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The Effect of Live Food on the Coloration and Growth in Guppy Fish, *Poecilia reticulata*

Elsah Arce Uribe[1*], Marco Polo Franco Archundia[2], Jorge Luna-Figueroa[1]

[1]Laboratorio de Acuicultura, Departamento de Hidrobiología, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México
[2]Maestría en Manejo de Recursos Naturales, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México
Email: *elsah.arce@uaem.mx, biol.marcofranco@hotmail.com, jluna@uaem.mx

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Abstract

Colour production in fish is due mostly to food. In conditions of captivity, the type of food is restricted, while various types of food are used in aquaculture, from processed dry food to small aquatic animals. In this study, we used nauplii of *Artemia franciscana,* "water fleas" *Moina wierzejski,* micro-worm *Panagrellus redivivus,* and commercial flakes. We used *Poecilia reticulata,* which is one of the most traded fish in ornamental aquaculture and hypothesise that if the live food influences the coloration and growth of *P. reticulata*, there must be differences in the intensity of colour pattern and growth rate in fish fed with different living animals. Consistent with our prediction, females and males of *P. reticulata* were more colourful when they were fed on *A. franciscana, P. redivivus* and *M. wierzejski* than when they were fed commercial flakes. Females and males of *P. reticulata* fed with *A. franciscana* grew in less time than fish fed with *P. redivivus, M. wierzejski,* or commercial flakes. We conclude that live food is an excellent way to affect growth and coloration in fish.

Keywords

Coloration, Growth Rate, *Artemia franciscana, Moina wierzejski, Panagrellus redivivus*

1. Introduction

Food is critical for all features of individuals. Colour production in fish is due mostly to food. In some animals like fish, coloration is used to indicate immune states [1], camouflage helps to detect predators and when stalking prey [2] [3] and it is a character used in sexual selection [4]. In many animals, colour is determined by the quality and quantity of food eaten, for instance, molluscs nurtured with carotenoid pigments [5] or crustaceans with *Hibiscus sabdariffa* in their food to increase their colour pattern [6]. In fish, feeding depends on several factors, such as the type of mouth, which is determined by the site of the water column in which the animal lives [7] [8]; age, which defines the size and type of food that the fish can ingest [9]; food availability, which is determined by the seasons in which the environment has a higher quantity and quality nutrients in the water [10]; and the number of individuals that compete for food resources in a specific area [11]. Colour is an attribute that is regulated by genetics and environmental conditions. Fish obtain coloration from carotenoids found in different foods [12]. When the animals eat, the pigment is saved directly to the skin, which is where the chromatophores are located; these cells reflect the coloration [13]. Feeding in fish is crucial from ecological and ornamental context. In conditions of captivity the most important animals used as live feeds are nauplii of *Artemia franciscana* [14], and mosquito larvae, *Culex quinquefasciatus* and *C. stigmatosoma* [15]. Other cultured animals for feeding are commonly known as "water fleas", *Moina wierzejski* and *Daphnia pulex* [16], and the micro-worm *Panagrellus redivivus* [17].

Poecilia reticulata is one of the most traded fish in ornamental aquaculture; there are many varieties which are characterised by the colour pattern. As in other species, coloration in *P. reticulata* could indicate immune status and is an important character in mate choice. This species is one of the most studied fish for their ecological and ornamental importance. We evaluated the effect of live food in the coloration and growth of *P. reticulata*, and we hypothesize that if live food influences the coloration and growth of *P. reticulata*, there will be differences in the intensity of the colour pattern and growth rate in fish fed with different living animals.

2. Materials and Methods

2.1. Experimental Diets

Four experimental diets were used containing different nutrients. Three of the experimental diets were cultured in the laboratory. The water flea, *M. wierzejski,* was grown in fiberglass ponds fertilised with chicken manure (50% protein, 19.37% lipids, and 4.12% carbohydrates) [18]. The micro-worm, *P. redivivus,* was grown in plastic containers with water-oatmeal (44.22% protein, 11.31% lipids, and 5.22% carbohydrates) [18]. Nauplii of *A. franciscana* were hatched in glass bottles in water at 35 ups (57.26% protein, 16.21% lipids, and 6.67% carbohydrates) [18]. The other diet was commercial flakes (45% protein, 4.5% lipids, and 5.0% carbohydrates). All cultures were maintained at 28˚C ± 2˚C.

2.2. Maintenance of the Animals

Fish were descendants by reproduction in the laboratory. Fish were obtained randomly at 30 days old from the same population of offspring of a pair of guppies, *P. reticulata*. Physical and chemical conditions of the water were: 25˚C ± 0.5˚C, pH 7.2 ± 0.1, 154.5 $mg \cdot L^{-1}$ $CaCO_3$ and 5.5 ± 0.5 $mg \cdot L^{-1}$ O_2. Food waste and feces were removed by siphon daily.

2.3. Experimental Protocol

Physical and chemical conditions of the water were the same as those used during acclimatization. Fish were randomly divided into four experimental groups and two replicates (n = 216). At the start of the experiment, fish were placed in 20 L tanks and fed daily *ad libitum* twice a day with each of the experimental diets. At 90 days the fish were removed of the experiment and the Absolute Growth Rate (AGR) was estimated as the difference between the final and the initial size, divided by the duration of the experiment. Fish were weights with a plate balance (OHAUS; 0.01 g), measured with callipers (0.01 mm), and sex was determined by the identification of gonophores.

2.4. Colour Measurements and Statistical Analyses

At the end of the experiment, each fish was introduced into a photographic tank (10 × 10 × 1 cm). All of the walls of this tank were white. Each fish was photographed 3 times in a plane parallel to the body using a camera (Panasonic DMC-GH4). All pictures were converted into 8-bit greyscale [19] and analysed for colour using ImageJ software [20]. This software detects intensity of the colour of an image in a grayscale; it is used in the digital image and in this case, the value of each pixel (PX) has a value equivalent to a grayscale. Each pixel is assigned a number between 0 and 255. The value 0 represents the black and the value 255 represents the white. Values closer to cero denote a higher intensity of colour and vice versa. The coloration and growth were evaluated using General linear models (GLM). To specify which means were significantly different from one another, we performed a Tukey post hoc test [21]. All statistical analyses were conducted using Statistics 10 Software.

3. Results

The survival rate of the test animals was 100%. Fish used in the analysis of colour at the end of the experiment did not different in size and weight in females or males ($P > 0.05$). Females and males of *P. reticulata* were more colourful when they fed with *A. franciscana, P. redivivus* and *M. wierzejski* than when they fed with commercial flakes ($F_{(3,116)} = 19.40$, $P < 0.001$; $F_{(3,100)} = 21.78$, $P < 0.001$; **Figure 1(A)** and **Figure 1(B)** respectively). Colour intensity between females and males did not differ in the animals fed with *A. franciscana, P. redivivus* and *M. wierzejski*; colour intensity between females and males was different in the fish fed with commercial flakes ($F_{(7,216)} = 19.10$, $P < 0.001$).

Females and males of *P. reticulata* fed with *A. franciscana* grew in less time than fish fed *P. redivivus* and *M. wierzejski.* The animals fed with commercial

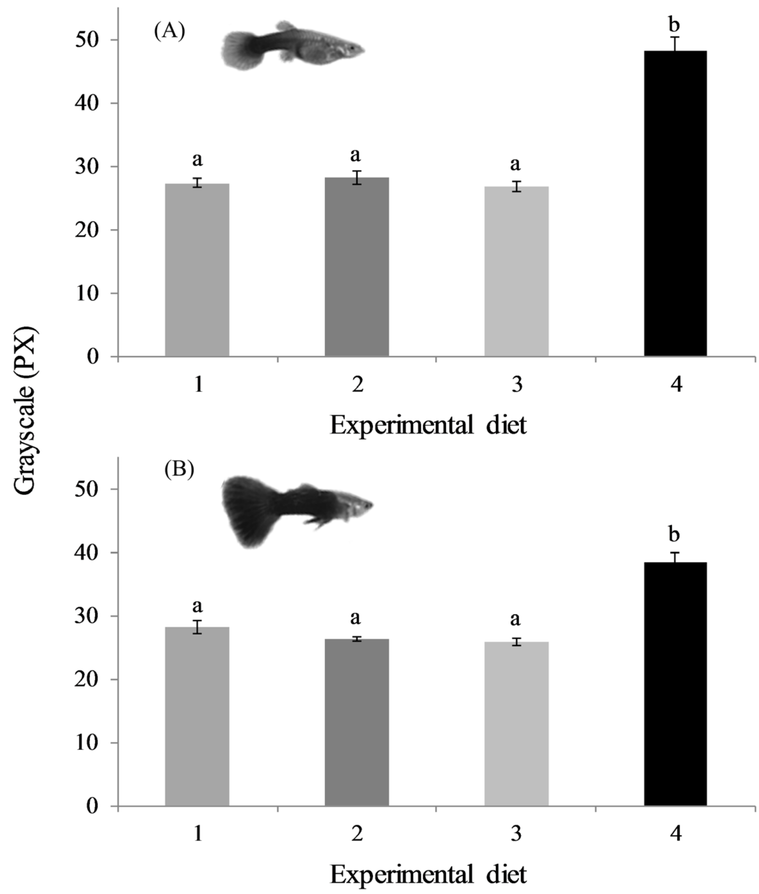

Figure 1. Coloration in *P. reticulata* fed with the four experimental diets: 1) *A. franciscana,* 2) *P. redivivus*, 3) *M. wierzejski*, and 4) commercial flakes. Letters indicated statistical differences ($P < 0.01$). Mean values and standard error are shown; a value closer to 0 represents a higher intensity of black color. A indicates females and B indicates males.

flakes in both genders grew longer than the other experimental diets ($F_{(3,116)} = 7.13$, $P < 0.001$; $F_{(3,100)} = 8.38$, $P < 0.001$; **Figures 2(A)-(B)**).

4. Discussion

High percentages of survival determine the success in the production of fish species with economic potential. The survival rate of the test animals was 100% for all diets used. Coloration is a cue for communication. Some animals, like fish, display carotenoid-based ornaments which are considered an honest signal of the body condition and parasite resistance [19] [22]. For example, studies showed that carotenoid-based coloration is related to social dominance [23]. Fish must obtain carotenoid pigments from their diet. Females and males of *P. reticulata* were more colourful when fed *A. franciscana, P. redivivus* and *M. wierzejski* than when they were fed with commercial flakes. Although flakes supplied in the experiments have a high nutritional value, coloration was more intense in fish that consume any live food. This difference could be the result of

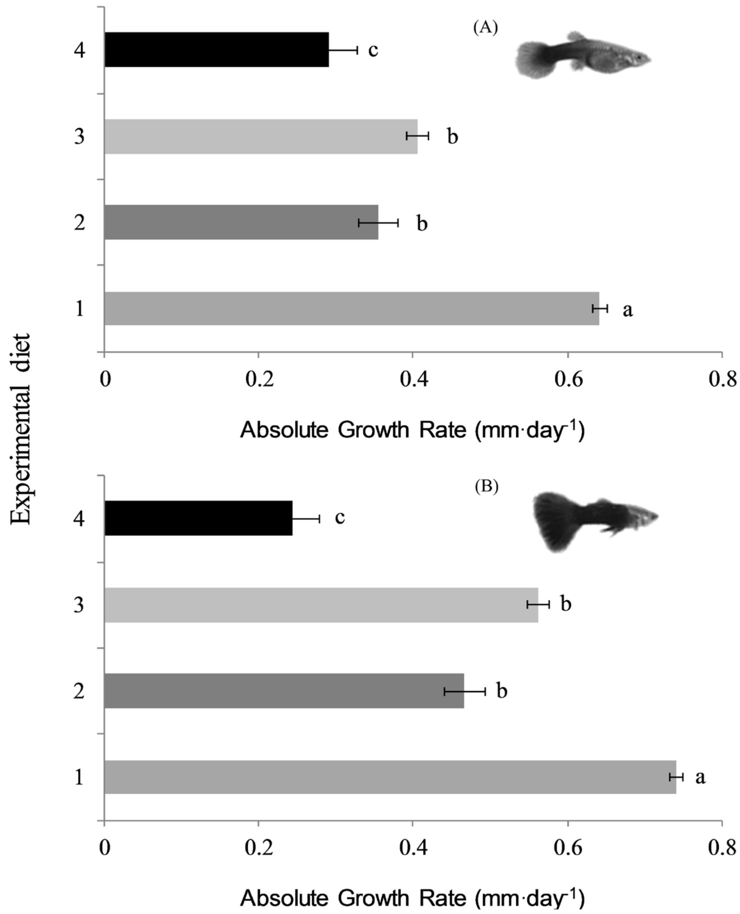

Figure 2. Growth in *P. reticulata* fed with the four experimental diets: 1) *A. franciscana,* 2) *P. redivivus*, 3) *M. wierzejski*, and 4) commercial flakes. Letters indicated statistical differences ($P < 0.01$). Mean values and standard error are shown; A indicates females and B indicates males.

the diet digestibility and in stimulating fish catches. Additionally, the live food does not undergo drying processes, freezing, or packaging, which decreases its original value [24] [25] [26]. On the other hand, increasingly colour intensity using live food in comparison with commercial flakes could be attributed to crustacean used (*M. wierzejski* and *A. franciscana*) which are filter feeders, so they obtain sources of carotenoids from microalgae present in cultured [27] [28] [29].

Growth is a good indicator of the health of an organism [30]. The growth rate of fish is affected by food quality, which is essentially determined by protein quality [31]. In this study, females and males of *P. reticulata* fed with *A. franciscana* grew in less time than the fish fed *P. redivivus* and *M. wierzejski.* In contrast, in both genders, animals fed with commercial flakes took longer to grow than the other experimental diets with live food. Live food is a capsule that contains the basic elements of a balanced diet with the advantage that it retains its value to be eaten by fish (*i.e.*, remains undissolved in contact with water).

Some studies have found that the growth is increased with live food in contrast with commercial flakes [12] [32] [33] [34]. Live food like *A. salina*, *Tubifex tubifex*, *D. pulex*, *Brachionus plicatilis*, and *C. pipiens* have differences in protein digestibility; these differences are due to the enzymes present in them. *Artemia* nauplii are an essential diet for fish with aquaculture potential [35]. However, *Artemia* nauplii quality varies with the strain, location, and season [36]. Additionally, *Artemia* nauplii are expensive and there is variation in availability, so we proposed *Moina* and *Panagrellus* as alternative of live food for aquaculture [37] [38]. *Moina* are crustaceans with high quality, and fast growth and reproduction. In this research, we showed that live food is an excellent alternative for aquaculture, all live food options are potentially usable for ornamental fish production, and we demonstrate a positive effect of live food in coloration and growth in *P. reticulata*.

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