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Dusky Grouper *Epinephelus marginatus* Growth and Survival When Exposed to Different Photoperiods

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Abstract: Photoperiod is considered an environmental factor that influences reproduction and the growth processes of fish throughout the year. In the present study, we subjected dusky grouper *Epinephelus marginatus* to different photoperiods in order to evaluate growth and survival. Juvenile dusky grouper were randomly distributed in twelve 100 L tanks containing four fish per aquarium. The fish were exposed to continuous light, normal photoperiod or continuous darkness for 50 days. Fish were fed 3% of their total biomass, twice a day, and the diet consisted of ground commercial dry pellets (42% crude protein, 12% humidity, 9% ether extract, 15% mineral matter, 4% crude fiber, 3.5% calcium and 3% vitamin C, in accordance with the manufacturer's instructions). There was no mortality during the experiment. After 50 days, the best performance was found for exposure to continuous light (24 h artificial light). The final weight of the fish reared under continuous light was significantly higher than that of the fish exposed to continuous darkness. The specific growth rate of the fish exposed to continuous light was significantly higher than that of the fish exposed to the natural photoperiod and to continuous darkness. Modifications to the photoperiod can be a key factor in increasing the efficacy of current production and the improvement of current aquaculture protocols.

Keywords: fish farm; marine; threat; extinction

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1. Introduction

The species *Epinephelus marginatus* (Lowe, 1834), popularly known as the dusky grouper, occurs in the eastern and southwestern Atlantic, the Mediterranean, the western Indian Ocean and south of the Bay of Biscay to the southern tip of Africa [1]. Its geographical distribution includes the coast of South America, from Rio de Janeiro to Argentina [1]. Overfishing, high commercial value and late maturation characteristics are factors that have caused a decline of the dusky grouper [2]. In Brazil, the dusky grouper is caught in artisanal fisheries in the southern and southeastern states of São Paulo and Rio de Janeiro [3]. The preferred habitat of this species is the rocky and sandy sea bottom around islands. Young fish are common in lagoons near mangroves [4], can exceed one meter in length and 40 kg in weight and are therefore considered to be an important resource in Brazil [5].

Fish of the family Serranidae, such as the grouper, are commercially important food fish in oriental countries [6]. The dusky grouper *E. marginatus* is a popular fish species for recreational and commercial fishery activities in Europe [7]. The dusky grouper integrates benthic habit and inhabits shallow-water habits when young, though it lives at greater depths when adult. It is a non-specialized carnivore, and its preferred diet includes fish, mollusks, crustaceans and zoobenthos [4].

In Asia and in the Mediterranean sea, groupers have become an emerging fish for aquaculture [7]. Several attempts have been made to reproduce dusky groupers under controlled conditions in the Mediterranean sea area for conservation programs and for consumption [8]. However, despite all efforts to increase the production of dusky groupers, they are still at an experimental stage with low larval and juvenile survival rates. At the present time, dusky grouper reproduction in captivity still poses many problems.

Many species of grouper are farmed in floating net cages or tanks after artificial breeding in hatcheries [7]. The results have shown that the fish are not suited to artificial breeding conditions, and sustained production rates for this species in aquaculture production systems might be difficult to achieve [9]. Studies on artificial induction of spawning among groupers have shown that these fish already had ovaries containing vitellogenic or full-grown mature oocytes [10,11].

Marino et al. [10] reported that, since 1995, true groupers have been on the list of threatened fishes (Berne Convention, Annex 3—Protocol for Mediterranean Biodiversity). These species have been included in the Red List of the International Union for Conservation of Nature and Natural Resources. In Brazil, these species are on the list of over-exploited or threatened marine fishes [2].

Ministry of the Environment Order No. 445/2014 (IBAMA, 2014), Brazil, prohibits fisheries for many fish species, including dusky grouper. A complex reproductive style, in which a protogynous hermaphrodite changes from female to male when it reaches a larger size, might be the main cause of its population decline. Based on the above-mentioned studies, the possibility of breeding this species in captivity has been suggested as an alternative to protect the natural stocks of this threatened fish [12].

The metabolic activity of fish may become altered through a variety of environmental factors that change seasonally, including growth [13]. In different locations, these factors may affect different metabolic processes. The growth of fish in ponds is directly related to environmental factors such as temperature, salinity and photoperiod [14]. Manipulation of environmental factors such as temperature, salinity and photoperiod is currently used to modulate farmed fish growth [14].

The photoperiod is considered a key environmental factor that influences the reproduction and growth of fish during the year [15]. Fish respond to changes in the photoperiod, and these changes may affect feeding rhythms and growth [16]. Photoperiod alterations can be used to stimulate or delay gonadal maturation and thus to change the spawning period or rate of somatic growth [17]. Thus, the influence of the photoperiod has been tested to seek improvement in growth rates in some species. Changes to the photoperiod and the directional effect of the photoperiod on growth and survival have not been investigated in relation to juvenile dusky groupers. This needs to be clarified in order to establish the light regime that will allow optimal growth for this species in aquaculture systems.

The aim of the present study was to determine the best growth and survival rates of the dusky grouper *E. marginatus* in response to different photoperiods (continuous light, normal photoperiod and continuous darkness).

2. Materials and Methods

Juvenile dusky grouper *E. marginatus* were distributed into twelve 100 L aquaria containing 4 fish per aquarium (four replicates per treatment). In these experimental units, pieces of polyethylene pipe were placed to serve as shelters and thus to reduce aggression. The fish were exposed to continuous light (24 h artificial light), normal photoperiod (10 h light and 14 h darkness) or continuous darkness (24 h darkness) for 50 days (3 tanks per

treatment containing 4 fish each). The aquarium water was maintained at 25.0 ± 1.0 °C and was continuously aerated by means of 40 W air pumps that promoted water circulation through a plastic mesh and stones in order to reduce water turbidity (5.8 ± 0.4 mg O_2 L^{-1}). The water temperature and dissolved oxygen were measured using an oxygen meter (YSI Model 55, Yellow Springs, OH, USA). pH was maintained between 5.8 and 6.2 and was measured using a pH meter (VWR Scientific Model 34100, West Chester, PA, USA).

Fish were fed with 3% of their total biomass, twice a day. The diet consisted of ground commercial dry pellets (42% crude protein, 12% humidity, 9% ether extract, 15% mineral matter, 4% crude fiber, 3.5% calcium and 3% vitamin C, in accordance with the manufacturer's instructions). All feces and pellet residues were removed daily through siphoning, and the water level was adjusted as necessary. Every 10 days after the start of the experiment, all the fish were anesthetized using tricaine methanesulfonate (MS-222, USA, 100 mg/L water) and were individually weighed. The total duration of anesthesia was 4–8 min, and the fish were then returned to their aquaria. All procedures were performed in accordance with the recommendations of the American Fisheries Society [18].

Fish growth performance among the treatments was evaluated using the following parameters: specific growth rate (SGR) [19] was calculated as SGR = (eg^{-1}) × 100, where g = (ln W2 − ln W1) × (T2 − T1) − 1; and W2 and W1 are weights (g) on days t2 and t1, respectively [20]. The coefficient of variation (CV) of the weight was then calculated. The data were expressed as means ± SE and were analyzed using one-way ANOVA [21].

3. Results

There was no mortality during the experiment with *E. marginatus* exposed to different photoperiods. The water temperature during the experiment was 25.0 °C. The salinity remained stable at 33.5 (±1.1) g L^{-1} and the oxygen level ranged from 8 to 10 mg L^{-1}. pH was 7.7 (±0.8). The data show that juvenile *E. marginatus* exposed to continuous light grew better than those exposed to the natural photoperiod and to continuous darkness. After 50 days, the final weight of the fish reared under continuous light was significantly higher than that of the fish exposed to continuous darkness (Figure 1). The SGR of the fish exposed to continuous light treatment was also significantly higher than that of the fish exposed to the natural photoperiod and to continuous darkness ($p < 0.05$) (Table 1).

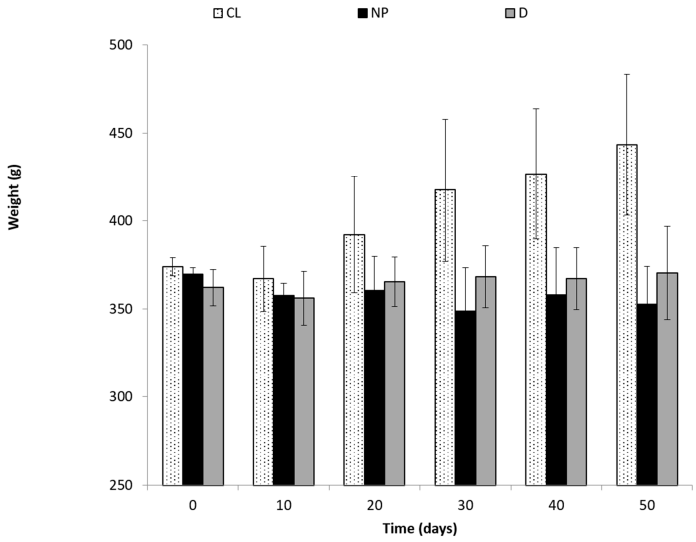

Figure 1. Growth performance of juvenile dusky grouper (*Epinephelus marginatus*) submitted to different photoperiods for 50 days. Continuous light (CL = 24 h light), normal photoperiod (NP = 10/14 light/dark) and continuous darkness (D = 24 h dark). Values indicated are means (SE) of four replicates.

Table 1. Juvenile dusky grouper *Epinephelus marginatus* growth performance submitted to different experimental photoperiods after 50 days. Continuous darkness—D; normal photoperiod—NP; and continuous light—CL. SGR is growth rate; CV is coefficient of variation for weight.

	CL	NP	D
Initial weight (g)	374.1 ± 5.2	369.7 ± 3.9	362.2 ± 10.3
Final weight (g)	443.4 ± 40.2	352.8 ± 21.4	370.4 ± 26.5
SGR (%)	0.09 *	−0.07	0.001 *
CV (%)	0.09 *	0.05	0.004

* statistical significance differences.

4. Discussion

Photoperiod regulates the circadian rhythm of fishes from continental and marine waters. In the present study, dusky grouper juveniles showed the best growth performance when exposed to continuous light. Other fishes, such as Atlantic halibut (*Hippoglossus hippoglossus* L.) also showed better growth rates when exposed to continuous light [22], but Arctic char (*Salvelinus alpinus*) may feed and grow even in complete darkness [23], which is also seen in tambaqui *Colossoma macropomum* [16] and catfish. These differences may be associated with the life habits of these different fish species.

Species such as leopard grouper *Mycteroperca rosacea* have shown changes in growth and biochemical composition in response to different photoperiods and temperatures [24], as was observed in the present study. For Coho salmon (*Oncorhynchus kisutch*), the photoperiod is widely manipulated in aquaculture systems, but continuous light may lead to increased total energy expenditure and may affect overall physiological performance and stress tolerance [25].

In the wild areas, the dusky grouper is characterized by slow growth and delayed maturity. This species has sedentary territorial behavior and a complex reproductive strategy with protogenetic hermaphroditism and external fertilization [1]. All these characteristics are reflected in the irregular growth of this species over time, as observed in the present study.

Additionally, groupers face different types of water visibility in the wild, with different light intensities, depending on depth. The improved performance of groupers under continuous light can be explained by the increased activity of individuals in response to this condition. According to Simensen et al., [22], fish may adjust to longer photoperiods by displaying higher feeding activity, growth and food utilization, as observed for groupers in the present study.

Despite the placement of pieces of polyethylene pipe in the present study (in an attempt to reduce aggression between individuals), the juvenile groupers that were exposed to continuous light presented damaged fins (more than 50% of the fish), possibly as a result of fights. This situation differed from that of the fish that were exposed to darkness and to the normal photoperiod. Damaged fins have also been observed in relation to silver catfish *Rhamdia quelen* [26] and tambaqui [16] that were exposed to continuous light.

According to Volpato and Fernandes [27], continuous light exposure can lead to the development of social stress for many fish species, which results in decreased food intake and increased aggression. For dusky groupers, despite occurrences of damaged fins in the present study, continuous light enabled greater weight gain. Continuous light is commonly used in commercial fish farming of Atlantic salmon (*Salmo salar*) [28] and Atlantic halibut (*Hippoglossus hippoglossus* L.) [29] in order to increase growth and influence the time of maturation.

The high coefficient of variation (CV) observed among groupers exposed to continuous light treatment also indicates that the levels of social interaction may have been increased through this treatment. This may be related to an increase in swimming activity due to greater exposure to light. This has also been observed for other fish species such as the African catfish *R. quelen* and the silver catfish [26]. Groupers have been described as a benthic species in wild areas [5], but in production systems, they are not usually considered

to be an aggressive species. The effects of the photoperiod on fish growth probably also relate to feeding habits, i.e., not just social habits or aggressiveness.

In the present study, the SGR was less than what was obtained in previous studies with the same species. This may have been related to the age of the fish, which were larger in size (older). In an evaluation on the SGR of other serranid species, Botero and Ospina [30] reported that for *Epinephelus itajara*, SGR was 0.13 when this species was fed with fish tails and 0.96 to 1.40 when fed with fish. Tucker [31] reported that the SGR for Nassau groupers *Epinephelus striatus* was 0.52 after feeding with fish tails. López et al. [32] evaluated different diets for dusky grouper and obtained a growth rate of 0.51 g day^{-1}, with juveniles weighing 22.6 g after 65 days of feeding.

With the larger size of the specimens (370 g) in the present study, the daily weight gain was 0.09 g day^{-1}. The greatest growth of groupers was achieved under continuous light, although this species does not have diurnal feeding habits. Therefore, it is possible to improve juvenile weight gain performance in groupers when they are kept under continuous light.

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