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Original Article

What kind of sheets does the fat sleeper fish prefer? Effect of pond bottom color on the growth and health of *Dormitator latifrons* (Richardson 1844)

Que tipo de lençol prefere o peixe dorminhoco gordo? Efeito da cor do fundo do tanque no crescimento e na saúde dos *Dormitator latifrons* (Richardson, 1844)

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Abstract

A little-studied characteristic of the Mexican native fish *Dormitator latifrons* is the effect that the color of the bottom or lining of ponds or tubs can have on their growth and blood parameters under controlled conditions. After a quarantine period in prophylactic treatment, an initial assay was performed. The organisms were grouped into four treatments (white, yellow, blue, and black) in triplicate, with 30 individuals with an average weight of 34.7± 2.5 g and average size of 12.5 ± 1.5 cm per tank. The trial lasted 60 days, after which a final biometry to all specimens and blood extractions to six random organisms per tank were performed. The following hematological and blood chemistry parameters were evaluated: erythrocytes, cell counts, and differential counts, as well as glucose, albumin, a/g ratio, and total proteins. Final weight, weight gain, and specific growth rate presented statistical differences between treatments ($p<0.05$), with dark bottoms (blue and black) above 80% of WG and above 1.0 of SGR. No statistically differences were found in hematological blood chemistry parameters ($p>0.05$). The growth results suggest that dark pond bottoms promote the adaptation of *Dormitator latifrons* by allowing it to avoid detection by predators through the adoption of a cryptic coloration. However, the species shows a great capacity for crypsis, being able to change its pigmentation to adapt to different bottom colors.

Keywords: blood chemistry, mimicry, Eleotridae, rural aquaculture, crypsis.

Resumo

Uma característica pouco analisada do peixe nativo *Dormitator latifrons* é o efeito que a cor do fundo ou revestimento de tanque, ou banheiras pode ter sobre seu crescimento e parâmetros sanguíneos em condições controladas. Após um período de quarentena em tratamento profilático, foi realizado um bioensaio inicial. Os organismos foram agrupados em 4 tratamentos (branco, amarelo, azul e preto) em triplicata, com 30 indivíduos por viveiro. O bioensaio durou 60 dias, após os quais foi realizada uma biometria final e extrações de sangue. Os seguintes parâmetros hematológicos e bioquímicos foram avaliados: eritrócitos, contagem de células, contagens de diferenciais, glicose, albumina, relação a/g, e proteínas totais. O peso final, o ganho de peso e a taxa de crescimento específico apresentaram diferenças entre os tratamentos, em fundos escuros (azul e preto), os parâmetros citados foram maiores. Não foram encontradas diferenças estatisticamente significativas nos parâmetros químicos hematológicos do sangue. Os resultados sugerem que os fundos escuros dos tanques promovem a adaptação e o crescimento dos *Dormitator latifrons*, permitindo que eles evitem a detecção por predadores através da adoção de uma coloração críptica. No entanto, a espécie apresenta grande capacidade de cripse, podendo alterar sua pigmentação para se adaptar a diferentes cores de fundo.

Palavras-chave: química do sangue, mimetismo, Eleotridae, aquicultura rural, cripse.

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1. Introduction

Today, aquaculture is considered one of the fastest-growing food production sectors (FAO, 2020). Worldwide, freshwater aquaculture has turned to the cultivation of exotic species (tilapia, catfish, and trout) due to their adaptability and higher growth rates compared to native species, as well as the significant advances in the technification of fish cultivation, (FAO, 2020). However, the introduction of exotic species outside their natural distribution area can cause alterations in native ecosystems. Even though in some cases these alterations are evident, they remain little studied for the most part. To prevent the harmful effects of aquaculture due to the indiscriminate use of introduced species, it is necessary to develop specific culture technologies for native species and create an aquaculture industry focused on sustainability (Basto-Rosales et al., 2019).

Dormitator latifrons (Richardson 1844) is a native species distributed along coastal areas from southern California (USA) to Peru. It can adapt to various ecosystems, marshes being one of its main habitats (Agualsaca-Ormaza, 2014; Vega-Villasante et al., 2021). It represents an alternative for aquaculture because it has the potential to be commercially exploited for direct human consumption and fishmeal production (Badillo-Zapata et al., 2018; Vega-Villasante et al., 2021). This species can be cultivated using artisanal methods, under rustic conditions and at low cost, with minimal environmental impact yielding a product of high quality and nutritional value (Castro et al., 2005; Lopez-Huerta et al., 2018; Basto-Rosales et al., 2020; Vega-Villasante et al., 2021).

The aquaculture industry uses materials of different colors for the coating of culture ponds, the following authors have researched the possible negative effects that a poor selection of pond bottom colors can have on fish development: Volpato et al. (2004), reported that white-colored environments are associated with lower frequency and intensity of reproduction in Nile tilapia compared to blue-colored environments; Kesbiç et al. (2016), found out that yellow or blue ponds are associated with lower economic gains in juvenile sea bass cultures compared to red- and green- colored ponds; Bayrami et al. (2017), suggested that ponds with a color other than black are associated with low biomass increase in sturgeon *Acipenser ruthenus*; and McLean (2021), reported that light-colored ponds increase aggressiveness in some fish species.

It is of utmost importance to consider whether the color of the materials used in aquaculture ponds produces stress or negative effects on the growth of cultured fish. The present study aimed to evaluate the effect of the color of culture pond bottoms on the growth, survival, and health of *Dormitator latifrons*.

2. Materials and Methods

2.1. Collection of organisms

The study was conducted at the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) of the University Center of the Coast of the University of Guadalajara, Puerto Vallarta, Jalisco, Mexico. One thousand juveniles of *D. latifrons* were captured in the Ermitaño estuary, located in the town of Cruz de Loreto in the municipality of Tomatlán, in the state of Jalisco, Mexico. The specimens were captured using a cast net (2" mesh size) and a spoon net. The organisms were transported in a 1000 L container with constant aeration and ice to reduce their metabolism while also decreasing the stress caused by overcrowding and transport. Once at the LACUIC facilities, the fish started a period of quarantine and acclimation to captivity of 20 days; they were subjected to an ectoparasite removal treatment during quarantine with Dimilin ® (diflubenzuron 22%, at a concentration of 300 µg L^{-1}) in a 200 L container for 10 minutes during the first day of quarantine, after parasite removal they were transferred to a 1700 L tank with a mean temperature of 29.5±1.0 °C, mean dissolved oxygen of 4.8±0.5 mg/L, and mean pH 7.8±0.2, during quarantine period (recorded with the use of an oximeter YSI® 550A). During quarantine the organisms were fed with commercial balanced feed (35% protein and 3.5% lipids; Grow fish Tilapia No. 2®) for juvenile tilapia. Constant aeration was provided and 75-80% of the water was changed every third day.

2.2. Experimental design

The experimental culture comprised four different bottom colors (four treatments) for the culture ponds: white (rgb 255,255,255), yellow (bright yellow, rgb 255,255,0), blue (royal blue, rgb 65,105,255), and black (rgb 0,0,0), each in triplicate. The system consisted of 12 experimental units (EU), each of which consisted of a 600 L reservoir (Rotoplas®) internally lined with plastic of the corresponding color. External pressurized canister-type biological filters (Sunny® model SCF-1200) were installed in all the EUs. A total of 360 fish were selected from the quarantine pond and weighed with an analytical balance (Rhino® precision of 0.2 g). The length (cm) of the selected fish was measured with an ichthyometer. The average initial weight was 34.7± 2.5 g and the average initial length was 12.5 ± 1.5 cm. Thirty specimens per EU were randomly selected and weighed together so that each EU contained an amount of biomass as homogeneous as possible. The experimental assay lasted 60 days.

During the experimental stage, the fish were fed once a day with the same feed used for their maintenance (Grow fish® stage 2), based on 4% of the biomass of each EU. The EU were filled to 90% of their capacity, maintained under constant aeration and 75-80% of the water volume was changed every third day using dechlorinated water.

The photoperiod was adjusted to the natural light corresponding to the time of the year (12 h light/12 h dark). The average temperature of the ponds was kept within a range of 28.2 - 30.1 °C. At the end of the 60 days of experimentation, the individual weight (g) and length (cm) of all specimens of each EU were recorded again.

2.3. Growth indices

Growth indices were determined for each treatment by evaluating weight gain and specific growth and survival

rates. The indices were calculated according to the following Formulas 1, 2 and 3:

$$Weight\ gain(WG): \left((final\ weight - initial\ weight)/initial\ weight\right)*100 \quad (1)$$

$$Specific\ Growth\ Rate(SGR) = 100 * \left((ln\ final\ weight - ln\ initial\ weight)\right)/time \quad (2)$$

$$Survival\ percentage = \left(Final\ number / Initial\ number\right)*100 \quad (3)$$

2.4. Collection of blood samples

After the end of the 60-days experiment, blood samples were taken from six randomly chosen organisms per experimental unit. The fish were fasted for 24 hours before collecting the blood samples. To avoid stress and follow good animal welfare practices, the fish were numbed (one fish at a time) before and during blood collection with an infusion of clove oil (0.3 mL/L) dissolved in ethanol (1:10), according to the method proposed by Javahery et al. (2012), until they showed erratic swimming. The fish were placed in a dissection tray and their heads were covered to prevent the fish from moving during caudal vein puncture. Blood samples were collected using a non-heparinized insulin syringe, following the technique of Stoskopf (1993). To determine the hematological parameters, three of the samples were placed in Microtainer® K2-EDTA tubes with anticoagulant and refrigerated at 15 °C. The rest of the blood samples were placed in vials without anticoagulant. The latter samples (without anticoagulant) were centrifuged for 5 minutes (Ika mini G 3958000® centrifuge) at 2000 x g to obtain the blood serum. Once the serum was obtained, it was transferred to plastic vials (Eppendorf®) and stored at 4 °C for later analysis. The above process was performed in the same way for each EU.

2.5. Respiratory burst analysis

The respiratory burst analysis was performed following the method reported by Ibrahem et al. (2010). A total of 100 µL of blood with K2-EDTA additive were placed in a well of a microplate, as well as 100 µL of a solution with 0.2% nitroblue tetrazolium (Nitroblue tetrazolium, NBT) (previously prepared in an amber container). The same procedure was performed for each blood sample with K2-EDTA additive in its respective well. Subsequently, the microplate was covered with aluminum foil and incubated for 30 minutes at room temperature, after which 50 µL of the mixture were taken and placed in an Eppendorf® tube containing 1mL of N, N-dimethyl formamide, and centrifuged at 2000 x g for 5 minutes. The supernatant was recovered and analyzed in a spectrophotometer at 620 nm using 1 mL quartz cells. The whole process was carried out in low light to avoid sample deterioration.

2.6. Hematological parameters

The hematocrit analysis (Hct %) was carried out in the following way: using a glass capillary, approximately 60 µL were collected from each of the blood samples with k2-EDTA additive, placed in a DM1424 Hematocrit Centrifuge (Scilogex®), and centrifuged for 10 minutes at 4000 x g. Once the process was finished, the hematocrit was measured in a circular glass capillary reader.

For the erythrocyte cell count (mm^3), 20 µL of blood with K2-EDTA additive were mixed with 4 mL of Natt-Herrick solution. A compound microscope (AmScope®) and a Neubauer chamber were used for the analysis. Erythrocytes were counted in the central quadrant of the Neubauer chamber, in five points (the four corners and the central point of the quadrant) with an area of 0.04 mm^2 each.

The mean corpuscular volume (MCV) is a measure of the size of erythrocytes according to the Formula 4:

$$MCV = \left(hematocrit \times 10 / erythrocyte\ count\right) \text{ (Table 2)} \quad (4)$$

2.7. Blood chemistry

The blood chemistry analysis was performed on the samples (serum) preserved in previously thawed vials. Total proteins were determined using the Biuret method with a Mexlab® kit. Albumin was determined using the BCG method with a Mexlab® kit. For glucose analysis, the GOD-PAD method was used, with a Mexlab® kit. A spectrophotometer (VE-5000V®) was used, at a wavelength of 620 nm, to record the absorbance values resulting from the above analyses.

2.8. Statistical analysis

Shapiro-Wilks (normality) and Bartlett (homoscedasticity) tests were performed. Once the data were found to conform to the above assumptions, a one-way analysis of variance (ANOVA) was used to evaluate significant differences between treatments for initial weight, final weight, weight gain, and specific growth rate. The survival data (expressed as percentages) were previously transformed by the arcsine root transformation method for statistical analysis. Statistical significance was set at $p<0.05$ and the results are expressed as means and standard deviation values. The hematocrit is expressed as arcsine-transformed percentage values. All data were analyzed statistically using the software SigmaPlot 11.0.

3. Results

3.1. Growth parameters

Regarding the growth parameters, there were significant differences ($P<0.05$) in weight gain (g) (Formula 1), weight increase (%), and specific growth rate (Formula 2). These differences were found when comparing ponds with light- (white and yellow) and dark-colored (black and blue) bottoms. The highest weight increase was observed in the black bottom treatment (67.8 ± 5.4), followed by the blue (63.3 ± 2.9), yellow (58.0 ± 1.6), and white (54.2 ± 4.6) bottom treatments. The specific growth rate (SGR) was

highest (1.1) in the black bottom treatment, followed by blue (1.0), yellow (0.9), and white (0.8). In all treatments, the survival rate at least 97% (Table 1) for all bottom colors (97% for yellow and white, and 100% for black and blue), there was not statistically differences (P>0.05) observed in the survival rate (Formula 3) during this experiment.

3.2. Hematology and blood chemistry analysis

Respiratory burst was low in blue bottom organisms (0.12 ± 0.02), and the highest RB was observed in yellow bottom (0.20 ± 0.07). Organisms in dark color ponds presented higher mean values of hematocrit (46.33 ± 9.45% in blue, and 43.50 ± 3.61% in black), than in light color pond (37.33 ± 12.24% in yellow, and 34.67 ± 10.12% in white). Glucose in blood during this experiment ranged from 85.54 ± 12.16 (yellow ponds), to 126.10 ± 26.66 mg dL^{-1} (blue ponds). Proteins in blood ranged from 4.76 ± 1.29 (blue ponds), to 5.33 ± 1.73 g dL^{-1} (yellow ponds). Albumins/Globulins ratio showed a mean value of 0.90 ± 0.97 in white pond organisms, 0.52 ± 0.32 in blue pond organisms, 0.51 ± 0.25 in yellow pond organisms, and 0.38 ± 0.12 in black pond organisms. However, no significant differences (P> 0.05) were found between the different treatments in any parameters of the hematological and blood chemistry analysis (Table 2). Results of hematological and blood chemistry obtained were within ranges of good health for the species.

4. Discussion

Recent studies in other fish species such as *Carassius auratus* (Linnaeus 1758) (Eslamloo et al., 2013), *Oncorhynchus mykiss* (Walbaum 1792) (Üstündağ and Rad, 2015), *Dicentrarchus labrax* (Linnaeus 1758) (Kesbiç et al., 2016), *Clarias gariepinus* (Burchell 1822) (Okomoda et al., 2017) and *Cyprinus carpio* (Linnaeus 1758) (Marandi et al., 2018) suggest that growth, age, biotopes, feed type, food habits, behavior, survival, diseases, and reproduction are directly related to the bottom color of culture ponds.

Regarding survival, the only mortalities recorded during the experiment were observed in the ponds with light-colored bottoms (white and yellow). However, there

Table 1. Growth indicators (mean ± SD): initial weight (g), final weight (g), initial length/ind (cm), final length/ind (cm), total increment (g/day), weight increment (%), specific growth rate, survival (%), observed in *Dormitator latifrons* juveniles subjected to different pond bottom colors during 60 days of experimentation. Different super index letters in rows indicate statistical significance (P<0.05).

Growth indicators	Experimental treatments			
	White	Yellow	Blue	Black
Initial weight/ind (g)	34.8 ± 0.1	34.7 ± 0.1	34.7 ± 0.1	34.8 ± 0.2
Final weight/ind (g)	54.2 ± 4.6[a]	58.0 ± 1.6[a]	63.3 ± 2.9[b]	67.8 ± 5.4[b]
Initial length/ind (cm)	11.5± 1.2	11.7± 3.2	10.5± 2.2	10.9± 1.2
Final length/ ind (cm)	14.5± 2.2	15.2± 3.2	16.5± 2.2	17.5± 3.2
Total increase (g)/day	0.33±0.04[a]	0.38±0.01[a]	0.47±0.02[b]	0.54±0.03[b]
Weight increase (%)	58.5±5.2[a]	66.9±4.8[a]	82.4±5.5[b]	93.9±6.2[b]
Specific growth rate	0.8	0.9	1.0	1.1
Survival (%)	97	97	100	100

Table 2. Hematological parameters: respiratory burst, hematocrit (%), Erythrocytes (mm^3) Mean corpuscular volume (fL), and blood chemistry parameters: glucose (mg dL^{-1}), proteins (g dL^{-1}), albumins (g dL^{-1}), globulins (g dL-1); observed in *Dormitator latifrons* juveniles subjected to different pond bottom colors. Mean ± SD.

Parameters	Experimental treatments			
	White	Yellow	Blue	Black
Respiratory burst	0.17 ± 0.03	0.20 ± 0.07	0.12 ± 0.02	0.16 ± 0.02
Hematocrit (%)	34.67 ± 10.12	37.33 ± 12.24	46.33 ± 9.45	43.50 ± 3.61
Erythrocytes X 106 (mm^3)	0.84 ± 0.58	0.80 ± 0.19	1.01 ± 0.34	1.01 ± 0.15
Mean corpuscular volume (fL)	425.93 ± 342.06	343.94 ± 80.81	356.89 ± 107.44	337.65 ± 62.10
Glucose (mg dL^{-1})	110.00 ± 34.32	85.54 ± 12.16	126.10 ± 26.66	95.71 ± 37.59
Proteins (g dL^{-1})	5.15 ± 2.23	5.33 ± 1.73	4.76 ± 1.29	5.31 ± 2.96
Albumins (g dL^{-1})	1.64 ± 0.40	1.62 ± 0.10	1.44 ± 0.29	1.30 ± 0.33
Globulins (g dL^{-1})	3.51 ± 2.62	3.72 ± 1.80	3.31 ± 1.46	4.01 ± 2.63
A/G Ratio	0.90 ± 0.97	0.51 ± 0.25	0.52 ± 0.32	0.38 ± 0.12

were no statistically significant differences between the treatments, suggesting that mortality in this species is not affected by tank color, just like results reported by Üstündağ and Rad (2015), Kesbiç et al. (2016), and Okomoda et al. (2017), in other cultivated species. In this experiment, the mortalities were probably not related to the effect of the pond bottom, and more likely were related to food competition or to management problems, like bad handling of the organisms while performing the initial biometric or during ponds cleaning in the first days.

In a study of *Carassius auratus*, Eslamloo et al. (2013) used ponds with different bottom colors (white, black, blue, and red). They reported a tendency for weight increase in ponds with white bottoms. Üstündağ and Rad (2015) evaluated the effect of four different bottom colors (beige, gray, dark green and light green) on rainbow trout fry (*Oncorhynchus mykiss*) and observed statistically significant differences between them, with beige being the most suitable color for fish rearing. In both cases, there was a tendency for the species under study to thrive in ponds with light colors, contrary to the results obtained in the present study, in which the best results were associated with dark colors. Growth results in our experiment are consistent with those reported by Bayrami et al. (2017) for juvenile sturgeon *Acipenser ruthenus* (Linnaeus 1758) kept in ponds with black, dark blue, gray, and white bottoms. They reported that dark colors (black and blue) were associated with better growth and survival rates.

It has been suggested that the bottom color of aquaculture ponds influences the stress response and health of cultured fish (McLean, 2021). McLean (2021) mentioned that hematology is an effective tool to assess fish health since blood components respond rapidly, depending on physiological factors such as species, age, life stage, sexual maturity stage, density, health condition and environmental variables, among others. Regarding *D. latifrons*, the existing information on its hematological and blood chemistry parameters can serve as a reference when trying to determine if the color of the pond has a stressful or negative effect on this species (Ruiz-González et al., 2020; Badillo-Zapata et al., 2022), however, not statistical differences in those parameters seems to suggest that there are not stress relate problems in this fish caused by the pond color. Eslamloo et al. (2013), found similar hematological results in goldfish with different background colors, however, they found statistical differences in blood chemistry, indicating an increase of stress in goldfish growing in ponds with dark color bottoms (red and dark blue).

Ruíz-González et al. (2020) proposed reference blood parameters for juvenile specimens of *D. latifrons* grown in ponds with white bottoms at a density of 10 fish m^{-2}. They reported the following results: respiratory burst interval (NBT) (0.23-0.55 abs), hematocrit (11.103-45.064%), erythrocytes (1.1×10^6-2.9×10^6 (mm^3)), leukocytes (21.01×10^3 - 49.05×10^3 (mm^3)), MCV (91.55-231.53 fL), glucose (34.63 - 68.30 mg dL^{-1}), protein (2.25-5.6 g dL^{-1}), albumin (0. 33-3.74 g dL^{-1}), globulin (1.85 - 2.9 g dL^{-1}) and albumin/globulin ratio (0.30-1.06). As can be seen, the results of the present study are within the reference intervals for hematological and blood chemistry parameters (Table 2). The results proposed by Ruiz-Gonzalez et al. (2020) indicates a good health in *D. latifrons*, this may suggest that in our experiment the organisms were healthy after cultivation and adaptation to background color does not increase stress.

In freshwater ecosystems, crypsis and mimicry are essential for the survival of many organisms, allowing them to avoid detection by potential predators and other threats (Nobre Carvalho et al., 2006; Magellan & Swartz, 2012; Magellan, 2020; Encel & Ward, 2021). In the present study, the crypsis behavior observed in *D. latifrons* did not significantly affect blood parameters. Since the hematological and blood chemistry parameters of all the organisms under study were within the normal intervals for the species (Ruiz-González et al., 2020), it is possible to conclude that the stress associated with the crypsis behavior is not a detrimental factor for the health of *D. latifrons* under captive conditions. The natural coloration of this species varies, but the most common is dark brown with some black in the dorsal area and gray or light brown in the ventral area (Vega-Villasante et al., 2021). This suggests that the fish can more easily camouflage with dark backgrounds, especially with black, which allows them to conserve energy for other activities such as food intake and digestion, leading to better growth (Magellan, 2020).

In conclusion, significant differences were observed in the growth parameters of the organisms under study in response to the different bottom colors of the culture ponds. Greater growth was recorded when darker bottoms were used. However, the hematological and blood chemistry parameters of juvenile specimens of *Dormitator latifrons* showed no effect in response to the possible stress caused by the color of the culture ponds, which suggests that changing the bottom color of the culture ponds does not affect the health of the cultured organisms.

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