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Short Communication

Background color effect on the pigmentation of prawn *Macrobrachium tenellum*

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ABSTRACT. In this research it was evaluated the effect of background color on the development of the chromatophores in *Macrobrachium tenellum* prawns, six juvenile prawns were exposed to five background colors: black, white, pink, red, white and green; each one in triplicate, for 60 days. The development of the chromatophores was evaluated by means of the chromatophores index using a scale of 1 (the maximum concentrated pigment) to 5 (the maximum dispersed pigment). The coloration was analyzed in four anatomical regions of the organisms with the HSB model (hue-saturation-brilliance). The results show that the prawns modify their pigmentation according to the different background colors of experimental aquariums.

Keywords: *Macrobrachium tenellum*, chromatophores, coloration, prawns, crypsis.

In the crustaceans, not all individuals of the same species can incorporate the same amount of pigments into their tegument, mainly because they acquire them through food (Menasveta *et al*., 1994; Meyers & Latscha, 1997) and individual differences may exist at the genetic level (Flores & Chien, 2011). For example, Bauer (1982) found polymorphisms in populations of caridean shrimp of the genus *Heptacarpus* because of their genetic variation. These changes appear to be an adaptation against predators in the habitat. In the wild, intraspecific genetic variation in pigment content has been reported in marine shrimp (Yanar *et al*., 2004) and in salmonids (Choubert, 2001).

The crustacean chromatophores have the functions of photoprotection, cryptosis and/or thermoregulation (Fuhrman *et al*., 2011). In photoprotection, chromatophores are used as shields to protect the animal's organs against radiation (Miner *et al*., 2000; Auerswald *et al*., 2008). Crypsis is a type of camouflage where it mixes with the background color and reduces the risk of being detected by a predator, this is achieved by matching the body color to the background color or using offensive color patterns that break the contour of the body and creates false lines (Merilaita, 1998). Background color adaptation has been found in several species of crustaceans (Fingerman, 1965, 1970; Johnson, 1974; Willmer *et al*., 1989) and represents one of the most common mechanisms of cryptic coloration (Endler, 2006; Stevens, 2007). An example of crypsis is seen in the amphipod *Apherusa glacialis*, which has the adaptation to different background colors and can prevent the detection of visual predators such as the polar cod (*Boreogadus saida*), which makes crypsis a reasonable advantage in the color change in *A. glacialis* (Fuhrman *et al*., 2011). In caridean shrimp, camouflage is considered the first function of chromatic adaptation for different habitats and background colors (Noël, 2000; Bauer, 2004). In addition, along with the effect of the background color on the crypsis, light intensity on organisms is added, as reported by Vega-Villasante *et al*. (2015) in *Macrobrachium tenellum*, where they find that at lower light intensities there is a lower number of chromatophores, and on the contrary, the greater the intensity of light, the higher the number of expressed chromatophores.

Size, shape, and color are the characteristics that determine the value of a fish or an organism in the market, therefore a production strategy is to change or increase its coloration using natural pigments in the diets. The high costs due to the supplementation of carotenoids in food, has led to a research on aspects that address alternative sources of pigments applicable in aquaculture (Imués-Figueroa *et al*., 2012). Synthetic products have been the most widely used pigment source in the aquaculture world, being standardized products, with a high concentration of carotenoids and easy to handle. However, these offer this unique contribution to the diet at an extremely high price, representing between 15 and 20% of the cost of the food (Sinnott, 1989). In view of the above scenario, the objective of this work is to observe if the background color can modify the coloration of the prawn *Macrobrachium tenellum* without the supplementation of pigments.

The bioassays were carried out at the Laboratorio de Calidad de Agua y Acuicultura Experimental, Centro Universitario de la Costa, Universidad de Guadalajara at 20°42'19"N and 105°13'16"W, at an altitude of 10 m above mean sea level in Puerto Vallarta, Jalisco, Mexico. Glass aquariums with 40 L capacity were used. To maintain the quality of the water cascade filters were used (Elite®). The temperature was maintained constant with thermostats (Sunny®) at a temperature of 28°C. The water was obtained from the municipal system, previously rested, to remove excess chlorine. The preadult of *M. tenellum* were collected in the waterway of the creek "El Zarco", in the municipality of Puerto Vallarta, Jalisco. All organisms were acclimatized for seven days prior to experimentation. Six preadult of *M. tenellum* were used, placed in each of the aquariums and exposed to five background colors (gravel): black, white, pink, red, white and green. Treatments were performed in triplicate. The experimental units were randomly distributed and had a duration of 60 days, with 12 h light and 12 h night.

For the base control diet, a balanced feed of initiation for tilapia (Purina®) with 45% protein, 14% moisture, 8% fat, 5% crude fiber, 10% ash was used. Daily feeding was carried out. The daily feed portion was adjusted to 10% of the body weight of the organisms according to Ponce-Palafox *et al.* (2002), the portion was not modified during the experimentation. To evaluate the effect of background color on the development of chromatophores, the chromatophores index proposed by Hogben & Slome (1931) was used, on a scale of 1 (the maximum concentrated pigment) to 5 (the maximum dispersed pigment). In the present study, a brightness close to 5 is considered as a more developed chromatophore. By treatment, 6 random organisms were taken to evaluate the development of chromatophores. With a Quasar™ microscope the chromatographs of the second abdominal segment and the lateral area of the cephalothorax were observed and three microscopic fields per organism were chosen. With a Canon T1I professional camera, photographs were taken on a white background from six randomly selected organisms. Image analysis was performed with the HSB (hue-saturation-brightness), of the color of the second abdominal segment and from the lateral side of the carapace were obtained through this model. The HSB model is a mathematical representation of color, in a similar way as to how the human eye perceives color. Brightness is the light emitted or reflected by a color in relation to other colors on which depends. It is the amount of white or black that has a color. Vertical displacement along the height of the pyramid, toward the black, involve a darkening of the color (0%), while at the white a clearing of the color it is presented in which the limit will be white (100%) (Georgieva *et al.*, 2005). The data of the index of chromatophores, the color value of the abdomen and cephalothorax, in each of the treatments, were compared to each other, for each case, variance analysis of one-way, previous tests of normality (Kolmogorov-Smirnov; $\alpha = 0.05$) and homoscedasticity (Bartlett; $\alpha = 0.05$), all through the statistical software, SigmaStat V3.1 (2004). The Kruskal-Wallis test for all data was applied. The significant differences between treatments were determined by Tukey multiple comparisons ($\alpha = 0.05$).

According to the index of chromatophores, the development of the chromatophores of the cephalothorax and abdomen of preadult prawns of *M. tenellum* was significantly different ($P < 0.05$) between prawns which were kept in a black and white background (Fig. 1). Prawns kept in a black background showed greater development ($P < 0.05$) in the abdomen chromatophores in regard to chromatophores of prawn kept in white, pink, red and green background, while the chromatophores development of the cephalothorax of the prawns kept in black background shares similarity ($P > 0.05$) with the prawns kept in red and green background, but not with the ones kept in white background, being these the least developed ($P < 0.05$) in the cephalothorax area (Fig. 1b). The brightness of the color in the abdomen of the prawns kept in black background was significantly lower ($P < 0.05$) than the rest of the treatments, while the value of color in the abdomen of the prawns kept in white, pink, red and green color background was significantly the same ($P > 0.05$) (Fig. 2a). The value of the color in the cephalothorax of prawns kept in all background colors did not show significant differences ($P > 0.05$) (Fig. 2b). The chromatophores of these prawns index coincide with chromatophores described by Arechiga *et al.* (2015) with *M. tenellum* juvenile prawns and Bauer (1981) with *Heptacarpus pictus* shrimps and *H. paludicola*. The type of chromatophores that describe the designated authors is red-yellow and the dispersion of pigments on the chromatosomes is, of soft red too intense for the center or soma and strong yellow for the ramifications, although in this study was also observed a red/orange for the ramifications.

The response to the background color have been observed in a wide variety of crustaceans, for example, in the marine shrimp, *Penaeus vannamei*, kept in dark tanks presented a blue/gray color, and when they were

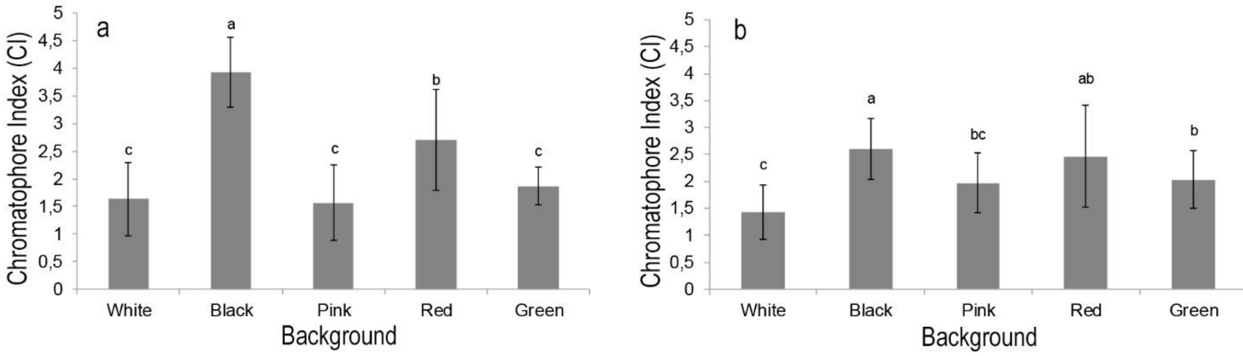

Figure 1. Evaluation of the development of chromatophores from the proposed chromatophor index by Hogben & Slome (1931) *M. tenellum* preadult prawns kept in aquariums with different background colors. a) Evaluation on the second abdominal segment, b) evaluation of the lateral side of the cephalothorax. Different superscripts show statistically significant differences between treatments ($P < 0.05$).

Figure 2. Evaluation of the effect of background color in the pigmentation of *M. tenellum* preadult prawns kept in aquariums with different background colors. a) Evaluation on the second abdominal segment, b) evaluation of the lateral side of the cephalothorax, c) prawn kept in white background, d) prawn kept in black background. The superscripts show statistically significant differences between treatments ($P < 0.05$).

cooked presented an orange color more intense than shrimp in white tanks (Parisenti *et al*., 2011). Raw shrimp kept in dark tanks were darker than the shrimp in white tanks. Similarly, Stevens *et al*. (2014) found that juveniles of *Carcinus maenas* are able to change significantly in brightness, being more shiny in white background and darker on a black background. The results of this study are consistent with what has mentioned above since the chromatophores of the abdomen of the prawns of *M*. *tenellum* kept on a black background were darker in regard to the prawns kept in white, pink, red and green backgrounds. The red chromatophores of the dwarf crayfish, *Cambarelus shifeldti*, kept over a dark color background showed a change in the degree of dispersion or concentration of the pigment. The red chromatophores, exposed to a white color background showed a maximum concentration of pigment and when they were exposed to a dark color background, they showed a maximum dispersion and changed their morphology (Fingerman & Lowe, 1957) in the scale of the development index of chromatophores proposed by Hogben & Slome (1931). The results of this study agree with the same response to the background color of the prawns kept both on white as dark background. Fingerman & Lowe (1957) mentioned that this morphological change also has an adaptive significance because the organisms became darker and are therefore more difficult to perceive in black backgrounds. And the same adaptive change happens to organisms kept on white backgrounds, where there is a maximum concentration of pigment in the central soma of the chromatophore.

The migration of granules of pigment inside crustaceans chromatophores provides an excellent model for understanding cytoplasmic movements, given the antagonist peptide neurosecretory of the granules translocation. Red pigment concentrating hormone (RPCH) induces aggregation of pigment in chromatophores of shrimp through an increase in the intracellular Ca^{++} (Reibero & McNamara, 2006). As an alternative to the pigmentation by the background color, it is proven that a red chromatophore it is completely dispersed, so it will be difficult to concentrate its pigment again. This inability to concentrate red pigment may be due in part to their morphology in the change of color (Fingerman & Lowe, 1957). If the red pigment in the chromatophore has increased, then all pigment could not have been able to fit in the central portion of the chromatophore, so that, despite the maximum concentration of the pigment, the chromatophore appears to be in a development stage 3 according to Hogben & Slome (1931) and Fingerman & Lowe (1957). In addition to the objective of this work, Parisenti *et al*. (2011) found no difference in the total of carotenoids in shrimp from *P. vannamei* kept in dark and white background of the cephalothorax and muscle. The conclusions made by Parisenti *et al*. (2011) indicate that the background color changes the color of the shrimp, but does not change a number of accumulated carotenoids. It is also important to note that the prawns brightnessof this study (≈50%) is close to *M. tenellum* in wild habitat, as reported by Aréchiga *et al*. (2015). Therefore, the use of the background color may be an alternative to intensify the color of the prawn being cultivated which are not fed with food containing carotenoids or pigments.

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