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SCIENTIFIC NOTE

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PRELIMINARY RESULTS OF FIRST PILOT PROJECT TO ASSESS THE BIOLOGICAL ADAPTABILITY AND ZOOTECHNICAL PERFORMANCE OF THE DUSKY GROUPER (Epinephelus marginatus) IN NORTHEASTERN BRAZIL

Resultados preliminares do primeiro projeto piloto para avaliar a adaptabilidade biológica e o desempenho zootécnico da garoupa-verdadeira (*Epinephelus marginatus*) no nordeste do Brasil

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ABSTRACT

This note records the preliminary results obtained during the course of the first pilot project to investigate the adaptability and zootechnical performance of dusky grouper *Epinephelus marginatus* in northeastern Brazil. The project started with first juvenile transport from Prime Seafood Marine Farm, Alcobaça – BA - Brazil, to Mirante Pescados, Cascavel – CE – Brazil, and is running with individuals maintained in a float net cage, enclosed in a frame tank padded with plastic liner, with a land-based Recirculating Aquaculture System (RAS). The biological adaptation was evaluated by fish survival and feed intake. Growth performance by zootechnical indexes was estimated through biometric measurements. During this initial report period (14th November 2021

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to 9th February 2022), fish were hand-fed twice daily (8:00 a.m. and 5:00 p.m.) at a rate of 1.5 % total biomass. The water quality parameters were monitored regularly, at least once a week, and commercial probiotic was added to the water tank. Water quality remained in the range tolerable for the species and to February 2022, survival is 100%. The feeding behavior has been immediate consumption of all offered food, demonstrating the biological adaptability of the dusk grouper. Over the 87-days report period, the juveniles showed a significant ($P < 0.05$) improvement in growth performance and reached an average weight and length of 290.2 ± 4.7 g and 25.03 ± 0.1 cm. Likewise, the mean apparent food ration converse and specific growth rate were 1.33 and 0.77 (% -day). The preliminary pilot project has already accomplished the first successful transport of groupers to Ceará – Brazil; attest the ability of the dusky grouper *E. marginatus* to be adapted to a new hot and dry environment and the preliminary growth performance indicates there is great potential of dusky grouper production in northeastern Brazil.

Keywords: Mariculture, marine finfish, fish farming.

RESUMO

Esta nota registra os resultados preliminares obtidos na execução do primeiro projeto piloto para investigar a adaptabilidade e o desempenho zootécnico da garoupa-verdadeira Epinephelus marginatus *no nordeste do Brasil. O projeto teve início com o primeiro transporte de juvenis da garoupa-verdadeira da Fazenda Marinha Prime Seafood, Alcobaça – BA - Brasil, para Mirante Pescados, Cascavel – CE – Brasil, e foi realizado com indivíduos mantidos em gaiola-rede flutuante, encerrados em tanque suspenso coberto com lona plástica, em Sistema de Recirculação de Água (SRA) no continente. A adaptação biológica foi avaliada através da sobrevivência dos peixes e consumo de ração. O desempenho zootécnico pelos índices zootécnicos foram estimado por meio de medidas biométricas. Durante o período inicial (14 de novembro de 2021 a 9 de fevereiro de 2022), os peixes foram alimentados manualmente duas vezes ao dia (8h e 17h) a uma taxa de 1.5% da biomassa total. Os parâmetros de qualidade da água foram monitorados regularmente, pelo menos uma vez por semana, e um probiótico comercial foi adicionado diretamente na água. A qualidade da água permaneceu na faixa tolerável para a espécie e a sobrevivência tem sido de 100%. O comportamento alimentar observado é de consumo imediato da ração ofertada, demonstrando a adaptabilidade biológica dos juvenis de garoupa-verdadeira. Durante o período de 87 dias, os juvenis apresentaram uma melhora significativa ($P < 0,05$) no desempenho do crescimento, atingindo peso e comprimento médios de 290,2 ± 4,7 g e 25,03 ± 0,1 cm. Da mesma forma, as médias do Conversão Alimentar Aparente (CAA) e da Taxa de Crescimento Específico (TCE) foram 1,33 e 0,77 (% -dia). O Projeto Piloto realizou o primeiro transporte, bem-sucedido, de garoupa-verdadeira para o Ceará – Brasil, atestou a capacidade da espécie E. marginatus de se adaptar ao ambiente quente e seco, característico do nordeste brasileiro, e o seu desempenho zootécnico preliminar apontam para o potencial da sua produção no Nordeste do Brasil.*

***Palavras-chave**: Maricultura, peixe marinho, piscicultura.*

INTRODUCTION

Groupers (*Epinephelus* sp.) are distributed in the seas and oceans of the planet (Pierre *et al*. 2008) and according to FAO (2022), commodities such as groupers, a high value coastal resource, have been overfished, including dusky grouper, being listed as vulnerable globally according to the IUCN Red List of Threatened Species (Pollard et al. 2018). In this sense, the prospects for the development of grouper aquaculture in order to reduce fishing on wild populations and meet market demand are welcome.

The subfamily Epineplhalinae has important species for aquaculture production, which occur mainly in Asia, the major supplier of cultivated grouper (Rimmer and Glamuzina 2017). *Epinephelus marginatus* occurs in the Atlantic Ocean, Indian Ocean and Mediterranean Sea (Condini *et al*. 2018) and due to its good taste, commercial value and importance for both coastal communities and local fisheries (Rimmer and Glamuzina 2017) it has become an emerging native species for Brazilian aquaculture in the last few years.

Marine finfish farming in Brazil has been running into challenges and it is in its initial phase, with a few small-scale production units (Valenti *et al*. 2021). However, currently, unlike most Asian producers (Pierre *et al*., 2008; Shapawi *et al*. 2018), grouper production initiatives in Brazil start mainly with grouper fingerlings supplied from commercial hatcheries, not from wild-caught juveniles. In 2020, the only Brazilian commercial marine finfish hatchery produced 30,000 grouper fingerlings (Claudia Ehlers Kerber, pers. comm.). Likewise, the main source of Brazilian finfish food is commercial feed and not trash fish, even though local industry has not yet developed continuous production. Both facts contribute significantly to the sustainable operation of Brazilian grouper aquaculture development.

Although some authors point out that the northeast region of Brazil would probably provide an environment for better zootechnical performance of this species, due to its continuous high temperature (Kerber 2012; Kerber *et al*. 2012), to date, there has been no concrete data on that hypothesis and no official data on zootechnical performance of captive dusky grouper in the northeast region of Brazil.

We have identified the absence and need for such information. In order to bridge these gaps, and support interest in developing grouper production and disseminating research findings, the aim of this report is to present the preliminary results of the first pilot project to assess the adaptability and feasibility of grouper aquaculture in northeastern Brazil.

MATERIAL AND METHODS

The project started with the first transport of dusky grouper (*E. marginatus*) to Ceará - Brazil. The first 150 juveniles (150.13 ± 3.0 g; 20.6 ± 0.03 cm) were transported from Prime Seafood Marine Farm, Alcobaça – BA - Brazil, to Mirante Pescados, Cascavel – CE – Brazil, between 12th and 14th November 2021, in open fish transport systems consisting of four 1000 L water-filled containers continuously supplied with an outside oxygen source by aerator stone under technical supervision.

After measuring container's initial water-dissolved oxygen, temperature, salinity, pH, total ammonia and alkalinity (T0), fish were distributed and stocking density was; three "*transfish*" with 6 kg/m^3 and one with 4.5 kg/m^3. Water quality parameters during fish transport, and on arrival, were monitored as follows; the dissolved oxygen (DO) and temperature (°C) were measured using an AT 155 Microprocessor Oximeter ALFAKIT, salinity (‰) using a portable refractometer, and pH using an EcoSense pH10A pen, alkalinity, total ammonia nitrogen (TAN) using a SERA water test, and dissolved carbon dioxide (CO_2) was deduced using a stoichiometric mathematical formula.

Upon arrival at the project's experimental location, fish were submitted to an

acclimatization process and then stocked (10 kg m^{-3}) into a floating net cage (1.5 m x 1.5 m x 1 m with a 4 mm mesh size) enclosed in a frame tank padded with a plastic liner (7 m x 7 m x 1.40 m), filled with marine water (35 g L^{-1}) with a land-based Recirculating Aquaculture System -RAS (biological and suspended solids filter). The marine water used to fill the tank was transported (32.3 km) to the experimental location using a water transport tank.

Fish were fed with commercial feed (GuabiTech Carnívoros Marinhos 9-11 mm, 45 % crude protein) twice daily (8:00 a.m. and 5:00 p.m.) at a rate of 1.5 % total biomass per day, adjusted according to biometric measurements. The portion of food corresponding to 1.5% of the biomass was offered gradually by hand until total consumption or apparent satiety. In the event of apparent satiety before total consumption, the uneaten wet pellets were weighed to determine apparent food consumption and calculate the supplied feed.

Dissolved oxygen (DO), temperature (°C), salinity (‰), pH, alkalinity, dissolved carbon dioxide (CO_2) and total ammonia nitrogen (TAN) were monitored with the same methodology used in fish transport. The nitrite (NO_2), nitrate (NO_3) and water transparency monitoring started in December 2020, using a SERA water test (NO_2 and NO_3) and Secchi disk (water transparency). To maximize water quality, a commercial probiotic (BM-PRO Biotrends) was added to the water tank according to the label's instructions.

Biological adaptation was evaluated by fish survival, and feed intake and growth performance in captivity was based on calculated zootechnical indexes through biometric measurements as follow;

- Weight gain (WG %) = $[(W_f - W_i)/W_i] \times 100$;

- Apparent Food Conversion Rate (AFCR) = *supplied feed/increase in fish weight*;

- Specific growth rate (SGR) (% day^{-1}) = $100 (\ln W_f - \ln W_i/t)$;

- Survival (%) = $(N_i - N_f) \times 100$.

Where "Wi" is the initial mean weight of fish (g), "W_f" is the final mean weight of fish (g), "t" is the time period in days, "Ni" is the initial number of fish and "Nf" is the final number of fish during the report period (days).

Data were expressed as mean ± standard error and tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene´s test). The means of the parameters evaluated were submitted to the analysis of variance (ANOVA) for comparison between the report period. In the case of significant difference, Tukey's test was applied ($p < 0.05$).

RESULTS

The transport of the dusky groupers to Ceará - Brazil lasted 40 hours and, during this period, nine water quality parameters measurements were made to monitor water quality and fish behavior. The overall mean water quality parameters remained within the following values: DO (mg O_2 $^{-}$L) 7.00 ± 0.5, Temperature (°C) 25.0 ± 0.1, Salinity (‰) 36.0 ± 0.0, pH 7.6 ± 0.01, Alkalinity (mg CaCO3 $^{-}$L) 106.0 ± 1.4, TAN (ppm) 0.6 ± 0.2, NH_3-N (ppm) 0.09 ± 0.01 and CO_2 (ppm) 4.5 ± 0.1. The 250 *E. marginatus* juveniles exhibited calm behavior, with an absence of frantic swimming, crashing against the container and frenzy. The fish transport survival rate was 100%.

The water quality parameters during the report period (14th November 2021 to 9th February 2022), are summarized in Table 1. Temperature, salinity, TAN and Secchi depth readings showed significant variations ($p < 0.05$) while other parameters remained statistically similar between months ($p > 0.05$).

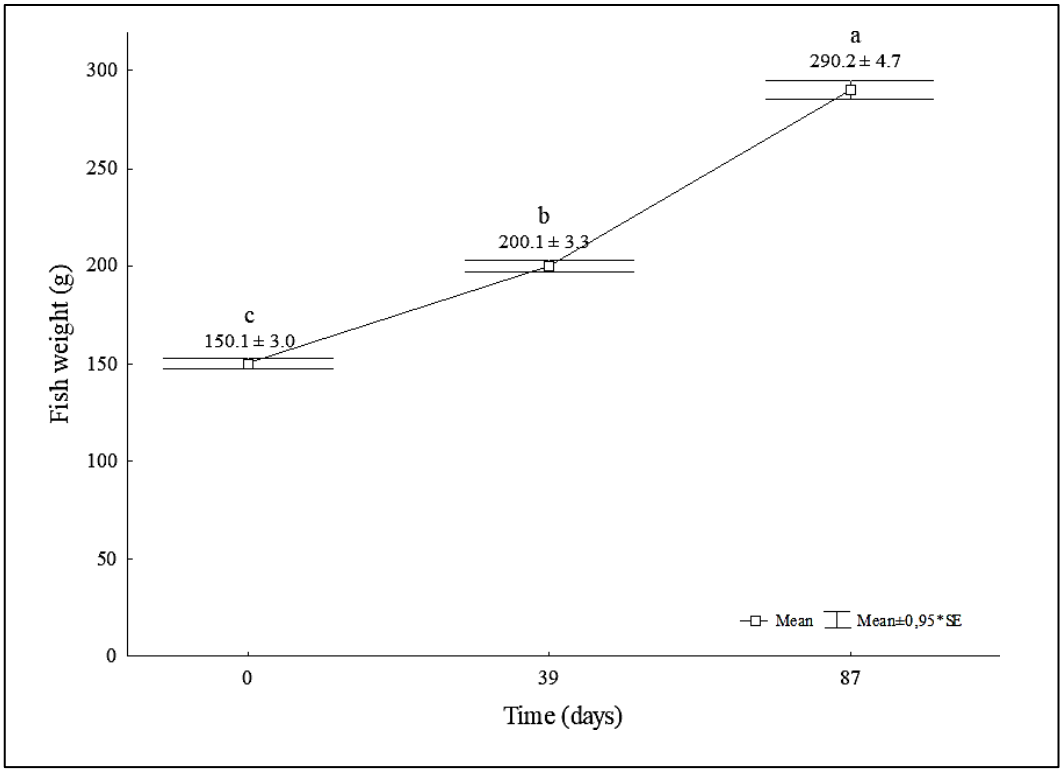
Table 1 – Water quality parameters (mean ± standard error) recorded during report period (14th November 2021 to 9th February 2022)

Parameters	Report period			
	November/2020	December/2020	January/2021	February/2021
DO ($mgO_2 \cdot L$)	6.4 ± 0.2	6.2 ± 0.2	6.1 ± 0.07	6.5 ± 0.05
Temperature (°C)	30.1 ± 0.2 [a]	29.0 ± 0.08 [b]	28.2 ± 0.08 [b]	28.8 ± 0.1 [ab]
Salinity (‰)	37.0 ± 0.6 [a]	38.0 ± 0.6 [a]	27.6 ± 1.8 [b]	25.0 ± 0.1 [b]
pH	8.1 ± 0.04	8.2 ± 0.02	8.0 ± 0.09	8.0 ± 0.1
Alkalinity (mg $CaCO_3 \cdot L$)	124.6 ± 3.8	120.1 ± 4.4	102.3 ± 4.45	98.0 ± 26.7
TAN (ppm)	1.0 ± 0.01	1.1 ± 0.5	0.4 ± 0.1	0.2 ± 0.07
NH_3-N (ppm)	0.08 ± 0.02	0.79 ± 0.4	0.05 ± 0.01	0.01 ± 0.01
CO_2 (ppm)	1.5 ± 0.1	1.2 ± 0.3	2.1 ± 0.3	2.0 ± 0.01
Secchi depth (cm)	x	52.0 ± 2.5	49.7 ± 2.7	43.0 ± 3.0
NO_2 (ppm)	x	6.6 ± 1.6 [b]	15.6 ± 2.3 [a]	0.8 ± 0.1 [b]
NO_3 (ppm)	x	100.0 ± 0.01 [b]	85.0 ± 8.6 [b]	22.0 ± 3.3 [a]

DO = Dissolved oxygen; TAN = Total ammonia nitrogen; NH_3-N = unionized ammonia; CO_2 = Dissolved carbon dioxide; NO_2 = Nitrite; NO_3 = Nitrate. Means with different letters are significantly different ($P < 0.05$). Means with absence of letters indicate not significantly different between experimental periods.

The preliminary juvenile growth performance is illustrated in Figure 1. There was a significant ($p < 0.05$) increase in fish weight and length over the 39- and 87-days, with an average, respectively, of 200.1 ± 3.3 g and 22.5 ± 0.1 cm and 290.2 ± 4.7 g and 25.03 ± 0.1 cm.

Figure 1 - Preliminary growth performance (mean ± standard error) of juveniles *E. marginatus* maintained in floating net cage enclosed in frame tank padded with plastic liner with a land-based Recirculating Aquaculture System (RAS) during report period (days) (n = 150). Different letters means significantly different between treatments ($P < 0.05$)



The zootechnical indexes (Table 2) AFCR (1.33) and SGR (0.77 % day $^{-1}$) mean values were similar during the period ($p > 0.05$), while WG demonstrated a significant ($p < 0.05$) increase. Until February 2022, survival rate remained 100%.

Table 2 - Zootechnical indexes (mean ± standard error) of *E. marginatus* maintained in floating net cage enclosed in frame tank padded with plastic liner with a land-based Recirculating Aquaculture System (RAS) during report period (days) (n = 150)

	Report period		
Zootechnical indexes	0 (days)	39 (days)	87 (days)
Weight (g)	150.1 ± 3.0 ^{c}	200.10 ± 3.3 ^{b}	290.17 ± 4.7 ^{a}
Lenght (cm)	20.6 ± 3.0 ^{c}	22.46 ± 0.1 ^{b}	25.08 ± 0.1 ^{a}
WG (%)	x	38.3 ± 2.0 ^{b}	93.4 ± 3.1 ^{a}
SGR (% day $^{-1}$)	x	0.8 ± 0.03	0.8 ± 0.01
AFCR	x	1.3 ± 0.1	1.3 ± 0.03
SR (%)	x	100	100

WG = Weight gain. SGR = Specific growth rate; AFCR: Apparent food conversion rate; SR =Survival rate. Means with different letters are significantly different ($P < 0.05$). Means with absence of letters indicate not significantly different between treatments.

DISCUSSION

Although marine fish were the first aquatic organisms reared in Brazil, to date, total production does not exceed 20 tons per year. In addition, although Brazil's northeast region has unquestionable potential for marine finfish aquaculture, the current restricted commercial production is mainly concentrated in the southeastern region (Valenti *et al.* 2021; Kuhnen *et al.* 2022).

This report details the first pilot project that aims to support an interest in developing Ceará's marine finfish production, with dusky grouper *Epinephelus marginatus*, a high commercial value species (Cabaleiro *et al.* 2018) and commercial fingerling supply (Redemar Alevinos –

Ilhabela- SP).

The first challenge for the project was fish acquisition, which was overcome via partnerships and collaboration deals and the first successful dusky grouper transport to Ceará. The water quality parameters during the journey remained within the safe range for the species (Gracia López and Castelló-Orvay 2003), with 100% survival.

Secondly, despite assumptions about grouper's adaptive propensity to the hot and dry climate of northeastern Brazil, there was a doubt about whether *E. marginatus* juveniles, a subtropical species, would in fact to be adaptable to such a new environment. The 100% survival rate and the observed spontaneous and regular food consumption that followed fish conditioning in the system attest the juvenile dusky grouper's adaptability to northeastern Brazil's environmental conditions.

Having overcome both of the initial challenges, the species responded well to the captive system. The "maturation system" (establishment of nitrifying bacteria) period was verified by the gradual total ammonia nitrogen decrease over time. Complementarily, nitrite began to increase and then to decrease, demonstrating a trend of stabilization by February 2022. However, despite the expected increase in nitrate concentration, there was an unexpected, but comprehensive, significant reduction of that parameter in February 2022, and the same reduction tendency was observed for temperature and salinity. This circumstance presented are comprehensive because the report period covers both the beginning of summer (November-December) and after this, the onset of the rainfall season (January -February). In the dry season, due to high evaporation, there were three occasions when salinity achieved 40. In contrast, the onset of the rainy season softened the temperature and, as initially there was no cover to prevent fresh water input into the system, it caused salinity and nitrate dilution. Both problems were resolved by the addition of fresh water to the system until salinity was reduced from 40‰ to 35‰, and the tank was covered with an aluminum roof. It must be noted that the water quality remained in the admissible range for the species (López and Castelló 2003).

The preliminary zootechnical performance observed corroborates other *E. marginatus* studies. Coelho *et al.* (2021), studying dusky grouper in the same weight range, between 143 and 322 g, reported an SGR (% ⁻day) of 0.8 to 0.5. while Gracia López and Castelló-Orvay (2003), Sousa *et al.* (2019) and Spandri *et al.* (2020), even though they were studying juveniles in a smaller weight range, between of 7- 64 g, reported an SGR (% ⁻day) of 0.1 to 2.0. Therefore, when considering the established physiological growth studies that indicate specific growth rates decrease with increased fish size (Jobbling 1994), a feasible growth improvement through environmental and nutritional optimization (Watanabe *et al.* 2004) and as an ectotherm species, a typical increase in fish growth as a response to an increase in temperature (Kingsolver 2009), the pilot project's preliminary growth performance indeed indicates auspicious results.

Likewise, the AFCR was at least similar, but mostly better, to values reported by several studies developed with the same species, with reported values between 1.2 and 2.2, for fish equally fed with commercial feed (López and Castelló-Orvay 2003; Sousa *et al.* 2019; Spandri *et al.* 2020; Coelho *et al.* 2021).

Despite all the challenges, the project has development potential, even though there is still a need of established strategies for sustainable dusky grouper aquaculture. We intend to develop technological feasibility solutions (aimed at different systems) to determine how long it takes to reach marketable size and evaluate the economic viability of dusky grouper production in northeastern Brazil.

CONCLUSIONS

On the basis of this pilot project, the critical challenge is to build strategies to create an environment that promotes and attracts collaborators, entrepreneurs, investors and suppliers to create a sustainable marine fish farming business. Initially this will be held in the northeast region,

but eventually it could be extended to other prone regions in Brazil.

From our preliminary results, we can conclude the following: in a responsible and innovative way, the pilot project has already accomplished the first successful transport of grouper to Ceará – Brazil, determined the biological adaptability of the dusky grouper to the climate of northeastern Brazil. We are currently seeking to evaluate the technical feasibility and economic viability of *E. marginatus* production

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