BOLETIM DO INSTITUTO DE PESCA
Scientific Article

Oxygen consumption of the dog snapper in captivity: implications of water quality and fish stage

Evandro Malanski[1]*, Ana Cecilia Gomes Silva Malanski[1], Luiz Fernando Loureiro Fernandes[1]

[1]Universidade Federal do Espírito Santo Centro de Ciências Humanas e Naturais, Departamento de Oceanografia e Ecologia, Base Oceanográfica – Aracruz (ES), Brazil.
*Corresponding author: evanmal@gmail.com

ABSTRACT

Respirometry represents the least invasive method to investigate fish physiology. Therefore, oxygen consumption (OC) of the dog snapper *Lutjanus jocu* was evaluated in two specific culture water conditions: the cleanest possible water, and accumulated nitrate water. Clean water represents the seawater collected straight from the environment with a basic treatment, and nitrate water represents the seawater kept at the rearing tanks using advanced water treatment in its recirculating system, in which nitrogen compounds accumulate. Fish were classified into stages, and median OC for juvenile, grow-out and broodstock stages were 322.4, 176.5 and 78.4 mg·(kg·h)$^{-1}$, respectively. Despite no significant differences in OC for the dog snapper were found during day and nighttime, this last condition represents a 5% increase on the measurements, possibly related to the circadian rhythm of this species, known for being active during the night. Remarkable increase in respirometry is observed in the dog snapper grow-out stage cultured in nitrate water. This condition demands extra management of the production to promote fish welfare, and consequently better growth.

Keywords: Respirometry; Nitrate water; Fish culture; Life stage.

Consumo de oxigênio pelo vermelho-dentão em cativeiro: implicações da qualidade da água e do estágio de desenvolvimento do peixe

Resumo

Respirometria representa o método menos invasivo para investigar a fisiologia de peixes. Sendo assim, o consumo de oxigênio (CO) de vermelho-dentão *Lutjanus jocu* foi avaliado em duas condições específicas de água de cultivo: água mais limpa possível, e água com acúmulo de nitrato. A água limpa representa a água marinha coletada diretamente do ambiente com um tratamento básico, e a água com nitrato representa a água marinha mantida nos tanques de cultivo usando tratamento avançado da água no sistema de recirculação, em que os compostos nitrogenados acumulam. Os peixes foram classificados em estágios, e as medianas de CO para os estágios juvenil, engorda e reprodutor foram 322,4, 176,5 and 78,4 mg·(kg·h)$^{-1}$, respectivamente. Apesar de não ter havido diferenças significativas no CO para o vermelho-dentão durante os períodos diurno e noturno, esta última condição representa aumento de 5% nas medições, possivelmente relacionado com o ritmo circadiano da espécie, conhecida por ser ativa durante a noite. Notável aumento da respirometria foi observado no estágio engorda do vermelho-dentão cultivado em água com nitrato. Essa condição demanda gerenciamento extra na produção para promover o bem-estar do peixe e, consequentemente, melhor crescimento.

Palavras-chave: Respirometria; Água com nitrato; Piscicultura; Estágio de vida.

Received: September 21, 2023 | **Approved:** June 6, 2024
Section editor: Fabiana Garcia

INTRODUCTION

Fishes of the family Lutjanidae, known as snappers, are of relevant commercial interest owed to its tasty white meat. Due to this demand, wild snapper stocks are suffering with overexploitation (Amorim et al., 2019), what should motivate their development in hatcheries and farms to supply the market, especially since they are reported as a potential candidate for domestication (FAO, 2019).

In the Southeast of Brazil, the dog snapper *Lutjanus jocu* is one of these relevant species with high demand, and commercial value, with a decline in its stocks. In estuarine systems, *L. jocu* is abundant in the juvenile stage, suggesting that the environmental conditions are favorable for developing to adulthood (Pimentel & Joyeux, 2010). However, finding larger mature adults of this species is not an easy task. Their behavior of creating spawning aggregations in places far from the coast (Bezerra et al., 2021) make them more susceptible to be captured, what may contribute to their stock decline.

In Brazil, few marine species have developed protocols to start a production enterprise (Valenti et al., 2021). There is a need to invest in marine farming, in both scientific and commercial aspects, to develop a profitable business (Lisboa et al., 2020) in which the supply of products and services surpasses the productivity challenges. Cultivating dog snapper could represent a step forward to the blue economy, reducing the pressure on its stocks due to overfishing, contributing to social, environmental, and food security issues when a high production is achieved. However, snappers have few sources of juvenile production for commercial aquaculture in Latin America (Ibarra-Castro et al., 2020), none in Brazil. Also, snappers are very resistant fishes, and their rearing has virtually no break during growth when established protocols are followed (Coniza et al., 2012).

Among the extremities for culturing marine fishes, the offshore extensive method produce fish in cages in which the water should be always suitable due to the exchange by ocean currents, while in the intensive method the fish is produced confined in tanks with high technological filtration packages to ensure water quality, but still the water accumulates dissolved compounds that may demand a physiological response from the cultured fish, even with basic or more sophisticated filtration models. Both types have advantages and disadvantages for aquaculture development, associated with their risks, costs and fluctuations in the production unit, and their possibility to use during different life stages. The type of enterprise should be carefully chosen, since water quality does affect fish metabolism (Wuenschel et al., 2005), impacting fish production. They must be also related to animal welfare, and understanding the water conditions has important implications for fish growth management.

Regarding the waste products when culturing fish, suspended particles should be quickly removed by mechanical retention, while the dissolved ones should be removed/converted to less toxic compounds as quickly as possible by the culture system. Ammonia is one of the dissolved waste products that need special attention in aquaculture due to its toxicity and consequences on fish physiology (Barbieri et al., 2019). Considering a hypothetical high-technology filtration system model coupled to the culturing fish tank, the ammonia excreted by the fish should be rapidly converted to the less toxic nitrogen compound, the nitrate, as a process of oxidation. As a consequence, nitrate still tends to accumulate, being controlled by the water renewal of the maintenance schedule. Despite this water condition being applied for fish culture, it would never be experienced by one fish confined in the extensive offshore cage and is expected to have different physiological demands and responses.

In animal physiology, several bounds of organization are investigated to understand the processes for life to exist, and it may include the whole animal since its reaction (behavior) has important implications for life maintenance. Animal behavior is affected by intrinsic and extrinsic mechanisms (Duque-Wilckens et al., 2019), such as circadian rhythm and photoperiod, respectively. Attention is given to that since, during the progress of domestication of dog snapper in the laboratory, it was observed different behavior throughout the day, even when the rearing condition was kept stable for months.

In this context, between the methodologies to investigate fish physiology, evaluating the oxygen consumption rate is one possible solution, being the least invasive method, and also the most practical to be used in production units (Kim et al., 1995). Attention is given to fish respirometry as the most basic condition to permit them to stay alive: breathe or die. In an environment without stress, the oxygen consumption rate is represented by the lesser demand possible, while any possible stressor requests extra oxygen demands by the fish. The oxygen demand represents for one species the possibility of an increase/decrease in stocking density, considering the type of cultivation chosen, and the controlled parameters that need continuously to be assessed, as well as the equipment and other resources available.

Herein, we aimed to present relevant information regarding snapper respirometry in different size/stage ranges, with two distinct rearing water conditions: the zero-nitrate water, and high-nitrate water. The zero-nitrate water represents the most natural seawater possible, while the high-nitrate water represents the rearing condition using the recirculating aquaculture system (RAS) technique.

Considering the different water qualities, the size/stage ranges, and the circadian rhythm of the species, it is hypothesized that a distinct mean on the oxygen consumption rates is achieved by each category. Consequently, the results will give directions for the maintenance of this species in captivity.

MATERIALS AND METHODS

Seawater was collected from a beach area near the laboratory, where basic treatment (particle retention and sterilization by chlorine solution) was done prior to its use in the rearing tanks.

Dog snappers (*L. jocu*) were sampled with a hook and line in the estuarine system near the laboratory, SISBIO collecting license numbers 64108-2 and 77273-1, and the experimental procedures approved by the Ethics Committee for Animal Research/Universidade Federal do Espírito Santo license number 02/2021. Larger individuals (n = 10) used herein are part of the broodstock kept in the laboratory for about three years in confinement, while the smaller individuals (n = 14) were sampled near the time of this experiment, but only used after quarantine procedures. As part of the quarantine, disinfection with freshwater and formaldehyde (10 $mg \cdot L^{-1}$) baths for 10–30 minutes in separate containers were performed every other day for two weeks, with a daily full exchange of seawater in the confinement tank. In the first five days, fish were not fed to empty their guts. After one month, if the fish had no signs of disease during the entire period, and were eating normally, they were tagged with a microchip (if possible due to their size), and moved to the rearing system.

The fish oxygen consumption measurements tested four different conditions: clean water *vs*. rearing system water, and daytime *vs*. nighttime. Clean water was the collected seawater that underwent a basic treatment, without accumulated nitrogenate compounds, while the rearing system water was based on the RAS1 system (recirculation system RAS1, described in Malanski et al., 2024), with the advanced recirculation treatment (particle retention, ultraviolet and ozone sterilization, biofiltration), with nitrogenate compounds accumulation; N-ammonium, N-nitrite and N-nitrate tested with colorimetric kits and photocolorimeter (Alfakit). For any of these two water conditions, oxygen consumption was tested during daytime and nighttime, since the circadian rhythm of the fish (behavior and resting) may affect measurements. Tentatively, every fish had the experiment done for the four different conditions tested. Throughout the experiment, some fish were lost between measurements and, in this case, not every fish identification (ID) had all the conditions tested, but these data are still used for analytical purpose within the specific classification categories.

All the fish were kept in the rearing system for at least two weeks prior to being transferred to the closed-flow chamber within the oxygen consumption (OC) system, in which respirometry measurements for each fish were taken. Also, since the same fish had the OC data taken in four different situations (daytime and nighttime for both the clean and rearing waters), there was at least a one-day feeding interval between each measurement, in which the fish could recover from any stress, and the water in the OC system could be changed. The chamber was either a smaller (10 L capacity, for fish between 25–450 g) or a larger (36 L, for fish weighing more than 450 g) device, with a coupled multiparameter probe (for water quality measurements).

Inside the chamber, the closed-loop water flow was kept with the use of a submerged pump. Consequently, the water movement maintained the fish swimming. This chamber was submerged in a 500-L tank, in which an air blower kept the oxygen saturation in the water, and the temperature was controlled by a submerged heater. Valves controlled the water exchange between the chamber and tank when needed. For the very small fish, up to 15 g, the respirometry measurements were taken in a 2-L Becker, filled with water to its top and covered with a plastic film to avoid any oxygen exchange with the air, and the movement of water inside was done with the use of a magnetic stirrer; the Becker was placed inside a small 7-L aquarium to avoid temperature fluctuations throughout the experiment.

Water quality data was obtained with a multiparameter probe (YSI Pro Plus model), with dissolved oxygen, conductivity/salinity, temperature, and pH sensors installed on it. Despite the dissolved oxygen sensor being from a galvanic model, meaning there is a little consumption of oxygen by the sensor, this little consumption was negligible compared to the fish respiration, since the measured consumption by the sensor was in the order of 0.03 $mg \cdot h^{-1}$.

The oxygen consumption experiment followed an eight-step protocol:

- The OC-system was filled with the water used for the experiment, with the nitrogen (N-ammonium, N-nitrite and N-nitrate) measured;
- The OC-system was turned on (multiparameter sensor, pump, heater, and air blower) for at least 1 hour before the fish was moved to the chamber, or until stabilization of the water parameters;

- The fish was gently moved from the rearing system to the chamber, with the valves in position to keep the water exchange between the chamber and tank for at least 1 hour, or until visually the fish was less stressed from the manipulation;
- Valves were positioned to stop the water exchange between chamber and tank, keeping the closed-loop in the chamber, with the first oxygen and temperature data taken;
- Oxygen and temperature data were taken every 5 minutes for up to 30 minutes of water isolation within the chamber, or until the oxygen concentration was down to 4 mg·L^{-1} (maximum of seven data reads per measurement), with the average of this data comprising the fish oxygen consumption measurement;
- Valves were repositioned to allow water exchange between the chamber and tank for a minimum of 5 minutes for chamber reoxygenation;
- Repeated steps 4, 5 and 6 until at least three measurements per fish were taken;
- Before fish was moved back to the rearing system, biometric measurements (size and weight) were taken.

The consumption data reads used Eq. 1, proposed in Kawamoto (1977 *apud* Kim et al., 1995), as follows:

$$OC_{fish} = \left(\frac{(DO_i - DO_f) \times V}{(t_f - t_i) \times W} \right) \quad (1)$$

where: OC_{fish}: the specific oxygen consumption by the fish in the time period, in mg·(kg·h)$^{-1}$; DO: the dissolved oxygen at the initial and final time, in mg·L^{-1}; V: the water volume within the chamber, in L; t: the time elapsed between DO measurements, in hours; W: the body weight of the fish, in kg.

For analytical purposes, all the fish were categorized as follows:

- Weight category: fish weighing less than 750 g had their categories varying each 50 g (*i.e.*, 150–200 g), while fish weighing more than 750 g had their categories varying each 250 g (*i.e.*, 750–1,000 g);
- Life stage category: fish weighing less than 100 g was categorized as juvenile stage (n = 4), fish between 100–750 g was categorized as grow-out stage (n = 10), and fish more than 750 g as adult/broodstock stage (n = 10).

Statistical analyses were performed using R software (R Core Team, 2022), with analysis of variance to evaluate differences in the oxygen consumption with the effects of day-night time (DN), cleaner-nitrate water (CN), fish ID category (ID), weight category (WT), and stage category (ST). The assumptions for using analysis of variance are normality and homogeneity of variances, tested with Shapiro-Wilk's test and Levene's test, respectively.

Homogeneity of variances is not achieved with the core independent factor, the categories ID, WT and ST, which is expected to have differences within each category, and consequently it does not produce normality of data. Considering there is the interaction between factors, and that homogeneity of variances is achieved with DN and CN, the unbalanced two-way analysis of variance was used herein for statistical purposes.

Acknowledging the limitations of the data analysis, related to the number of samples available, the statistical models follow the functions show in Eq. 2:

$$OC \sim DN \times CN \times ID; \quad (2)$$
$$OC \sim DN \times CN \times ST.$$

A post-hoc TukeyHSD test was done when significant differences were observed. Considering the same fish stage when analyzing the oxygen consumption, the *i*-th stage was selected to evaluate the differences with the function $OC_i \sim DN_i \times CN_i$, and the fish weight category was used to observe differences within the fish stage using the Wilcoxon signed-rank test, with the Bonferroni's correction.

Also, the measurement of oxygen consumed by the dissolved oxygen sensor of the multiparameter was done three times for each of the water conditions, the clean and the nitrate water, following the 30-minute protocol as with the fish. The data obtained was tested to verify if the sensor performed differently during its measurements between the cleaner-nitrate water conditions, where a Student's t-test was performed. The mean value related to the oxygen consumed by the sensor is used as a correction factor for the oxygen consumed by the fish.

RESULTS

The seawater used throughout the experiment had a slight variation in its characteristics, with salinity ranging from 30.4–32.5, and temperature from 24.1–26.0°C, without ammonia being detected neither in the cleaner nor in the rearing waters, while nitrite was not detected only in the cleaner water (Table 1). Nitrite and nitrate had variations in the rearing water throughout the experiment due to the maintenance of the rearing system since the experiment lasted more than two months.

The dissolved oxygen sensor from the multiparameter had an average oxygen consumption of 0.0358 and 0.0319 mg·h^{-1} for the cleaner and culture water, respectively. However, this

Table 1. Water characteristics throughout the oxygen consumption experiment for the two conditions tested: cleaner water and nitrate water from the rearing system.

Condition	Temperature (°C)	Salinity	NH_4 ($mg \cdot L^{-1}$)	NO_2 ($mg \cdot L^{-1}$)	NO_3 ($mg \cdot L^{-1}$)
Cleaner water	24.20–25.90	31.20–32.40	0.00	0.00	< 1.00
Nitrate water	24.10–26.00	30.40–32.50	0.00	0.09-0.65	155.80–368.40

variation was not significant (Student's t-test, $p = 0.79$), and these low values have the meaning that the oxygen consumed by the sensor is negligible when compared to any of the oxygen consumed by the fish.

The oxygen consumption in *L. jocu* ranged between 53.2 and 551.6 $mg \cdot (kg \cdot h)^{-1}$ (Fig. 1). When evaluating the oxygen consumption individually, they did have differences between each other as expected (analysis of variance, df = 1, F = 1149.30, $p < 0.001$), and between CN water (analysis of variance, df = 1, F = 4.62, $p = 0.03$), despite no differences were observed individually for DN condition (analysis of variance, df = 1, F = 1.93 $p = 0.16$). Differences remain (analysis of variance, df = 1, F = 6.33, $p = 0.01$) when analyzing the interactions between individuals, DN and CN, and with the interactions between DN and CN (analysis of variance, df = 1, F = 4.93, $p = 0.02$), however no differences were observed in the interactions between individuals and DN (analysis of variance, df = 1, F = 2.27, $p = 0.13$), and individuals and CN (analysis of variance, df = 1, F = 0.12, $p = 0.72$).

Evaluating fish life stage categories, the median consumption by juveniles, grow-out and adult stages were 322.4, 176.5 and 78.4 $mg \cdot (kg \cdot h)^{-1}$, respectively. There are differences in oxygen

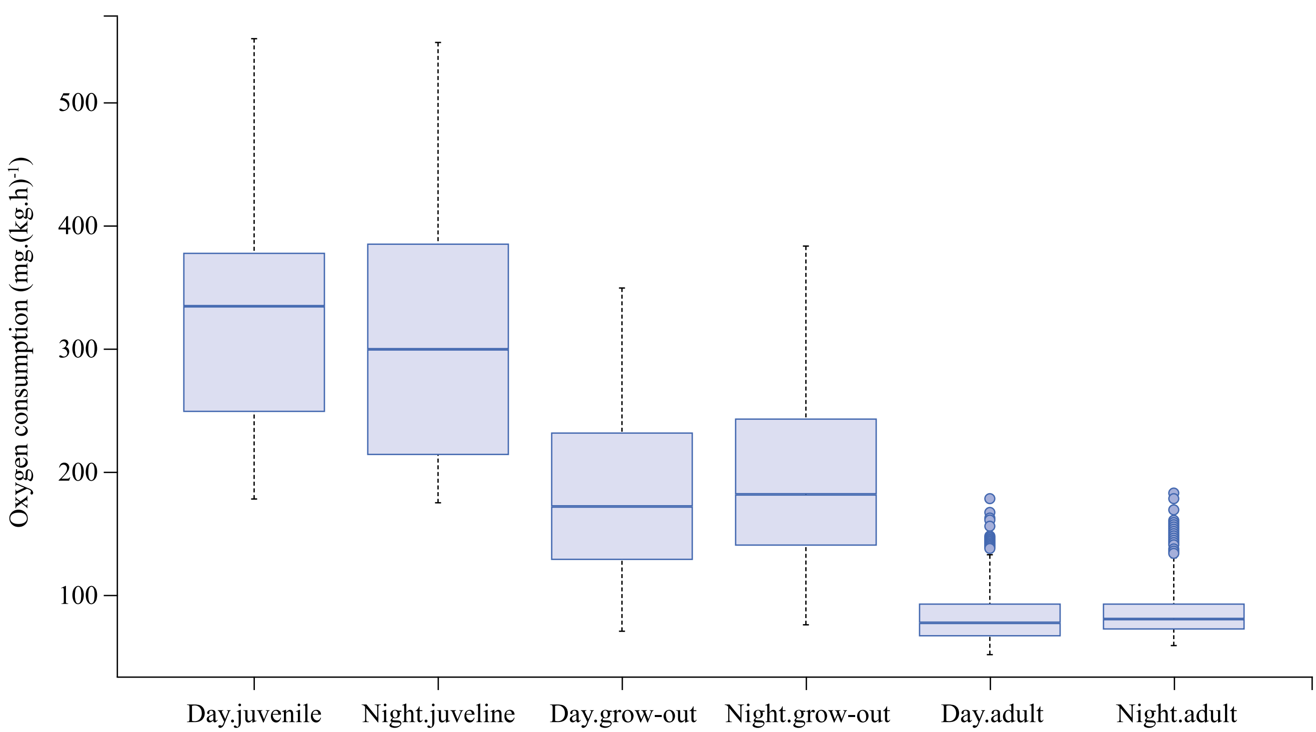

Figure 1. Oxygen consumption, in $mg \cdot (kg \cdot h)^{-1}$, for the three stages of *Lutjanus jocu* during the day and nighttime. In the boxplot within each category, its median value is indicated by the thick black line; 50% of measured data is represented in the limits of the gray area, where most of the measurements are concentrated in the lowest data interval; the remaining valid data is represented by the whisker limits; and outliers, values discrepant of the normal data tendency and very infrequent to be measured, are represented by the points outside the whiskers.

consumption between all stages (analysis of variance, df = 2, F = 1,728.03, $p < 0.001$) and between CN condition (analysis of variance, df = 1, F = 16.71, $p < 0.001$), while no differences were observed for the DN condition (analysis of variance, df = 1, F = 0.41, $p = 0.52$). The results showed that interactions between stage, DN and CN had no significant difference (analysis of variance, df = 2, F = 1.07, $p = 0.34$), even between DN and CN (analysis of variance, df = 1, F = 2.79, $p = 0.09$). However, significant differences were observed when considering the interactions between stage and DN (analysis of variance, df = 2, F = 5.42, $p < 0.01$), and stage and CN (analysis of variance, df = 2, F = 134.18, $p < 0.001$).

When looking at the source of differences in the interaction between life stage and DN, no differences were found in the oxygen consumption within the same life stage during day and night (juveniles and DN, TukeyHSD, $p = 0.07$; grow-out and DN, TukeyHSD, $p = 0.19$; adults and DN, TukeyHSD, $p = 0.93$), but all the other interactions *(i.e.*, juvenile during daytime *vs.* adult during daytime, etc.) did have significant differences (TukeyHSD, $p < 0.001$).

Despite this analysis showed no significant difference within the life stages during day and nighttime, the data demonstrated that during nighttime the oxygen consumption increased 5% for the grow-out and adult stages (Fig. 1). Looking into the source of differences in the interaction between life stage and CN, no differences were found only for the oxygen consumption of adults in cleaner and nitrate water (TukeyHSD, $p = 0.41$), while all the other interactions had significant differences (TukeyHSD, $p < 0.001$). However, when the data is shown, in nitrate water condition the oxygen consumption was 12% higher in the adult stage, 39% higher for the grow-out stage, but interestingly 15% lower for the juvenile stage (Fig. 2).

Considering the differences in the fish stage, when looking at the grow-out stage there were no significative differences in the oxygen consumption between DN conditions (analysis of variance, df = 1, F = 2.18, $p = 0.14$), but there were differences between CN condition (analysis of variance, df = 1, F = 204.66, $p < 0.001$). The interactions between DN and CN indicate no differences (analysis of variance, df = 1, F = 2.93, $p = 0.08$). The analysis within the fish stage indicates the weight category 400–450 g being the only one without differences between

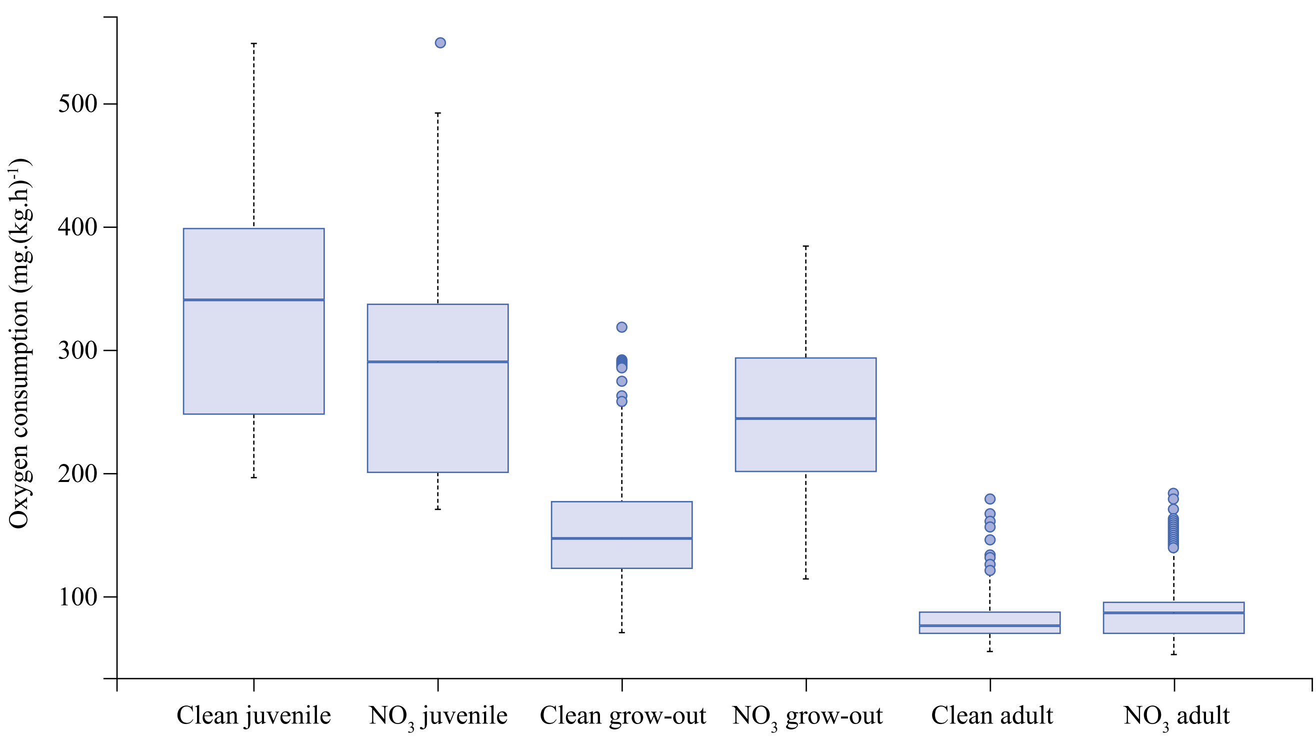

Figure 2. Oxygen consumption, in mg·(kg·h)$^{-1}$ for the three stages of *Lutjanus jocu* in cleaner and nitrate water. In the boxplot within each category, its median value is indicated by the thick black line; 50% of measured data is represented in the limits of the gray area, where most of the measurements are concentrated in the lowest data interval; the remaining valid data is represented by the whisker limits; and outliers, values discrepant of the normal data tendency and very infrequent to be measured, are represented by the points outside the whiskers.

the other weight categories with all p-values higher than 0.10 (Wilcoxon, $p > 0.05$), possibly being the best representative of the oxygen consumption readings for the stage.

Now, at the adult stage, significant differences were observed in the oxygen consumption between the DN condition (analysis of variance, df = 1, F = 6.27, $p = 0.01$), and between the CN condition (analysis of variance, df = 1, F = 15.07, $p < 0.001$), but the interactions between DN and CN indicated no differences (analysis of variance, df = 1, F = 0.24, $p = 0.61$). The analysis within the fish stage indicates no differences observed only between the weight categories 1,250–1,500 and 1,500–1,750 (Wilcoxon, $p = 0.06$), and 1,500–1,750 and 1,750–2,000 (Wilcoxon, $p = 1.00$); all the other comparisons did present differences (Wilcoxon, $p < 0.05$).

DISCUSSION

Water quality is a key factor when culturing fish (Zhang et al., 2011), and, with the development of intensive mass culture systems, the management and control of water parameters play an important role in the sustainability of the enterprise. Together, the operating procedures of a RAS and the metabolism knowledge of a species represent the most significant costs that need to be considered when building the infrastructure. Fish welfare must be also measured, since it relates directly to the fish health and performance (Beltrán et al., 2023; León-Ramírez et al., 2022), showing that the economics are not only related to the infrastructure built, but also to the observations throughout the culturing period.

On the respirometry knowledge of a fish, the data provided might infer on its stocking density, as a significant information to promote better growth and profitability (Garcia Trejo et al., 2016), even having consequences on building the infrastructure and acquiring the necessary equipment, as the design and size affect both the capital and the labor costs when culturing the species (Engle et al., 2020). The water treatment unit also plays a role in the oxygen demands on the culture system, in which dissolved toxic compounds should be rapidly oxidized to promote a better culture environment.

Although oxygen demands are crucial in any aquaculture enterprise, the operational management should consider all aspects of production, including even less problematic issues, what justifies the daily demands in the production environment. For example, the nitrogen cycle in the RAS system is well studied (Hagopian & Riley 1998), in which the ammonia excreted by fish is oxidized, and the accumulation of nitrate is the last product of nitrification. Somehow, a lot of attention is given to ammonia, possibly due to its acute impact on the culture (Barbieri et al., 2019).

However, despite the fact that nitrate is the friendlier version of a nitrogen compound in the water, fish tolerance has also limits to the accumulation of this compound, as nitrate impacts the oxygen-carrying capacity of the blood (Gomez Isaza et al., 2020; Rodrigues et al., 2011), potentially becoming toxic. This explains the differences in oxygen consumption by grow-out and broodstock stages of dog snapper, being higher in the RAS culture water than in cleaner water. It might require extra daily resources and demands to be managed, depending on the production step of the enterprise, since fish stage and water condition do correspond to differences in oxygen consumption. However, adults might require less management because, within this stage, the oxygen consumption has no differences between water qualities, being the most stable stage in terms of oxygen consumption, as seen in Figs. 1 and 2.

However, when the decreased respirometry of dog snapper juveniles at RAS culture water is observed, is this information a good reason to increase its stocking density? When the nitrate effect was evaluated in juvenile cobia *Rachycentron canadum*, acute exposure induced gill damage (Rodrigues et al., 2011), and in medaka fish *Oryzias latipes*, short-term exposure led to cellular disorder and metabolic disturbances (Shimura et al., 2004). It is suggested that nitrate may act as a narcotic compound in juvenile fish, decreasing respirometry, and having consequences to the whole body in long-term exposure such as the curvature of the spinal cord (Shimura et al., 2004). Such evaluations were not done for the dog snapper in the current investigation, but they represent the possible consequences of making culture decisions when considering a single information without the possible interactions with the subsequent culture condition.

The evidence that nitrate elevates steroid concentrations, as observed in female Siberian sturgeon *Acipenser baerii* (Hamlin et al., 2008), indicates another chronic consequence of the water quality when culturing fish. Hormones can be associated with reproduction stimuli, and since in nature snappers are shoaling usually for reproductive purposes, that condition may be correlated with this study as the dog snapper kept in our RAS system expressed some kind of excitability, a condition that might be promoted by the increase in nitrate concentration. Hormones were not measured in our investigation neither in the fish nor in the water, but the elevated respirometry metabolism in nitrate water (Fig. 2) can indicate, indirectly, that the dog snapper got more excited. Such excitement suggests hormone accumulations in the RAS system, and the observation of constant reproductive events for the dog snapper kept in captivity (Malanski et al., 2024) indicates the production of hormones by the broodstock. Hormones play a key role in the behavior of any organism

(Duque-Wilckens et al., 2019), which also might explain the aggressive mating behavior observed in the laboratory, where the dominant female kept its hegemony in the tank (data not shown).

When attaining to snapper daily behavior in nature, it is highlighted that adults are shoaling in the early evening, starting their reproductive activity at nighttime (Grimes, 1987); such circadian rhythm is also observed in the cultured dog snapper inside a laboratory facility (Malanski et al., 2024). This information led us to verify if any difference in respirometry was detectable between day and nighttime. Despite no significant statistical differences found for such a scenario within the life stage, there was an increase in oxygen consumption by the dog snapper in the order of 5% during nighttime (Fig. 1). Taking that into consideration, and from the snapper's circadian rhythm found in literature, the higher oxygen consumption during nighttime may be related to some kind of synchronicity to their mating behavior, when the mating act is related to fast sprint swim, consequently with the need to uptake more oxygen.

Another ecological aspect that might be inferred by our results is related to the juvenile stage of dog snapper. Despite the limitation of samples at this stage, its higher respirometry data indicates the need to occupy waters with higher oxygen concentrations. Considering their distribution in nature, they are found in the intertidal rocky shores where the waves promote most oxygenation (E. Malanski, personal observation). Consequently, this is probably the best place to stay when in need of more oxygen to uptake, and even protect them from large predators.

CONCLUSION

This investigation attempted to link the respirometry knowledge of a species that has been domesticated to the possible challenges promoted by its production technique, in which the water and species characteristics play important roles in operational procedures in any enterprise. No significant differences on oxygen demand happened during daytime and nighttime within life stages (except for adults) or size classes. Cleaner and nitrate water conditions showed significant differences within life stages and size classes.

The adult stage of dog snapper performing the steadiest oxygen consumption rates between any conditions analyzed (DN and CN), showed that they are less impacted by acute changes. Although these adults analyzed here composed the broodstock kept in the laboratory facility for three years, conditioned to confinement and manipulation, it should be less stressful for them to experience both water conditions tested.

Regarding juveniles, this stage had the highest oxygen demand, as it does also in other species, and the oxygen consumption obtained here will support the need for infrastructure and protocols to be applied in any hatchery and rearing facility.

In the grow-out stage, which corresponds to fish between 100–750 g in weight, a remarkable oxygen consumption rate has been shown when cultured in water that has accumulated nitrogen using recirculation systems. Despite lowering water usage, the consequences of applying such a technique may surpass other costs, related to production profits, infrastructure needed, and energetic demands. This situation claims for new investigation detailing the benefits and constraints of using RAS technology at this specific stage, as it should count as the period of highest growth rate in which the aquaculture industry usually spends most of its money.

As far as the results, they are related to individuals obtained from nature, that are still being domesticated. It would be beneficial to verify differences between wild individuals and domesticated ones (F0 to Fn). Such evaluation would represent the benefits of keeping the broodstock in captivity, and consequently the maintenance characteristics throughout generations such as the circadian rhythm and the respirometry.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTIONS

Conceptualization: Malanski E; Methodology: Malanski E; **Investigation:** Malanski E, Malanski ACGS; **Data acquisition:** Malanski E, Malanski ACGS; **Data curation:** Malanski E; **Formal Analysis:** Malanski E, Malanski ACGS; **Funding acquisition:** Malanski E; **Project administration:** Malanski E; **Supervision:** Fernandes LFL; **Writing – original draft:** Malanski E, Malanski ACGS; **Writing – review & editing:** Fernandes LFL.

FUNDING

Fundação de Amparo à Pesquisa do Espírito Santo
Grant No: 522/2020
Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grant No: 316224/2020-0

ACKNOWLEDGMENTS

We thank the Laboratório de Cultivo de Organismos Marinhos, at Base Oceanográfica/Universidade Federal do Espírito Santo, for providing the infrastructure to the development of this project; and the reviewers for improving this paper with the valuable discussion.

REFERENCES

Amorim, P.; Sousa, P.; Jardim, E. & Menezes, G.M. (2019). Sustainability Status of Data-Limited Fisheries: Global Challenges for Snapper and Grouper. *Frontiers in Marine Science*, 6: 654. https://doi.org/10.3389/fmars.2019.00654

Barbieri, E.; Lenz, R.M.; Andrade de Nascimento, A.; Lopes de Almeida, G.; Yoshida Roselli, L. & Barbosa Henriques, M. (2019). Efeito letal e subletal da amônia sobre o Lambari (Deuterodon iguape, Eigenmann 1907), espécie potencial para a Aquicultura Brasileira. *Boletim do Instituto de Pesca*, 45(1): e440. https://doi.org/10.20950/1678-2305.2019.45.1.440

Beltrán, A.; Vela Magaña, M.A.; Dumas, S. & Peñalosa Martinell, D. (2023). Rearing performance of juvenile yellowtail snapper, *Ocyurus chrysurus*, in a sea water recirculation system at two different stocking densities. *Journal of the World Aquaculture Society*, 54(6): 1430-1446. https://doi.org/10.1111/jwas.13007

Bezerra, I.M.; Hostim-Silva, M.; Teixeira, J.L.S.; Hackradt, C.W.; Félix-Hackradt, F.C. & Schiavetti, A. (2021). Spatial and temporal patterns of spawning aggregations of fish from the Epinephelidae and Lutjanidae families: An analysis by the local ecological knowledge of fishermen in the Tropical Southwestern Atlantic. *Fisheries Research*, 239: 105937. https://doi.org/10.1016/j.fishres.2021.105937

Coniza, E.; Catacutan, M. & Caballero, P. (2012). *Grow-out culture of mangrove red snapper (Lutjanus argentimaculatus* Forsskal, 1775*) in ponds*. Southeast Asian Fisheries Development Center.

Duque-Wilckens, N.; Trainor, B.C.; Marler, C.A. (2019). Aggression and Territoriality. In: Choe J.C. (Ed.). *Encyclopedia of Animal Behavior*. Elsevier. p. 539-546. https://doi.org/10.1016/B978-0-12-809633-8.90064-5

Engle, C.R.; Kumar, G. & Senten, J. (2020). Cost drivers and profitability of U.S. pond, raceway, and RAS aquaculture. *Journal of the World Aquaculture Society*, 51: 847-873. https://doi.org/10.1111/jwas.12706

Food and Agriculture Organization (FAO). (2019). *The State of the World's Aquatic Genetic Resources for Food and Agriculture*. Rome: FAO. https://doi.org/10.4060/CA5256EN

Garcia Trejo, J.F.; Pena Herrejon, G.A.; Soto Zarazua, G.M.; Mercado Luna, A.; Alatorre Jacome, O. & Rico Garcia, E. (2016). Effect of stocking density on growth performance and oxygen consumption of Nile tilapia (*Oreochromis niloticus*) under greenhouse conditions. *Latin American Journal of Aquatic Research*, 44(1): 177-183. https://doi.org/10.3856/vol44-issue1-fulltext-20

Gomez Isaza, D.F.; Cramp, R.L. & Franklin, C.E. (2020). Simultaneous exposure to nitrate and low pH reduces the blood oxygen-carrying capacity and functional performance of a freshwater fish. *Conservation Physiology*, 8(1): coz092. https://doi.org/10.1093/conphys/coz092

Grimes, C.B. (1987). Reproductive biology of the Lutjanidae: a review. In: Polovina, J.; Ralston, S.; Munro, J.L.; Powers, J.E.; Fox Jr., W.W. (Eds.). *Tropical snappers and groupers: biology and fisheries management*. Westview Press. p. 239-294.

Hagopian, D.S. & Riley, J.G. (1998). A closer look at the bacteriology of nitrification. *Aquacultural Engineering*, 18(4): 223-244. https://doi.org/10.1016/S0144-8609(98)00032-6

Hamlin, H.J.; Moore, B.C.; Edwards, T.M.; Larkin, I.L.V.; Boggs, A.; High, W.J.; Main, K.L. & Guillette, L.J. (2008). Nitrate-induced elevations in circulating sex steroid concentrations in female Siberian sturgeon (*Acipenser baeri*) in commercial aquaculture. *Aquaculture*, 281(1-4): 118-125. https://doi.org/10.1016/j.aquaculture.2008.05.030

Ibarra-Castro, L.; Ochoa-Bojórquez, M.O.; Sánchez-Téllez, J.L.; Rojo-Cebreros, A.H. & Alvarez-Lajonchère, L. (2020). A new efficient method for the mass production of juvenile spotted rose snapper *Lutjanus guttatus*. *Aquaculture Reports*, 18: 100550. https://doi.org/10.1016/j.aqrep.2020.100550

Kim, I.N.; Chang, Y.J. & Kwon, J.Y. (1995). The Patterns of Oxygen Consumption In Six Species of Marine Fish. *Korean Journal of Fisheries and Aquatic Sciences*, 28(3): 373-381.

León-Ramírez, J.J.; García-Trejo, J.F.; Felix-Cuencas, L.; López-Tejeida, S.; Sosa-Ferreyra, C.F. & González-Orozco, A.I. (2022). Effect of the water exchange rate in a recirculation aquaculture system on growth, glucose and cortisol levels in *Oreochromis niloticus*. *Latin American Journal of Aquatic Research*, 50(2): 267-275. https://doi.org/10.3856/vol50-issue2-fulltext-2790

Lisboa, V.; Rodrigues Freire Eloy, H.; Catter, K.M.; Cruz Araújo Bezerra Vidigal, R.; Lelis Muniz Souza, R. & Nogueira Matias, J.F. (2020). Piscicultura marinha brasileira: desafios e perspectivas do seu desenvolvimento no estado do Ceará. *Sistemas & Gestão*, 15(2): 113-122. https://doi.org/10.20985/1980-5160.2020.v15n2.1636

Malanski E.; Malanski A.C.G.S. & Fernandes L.F.L. (2024). Reproduction of the dog *Lutjanus jocu* in captivity, linking ecological and production observations. *Boletim do Instituto de Pesca*, 50 (1) e.878. https://doi.org/10.20950/1678-2305/bip.2024.50.e878

Pimentel, C.R. & Joyeux, J.C. (2010). Diet and food partitioning between juveniles of mutton Lutjanus analis, dog Lutjanus jocu and lane *Lutjanus synagris* snappers (Perciformes: Lutjanidae) in a mangrove-fringed estuarine environment. *Journal of Fish Biology*, 76(10): 2299-2317. https://doi.org/10.1111/j.1095-8649.2010.02586.x

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Core.

Rodrigues, R.V.; Schwarz, M.H.; Delbos, B.C.; Carvalho, E.L.; Romano, L.A. & Sampaio, L.A. (2011). Acute exposure of juvenile cobia Rachycentron canadum to nitrate induces gill, esophageal and brain damage. *Aquaculture*, 322-323: 223-226. https://doi.org/10.1016/j.aquaculture.2011.09.040

Shimura, R.; Ma, Y.X.; Ijiri, K.; Nagaoka, S. & Uchiyama, M. (2004). Nitrate Toxicity on Visceral Organs of Medaka Fish, Oryzias latipes: Aiming to Raise Fish from Egg to Egg in Space. *Biological Sciences in Space*, 18(1): 7-12. https://doi.org/10.2187/bss.18.7

Valenti, W.C.; Barros, H.P.; Moraes-Valenti, P.; Bueno, G.W. & Cavalli, R.O. (2021). Aquaculture in Brazil: past, present and future. *Aquaculture Reports*, 19: 100611. https://doi.org/10.1016/j.aqrep.2021.100611

Wuenschel, M.J.; Jugovich, A.R. & Hare, J.A. (2005). Metabolic response of juvenile gray snapper (*Lutjanus griseus*) to temperature and salinity: Physiological cost of different environments. *Journal of Experimental Marine Biology and Ecology*, 321(2): 145-154. https://doi.org/10.1016/j.jembe.2005.01.009

Zhang, S.Y.; Li, G.; Wu, H.B.; Liu, X.G.; Yao, Y.H.; Tao, L. & Liu, H. (2011). An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacultural Engineering*, 45(3): 93-102. https://doi.org/10.1016/j.aquaeng.2011.08.001