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RESEARCH ARTICLE

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## EFFECT OF ENVIRONMENTAL ENRICHMENT ON THE BEHAVIOR OF BETTA FISH EXPOSED TO INCIDENT SUNLIGHT

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### ABSTRACT

The aim of this study was to evaluate the effect of using environmental enrichment on the behavior of Betta fish exposed to sunlight. For the experiment, 36 adult females of Betta fish were used. The study was carried out in a completely randomized design (DIC) with three treatments, with each fish defined as one repetition, totaling 12 repetitions in each treatment. Each aquarium represented a treatment, being (1) Without environmental enrichment; (2) Stacked stones and (3) Stacked stones and aquatic plants. In each of the treatments, the behavioral observations of the animals were collected and exposed to the sun every five minutes in an 8 h/daily period. The data were analyzed by the IBM® SPSS® Statistics Subscription Software using Friedmann's ANOVA for non-parametric data followed by the Kruskal-Wallis test, at 5% significance. A total of 672 behavioral observations were made. The observed results demonstrate the need to use environmental enrichment in cultivation aquariums, in addition to verifying their low preference in relation to exposure to light. The use of environmental enrichment in Betta fish tanks favors greater well-being. The exposure of these fishes to incident light increases the stress and must happen in a controlled and not a direct way.

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### INTRODUCTION

Fish are ectothermic animals and do not have an internal mechanism that regulates their body temperature, that is, they remove heat from the environment where they live to maintain thermal equilibrium (Costa *et al.*, 2009). Daily exposure to the sun brings several benefits to the health of mammals, which does not escape the rule of fish, especially those classified as ornamental, as it stimulates the production of vitamin D, which is essential for various activities of the body, in addition to stimulating the disease prevention and increase the feeling of well-being. Terrestrial animals that receive exposure to sunlight generally do not need vitamin D in their diet, since this compound is synthesized naturally (Trautenmüller *et al.*, 2019). However, in places where solar activity is low, there is a lack of this vitamin that can be obtained from supplementation in the diet. Vitamin D supplementation in the diet becomes even more essential for fish that receive little ultraviolet radiation from the sun's rays.

The contact of fish to sunlight is necessary, because ultraviolet rays activate the synthesis of vitamin D3, responsible for the assimilation of calcium, which in turn is important for the formation of the skeleton (Khan *et al.*, 2020), however, if you pay attention to the frequency and the way these animals will be exposed to sunlight. In view of the benefits, sunbathing (natural or artificial) makes the animals eat better, become more active and resistant. They are also important for preventing fungi and helping small wounds to heal. In contrast, when the aquarium cannot be exposed to the sun's rays on a regular basis, they must be replaced by artificial lighting specific to fish (Czarnecka *et al.*, 2019). The lamps must provide the full spectrum of light, especially ultraviolet radiation. The ideal power of the lamp will depend on the size of the aquarium. The lighting for fish must respect the photoperiod of 12 hours of light, that is, when choosing to use lamps, they must be turned off daily for 12 hours (Imsland *et al.*, 2019).

The amount of ultraviolet radiation emitted by these lamps decreases with use, so they must be replaced annually (Rodrigues *et al.*, 2019). The way ornamental fish are exposed to the sun should always allow them to choose to stay to be directly under the incident rays. The use of environmental enrichment is a procedure that aims to stimulate the natural behaviors of each species, using methods that alter the environment, obtaining a result of improvement in the quality of life of the species when fulfilling their behavioral needs (Silva *et al.*, 2019). By providing an environment enriched with artifacts for the interaction of animals, one can observe variable behaviors coming from them, however, information on the effect of using environmental enrichment on the behavior of ornamental fish exposed to sunlight is still incipient. Thus, the aim of this study was to evaluate the effect of using environmental enrichment on the behavior of Betta fish (*Betta splendens*) exposed to incident sunlight.

## MATERIALS AND METHODS

The study was carried out at the Marine Biology Station of the Institute of Zootechnics belonging to the Federal Rural University of Rio de Janeiro, in the district of Itacuruçá, municipality of Mangaratiba, State of Rio de Janeiro, with a seven-day trial period, determined according to the meteorological data collected from the National Meteorological Institute (INMET) for days with less probability of rain. 36 adult females of Betta fish were used, distributed in three aquariums of translucent material, with a total capacity of 18 liters and volume in use of 12 liters each, with dimensions of 25 cm in height, 35 cm in width and 10.5 cm in depth of water column, being determined the proportion of 12 fish per aquarium and the spacing of 1 L / fish. Feeding was performed daily, twice a day, at the beginning and at the end of the observational collections.

The water quality parameters (pH and toxic ammonia) were evaluated at the beginning and at the end of the study, with the water temperature being monitored daily by a floating thermometer. The ambient temperature and relative humidity of the air was obtained with the aid of a digital hygrometer HTC-2A in Celsius scale (°C) and to put an end to the radiation it was obtained with the help of data from the automatic surface observation station of Restinga da Marambaia, located 17 km from the experimental site. The study was carried out in a completely randomized design with three treatments, with each fish defined as one repetition, totaling 12 repetitions in each treatment.

The aquariums contained 50% of their area with sunlight and 50% shading, in addition to environmental enrichment composed of stones or stones plus aquatic plants in two of the evaluated treatments. Each aquarium represented a treatment, where it was characterized by: Treatment 1 (SEA) - Aquarium without environmental enrichment; Treatment 2 (EP) - Aquarium with stacked stones and Treatment 3 (EPP) - Aquarium with stacked stones and aquatic plants. In each of the treatments, the behavioral observations of the animals were exposed to exposure to the sun, using an instantaneous collection route at each five-minute interval over a period of 8 h / daily (8:00 am to 4:00 pm) and noted on an appropriate form through the focal sampling route. The data were analyzed using the IBM® SPSS® Statistics Subscription Software using Friedmann's ANOVA for non-parametric data followed by the

Kruskal-Wallis test, at 5% significance level for the interaction of behavioral observations.

## RESULTS AND DISCUSSION

During the experimental period, the water quality parameters remained within the ideal range for aquaculture, as recommended by Boyd (2000) with average values of water temperature, pH, total ammonia and nitrite:  $25.6 \pm 1.4$  °C;  $6.8 \pm 0.2$ ;  $0.20 \pm 0.05$  mg / L;  $0.008 \pm 0.001$  mg / L respectively. In table 2, we can see the average values for the variables Ambient Temperature, Relative Air Humidity, Solar radiation and Water temperature. The ideal room and water temperature for breeding Betta fish is in the ideal acceptable range of 24 °C to 28 °C, and these temperatures are not very variable when the breeding is carried out indoors and without the influence of weather conditions. In this study, the ambient temperature ranged from 21°C in the morning to 31 °C in the afternoon, with averages of  $23.29 \pm 2.75$  °C and  $29.08 \pm 1.32$  °C, respectively, while the temperature of water varied between 20 °C in the morning shift to 30 °C in the afternoon shift, with averages of  $22.10 \pm 1.56$ °C and  $27.17 \pm 2.30$ °C, respectively. The use of suitable facilities for the production and breeding of Betta fish helps to control the temperature of the environment and, consequently, the water temperature in the production systems more dynamically.

Regarding the relative humidity of the air, this variable acts according to the temperature of the environment, this parameter being within the expected range for a day with low probability of precipitation. Environmental factors such as temperature, environment, water salinity, surface illumination, among others, influence physiological responses to stress (Barton, 1997). The parameters of water quality are an extremely important factor in the creation of Betta fish and should not be neglected. Lima *et al.* (2017), reported that the water temperature for breeding Betta splendens varies from 25 °C to 28 °C, however Faria *et al.* (2006) notes that temperatures between 23 °C and 34 °C are tolerable for these animals, with the ideal range being around 27.5 °C. Since they are ectothermic animals, the temperature of the environment in which they are raised has profound effects on their physiology and consequently on their behavior. The experimental period comprised days with high levels of solar luminosity verified by the constant energy of solar radiation emitted. The behavioral frequencies of Betta fish in aquariums with different environmental enrichments and exposed to incident sunlight are shown in table 3.

Behavioral frequencies numerically demonstrate the number of times the animal or group of animals performed a certain activity in the environment in which they are raised. A total of 672 behavioral observations of groups of Betta fish were made. The behaviors observed in these animals demonstrate the need for the use of environmental enrichment in cultivation aquariums, in addition to verifying their low preference in relation to exposure to light. When exposed to sunlight for a long period, Betta fish, when raised in groups, like females of this species, showed greater agitation and aggressive behavior compared to those that are kept in the shade or with environmental enrichment in aquariums or tanks. of production. Isolated bred animals, such as Betta males, may have a higher degree of stress associated with exposure to incident light.

**Table 1. Etogram of the behaviors observed in the present study.**

N°	Behavior	Description
1	Tranquil in the Shadow	When the fish were observed in idle, standing in the shade, without performing any agitation, interaction or changes in the behavioral aspect, they were calm and undisturbed.
2	Agitated	When the fish were observed chasing another fish, pinching another fish, swimming in a disorderly manner.
3	Hidden	When the fish remains non-visible, be it between rocks or plants.
4	Tranquil in the Sun	When the fish were observed standing in the sun, without any agitation or changes in behavior, they were calm and undisturbed.
5	Interacting	The fish were observed swimming together peacefully, and / or exploring the aquarium environment.

**Table 2. Variables environmental noted in the morning and afternoon experimental period**

Variables	Morning	Afternoon	CV (%)
	08:00-12:00h	12:05-16:00	
RT, °C	23.29 ± 2.75	29.08 ± 1.32	14.13
RH, %	73.15 ± 6.09	66.00 ± 5.03	15.22
SR, kJ/m <sup>2</sup>	501.62 ± 622.82	2418.19 ± 321.40	81.02
WT, °C	22.10 ± 1.56	27.17 ± 2.30	16.02

RT = Room Temperature; RH = Relative Humidity; SR = Solar Radiation; WT = Water Temperature; CV = Coefficient of Variation.

**Table 3. Behavioral frequencies of Betta fish in aquariums with different environmental enrichments and exposed to incident sunlight**

Variables	Treatments		
	SEA	EP	EPP
TSM	637 (94.8%)	637 (94.8%)	663 (98.7%)
AG	113 (16.9%)	44 (06.5%)	9 (01.3%)
ES	-	340 (50.6%)	445 (66.2%)
TSL	201 (29.9%)	323 (48.1%)	279 (41.6%)
INT	253 (37.7%)	340 (50.6%)	271 (40.3%)
OT	672	672	672

SEA = Treatment Without Environmental Enrichment; EP = Enriched Stone Treatment; EPP = Enriched Stone and Plant Treatment; TSM = Tranquil in the Shadow; AG = Agitated; ES = Hidden; TSL = Tranquil in the Sun; INT = Interacting; OT = Total observations in each treatment.

**Table 4. Differences between the average occurrences of behaviors between the treatments of the experiment, considering the averages of observations in the morning and in the afternoon**

Behaviors	Treatments		
	SEA	EP	EPP
TSM	3.42 a	3.42 a	3.15 a
AG	6.84 a	2.63 b	1.56 b
ES	-	4.33 a	6.58 b
TSL	2.50 a	4.02 b	3.47 b
INT	2.90 a	3.93 b	2.98 a

Different letters in the lines indicate significant differences by the Kruskal-Wallis test at 5% probability.

The use of environmental enrichment in the form of stacked stones favors animals to feel safer, using these as shelter. When aquatic plants are added, the behavior of hiding becomes even more frequent, demonstrating the preference of these animals to be in places with less incidence of direct light. Behavior is one of the most important early indicators of an individual's well-being and adaptation to the environment and reflects the immediate response to the interaction between the animal and its environment. Hedayatirad *et al.* (2017) were able to prove through behavioral analysis that at high temperatures (closer to 30 °C) *Betta splendens* are more active fish with a general increase in aggressiveness. In females of *Betta splendens* the effect of temperature can also affect their natural behavior, making them more aggressive, since females do not routinely show stereotypical aggressive behavior and demonstrate a social behavior similar to that of schools under specific conditions, as in laboratories (Blaskelee *et al.*, 2009). Table 4 shows the differences between the average occurrences of behaviors between the treatments of the

well-being, considering that these fish also use it as a form of shelter for their own defense or defense against predation spawning and in breeding activities (Zuanon *et al.*, 2015). Compared to individuals reared in a conventional breeding system, Atlantic Cod reared by varying the form of food and with environmental enrichment (stones and algae) had a faster recovery after the stressor, greater exploratory activity, showing greater behavioral plasticity in general (Braithwaite and Salvanes, 2005). In question, the exposure to incident sunlight, not yet and has a lot of information about the effects caused on the behavior of *Betta* fish, however depending on the fish species, the characteristics of the environment and their specific abilities, the light spectrum can affect several biological processes such as feeding, reproduction, growth, behavior and the neurohormonal system (Ruchin, 2004). Exposure of Atlantic salmon (*Salmo salar*) to blue LED light resulted in an increase in cortisol and glucose levels in 3 hours, returning to baseline levels in 24 hours. This typical stress response was not observed in fish exposed to white LED light and blue light of lower intensities, suggesting differential

sensitivity to the spectral content of light (Migaud *et al.*, 2007). The spectrum of incident light, which can be different in indoor systems, can also cause stress. Jundiá (*Rhamdia quelen*) exposed to green light for 10 days had an increased stress response (increased cortisol) compared to those kept under white and blue light (Barcellos *et al.*, 2006), probably due to the water column function as a chromatic filter and as the depth increases, there is a predominance of the blue wavelength (Jerlov, 1968). Excessive light can also cause damage to the fish's retina and the lack can interfere with the capture of food, especially in visual fish. In general, studies related to the use of environmental enrichment to promote natural behaviors and assist in the greater well-being of ornamental fish are fundamental at a time when issues of improvement of livestock systems are so debated.

## Conclusion

The use of environmental enrichment in Betta fish tanks favors greater well-being. The exposure of these fishes to incident light increases the stress and must happen in a controlled and not a direct way.

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