BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Note

SHELTERS INFLUENCE IN DUSKY GROUPER CULTURE

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Received: December 09, 2018
Approved: May 03, 2019

ABSTRACT

Environmental enrichment can be an important tool to improve the productive performance of dusky grouper *Epinephelus marginatus* (Lowe, 1834). The goal of this study was to evaluate the preference for shelters and the effect of the availability of shelters on the productive performance of dusky groupers juveniles in salt water recirculation system. Two trials were conducted, the first one to determine the preference for different types of shelters (rocks, bricks and PVC pipes of different colors) and the second one on the use of shelters, with three treatments (T1 = without shelter; T2 = one shelter per fish and T3 = two shelters per fish). The dusky grouper showed preference for shelters of PVC tubes of brown color. The use of shelters favored better productive performance of the species in salt water recirculation systems.

Key words: environmental enrichment; *Epinephelus marginatus*; zootechnical performance; mariculture; aquaculture.

INFLUÊNCIA DE ABRIGOS NA CRIAÇÃO DA GAROUPA-VERDADEIRA

RESUMO

O enriquecimento ambiental pode ser uma ferramenta importante para melhorar o desempenho produtivo da garoupa-verdadeira *Epinephelus marginatus* (Lowe, 1834). O objetivo deste estudo foi avaliar a preferência por abrigos e o efeito da disponibilidade de abrigos sobre o desempenho produtivo de juvenis de garoupa-verdadeira em sistema de recirculação de água salgada. Foram realizados dois ensaios, o primeiro para determinar a preferência por diferentes tipos de abrigos (pedras, tijolos e tubos de PVC de diferentes cores) e o segundo sobre o uso de abrigos, com três tratamentos (T1 = sem abrigo; T2 = um abrigo por peixe e T3 = dois abrigos por peixe). A garoupa-verdadeira mostrou preferência por abrigos de tubos de PVC de cor marrom. O uso de abrigos favoreceu um melhor desempenho produtivo da espécie em sistemas de recirculação de água salgada.

Palavras-chave: *Epinephelus marginatus*; enriquecimento ambiental; desempenho zootécnico; maricultura; aquicultura.

INTRODUCTION

Marine fish farming is still under development in Brazil. Therefore, raising threatened marine fish species can be an important tool in the conservation of fish stocks. The development of this productive chain can diversify mariculture in Brazil and provide employment and generate income for coastal communities (Sanches et al., 2013). The dusky grouper *Epinephelus marginatus* (Lowe, 1834) is a species of marine fish with high commercial value that has demonstrated vulnerability to human exploitation (Cunha et al., 2013). It is currently listed as endangered on the red list of the International Union for Conservation of Nature and Natural Resources (IUCN, 2017). Young specimens inhabit coastal areas with great availability of shelters, where they seek for shelter and food (Machado et al., 2003). However, the breeding of dusky grouper has been carried out in net cages or in recirculation systems without the use of shelters (Ramos et al., 2012; Sanches et al., 2014).

The success in domestication of wild fish is characterized by the ability of a species to adapt to the captivity environment (Kristiansen et al., 2004). In the case of the dusky grouper, there is already available knowledge in the areas of pathology (Roumbedakis et al., 2013, Souza et al., 2014), food management (Ramos et al., 2012; Sanches et al., 2014) and reproduction (Andrade et al., 2003; Sanches et al., 2009; Kerber et al. 2012;

Cunha et al., 2013). However, there is still a gap in studies that consider the biological preferences in the species when defining productive systems. Considering the principles of animal production, the biological characteristic of the species should be taken into account when defining production systems in captivity in order to provide an environment as close as possible to what the individual is accustomed to in the natural environment. The habit of using shelters, therefore, needs to be evaluated.

Environmental enrichment consists of a series of measures that modify the physical or social environment, improving the quality of life of captured animals, thus, providing conditions to carry out their ethological activities (Volpato et al., 2007). The availability of shelters can be considered a form of environmental enrichment. The preparation of shelters may vary according to size, type and material chosen, always considering the local availability of material and the cost of purchase. The materials used should be easily found, have a low cost, high durability and are non-toxic (Ramos et al., 2013). The use of shelters has already been evaluated in aquaculture with several species of organisms with positive results. Studies with the *Lota lota* fish concluded that the combination of high light intensity, uncovered cages and insufficient shelter availability negatively influence the productive performance of the species (Wocher et al., 2011). The use of tires in the cultivation of greasy grouper (*Epinephelus salmoides*) in net cages has shown that when the animal uses shelters, its energy is conserved and directed towards growth (Teng and Chua, 1979).

The low productive performance demonstrated by the dusky grouper may be related to the lack of knowledge of their biological needs. Considering the little availability of information on dusky grouper performance in recirculation systems and the lack of information on shelter preferences and availability, studies are needed concerning this form of environmental enrichment in this productive system so that the species can reach its growth in captivity.

The objective of this study was to evaluate dusky grouper preference for different types of shelters and the effect of shelter availability on their production in salt water recirculation systems. Our hypothesis is that the dusky grouper will have a preference for the stone shelter, because this type simulates the conditions of the natural environment. In parallel, our other hypothesis, considering the use of different shelters by the species in the natural environment, is that the availability of more shelters per fish will provide better productive results for the cultivation of this species.

MATERIAL AND METHODS

The experiments were conducted at the Marine Fisheries Laboratory of the Fisheries Institute, located in Ubatuba, SP, Brazil. The experiments were approved by the Fisheries Institute Ethics Committee in Animal Experimentation (001/2017). The dusky grouper juveniles were obtained by breeding in captivity from Redemar Alevinos, Ilhabela, Brazil.

Experiment I. Shelter Preference

In this experiment four types of materials used as shelters for the dusky grouper were evaluated: (i) stuck brick, (ii) flat stones, (iii) white PVC pipe (iv) brown PVC pipe. The shelter of stones was formed by three flat stones, one upper stone being supported in two lateral ones. The PVC tubes received two drops of silicone laterally to prevent dislocation by slipping. All shelters had a similar volume of 100 cm^3 and were randomly and simultaneously placed in a 100-liter glass aquarium with no bottom substrate, with the sides and bottom covered by black plastic to avoid external interference. The aquarium did not have aeration to avoid influencing the shelters with the movement of the water.

Ten specimens of dusky grouper (2.52±0.78 g and 5.3±0.6 cm total length) were individually introduced into the aquarium and recorded the time in which they remained within each shelter for a period of four hours. The evaluations were conducted by a single observer. The fish were fed 12 hours before the start and after the end of the experiment. For each fish the aquarium water was replaced to avoid interference. The temperature and dissolved oxygen content of the water in the aquarium was monitored with a YSI model 51 oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA). The total ammonia was monitored by a colorimetric method, with a Tetratest® Kit (Tetra Werke, Melle, Germany) and the salinity with an F3000 optical refractometer (BernauerAquacultura, Blumenau, Brazil). Oxidation-reduction potential (ORP) was measured using the PH-ORP Controller (PH-303) (Omega Engineering Inc., Taiwan). The water parameters of the aquarium were: temperature 28.1±0.6 ºC; salinity 31.8±0.8; dissolved oxygen 6.0±0.7 g L^{-1}; total ammonia 0.1±0.1 mg L^{-1}; pH 8.0±0.5; oxide-reduction potential (ORP) 242.6±9.4 µS cm^{-1}.

Experiment II. Determining shelter use

The fish (06 individuals per tank) (2.52±0.78 g and 5.3±0.6 cm total length) were divided into three treatments and submitted to different shelter availabilities: without shelters; one shelter per fish and two shelters per fish. Each treatment had three replicates, distributed randomly in nine circular cages with 150 liters, in a circular tank (5,000 L) of salt water recirculation system, equipped with a mechanical filtration, skimmer and water sterilization through ultraviolet lamps. The circulation rate of the cages was 200% by water pumps (the total volume of water in the cages was renewed twice a day). Before entering the system all the fish passed through a freshwater bath for 5 minutes (Kerber et al., 2011) in order to eliminate any ectoparasites. As a practice of operation, the evaporation losses of the system were daily replenished with deionized water. After the last daily feeding, the bottom of the cages was cleaned by siphoning to remove solid wastes. On a weekly basis, 10% of the total volume of the recirculation system has been replaced, aiming to eliminate components not retained or eliminated by the filtration system. The experiment lasted 60 days.

As a nutritional diet, commercial feed was used with the centesimal composition described by the manufacturer (Crude Protein 41.8%, Ethereal Extract 8.75%, Ash 6.77%, Crude Fiber 1.96%). This ration presented granulometry between 1 and 2 mm in diameter. The fish were fed three times a day (09:00 AM, 12:00 PM and 5:00 PM) with a food rate of 10% PV day^{-1}. In this situation, the total weight of the food offered was measured after each feeding.

Daily consumption of food (by weight difference between the food supplied and the food remaining at the end of the feeding) and the occurrence of mortality were verified. In order to obtain the biometric data, the fish of all treatments were anesthetized with benzocaine (0.05 g L^{-1} water, ChenCenter Inc.) and then measured with an ichthyometer (cm) and weighed (g) individually on a digital electronic scale (0.01 g precision) at the beginning and every 20 days until the end of the experimental period.

Zootechnical parameters were calculated as follows: Survival (S,%) = (Px_f / Px_i).100, where Px_f = number of fish at the end of period; Px_i = number of fish at the beginning trial. Specific Growth Rate SGR (% body weight day^{-1}) = 100.((ln W_2 - ln W_1) Δt^{-1}), where W_2 = average final weight; W_1 = average initial weight; Δt = rearing period (days). Body weight gain BWG (g day^{-1}) = (W_2 - W_1) Δt^{-1}, where W_2 = average final weight; W_1 = average initial weight. Feed Conversion Ratio FCR = C_f / D_f, where C_f = Total amount of food consumed during the period; D_f = weight gain during the experimental period.

All water quality parameters were measured daily in the center of tank (5,000 L). The water quality of the recirculation system remained within the range acceptable for the cultivation of the dusky grouper: temperature 28.3±0.9 ºC; salinity 30.5±2.3; dissolved oxygen 6.5±0.9 g L^{-1}; total ammonia 0.1±0.1 mg L^{-1}; pH 8.0±0.5; oxide-reduction potential (ORP) 283.1±8.2 µS cm^{-1}.

Statistical Analyses

Experiment I counted on four treatments and ten randomized repetitions (where the fish were considered the replica) by lottery through Excel. Experiment II had three treatments and three replicates (the tank being a replicate). All data from both experiments were tested for normality and homogeneity of variances (Shapiro Wilk test and Bartlett test). For comparison between treatments, the means of the evaluated parameters were submitted to analysis of variance (ANOVA). The values expressed as percentage (survival) were transformed according to the formula: transformed variable = arcsen$\sqrt{x}$. In case of significant difference, the Tukey test ($P < 0.05$) was applied.

RESULTS

The data obtained in the shelter preference experiment showed that the dusky grouper, when released in the aquarium, was quickly adapted. A significant preference of the species was observed for the use of shelters, where they remained in them for more than 80% of the time of observation. After 20 to 40 minutes of introduction into the aquarium, the fish started to look for different shelters, with a clear preference for the brown PVC tube (Figure 1).

In the second experiment, in a water recirculation system, with the supply of shelters, the dusky grouper presented differences in productive performance. No mortality was recorded, indicating the species' adaptability to the production system in water recirculation. The fish from the cages in which shelters were made available presented better productive performance for final weight, specific growth rate, daily weight gain and apparent feed conversion when compared to the control treatment (without shelter availability) (Table 1). However, there was no statistical difference between the treatments that provided different amounts of shelters.

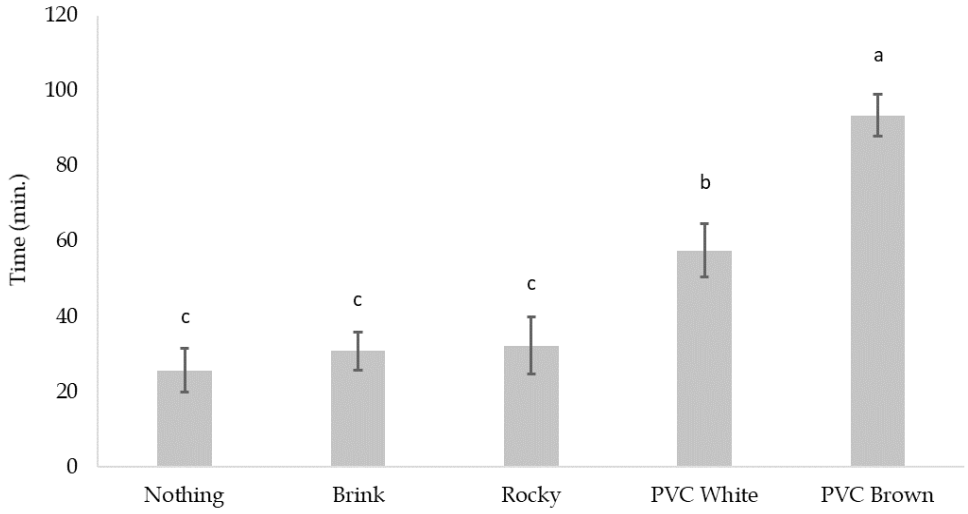

Figure 1. Time (in minutes, mean ± SE) of the dusky grouper *Epinephelus marginatus* in each shelter during the observation time of four hours (n=10 peixes). Bars sharing different letters indicate significant difference among treatments ($P<0.05$).

Table 1. Productive performance parameters of dusky grouper Epinephelus marginatus submitted to different shelters availability for 60 days (n = 3)[1].

Parameter	Without shelter	One shelter	Two shelters
Length (cm)	6.30 ± 0.60	6.60 ± 0.60	6.70 ± 0.70
Weight (g)	4.18 ± 0.38 [b]	4.97 ± 0.49 [a]	4.77 ± 0.42 [a]
Final Biomass (g)	24.98 ± 5.61	29.79 ± 6.47	28.00 ± 5.94
Survival (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Specific Growth Rate (%BW dia^{-1})	1.03 ± 0.02 [b]	1.23 ± 0.05 [a]	1.20 ± 0.05 [a]
Body Weight Gain (g dia^{-1})	0.02 ± 0.01 [b]	0.05 ± 0.01 [a]	0.05 ± 0.02 [a]
Feed Convertion Ratio	1.50 ± 0.10 [b]	1.10 ± 0.10 [a]	1.20 ± 0.10 [a]

[1]Lines with different letters indicate significant differences between treatments (P <0.05).

DISCUSSION

The dusky grouper is a species associated with consolidated rocky beds (Machado et al., 2003). The option for the different types of shelters showed that environmental enrichment can contribute to the maintenance of the species in captivity. Contrary to the hypothesis formulated, the dusky grouper showed no preference for the stone shelter, or the similar brick shelter, opting for the brown PVC pipe. This preference may be associated with the stone shelter being composed of three flat stones and the fish's supporting base was constituted with the glass of the aquarium which may not favor the best adaptation. On the other hand, the brown PVC tube provided a greater sense of security, given its less refraction of light. The variety of shelters has also shown that the dusky grouper has a preference in selection and the choice of a darker PVC tubing may be related to the greater sensation of concealment provided by this type of shelter. In the wild, young specimens are constantly searching for darker and deeper moors to shelter themselves from larger fish (Machado et al., 2003).

The remaining for a longer time in PVC shelters, especially in brown, is contrary to what was observed by Ramos et al. (2013), which, when assessing different shelters for the zebra pleco, *Hypancistrus zebra*, observed that this species had a longer lodging time in the rock shelter. These results demonstrated that different species may have preferences for different types of shelters, independent of the characteristics of their natural habitat. The study with *H. zebra* demonstrated that the selection of the shelter is not random and indicates that the nature of the material can significantly affect the acceptance of the shelter by the animal.

Environmental enrichment can be an important tool for the maintenance of species in the domestication process (Galhardo and Oliveira, 2006). Several experiments addressing the efficiency of environmental enrichment in fish reported a number of behavioral benefits (Salvanes et al., 2013). The presence of shelters caused differences in the aggressive behavior of the Atlantic cod, *Gadus morhua*, in cages with environmental enrichment; a greater social domain was observed (Salvanes and Braithwaite, 2005). The environmental enrichment for this same species improved the way the animals respond to new situations, presenting greater ease in learning in captivity conditioning (Strand et al., 2010). Work with crustaceans found an improvement in zootechnical performance when environmental enrichment was used. Considering that the dusky grouper is still a species in the process of domestication and considering the results of the preference test, further studies along this line of research are necessary, focusing on indicators of stress, such as cortisol and lactate, to obtain better results in the cultivation of this species.

Atlantic salmon juveniles, *Salmo salar*, in environmentally enriched cages showed significantly higher growth compared to the control treatment. However, fish reared under enriched environmental conditions were less willing to leave the shelter, clearly demonstrating a behavioral transformation compared to wild individuals (Roberts et al., 2011). In general, shelters reduce activity and potentially increase energy savings, but can also reduce food detectability and decrease forage time (Näslund and Johnsson, 2016). In the case of the dusky grouper, the presence of different amounts of shelter per fish did not imply behavioral changes for food intake.

In some situations, environmental enrichment may have a negative effect and increase the rate of individual aggressiveness. Nile tilapia, *Oreochromis niloticus*, when introduced in cages with environmental enrichment showed a more aggressive behavior, affecting even the way the specimen fight (Barreto et al., 2011). In the present study no aggressive or territorialistic behaviors were observed for the dusky grouper.

Significant differences in the zootechnical parameters of the fish in the water recirculation system demonstrated that the availability of a shelter per fish was enough to promote less stress in breeding conditions, providing better zootechnical performance. Stress has a negative economic impact, damaging the systems, affecting fish health, productivity and quality of the final product (Eissa and Wang, 2016). Acute and chronic stress can also trigger a series of defense mechanisms that require energy, thus inducing an elevation of the animal's metabolic rate (Lupatsch et al., 2010). Fish respond to conditions of confinement by triggering physiological mechanisms, adapting to the new conditions. However, when the physiological response exceeds the critical limit, there will be a change in swimming behavior and an increase in the rate of aggressiveness (Ramos et al., 2013).

The improvement in the productive performance of the fish that had available shelters may also be related to their mobility. Observations showed that shelter fish remained at rest in the shelter for longer while homeless fish were more mobile. The best performance in sheltered environments is therefore probably caused by reduced metabolic costs due to longer resting periods (Benhaïm et al., 2009). In the natural environment the dusky grouper passes an expressive part of the time inside the shelters only leaving to feed (Machado et al., 2003). Thus, the species conserves energy, directing the nutrients of the food to the growth. Even species that are less dependent on shelters in natural environment conditions may benefit from them, based on the issue of energy conservation. Significant improvement in the performance of juveniles of Atlantic salmon, *Salmo salar*, were observed when shelters were available, and the authors reported a reduction in metabolic costs, since the species in winter usually have negative energy budgets, and fish depend on a large part of the stored energy to survive (Finstad et al., 2007).

Another relevant point is that the group is in the habit of sheltering after food intake, and in this situation, the shelters recreate the natural environment, reducing the stress caused by confinement and increasing the growth of the fish (Teng and Chua, 1979). Another species of the Serranidae family, *Centropristis striata*, showed a significant difference in growth in specimens that had tank shelters, growing 1.7 times more than those housed in cages without shelters (Gwak, 2003). On the other hand, this same author reported that the presence of shelters increased intraspecific competition, territorialism and cannibalism, leading to the death of several individuals. Shelters promote survival by decreasing the interaction between the animals, increasing the area of the pond and decreasing the effect of density, but each species reacts in a way that can have positive and negative effects. The high survival obtained in this study with the dusky grouper demonstrated that the species does not present, regarding shelter availability, elevation in intraspecific competition, greater territorial disputes or cannibalism. Therefore, future studies could approach the cultivation of the dusky grouper in higher densities with shelter availability, evaluating zootechnical performance.

Daily weight gain, specific growth rate, and feed conversion were significantly better for shelter availability conditions. The existence of shelters would provide this metabolic benefit to fish, consolidating the goal of providing greater well-being to farmed fish (Martins et al., 2012). Fish welfare can be defined as the internal state of a fish when it remains under freely chosen conditions (Volpato et al., 2007). Considering the practicality in the operation of breeding systems and increased performance, the availability of only one shelter per fish proved adequate for the dusky grouper, providing satisfactory results of well-being and productivity.

In addition to the great complexity in providing shelters in productive systems, the results presented here demonstrated that in the weight range evaluated, shelter availability provided a better performance. Therefore, future studies should address physiological aspects of well-being in the dusky grouper, notably by measuring cortisol and lactate, in order to increase understanding about the breeding of this species in intensive production systems.

CONCLUSION

The dusky grouper showed preference for shelters of PVC tubes of brown color. Environmental enrichment (use of shelters) favored better productive performance of the species in water recirculation systems. The use of a one shelter for dusky grouper should be adopted in the maintenance of this species in production systems.

ACKNOWLEDGMENT

The authors would like to thank FAPESP (Foundation of São Paulo Research) for financing this project (process 2014/07886-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship awarded to Nayara Yoshimini de Oliveira for her Master's.

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