEN

Los animales obtienen su energía a través de los nutrientes encontrados en su alimento, la alimentación está relacionada con el color y la tasa de crecimiento en los animales acuáticos, ambas condiciones son aspectos influyentes en el valor de mercado de peces de ornato. En este estudio, se utilizó alimento vivo -pre-adultos de *Culex quinquefasciatus*- para probar la coloración y el crecimiento en peces ángel -*Pteryphyllum scalare*- que es una especie importante para la acuicultura ornamental en todo el mundo. Se utilizaron 60 individuos de *P. scalare* de la variedad negra, durante un período de 90 días. En este tiempo, los peces se alimentaron con pre-adultos de mosquito y hojuelas comerciales. Nosotros hipotetizamos que el alimento vivo influye en la coloración y el crecimiento en los peces ángel, esperando diferencias en la intensidad del patrón de color y la tasa de crecimiento en los peces alimentados con pre-adultos de mosquito. De acuerdo con esta predicción, se encontró diferencias en los peces alimentados con *C. quinquefasciatus* y hojuelas comerciales, mostrando mejores resultados en crecimiento e intensidad del color en peces alimentados con pre-adultos de mosquito. Los peces *P.* scalare fueron más coloridos cuando se alimentaron con *C. quinquefasciatus* que cuando se alimentaron con hojuelas comerciales. Se concluye que el alimento vivo (pre-adultos de *C. quinquefasciatus*) es una excelente forma de potenciar el crecimiento y la coloración de los peces ornamentales.

Palabras clave: Pterophyllum scalare, alimento vivo, coloración, tasa de crecimiento, Culex quinquefasciatus.

INTRODUCTION

Animals obtain their energy through nutrients found in their food (Schmidt, 1983). The quantity and the quality of these nutrients determine factors that are vital to their survival, such as growth, movement, reproductive rate, and color (Schmidt, 1983). Coloration is a highly plastic phenotype and is used in crypsis, communication, sexual selection, deimatic behavior, ultraviolet light protection, and thermoregulation (Proctor and McGinness, 1986; Sheridan and Pomiankowski, 1997; Stevens, 2005; Tattersall *et al*., 2006; Marshall *et al*., 2016). Several studies approach color in fishes for different lines of research, for instance, *Poecilia latipinna* (Lesueur, 1821) has the capacity to discriminate individuals in order to form groups of similar color patterns (McRobert and Bradner, 1998); *Betta splendens* (Regan, 1910) shows a sexual selection behavior that is based on their male red coloration (Clotfelter *et al*., 2007). Male three-spined sticklebacks -*Gasterosteus aculeatus* (Linnaeus, 1758) are capable of changing the color of their throats in order to drive off predators (Rowe *et al*., 2004).

Color expression and production in fishes is due mostly to carotenoid compounds (Sefc *et al*., 2014). Carotenoids exposure in fishes is considered an honest and costly signal. Due to the high amount of carotenoids in their diet, they are capable of using them for exposure in their tissue, aside from using them for their physiological functions, this ability of showing carotenoids in their skin is a reflection of their physical health (Sefc *et al*., 2014). In studies related to aquaculture, carotenoids have been focused mainly on the appealing properties of fish muscle for human consumption (Rajasingh *et al*, 2006; Valente *et al*., 2016). Considering that color determines the price of ornate fish, among other aspects such as size and body shape, there are few studies regarding this subject (Marañón *et al*., 1999).

Angel fish, *Pterophyllum scalare* (Schultze, 1823), has an important role in ornate fish economy (García-Ulloa and Gómez-Romero, 2005). Under production, its diet consist of small crustaceans of the order Cladocera and Anostraca (*Moina, Daphnia,* and *Artemia*), nematodes (*Panagrellus redivivus*; Goodey, 1945), annelids such as tubifex worms (*Tubifex tubifex*; Müller, 1774), pre-adults of mosquito (*Culex* *quinquefasciatus*; Say, 1823), and commercially available artificial feeds (Lim and Wong, 1997; Luna-Figueroa *et al*., 2010). *C. quinquefasciatus* is a cosmopolitan insect, its development from egg to adult takes 10 to 14 days (Manimegalai and Sukanya, 2014). During their pre-adult stage, they serve as diet for fishes. The effect in growth rate of using mosquito pre-adults as feed has been studied in *P. scalare* before with good results (Luna-Figueroa *et al.*, 2000; Luna-Figueroa *et al*., 2010), and their effect in color has been studied in the species *Labidochromis caeruleus,* Fryer, 1956 (Maleknejad *et al*., 2014). However, there has been no study about *C. quinquefasciatus* pre-adults and their effect in color intensity in *P. scalare*. In this work, we evaluated the effect of live feed (*C. quinquefasciatus*) in the coloration and growth rate of *P. scalare* under laboratory conditions.

MATERIALS AND METHODS

Collection and maintenance

Individuals (n =60) from the species *P. scalare*, of the black variety (TL= 25 ± 5.0 mm), were obtained from different adult pairs that came from ornate fish farms. The fish were introduced in a 200 L glass tank with constant aeration (26 ± 0.5 °C and a pH of 7.0 ± 0.2; Pérez *et al*., 2003) for a period of 15 days previous to the experiment. All fish were fed with a diet that consisted of 50% of *C. quinquefasciatus* pre-adults and 50% commercial flakes during acclimation (Luna-Figueroa *et al*., 2010).

Experimental protocol diets

Fish were starved for 48 h after acclimation and their weights were recorded with a plate balance (OHAUS; 0.01g), measured with digital calipers (INSIZE; 0.01 mm). Physical and chemical conditions of water remained the same as in the acclimation period. At the start of the experiment, angel fish were randomly divided into two experimental groups and three replicates (10 fish per tank; 6 tanks -20 L-). Fish were fed daily *ad libitum* once a day with each of the experimental diets, *C. quinquefasciatus* pre-adults and commercial flakes, for a period of 90 days.

Color measurements, growth rate and statistical analyses

At the beginning and at the end of the experiment, each fish was introduced in a light boot for photo shooting. The walls of the light boot where white and its measurements were 30 cm x 30 cm x 30 cm. The fish were placed one by one in plastic containers in order to take the photos. Each fish was photographed three times in the same position (ventral) using a Canon 60D professional camera. All pictures were converted into an 8-bit greyscale format and analyzed for color using ImageJ software (Schneider *et al*., 2012).

The daily growth rate or Absolute Growth (AG) of the angel fish was estimated as the difference between the initial and the final mass (M in g) and total length (TL in mm) divided by 90 days.The Absolute Growth Rate (AG) was estimated as the difference between the final and the initial size, divided by the duration of the experiment (90 days). The Relative Growth Rate (RGR) was calculated as the difference between the final and the initial size, divided by the duration of the experiment multiplied by the initial size and by 100. The Specific Growth Rate was estimated as the difference of the natural logarithm of the final and initial size, multiplied by 100 and divided by the duration of the experiment.

The coloration, final weight, and growth rate were evaluated using General Linear Models (GLM; Zar, 2010). Differences in the daily growth rate of the fish, using the diets as a variable, were examined at the end of the experiment using the Kruskal–Wallis test and conducted with Statistical software.

RESULTS AND DISCUSSION

Fish used in the analysis of growth rate and colour did not differ in size, weight, and coloration at the start of the experiment (P > 0.05). Individuals of *P. scalare* were more colourful when they fed on the *C. quinquefasciatus* diet than when they were fed with commercial flakes ($F_{(1,58)} = 4.49$, $P< 0.05$; Figure 1). The studies regarding dietary effects in coloration have used biomass that consisted of the microalgae *Chlorella vulgaris* (Beijerinck, 1890), *Haematococcus pluvialis* (Flotow, 1844), and also the Cyanobacteria *Arthrospira maxima* (Setchell and Gardner, 1917), as diets in the species *Cyprinus carpio* (Linnaeus, 1758) and *Carassius auratus* (Linnaeus, 1758), showing good results in comparison with commercial feed without added pigments (Gouveia *et al*., 2003).

Final mass and final total length were different among fish fed with commercial flakes (control) and the *C. quinquefasciatus* diet ($F_{(1,58)} = 29.79$, $P< 0.001$; $F_{(1,58)} = 24.87$, $P< 0.001$; respectively; Figure 2). The Absolute Growth Rate (AGR) differed among the fish fed with commercial flakes (control) and *C. quinquefasciatus* ($P < 0.01$). The Absolute Growth (AG) of the fish fed with *C. quinquefasciatus* was higher than the AG of the fish feeding with commercial flakes ($P <$ 0.01; Table 1). The relative Growth Rate (RGR) was 73.08% in mass and 73.33% in total length, higher for angel fish fed with *C. quinquefasciatus* in comparison to fish fed with commercial flakes. The Specific Growth Rate (SGR) was 85.71% in mass and 78.72% in total length higher in angel fish fed with *C. quinquefasciatus* in comparison to fish fed with commercial flakes (Table 1).

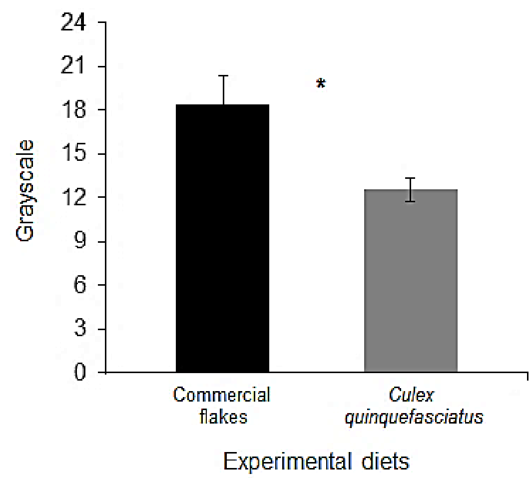

Figure 1. Coloration in *P. scalare* fed with commercial flakes (control) and *C. quinquefasciatus*.
Asterisk show significant difference (P < 0.05). Mean values and standard error are shown. A value closer to 0 represents a higher intensity of black color.

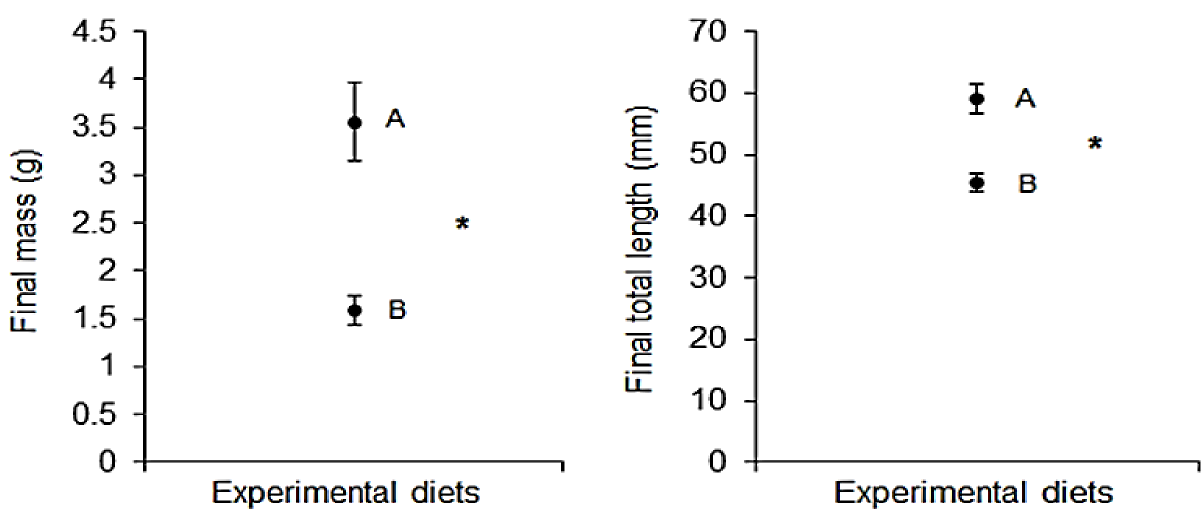

Figure 2. Final mass and total length in *P. scalare* fed with *C. quinquefasciatus* (A) and commercial flakes (B). Asterisk show significant difference (P < 0.001). Mean values and standard error are shown.

Table 1. Absolute Growth (AG), Absolute Growth Rate (AGR), Relative Growth Rate (RGR), and Specific Growth Rate (SGR), in *P. scalare* fed with commercial flakes and *C. quinquefasciatus*. Total length and mass were used.

		AG	AGR	RGR	SGR
Weight (g)	Commercial flakes	1.23	0.01	3.80	1.62
	C. quinquefasciatus	2.94	0.03	5.20	1.89
Total length (mm)	Commercial flakes	13.32	0.14	0.44	0.37
	C. quinquefasciatus	21.00	0.23	0.60	0.47

Live feed as *Tubifex*, *Gammarus*, *D. magna*, *Artemia* sp, and *Brachionus plicatilis* (Pallas, 1766) (Koca *et al*., 2009; Ortega-Salas *et al*., 2009; Kasiri *et al*., 2012) have been tested as diets measuring fish growth for *P. scalare* in comparison with commercial feed, showing good results

The use of *C. quinquefasciatus* as a diet compared to commercial flakes showed a difference in color and growth in *P. scalare* individuals. *C. quinquefasciatus* diets have been tested before in angel fish measuring growth rate, showing that mosquito pre-adults have a positive effect in this aspect and in reproduction frequency (Luna-Figueroa *et al*., 2000; Luna-Figueroa *et al*., 2010). Increasingly color intensity using mosquito pre-adults in comparison with commercial flakes could be attributed to *C. quinquefasciatus* pre-adults being filter feeders, meaning they feed on organic matter that is dissolved in the medium they are raised in (Manimegalai and Sukanya, 2014). *C. quinquefasciatus* used in the experimental diet were cultivated in an enriched organic matter environment (poultry waste fertilizer), which is an ideal medium for some types of algae to grow, such as *Chlorella* sp. (Agwa and Abu, 2014). Since mosquito pre-adults are prone to feed on these algae, the carotenoid contents algae carry could also be stored in *C. quinquefasciatus* pre-adults. Life feed has more nutritional benefits for fish digestion such as easy to digest proteins, enzymes that help macronutrients breakdown, and a higher protein content than commercial flakes feed (Luna-Figueroa *et al*., 2010). The results of this study showed *C. quinquefasciatus* diet to be more efficient in terms of color intensity and growth rate.

CONCLUSION

This work shows the potential use of mosquito pre-adults for angel fish production. Live feed (*C. quinquefasciatus*) is an excellent alternative for aquaculture because it demonstrates a positive effect in two of the most sought out aspects in the market: size and color.

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