

Original article

## Hematological and biochemical characteristics during the transport of dog snapper *Lutjanus jocu* (Perciformes: Lutjanidae)

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The objective of this study was to evaluate stress responses in dog snapper (*Lutjanus jocu*) during transport by evaluating their hematological and biochemical responses. Twenty-five wild dog snapper specimens were used in the experiment ( $220 \pm 68$  g and  $24.5 \pm 2.5$  cm total length). Blood samples were collected prior to transport (control), and fish were placed in two transport boxes, one with anesthetic and one without anesthetic. Immediately after transport and after 24 h, blood was collected from the fish that underwent each treatment (with anesthetic and without anesthetic). Biochemical and hematological results demonstrated the inefficiency of benzocaine as a stress reliever during handling and transport. Biochemical parameters revealed the effects of stress during transport, and after 24 h, glucose levels and hematological parameters (hemoglobin, erythrocytes, leukocytes, neutrophils and MCH) showed a tendency to return to control levels. This study is the first to report stress response measurements of hematological and biochemical indicators in dog snapper, representing an important basis for the planning of future experiments involving the transport and handling of this fish species.

**Keywords:** Anesthetic, Benzocaine, Glucose, Lactate, Marine fish.

O objetivo deste estudo foi avaliar as repostas de estresse em dentão (*Lutjanus jocu*) durante o procedimento de transporte, através de repostas hematológicas e bioquímicas. Vinte e cinco exemplares selvagens de dentão foram utilizados no experimento ( $220 \pm 68$  g e  $24.5 \pm 2.5$  cm de comprimento total). Foram realizadas coletas de sangue previamente ao transporte (controle), e os demais peixes foram acondicionados em duas caixas de transporte, uma com anestésico e outra sem anestésico. Imediatamente após o transporte e após 24 h, houve coleta de sangue para cada tratamento (com anestésico e sem anestésico). Os resultados bioquímicos e hematológicos apontam a ineficiência da benzocaína como mitigador do estresse durante o manuseio e transporte. Os parâmetros bioquímicos foram capazes de detectar o efeito do estresse durante o transporte, e após 24 h os níveis de glicose e alguns parâmetros hematológicos (hemoglobina, eritrócitos, leucócitos, neutrófilos e MCH) demonstraram uma tendência de retorno aos níveis do controle. Este trabalho é o primeiro a informar os níveis de resposta basal e de estresse para indicadores hematológicos e bioquímicos em dentão, representando uma base importante para o planejamento de futuras experiências com transporte e manejo dessa espécie.

**Palavras-chave:** Anestésico, Benzocaína, Glicose, Lactato, Peixe marinho.

### Introduction

The development of marine fish farming through the use of fish with economic value is essential for the preservation of fish stocks and has increased steadily in recent years (FAO, 2016). The success of rearing depends on the successful transport of wild fish to the breeding

laboratory, including capture, transport, prophylaxis and acclimation to rearing conditions (Bar *et al.*, 2015; Sampaio, Freire, 2016; Stieglitz *et al.*, 2017).

There is little scientific information on the transport of wild-caught fish and a growing demand for data to enable the establishment of specific and scientifically grounded protocols (Harmon, 2009; Sampaio, Freire, 2016). Blood

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analysis can be a rapid, non-lethal and low-cost tool for the early detection of malnutrition, stress and infection in fish, but the lack of hematological and biochemical reference values in healthy animals has limited its application (Peres *et al.*, 2014). Capture, handling and transport procedures can cause various changes in fish, with negative effects on growth, reproduction and immunity, and can even lead to death (Cnaani, McLean, 2009; Cunha *et al.*, 2010; Stieglitz *et al.*, 2012; Hohlenwerger *et al.*, 2016; Shabani *et al.*, 2016; Teixeira *et al.*, 2017).

The use of anesthetics at appropriate concentrations may be a means to reduce fish stress and mortality during transport and handling (Mamangkey *et al.*, 2009; Cunha *et al.*, 2011). Benzocaine is classified as a local anesthetic, although in fish, it acts systemically on the central nervous system (Okamura *et al.*, 2010), where its effects range from mild sedation to medullary collapse (Holloway *et al.*, 2004). However, the use of high concentrations of benzocaine is uneconomical and can cause undesirable effects such as anesthetic induction or mortality (Teixeira *et al.*, 2017).

Snappers (Lutjanidae) are considered important fishery resources worldwide (Frédou *et al.*, 2006; Ibarra-Castro, Duncan, 2007), with high commercial potential for aquaculture (Vettorazzi *et al.*, 2010; Leaw *et al.*, 2012; Souza *et al.*, 2015). Dog snapper, *Lutjanus jocu* (Bloch & Schneider, 1801), is distributed from Florida (USA) to southeastern Brazil and is one of the most important Lutjanidae in Brazilian fisheries (Frédou *et al.*, 2009; Previero *et al.*, 2011). Due to its high market value and its currently declining population (Lindeman *et al.*, 2016), dog snapper is an important species from which to obtain biochemical and hematological information. The establishment of reference blood parameters for *L. jocu* can provide a practical tool for monitoring well-being, allowing the safe evaluation of handling procedures that may induce stress (*e.g.*, transport, stocking density, water physicochemical parameters and disease). The objective of this study was to characterize and evaluate the effects of handling and transport on both anesthetized and non-anesthetized dog snapper and on stress levels prior to transport, after the arrival of specimens in the laboratory and 24 h later (recovery) by assessing hematological and biochemical responses.

## Material and Methods

**Experimental design and sampling.** The twenty-five dog snapper (*Lutjanus jocu*) specimens used in this experiment ( $220 \pm 68$  g and  $24.5 \pm 2.5$  cm in total length) were captured by fishermen in the Piraquimirim river estuary, Aracruz, state of Espírito Santo (ES), southeastern Brazil ( $19^{\circ}56'S$   $40^{\circ}10'W$ ), using a gillnet with a length of 100 m, a height of 1.5 m and a mesh size of 80 mm. The fish were then placed near the capture site in a cage with a 4 m<sup>3</sup> (2 x 2 x 1 m) working volume. The

cage was deployed in an estuarine region, approximately 6 km distance from the mouth of the river and at an average depth of 4 m. The time between the first and last capture was 30 days. The fish placed in the cage were fed daily with fresh fish provided by a local fisherman. After the last capture, 20 days elapsed before the experiment was performed, with feeding being interrupted one day before the beginning of the experiment. There was no mortality during the period of time in which the fish were kept in the cage.

After the acclimation period, 5 specimens were collected from the cage (Control; T-0) and sedated with benzocaine (50 mg L<sup>-1</sup>) for immediate blood collection and biometric measurement. Afterward, the remaining fish were placed in two 80-liter transport boxes with constant aeration, 10 in a box containing 10 mg L<sup>-1</sup> benzocaine and 10 in a box without anesthetic, and transported by boat to the Marine Organisms Rearing Laboratory of the Universidade Federal do Espírito Santo, a travel time of 2 h. Immediately after transport, 5 fish from each treatment group (with anesthetic: A-1, without anesthetic: C-1) were taken for blood collection and biometric measurements. After 24 h, the remaining fish were collected for the same procedures (with anesthetic: A-24, without anesthetic: C-24).

The fish subjected to a 24 h recovery treatment were placed in two 1,000 L tanks (with and without anesthetic), with water collected from the mouth of the same river where the fish were captured and caged, filtration through 3 filters of 15, 10 and 5 microns, an ultraviolet (UV) sterilization system and constant aeration. Temperature, salinity, dissolved oxygen and pH data were collected with a portable multiparameter probe. Ammonia was measured using a commercial colorimetric kit (Alfakit®, Brazil). These parameters were collected during transport and in the laboratory. The water was not changed due to the low stocking density and short duration of captivity (24 h). All research was conducted in accordance with the policies of the Ethical Conduct Committee on Animal Use (CEUA) as administered by the Universidade Federal do Espírito Santo (Protocol 96/2015). The collection license was provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; license number SISBIO 52082-2). The specimen was vouchered at the ichthyology collection of the Universidade Federal do Espírito Santo (CIUFES 3552).

**Analytical procedures.** Blood was collected by caudal puncture using a 1-mL EDTA-containing syringe, a procedure lasting less than 2 min, to avoid elevation of any biochemical parameters by handling during collection. The extracted blood samples were divided among two sets of tubes, one containing EDTA (10%) as an anticoagulant for sample preservation for hematological analyses and another heparinized for the determination of plasma glucose and lactate levels.

The hematocrit (%) was measured after the blood samples were centrifuged in heparinized microcapillary tubes. To perform an erythrocyte count, 1:200 dilutions in staining solution (Na<sub>2</sub>EDTA, formaldehyde, NaCl, NaOH, methylene blue) were performed, and cells were counted directly in a Neubauer chamber under an optical microscope (Olympus CX41).

To perform differential and total counts of leukocytes and thrombocytes, blood smears were prepared, dried at room temperature, fixed and stained with quick panoptic stain (commercial kit RENYLAB®, Brazil), and indirect counting was performed according to Hrubc, Smith (1998). From these results, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (1934). Plasma glucose and hemoglobin concentrations (commercial kit Bioclin®, Brazil) and plasma lactate concentrations (commercial kit BioTecnica®, Brazil) were measured by colorimetric and spectrophotometer analysis.

**Statistical analysis.** Before statistical analyses were conducted, the homogeneity and normality of the raw data were assessed. Statistical analyses were performed separately for each parameter using analysis of variance (ANOVA), and comparisons between groups (treatments) were performed using Tukey's test ( $p < 0.05$ ). The results are presented as the means and standard deviations, and all analyses were performed using software PAST v.3.15 (Hammer *et al.*, 2001).

## Results

**Water quality.** The water quality parameters in the boxes before the fish were introduced until their arrival in the laboratory were as follows: salinity 27, temperature  $27.7 \pm 0.6$  °C, dissolved oxygen  $6.8 \pm 0.8$  mg L<sup>-1</sup>, pH  $6.3 \pm 0.15$ , ammonia  $0.4 \pm 0.2$  mg L<sup>-1</sup>. The water quality parameters in the laboratory were as follows: salinity 28, temperature  $27.5 \pm 0.3$  °C, dissolved oxygen  $7.3 \pm 0.2$  mg L<sup>-1</sup>, pH  $7.3 \pm 0.1$ , and ammonia  $0.6 \pm 0.2$  mg L<sup>-1</sup>.

**Cell morphology.** The mature erythrocytes of *Lutjanus jocu* are oval to ellipsoidal in shape with a central nucleus and clear and homogeneous eosinophilic cytoplasm (Fig. 1a). In the young erythrocyte, the cytoplasm presents intense basophilia and dense chromatin (Fig. 1b). The thrombocytes were predominantly elliptical, with an elongated nucleus extending along almost the entire cell; oval cells were occasionally observed. The cytoplasm was scarce, with clear eosinophilic staining (Fig. 1c).

Of the leukocytes that are responsible for immune defense, neutrophils, lymphocytes and monocytes were found, but no eosinophils, basophils and special granulocytic cells were observed. The neutrophils were spherical in shape and their nuclei were most often round or oval, with compact chromatin and no visible nucleolus.

The cytoplasm was generally abundant, occupying almost the entire cell and containing slightly basophilic fine granules (Fig. 1d). There was a significant difference in neutrophils levels between control and other treatments.

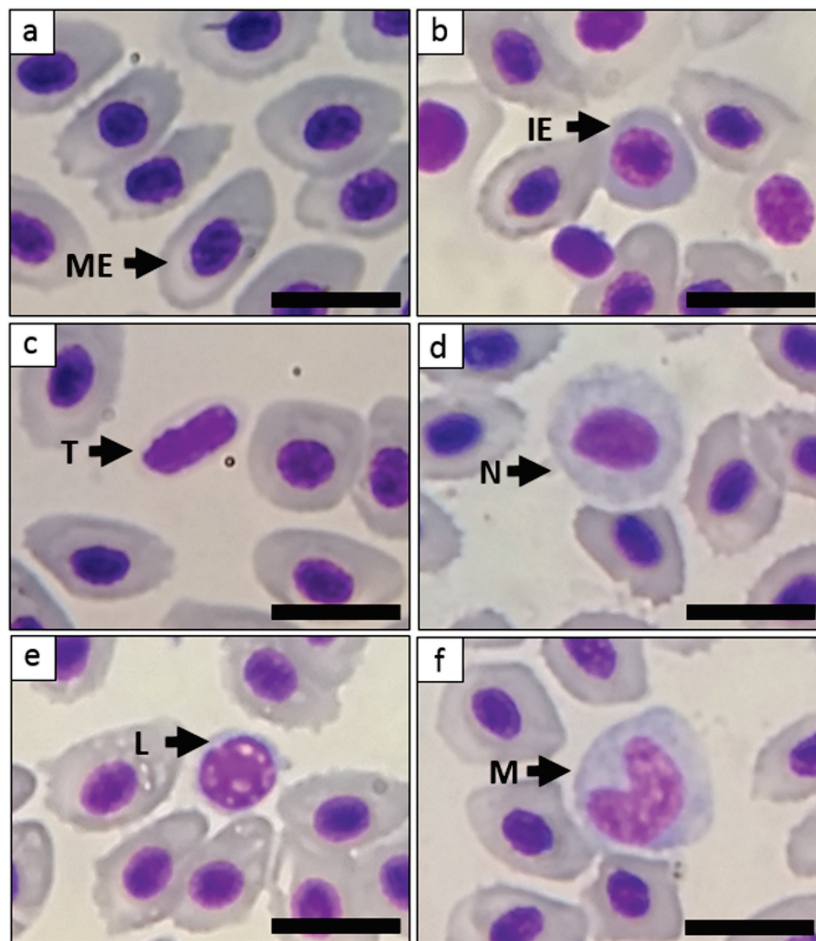
The lymphocytes were predominantly spherical, of varied size, with clear basophilic cytoplasm, presenting cytoplasmic projections and without visible granules. The nucleus had a rounded form, dense chromatin, and a high nucleus-to-cytoplasm ratio (Fig. 1e). For lymphocytes, there was a significant difference before and 1 h after transport, but did not exhibit significant differences after 24h. The observed monocytes were large, spherical and polymorphic. Eccentric nuclei predominated, with the occasional reniform nucleus, compact chromatin, intensely basophilic cytoplasm and vacuolization (Fig. 1f).

**Hematological analysis.** There was no mortality during the experiment. The reference intervals for dog snapper blood parameters are summarized (Tab. 1). There were no significant differences in parameters between treatments with or without anesthetic, both immediately after transport (A1 and C1) and after a recovery period of 24 h after transport (A24 and C24).

Hematological parameters were compared between fish before transport, immediately after transport and 24 h after transport (recovery). Hemoglobin values varied for the dog snapper and did not exhibit significant differences ( $p < 0.05$ ) between control and treatments immediately after transport (A-1 and C-1), but differences were observed between A-1 and C-1 and recovery values (A-24 and C-24). For the erythrocyte count, only the C-24 treatment (without anesthetic) exhibited high values that differed significantly from the other treatments. Hematocrit differed significantly only in C-1.

For the leukocyte count, only the C-24 treatment showed a significant difference from the other groups. Lymphocytes and neutrophils were more frequent among the leukocytes, with a low frequency of neutrophils in the control treatment, whereas monocytes and thrombocytes had similar counts between the treatments. For MCV, there was a significant difference between the control and recovery (24 h) treatments; MCH showed differences only between the immediately after transport and recovery treatments and MCHC did not present differences between any of the treatments.

**Biochemical analysis.** Tab. 1 shows no significant differences ( $p < 0.05$ ) in glucose and lactate levels between the treatments with and those without anesthetic. There was a significant increase in glucose levels immediately after transport, and a decrease after 24 h of transport, reaching levels close to those of the control group. Lactate concentrations did not show significant differences between the group sampled immediately after transport and the group sampled after 24 h of recovery.



**Fig. 1.** Photomicrographs of a peripheral blood smear of *Lutjanus jocu* showing a corresponding sequence of images: **a.** Mature Erythrocyte (ME); **b.** Immature Erythrocyte (IE); **c.** Thrombocyte (T); **d.** Neutrophil (N); **e.** Lymphocyte (L); and **f.** Monocyte (M). Scale bars = 10 μm.

**Tab. 1.** Biochemical and hematological parameters of *Lutjanus jocu*. Mean values  $\pm$  SEM. Different letter (a, b or c) indicates significant differences ( $p < 0.05$ ) between fish groups. Control: fish collected in the cage prior to transport; A1: collection 1 h after transport to the laboratory with anesthetic; C1: collection 1 h after transport to the laboratory without anesthetic; A24: collection after 24 h in the laboratory of the fish transported with anesthetic; C24: collection after 24 h in laboratory of fish transported without anesthetic.

Parameter	Before transport		After transport		24h after transport		P value
	Control	A1	C1	A24	C24		
Hematological							
Hemoglobin (g dL <sup>-1</sup> )	13.93 $\pm$ 1.30 ab	15.32 $\pm$ 1.03 a	15.89 $\pm$ 1.26 a	12.83 $\pm$ 0.90 b	13.02 $\pm$ 1.17 b	0.0009	
Erythrocytes (10 <sup>6</sup> mm <sup>3</sup> )	2.6 $\pm$ 0.52 b	2.6 $\pm$ 0.58 b	2.6 $\pm$ 0.57 b	3.5 $\pm$ 0.46 ab	4.0 $\pm$ 0.53 a	0.0006	
Hematocrit (%)	45.7 $\pm$ 4.5 a	34.2 $\pm$ 6.4 ab	34.4 $\pm$ 9.0 b	31.9 $\pm$ 9.2 ab	35.2 $\pm$ 4.6 ab	0.0456	
Leukocyte (10 <sup>3</sup> μL <sup>-1</sup> )	47.07 $\pm$ 18.86 b	44.35 $\pm$ 12.47 b	44.21 $\pm$ 15.33 b	73.89 $\pm$ 7.81 ab	94.28 $\pm$ 45.36 a	0.0101	
Neutrophils (%)	14.60 $\pm$ 4.34 c	53.60 $\pm$ 6.19 a	40.20 $\pm$ 5.20 ab	31.80 $\pm$ 7.60 b	35.20 $\pm$ 11.86 b	< 0.0001	
Lymphocytes (%)	75.40 $\pm$ 5.18 a	36.40 $\pm$ 10.01 c	49.60 $\pm$ 7.80 bc	57.60 $\pm$ 6.69 ab	56.80 $\pm$ 15.87 ab	0.0001	
Monocytes (%)	10.00 $\pm$ 6.16 a	10.60 $\pm$ 4.88 a	10.20 $\pm$ 3.83 a	9.80 $\pm$ 3.42 a	8.00 $\pm$ 4.18 a	0.7726	
Thrombocytes (10 <sup>3</sup> μL <sup>-1</sup> )	29.48 $\pm$ 10.64 a	33.01 $\pm$ 19.48 a	30.27 $\pm$ 27.39 a	42.89 $\pm$ 20.12 a	40.89 $\pm$ 22.15 a	0.7726	
MCV (μL <sup>-1</sup> )	182.54 $\pm$ 47.90 a	125.59 $\pm$ 49.57 ab	134.97 $\pm$ 25.54 ab	91.17 $\pm$ 23.90 b	88.38 $\pm$ 12.65 b	0.0024	
MCH (%)	55.96 $\pm$ 15.50 ab	62.52 $\pm$ 14.06 a	65.04 $\pm$ 18.95 a	37.13 $\pm$ 4.47 b	33.08 $\pm$ 6.08 b	0.0018	
MCHC (g dL <sup>-1</sup> )	30.72 $\pm$ 4.74 a	46.36 $\pm$ 10.57 a	48.85 $\pm$ 13.05 a	44.11 $\pm$ 17.11 a	37.50 $\pm$ 5.51 a	0.1112	
Biochemical							
Glucose (mg dL <sup>-1</sup> )	74.36 $\pm$ 15.52 b	106.94 $\pm$ 8.91 a	103.57 $\pm$ 14.65 a	64.81 $\pm$ 10.73 b	67.22 $\pm$ 8.89 b	< 0.0001	
Lactate (mg dL <sup>-1</sup> )	9.33 $\pm$ 1.77 b	14.98 $\pm$ 2.76 a	13.77 $\pm$ 2.49 a	10.71 $\pm$ 2.30 ab	11.44 $\pm$ 1.95 ab	0.0055	



## Discussion

Hematological parameters assessed during the handling and transport of dog snapper provided important information on physiological conditions and health status, and this is the first study of this kind in this species. The cells identified in *L. jocu* blood smears are morphologically similar to those described by Del Rio-Zaragoza *et al.* (2011) for rose snapper (*Lutjanus guttatus*). The same cell types (erythrocytes, neutrophils, lymphocytes, monocytes and thrombocytes) and morphological patterns were also characterized by Santos *et al.* (2009) in wild fat snook (*Centropomus parallelus*). There is an urgent need to provide reliable reference intervals for fish (Del Rio-Zaragoza *et al.*, 2011), mainly because of the economic importance of dog snapper.

Together with morphological characterizations, physiological responses were evaluated before, during and after transport and handling. The mean hemoglobin values were close to those reported by Del Rio-Zaragoza *et al.* (2011) for rose snapper juveniles reared in tanks in the laboratory, indicating similarities under different environmental conditions. There was no significant difference in hemoglobin levels before and 1 h after transport; however, Roque *et al.* (2010) found significant differences between the control group and a group exposed to hydrogen peroxide in sea bass (*Dicentrarchus labrax*). Hemoglobin levels in the group sampled after 24 h were significantly lower than those in the group sampled immediately after transport, demonstrating rapid recovery. Similar results were reported by Acerete *et al.* (2004) for the handling of Eurasian perch (*Perca fluviatilis*), with no significant difference between the control group and measurements taken 0.5 and 24 h after transport. For erythrocytes, two studies with *Lutjanus* fish found means close to those obtained in this study; Dotta *et al.* (2015) assessed wild red snapper (*Lutjanus analis*) transported to floating cages, and Del Rio-Zaragoza *et al.* (2011) studied rose snapper in laboratory. As with hemoglobin, the different stress responses among wild and farmed *Lutjanus* fish are indicative of good adaptive capacity.

There were no significant differences in hematocrit values between groups before transport and 24 h after transport, and only the C1 treatment exhibited differences compared to the control. No differences were found between treatments for juvenile pompano (*Trachinotus marginatus*) subjected to different salinities (Anni *et al.*, 2016) and cobia (*Rachycentron canadum*) anesthetized with 150 mg L<sup>-1</sup> benzocaine (Trushenski *et al.*, 2012). However, other authors have found significant differences in different species under acute stress (Roque *et al.*, 2010; Karakatsouli *et al.*, 2012; Rodrigues *et al.*, 2015). Because hematocrit is a secondary response to acute stress, its values may indicate inconsistent responses and should be evaluated in conjunction with other indicators.

Lymphocytes, which were the most frequently observed leukocytes, are cells involved in immunoglobulin production and defense modulation, while neutrophils are the primary phagocytic leukocytes, which proliferate in the circulation in response to infections, inflammation and stress (Davis *et al.*, 2008), demonstrating their importance in the immune defense of dog snapper. The lymphocyte values found by Del Rio-Zaragoza *et al.* (2011) in rose snapper were higher (between 81 and 92%) than those found in dog snapper (between 36 and 75%). Neutrophils before transport had a mean value of 14%, which is smaller and significantly different from the neutrophil values measured under other treatments (*e.g.*, 53% in the A1 treatment group). This leukocyte class is a first-line defense cell, the most important type of leukocyte in the peripheral blood, and is highly sensitive to environmental changes (Silva, Soriano, 2009).

The frequency of monocytes (8 to 10%) was high when compared to rose snapper (2%), as reported by Del Rio-Zaragoza *et al.* (2011), and juvenile pirarucu, *Arapaima gigas* (4.6%) (Drumond *et al.*, 2010). However, there were no significant differences between treatments, as seen for thrombocytes. There were no significant differences between treatments in MCV, MCH and MCHC, consistent with other studies reporting similar patterns (Del Rio-Zaragoza *et al.*, 2011; Michelato *et al.*, 2017).

Dotta *et al.* (2015) compared the hematological parameters of red snapper (*Lutjanus analis*) individuals who were caught in the wild and later transported to floating cages and found significant differences in hematocrit, leukocytes and monocytes, but no differences in erythrocytes, thrombocytes, lymphocytes and neutrophils. Pinho *et al.* (2016) reported that fat snook (*Centropomus parallelus*) maintained at higher stocking densities presented significantly higher ( $p < 0.05$ ) values of erythrocytes and thrombocytes, whereas hematocrit, leukocyte, neutrophil, monocyte and lymphocyte counts did not differ significantly. These different patterns of responses show the importance of evaluating and interpreting different stressors and environmental conditions in a compartmentalized way. These quantitative differences in hematological parameters should be considered when implementing aquaculture practices for the selection of broodstock for laboratory rearing.

The occurrence of stress during transport was confirmed by an increase in plasma glucose concentration immediately after transport, with values again approaching control values after 24 h of recovery. High glucose production helps animals metabolically cope with the increased energy demands of stress-responsive tissues, and the return to control levels is a common occurrence during transport in fish (Sena *et al.*, 2016). Cnaani, McLean (2009) exposed cobia to hypoxia and found that glucose levels peaked in the first few hours, decreasing to control levels after 24 h. Pedron *et al.* (2016) studied the transport of cobia sedated with benzocaine (6 mg L<sup>-1</sup>)

and found high glucose levels up to 2 h after transport but were similar to the control values after 48 h.

Lactate levels exhibited sensitivity to stress immediately after transport. This increase may be related to the intense work performed by the anaerobic metabolism, in which so much energy is demanded that the aerobic metabolism alone is not able to sustain energy requirements. A similar response was found by Shabani *et al.* (2016) in the transport of rainbow trout (*Oncorhynchus mykiss*), with high lactate levels immediately after transport, but with a significant reduction in the recovery periods (24 and 48 h). Fanouraki *et al.* (2011) found an increase in lactate concentrations at 30 min after stress (air exposure) in the species *Sparus aurata*, *Dentex dentex*, *Pagellus erythrinus*, *Diplodus puntazzo* (Sparidae), *Argyrosomus regius* (Sciaenidae) and *Dicentrarchus labrax* (Moronidae). The measurement of glucose and lactate levels is an agile and practical method for assessing stress in fish, both in the field and in the laboratory.

Since glucose and lactate levels effectively respond to acute stress levels after transport, then the hypothesis that adding anesthetic will reduce stress could not be confirmed, either immediately after stress or 24 h later. Moreira *et al.* (2015) added 15 mg L<sup>-1</sup> of eugenol to the water during the transport of juvenile Nile tilapia (*Oreochromis niloticus*) and did not observe minimized stress responses according glucose and lactate analysis.

The biochemical and hematological results demonstrate the inefficiency of benzocaine at 10 mg L<sup>-1</sup> as a stress reliever during the handling and transport of dog snapper. Other studies have also indicated that anesthetics are not able to reduce transport stress in pacu, *Piaractus mesopotamicus* (Feitosa *et al.*, 2013) and fat snook, *C. parallelus* (Tondolo *et al.*, 2013). The use of benzocaine (2 and 6 mg L<sup>-1</sup>) did not reduce the stress response of cobia (Pedron *et al.*, 2016). However, Carneiro *et al.* (2002) and Pramod *et al.* (2010) reported that benzocaine was able to minimize transport stress in some species, such as *Brycon amazonicus* (5 to 20 mg L<sup>-1</sup>) and *Puntius filamentosus* (20 mg L<sup>-1</sup>). The use of anesthetic also reduced activity and stress during transport in Atlantic salmon (*Salmo salar*) (Iversen *et al.*, 2009). Therefore, other concentrations and anesthetics should be tested to evaluate the well-being of dog snapper during transport.

This paper is the first to report stress response levels for hematological and biochemical indicators in dog snapper, representing an important basis for the planning of future experiments involving transport and handling. Changes in biochemical parameters revealed the effect of stress during transport, and after 24 h, glucose levels and some hematological parameters (hemoglobin, erythrocytes, leukocytes, neutrophils and MCH) showed a tendency to return to control levels. This physiological resistance of *L. jocu* to stress is an important characteristic for aquaculture, and thus, the use of *L. jocu* in aquaculture is promising.

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## References

- Acerete L, Balasch JC, Espinosa E, Josa A, Tort L. Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. Aquaculture [serial on the internet]. 2004; 237(1-4):167-78. Available from: <https://doi.org/10.1016/j.aquaculture.2004.03.018>
- Anni ISA, Bianchini A, Barcarolli IF, Varela Junior AS, Robaldo RB, Tesser MB, Sampaio LA. Salinity influence on growth, osmoregulation and energy turnover in juvenile pompano *Trachinotus marginatus* Cuvier 1832. Aquaculture [serial on the internet]. 2016; 455:63-72. Available from: <https://doi.org/10.1016/j.aquaculture.2016.01.010>
- Bar I, Dutney L, Lee P, Yazawa R, Yoshizaki G, Takeuchi Y, Cummins S, Elizur A. Small-scale capture, transport and tank adaptation of live, medium-sized Scombrids using “Tuna Tubes”. SpringerPlus [serial on the internet]. 2015; 4(604):1-12. Available from: <https://doi.org/10.1186/s40064-015-1391-y>
- Carneiro PCF, Urbinati EC, Martins ML. Transport with different benzocaine concentrations and its consequences on hematological parameters and gill parasite population of matrinxã *Brycon cephalus* (Günther, 1869) (Osteichthyes, Characidae). Acta Sci. 2002; 24(2):555-60.
- Cnaani A, McLean E. Time-course response of cobia (*Rachycentron canadum*) to acute stress. Aquaculture [serial on the internet]. 2009; 289(1-2):140-42. Available from: <https://doi.org/10.1016/j.aquaculture.2008.12.016>
- Cunha MA, Barros FMC, Garcia LO, Veeck APL, Heinzmann BM, Loro VL, Emanuelli T, Baldisserotto B. Essential oil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen*. Aquaculture [serial on the internet]. 2010; 306(1-4):403-06. Available from: <https://doi.org/10.1016/j.aquaculture.2010.06.014>
- Cunha MAD, Silva BF, Delunardo FAC, Benovit SC, Gomes LC, Heinzmann BM, Baldisserotto B. Anesthetic induction and recovery of *Hippocampus reidi* exposed to the essential oil of *Lippia alba*. Neotrop Ichthyol [serial on the internet]. 2011; 9(3):683-88. Available from: <https://doi.org/10.1590/s1679-62252011000300022>
- Davis AK, Maney DL, Maerz JC. The use of leukocytes profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol [serial on the internet]. 2008; 22(5):760-72. Available from: <https://doi.org/10.1111/j.1365-2435.2008.01467.x>

- Del Rio-Zaragoza OB, Fajer-Ávila EJ, Almazán-Rueda P, Abdo de la Parra M. Hematological characteristics of the spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869) healthy and naturally infected by dactylogyrid monogeneans. *Tissue Cell* [serial on the internet]. 2011; 43(3):137-42. Available from: <https://doi.org/10.1016/j.tice.2011.01.002>
- Dotta G, Roubbedakis K, Sanches EG, Jerônimo GT, Cerqueira VR, Martins ML. Hematological profile of the red snapper *Lutjanus analis* captured in Florianópolis, SC, Brazil, and cultured in floating net cages. *Bol Inst Pesca*. 2015; 41(1):183-89.
- Drumond GVF, Caixeiro APA, Tavares-Dias M, Marcon JL, Affonso EG. Características bioquímicas e hematológicas do pirarucu *Arapaima gigas* Schinz, 1822 (Arapaimidae) de cultivo semi-intensivo na Amazônia. *Acta Amazon*. 2010; 40(3):591-96.
- Fanouraki E, Mylonas CC, Papandroulakis N, Pavlidis M. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. *Gen Comp Endocrinol* [serial on the internet]. 2011; 173(2):313-22. Available: <https://doi.org/10.1016/j.ygcen.2011.06.004>
- Food and Agriculture Organization of United Nations (FAO). The state of world fisheries and aquaculture 2016: contributing to food security and nutrition for all. Rome; Food and Agriculture Organization of United Nations; 2016.
- Feitosa KCO, Povh JA, Abreu JS. Physiological responses of pacu (*Piaractus mesopotamicus*) treated with homeopathic product and submitted to transport stress. *Homeopathy* [serial on the internet]. 2013; 102(4):268-73. Available from: <https://doi.org/10.1016/j.homp.2013.07.004>
- Frédou T, Ferreira BP, Letourneur Y. A univariate and multivariate study of reef fisheries off northeastern Brazil. *ICES J Mar Sci* [serial on the internet]. 2006; 63(5):883-96. Available from: <https://doi.org/10.1016/j.icesjms.2005.11.019>
- Frédou T, Ferreira BP, Letourneur Y. Assessing the stocks of the primary snappers caught in Northeastern Brazilian reef systems. 1: Traditional modelling approaches. *Fish Res* [serial on the internet]. 2009; 99(2):90-96. Available from: <https://doi.org/10.1016/j.fishres.2009.05.008>
- Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electronica* [serial on the internet]. 2001; 4(1):1-9. Available from: [http://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm)
- Harmon TS. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture* [serial on the internet]. 2009; 1(1):58-66. Available from: <https://doi.org/10.1111/j.1753-5131.2008.01003.x>
- Hohlenwerger JC, Copatti CE, Sena AC, Couto RD, Baldisserotto B, Heinzmann BM, Caron BO, Schmidt D. Could the essential oil of *Lippia alba* provide a readily available and cost-effective anaesthetic for Nile tilapia (*Oreochromis niloticus*)?. *Mar Freshw Behav Physiol* [serial on the internet]. 2016; 49(2):119-26. Available from: <https://doi.org/10.1080/10236244.2015.1123869>
- Holloway AC, Keene JL, Noakes DG, Moccia RD. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquac Res* [serial on the internet]. 2004; 35(11):1025-30. Available from: <https://doi.org/10.1111/j.1365-2109.2004.01108.x>
- Hrube TC, Smith SA. Hematology of fish. In: Feldman BF, Zinkl JG, Jain NC, editors. *Schalm's Veterinary Hematology*. 5th ed. Blackburg: Wiley-Blackwell; 1998.
- Ibarra-Castro L, Duncan NJ. GnRH $\alpha$ -induced spawning of wild-caught spotted rose snapper *Lutjanus guttatus*. *Aquaculture* [serial on the internet]. 2007; 272(1):737-46. Available from: <https://doi.org/10.1016/j.aquaculture.2007.09.007>
- Iversen M, Eliassen RA, Finstad B. Potential benefit of clove oil sedation on animal welfare during salmon smolt, *Salmo salar* L. transport and transfer to sea. *Aquac Res* [serial on the internet]. 2009; 40(2):233-41. Available from: <https://doi.org/10.1111/j.1365-2109.2008.02091.x>
- Karakatsouli N, Katsakoulis P, Leondaritis G, Kalogiannis D, Papoutsoglou SE, Chadio S, Sakellariadis N. Acute stress response of European sea bass *Dicentrarchus labrax* under blue and white light. *Aquaculture* [serial on the internet]. 2012; 364-365:48-52. Available from: <https://doi.org/10.1016/j.aquaculture.2012.07.033>
- Leaw YY, Faizah S, Anil C, Kua BC. Prevalence, mean intensity and site preference of *Caligus rotundigenitalis* Yü, 1933 (Copepoda: Caligidae) on cage cultured crimson snapper (*Lutjanus erythropterus* Bloch, 1790) from Bukit Tambun, Penang, Malaysia. *Vet Parasitol* [serial on the internet]. 2012; 187(3-4):505-10. Available from: <https://doi.org/10.1016/j.vetpar.2012.02.003>
- Lindeman K, Anderson W, Carpenter KE, Claro R, Cowan J, Padovani-Ferreira B, Rocha LA, Sedberry G, Zapp-Sluis M. The IUCN Red List of Threatened Species. *Lutjanus jocu*: e.T190221A1944443 [internet]; 2016 [updated 2017 Feb 23; Cited 2017 Feb 26]. Available from: <http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T190221A1944443.en>
- Mamangkey NGF, Acosta-Salmon H, Southgate PC. Use of anaesthetics with the silver-lip oyster, *Pinctada maxima* (Jameson). *Aquaculture* [serial on the internet]. 2009; 288(3-4):280-84. Available from: <https://doi.org/10.1016/j.aquaculture.2008.12.008>
- Michelato M, Zaminhan M, Boscolo WR, Nogaroto V, Vicari M, Artoni RF, Furuya VRB, Furuya WM. Dietary histidine requirement of Nile tilapia juveniles based on growth performance, expression of muscle-growth-related genes and haematological responses. *Aquaculture* [serial on the internet]. 2017; 467:63-70. Available from: <https://doi.org/10.1016/j.aquaculture.2016.06.038>
- Moreira AGL, Coelho AAC, Albuquerque LFG, Moreira RT, Farias WRL. Efeito do eugenol como agente mitigador do estresse no transporte de juvenis de tilápia do Nilo. *Pesqui Vet Bras* [serial on the internet]. 2015; 35(11):893-98. Available from: <https://doi.org/10.1590/s0100-736x2015001100004>
- Okamura D, Araújo FG, Rosa PV, Freitas RTF, Murgas LDS, Cesar MP. Influência da concentração de benzocaina e do comprimento dos peixes na anestesia e na recuperação de tilápias-do-nilo. *R Bras Zootec*. 2010; 39(5):971-76.



- Pedron JS, Miron DS, Rodrigues RV, Okamoto MH, Tesser MB, Sampaio LA. Stress response in transport of juvenile cobia *Rachycentron canadum* using the anesthetic benzocaine. *Lat Am J Aquat Res*. 2016; 44(3):638-42.
- Peres H, Costas B, Perez-Jimenez A, Guerreiro I, Oliva-Teles A. Reference values for selected hematological and serum biochemical parameters of Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles under intensive aquaculture conditions. *J Appl Ichthyol* [serial on the internet]. 2014; 31(1):65-71. Available from: <https://doi.org/10.1111/jai.12641>
- Pinho SM, Brol J, Almeida EJ, Mello GL, Jerônimo GT, Emerenciano MGC. Effect of stocking density and vertical substrate addition on growth performance and health status of fat snook *Centropomus parallelus*. *Aquaculture* [serial on the internet]. 2016; 457:73-78. Available from: <https://doi.org/10.1016/j.aquaculture.2016.02.016>
- Pramod PK, Ramachandran A, Sajeevan TP, Thampy S, Pai SS. Comparative efficacy of MS-222 and benzocaine as anesthetics under simulated transport conditions of a tropical ornamental fish *Puntius filamentosus* (Valenciennes). *Aquac Res* [serial on the internet]. 2010; 41(2):309-14. Available from: <https://doi.org/10.1111/j.1365-2109.2009.02333.x>
- Previero M, Minte-Vera CV, Freitas MO, Moura RL, Tos CD. Age and growth of the dog snapper *Lutjanus jocu* (Bloch & Schneider, 1801) in Abrolhos Bank, Northeastern Brazil. *Neotrop Ichthyol* [serial on the internet]. 2011; 9(2):393-401. Available from: <https://doi.org/10.1590/s1679-62252011005000024>
- Rodrigues RV, Pedron JS, Romano LA, Tesser MB, Sampaio LA. Acute responses of juvenile cobia *Rachycentron canadum* (Linnaeus 1766) to acid stress. *Aquac. Res* [serial on the internet]. 2015; 46(5):1241-47. Available from: <https://doi.org/10.1111/are.12282>
- Roque A, Yildiz HY, Carazo I, Duncan N. Physiological stress responses of sea bass (*Dicentrarchus labrax*) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) exposure. *Aquaculture* [serial on the internet]. 2010; 304(1-4):104-07. Available from: <https://doi.org/10.1016/j.aquaculture.2010.03.024>
- Sampaio FDF, Freire CA. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish Fish* [serial on the internet]. 2016; 17(4):1055-72. Available from: <https://doi.org/10.1111/faf.12158>
- Santos AA, Egami MI, Ranzani-Paiva MJT, Juliano Y. Hematological parameters and phagocytic activity in fat snook (*Centropomus parallelus*): Seasonal variation, sex and gonadal maturation. *Aquaculture* [serial on the internet]. 2009; 296(3-4):359-66. Available from: <https://doi.org/10.1016/j.aquaculture.2009.08.023>
- Sena AC, Teixeira RR, Ferreira EL, Heinzmann BM, Baldisserotto B, Caron BO, Schmidt D, Couto RD, Copatti CE. Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). *Aquaculture* [serial on the internet]. 2016; 465:374-9. Available from: <https://doi.org/10.1016/j.aquaculture.2016.09.033>
- Shabani F, Erikson U, Beli E, Rexhepi A. Live transport of rainbow trout (*Onchorhynchus mykiss*) and subsequent live storage in market: Water quality, stress and welfare considerations. *Aquaculture* [serial on the internet]. 2016; 453:110-15. Available from: <https://doi.org/10.1016/j.aquaculture.2015.11.040>
- Silva FP, Soriano FG. Neutrophils recruitment during sepsis: critical points and crossroads. *Front Biosci*. 2009; 14:4464-76.
- Souza RLM, Vettorazzi MB, Kobayashi RK, Furtado-Neto MAA. Eugenol como anestésico no manejo de ariacó, *Lutjanus synagris* (Linnaeus, 1758), cultivado. *Cienc Agron*. 2015; 46(3):532-38.
- Stieglitz JD, Benetti DD, Serafy JE. Optimizing transport of live juvenile cobia (*Rachycentron canadum*): effects of salinity and shipping biomass. *Aquaculture* [serial on the internet]. 2012; 364-365:293-97. Available from: <https://doi.org/10.1016/j.aquaculture.2012.08.038>
- Stieglitz JD, Hoenig RH, Kloeblen S, Tudela CE, Grosell M, Benetti DD. Capture, transport, prophylaxis, acclimation, and continuous spawning of Mahi-mahi (*Coryphaena hippurus*) in captivity. *Aquaculture* [serial on the internet]. 2017; 479:1-6. Available from: <https://doi.org/10.1016/j.aquaculture.2017.05.006>
- Teixeira RR, Souza RC, Sena AC, Baldisserotto B, Heinzmann BM, Couto RD, Copatti CE. Essential oil of *Aloysia triphylla* in Nile tilapia: anaesthesia, stress parameters and sensory evaluation of fillets. *Aquac Res* [serial on the internet]. 2017; 48(7), 3383-92. Available from: <https://doi.org/10.1111/are.13165>
- Tondolo JSM, Amaral LP, Simões LN, Garlet QI, Schindler B, Oliveira TM, Da Silva, BF, Gomes LC, Baldisserotto B, Mallmann CA, Heinzmann BM. Anesthesia and transport of fat snook *Centropomus parallelus* with the essential oil of *Nectandra megapotamica* (Spreng.) Mez. *Neotrop Ichthyol* [serial on the internet]. 2013; 11(3):667-74. Available from: <https://doi.org/10.1590/s1679-62252013000300020>
- Trushenski JT, Bowser JC, Bowker JD, Schwarz MH. Chemical and electrical approaches to sedation of cobia: induction, recovery, and physiological responses to sedation. *Mar Coast Fish* [serial on the internet]. 2012; 4(1):639-50. Available from: <https://doi.org/10.1080/19425120.2012.728182>
- Vettorazzi MB, Teixeira EG, Souza RLM, César JRO, Furtado-Neto MAA. Motilidade espermática do sêmen do ariacó, *Lutjanus synagris*. *Arquivos de Ciências do Mar*. 2010; 43(2):21-26.
- Wintrobe MM. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. *Folia Haematologica*. 1934; 51:32-49.

