

RESILIENCE OF THE SHORTRNOSE GUITARFISH (*Zapteryx brevirostris*): COMPLETE COMPENSATORY GAIN, HEMATOLOGY AND HISTOPATHOLOGY*

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ABSTRACT

Compensatory gain has been used to evaluate the plasticity of species, in adverse situations such as food deprivation. The aim of the present study was to identify the type of compensatory gain achieved by the shortnose guitarfish (*Zapteryx brevirostris*), in situations of reduction of food resources. Three treatments were used: seven days of food deprivation and fourteen days of refeeding (T_{7x14}); fourteen days of food deprivation and fourteen days of refeeding (T_{14x14}); and feeding every day ($T_{Control}$). Zootechnical performance, blood samples and histological samples were evaluated. We demonstrated that this species presented complete compensatory gain and that some blood parameters and histological alterations were associated with fasting.

Key words: blood parameters; histology; nutrition; zootechnical performance.

RESILIÊNCIA DA RAIA VIOLA-DE-CARA-CURTA (*Zapteryx brevirostris*): GANHO COMPENSATÓRIO COMPLETO, HEMATOLOGIA E HISTOPATOLOGIA

RESUMO

O ganho compensatório vem sendo utilizado para avaliar a plasticidade de uma espécie, frente a uma injúria, como a privação de alimento. O objetivo do presente trabalho foi identificar qual o tipo de ganho compensatório da raia viola (*Zapteryx brevirostris*), frente à redução de recursos alimentares. Os tratamentos empregados foram sete dias de privação alimentar e quatorze dias de realimentação (T_{7x14}), quatorze dias de privação alimentar e quatorze dias de realimentação (T_{14x14}) e alimentação todos os dias ($T_{Control}$). Foram avaliados o desempenho zootécnico, amostras sanguíneas e histológicas. Demonstramos que a espécie apresenta ganho compensatório completo e existem parâmetros sanguíneos e alterações histológicas associados ao jejum.

Palavras-chave: parâmetros sanguíneos; histologia; nutrição; desempenho zootécnico.

INTRODUCTION

The species *Zapteryx brevirostris* (Müller & Henle, 1841), known as the shortnose guitarfish or lesser guitarfish, is the only species in its genus and has been classified as vulnerable (VU) by the International Union for Conservation of Nature (IUCN) (Vooren et al., 2006). It is mainly caught accidentally in trawling for shrimps (Pinheiro and Martins, 2009). It has low economic value and therefore specimens that are caught in this manner are not retained for commercialization but are returned to the sea. However, little is known regarding the survival of these individuals. It was observed, comparing specimens that were caught in 1994 and 1999, that the biomass of this species decreased by 86% (Vooren et al., 2006). The potential of this species for recovery of its population size is low, given that an average of only three offspring are produced per year, per mature female (Vooren et al., 2006), and that it reaches sexual maturity late (Abilhoa et al., 2007).

Little is known about the physiological alterations and resilience of shortnose guitarfish in situations of reduction of food resources. The term “resilience” refers to the persistence of relationships within a system and this parameter measures the

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capacity to absorb changes. It results in a measurement of the likelihood of extinction of a species (Holling, 1973). It is known that some species of teleosts cope well with food deprivation because they have physiological mechanisms that enable recovery of physical condition or growth after periods of food deprivation (Hayward et al., 1997; Cho et al., 2006). However, the presence of these physiological mechanisms, which are known as “compensatory gain”, has not been proven in elasmobranchs.

Compensatory gain can be classified into three categories, according to Ali et al. (2003): a) “partial compensatory gain”, when individuals that were deprived of food only recover part of the expected weight, because they were profoundly affected by the fasting and were unable to recover the lost weight or stagnated growth (Ribeiro and Tsuzuki, 2010); b) “complete compensatory gain”, when individuals recover all of the weight that was lost and normal growth is restored after the fasting, such that their development becomes the same as that of individuals that had been fed every day (Sogard and Olla, 2002; Cho et al., 2006; Foss et al., 2009; Türkmen et al., 2012); and c) “overcompensation”, when the weight and growth performance of individuals that were subjected to food deprivation exceeds that of individuals that had been fed continuously (Hayward et al., 1997; Chatakondi and Yant, 2001).

A period of adverse conditions is necessary for compensatory gain to be triggered. Compensatory gain results from a variety of sequential endocrine responses during the anabolic (physiological) state, catabolic (fasting) state and hyperanabolic (refeeding) state, thus giving rise to an increased specific growth rate (SGR) and decreased apparent food conversion (AFC) (Won and Borski, 2013).

The objective of this study was to evaluate the physiological response of *Z. brevirostris*, a species classified as vulnerable by the IUCN, in situations of cyclical periods of food deprivation, and to ascertain whether there might be any hematological parameters and histological alterations that would elucidate the physiology of this species.

MATERIAL AND METHODS

Subjects

Twenty-one specimens of shortnose guitarfish (*Z. brevirostris*), of mean total length 44.48 ± 3.34 cm and mean weight 486.56 ± 112.52 g, were collected as accompanying fauna in trawling for shrimp along the north coast of the state of São Paulo, in the municipality of Ubatuba. This was done under authorization: SISBIO no. 49980-3 and Ethics Committee for Animal Experimentation of the Fisheries Institute (CEEAI) no. 12/2016. To promote better welfare in catching and transporting rays, only animals caught in the last haul of the trawl were transferred to a 200L Styrofoam box, which contained a water pump that maintained the continuous flow of water 26 ± 2 °C, Salinity 35, Dissolved Oxygen > 5 mg L⁻¹). As soon as they landed, the Styrofoam with the animals was transported by hand to the Marine Fisheries Laboratory of the Fisheries Institute, which is located in front of the wharf where the fishing landing takes place. The animals were acclimatized and released in 2500 L fiberglass tanks with 112cm diameter (Temperature 25 ± 1 °C, Salinity 33, Dissolved

Oxygen > 5 mg L⁻¹) and the experiment only started when all the animals were feeding.

On the first day of the experiment, all of these fish were anesthetized through immersion in eugenol (63 mg L⁻¹), using a stock solution at a dilution of 1:5 in reagent-grade (p.a.) ethyl alcohol (Griffiths, 2000). The mean time taken for anesthesia to be induced was three minutes. The specimens were then removed from the anesthetic solution and each individual was fitted with a microchip so that its development could be monitored.

Maintenance system

The fish were then divided into three groups and each group was kept in a circular fiberglass tank of capacity 2500 L, with a water depth of 93 cm, without sediment and with an individual filtration system. The filtration setup consisted of a plastic reservoir of capacity 100 L containing a skimmer. To maintain water circulation, a pump immersed in the fish tank was used. Its extraction rate was 4000 L h⁻¹ and, thus, 100% of the water in the tank was recycled every 45 minutes. The stocking density of each tank was seven fish or approximately 1.16 kg m⁻³. The fish were kept under these conditions for at least 60 days, as an adaptation period, and were fed daily with fish and shrimp ad libitum. The photoperiod cycling comprised 12 hours of light followed by 12 hours of darkness. The water quality was monitored through daily measurements of temperature (26 ± 2 °C) using a mercury thermometer and salinity 30 ± 2 using a manual refractometer (BRIX®); and weekly measurements of dissolved oxygen (7 ± 1 g L⁻¹) using a multiparameter device (Hanna HI 9829) and total ammonia (0.1 ± 0.1 mg L⁻¹) and pH (8.3 ± 0.3) using colorimetric kits (Red Sea).

Experimental design

The duration of the experiment was 76 days, from December 2015 to February 2016. The following three treatments were used: seven days of food deprivation and fourteen days of refeeding (T_{7x14}); fourteen days of food deprivation and fourteen days of refeeding (T_{14x14}); and feeding every day (T_{Control}). The food that was made available consisted of a combination of 70% frigate tuna (*Auxis thazard*), in cubes, and 30% Atlantic seabob prawns (*Xiphopenaeus kroyeri*), without the head, in the quantity of 3% of the biomass of the tank. This was provided once a day at 8:30 am. With the aims of not impairing the water quality and conditioning the fish, the food was left in the tank for five minutes, which was enough time for all the fish to have moved towards the food and to have consumed it until reaching apparent satiety, such that they then returned to a resting position. The leftovers were removed with the aid of a syphon and were weighed while still wet (but without excess water), to determine the weight of food consumed by the fish in each tank every day. Thus, the quantity of food ingested per tank was obtained as the weight of the food offered minus the weight of the leftovers. A partial exchange of water (5%) was performed every day, to remove the oil that came from the fish that was provided as food, which remained on the water surface.

Zootechnical performance

The biometry of the fish was assessed at the start and end of the experiment, with measurement of the weight and total length. The following performance parameters were calculated: final biomass [sum of all the weights in the tank]; specific growth rate (SGR) [$(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{total duration of the experiment in days} \times 100$]; daily weight gain (DWG) [$(\text{final weight} - \text{initial weight}) / \text{total duration of the experiment in days}$]; apparent food conversion (AFC) [total quantity of food provided over the period/weight gain over the period of the experiment]; and survival [(number of fish still alive at the end of the experiment/initial number of individuals) $\times 100$].

On the last day of the experiment, the fish were sacrificed using eugenol (168 mg L⁻¹) in order to individually weigh the viscera and collect material for histopathological evaluation. The following performance indices were determined: viscerosomatic index [$(\text{weight of viscera} / \text{weight of fish}) \times 100$]; hepatosomatic index [$(\text{weight of liver} / \text{weight of fish}) \times 100$]; gastrosomatic index [$(\text{weight of stomach} / \text{weight of fish}) \times 100$]; and enterosomatic index [$(\text{weight of intestine} / \text{weight of fish}) \times 100$]. The fish were kept in a state of fasting for 48 hours before this, so that the stomach and intestine would be empty.

Hematological evaluation

Hematological evaluations were performed at the start and end of the experiment. After anesthesia was induced, blood samples were collected by means of puncturing the caudal vein. This was done with the aid of 20 \times 5.5 mm hypodermic needles moistened in 5000 UI of heparin, which were attached to disposable 3 mL syringes. The needle was inserted perpendicularly into the ventral region of the caudal vein. A sample of 2 mL of blood was withdrawn and, immediately afterwards, three blood smears were set up: two stained with May-Grünwald and Giemsa (Rosenfeld, 1947) and one using the rapid panoptic kit (methanol, eosin and hematoxylin), in order to obtain total leukocyte, thrombocyte and differential leukocyte counts. Erythrocyte counts were obtained using a Neubauer chamber after 1:200 dilution in methyl violet solution (Natt and Herrick, 1952).

Hematocrit was measured using the microhematocrit method, in a centrifuge at 12000 rpm, for 3 min. The total plasma protein (TPP) concentration was measured with the aid of a manual refractometer (301; Snt Lydia). The hemoglobin concentration was determined using the photometry method in an automated hematological analyzer (Bioclin Mindray; BC-2800 Vet).

Alkaline phosphatase and creatinine were measured by means of specific kits (Labtest®), with the aid of a spectrophotometer (Bioplus; BIO 200). The hematimetric indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Wintrobe (1934). Because of the large variety of nomenclatures that exists in the literature for the granulocytes of elasmobranchs, we adopted the nomenclature recommended by Ranzani-Paiva et al. (2013).

Histological analyses

For the histological analyses, the second pair of gills, the heart and the ventral portion of the liver were collected and fixed in 10% formalin for 24 hours, followed by conservation in 70% alcohol. These specimens were sent to the pathology sector of the Federal University of Rio Grande do Sul (UFRGS), where they were processed using routine histological procedures, to embed the material in paraffin and produce histological sections of thickness 5 micrometers. These sections were then stained with hematoxylin and eosin (HE) and images were obtained using an optical microscope (Leica ICCSO HD) and the Leica Las EZ 2.0 software. One slide was made from each organ of each individual, in accordance with the laboratory's standard routine. The number of fields analyzed was not counted; the classification was done subjectively; and the frequency of occurrence of lesions was not measured.

Statistical analysis

Because of space and resource limitations, each fish was taken to be an experimental unit, thus resulting in seven repetitions per treatment. Not all the data met the prerequisites for ANOVA, and for this reason, the nonparametric Kruskal-Wallis test was used. In cases of significant differences ($p < 0.05$), comparisons were made using Dunn's test. Regarding survival, the G test was used for comparisons between the treatments.

RESULTS

Zootechnical performance

The parameters for productive performance are presented in Table 1. The treatment T_{7x14} and T_{Control} presented similar performance. However, the treatment T_{14x14} present inferior performance to the T_{Control}.

Due to the low number of individuals available and the large variety of animals, with a maximum of 816.8 g and 53 cm and a minimum of 317.6 g and 37.5 cm, it was only possible to form homogeneous lots by discarding one individual from each treatment, totaling six animals from each treatment.

Hematological analysis

The hematological parameters are presented in Table 2 in which the data of the red series and the hematimetric indexes are listed, and in T_{14x14} group presented hypochromic microcytic anemia, with hypoproteinemia and low creatinine.

In Table 3 demonstrated the data of the white series and in the case of T_{14x14} there was eosinopenia. Figure 1 shows in detail the leukocyte differential.

Histological analysis

The histopathological analysis showed that there were no alterations in the gills (Figure 2a) or in the striated cardiac muscle tissue (Figure 2b). The control group (T_{Control}) presented moderate to severe hepatic vacuolar degeneration (Figure 2d). The groups

Table 1. Productive performance parameters for specimens of shortnose guitarfish (*Zapteryx brevirostris*) that were subjected to different durations of food deprivation over a 76-day period. Mean and standard deviation. Different letters on the same line of data indicate significant differences ($p < 0.05$).

Parameters	T _{Control}	T _{7x14}	T _{14x14}
Feeding (days)	74	49	32
Initial weight (g)	464.27 ± 47.84	435.80 ± 67.14	559.61 ± 159.98
Final weight (g)	471.17 ± 56.2	441.86 ± 70.2	569.00 ± 126.9
Initial total length (cm)	44.44 ± 2.48	41.14 ± 2.73	46.36 ± 3.26
Final total length (cm)	44.67 ± 2.5	41.86 ± 2.7	46.60 ± 2.3
Final biomass (g)	2827	3093	2276
Survival (%)	85.7 ^a	100 ^a	57.1 ^b
SGR weight (%W.day ⁻¹)	0.015 ± 0.09 ^a	0.017 ± 0.04 ^a	0.002 ± 0.02 ^b
DWG (g.day ⁻¹)	0.076 ± 0.44 ^a	0.080 ± 0.17 ^a	0.021 ± 0.11 ^b
Daily food consumption (g)	53.31 ^b	49.69 ^b	29.73 ^a
Mean AFC	113.7 ^b	57.4 ^c	148.7 ^a
Viscerosomatic index	7.45 ± 4.78	4.75 ± 0.69	4.88 ± 2.02
Hepatosomatic index	2.09 ± 0.5 ^a	1.26 ± 0.14 ^b	0.93 ± 0.19 ^c
Gastrosomatic index	1.10 ± 0.14 ^a	1.00 ± 0.06 ^a	0.78 ± 0.07 ^b
Enterosomatic index	1.10 ± 0.08 ^a	1.07 ± 0.15 ^a	0.78 ± 0.07 ^b

T_{Control} = feeding every day; T_{7x14} = 7 days of fasting and 14 days of feeding; T_{14x14} = 14 days of fasting and 14 days of feeding; SGR = specific growth rate; W = weight; DWG = daily weight gain; Mean AFC = mean apparent food conversion. Lines with different letters indicate significant differences between the treatments ($p < 0.05$).

Table 2. Hemogram and biochemical tests on shortnose guitarfish (*Zapteryx brevirostris*) subjected to different diets. Means and standard deviations are presented. Different letters on the same line indicate significant differences ($p < 0.05$) according to the Kruskal-Wallis and Dunn tests.

	T _{Control}	T _{7x14}	T _{14x14}
Erythrocytes (10 ⁶ mm ⁻³)	0.38 ± 0.11	0.37 ± 0.10	0.48 ± 0.17
Hematocrit (%)	19.00 ± 2.23 ^a	18.00 ± 1.80 ^{a,b}	13.00 ± 3.00 ^b
Hemoglobin (mg dL ⁻¹)	6.40 ± 0.91	5.80 ± 0.69	5.30 ± 0.87
Mean corpuscular volume (fL)	545.58 ± 114.15 ^a	489.80 ± 89.67 ^{a,b}	290.13 ± 59.47 ^b
Mean corpuscular hemoglobin concentration (g dL ⁻¹)	32.33 ± 2.26	31.18 ± 3.47	39.30 ± 7.74
Mean corpuscular hemoglobin (pg)	176.15 ± 29.68 ^a	157.66 ± 24.36 ^{a,b}	110.74 ± 18.93 ^b
Total plasma protein (g dL ⁻¹)	5.30 ± 0.46 ^a	4.80 ± 0.44 ^{a,b}	3.80 ± 1.10 ^b
Alkaline phosphatase (U L ⁻¹)	53.85 ± 8.16	58.00 ± 6.53	49.70 ± 12.45
Creatinine (mg dL ⁻¹)	1.95 ± 0.33 ^a	1.28 ± 0.30 ^{a,b}	1.02 ± 0.19 ^b

T_{Control} = feeding every day; T_{7x14} = 7 days of fasting and 14 days of feeding; T_{14x14} = 14 days of fasting and 14 days of feeding. Lines with different letters line indicate significant differences between the treatments ($p < 0.05$).

Table 3. Total leukocyte and thrombocyte counts and differential leukocyte counts (μL⁻¹) of shortnose guitarfish (*Zapteryx brevirostris*) subjected to different diets. Means and standard deviations are presented, along with relative numbers (%; in parentheses). Different letters on the same line indicate significant differences ($p < 0.05$) according to the Kruskal-Wallis and Dunn tests.

	T _{Control}	T _{7x14}	T _{14x14}
Leukocytes (μL ⁻¹)	18958 ± 9010	23511 ± 7550	45403 ± 24087
Thrombocytes (μL ⁻¹)	2687 ± 1740	4495 ± 1751	5998 ± 4105
Lymphocytes (μL ⁻¹)	11836 ± 5417 (62)	14442 ± 6113 (61)	27646 ± 26418 (61)
Neutrophils (μL ⁻¹)	5615 ± 4581 (30)	7766 ± 5490 (33)	12324 ± 10544 (27)
Eosinophils (μL ⁻¹)	880 ± 572 ^a (5)	831 ± 442 ^a (4)	5242 ± 1178 ^b (12)
Monocytes (μL ⁻¹)	368 ± 373 (2)	277 ± 323 (1)	949 ± 1145 (2)
SGC* (μL ⁻¹)	259 ± 339 (1)	141 ± 165 (1)	211 ± 423 (0)
Basophils (μL ⁻¹)	0 ± 0 (0)	26 ± 53 (0)	0 ± 0 (0)

*Special granulocytic cell; T_{Control} = feeding every day; T_{7x14} = 7 days of fasting and 14 days of feeding; T_{14x14} = 14 days of fasting and 14 days of feeding.

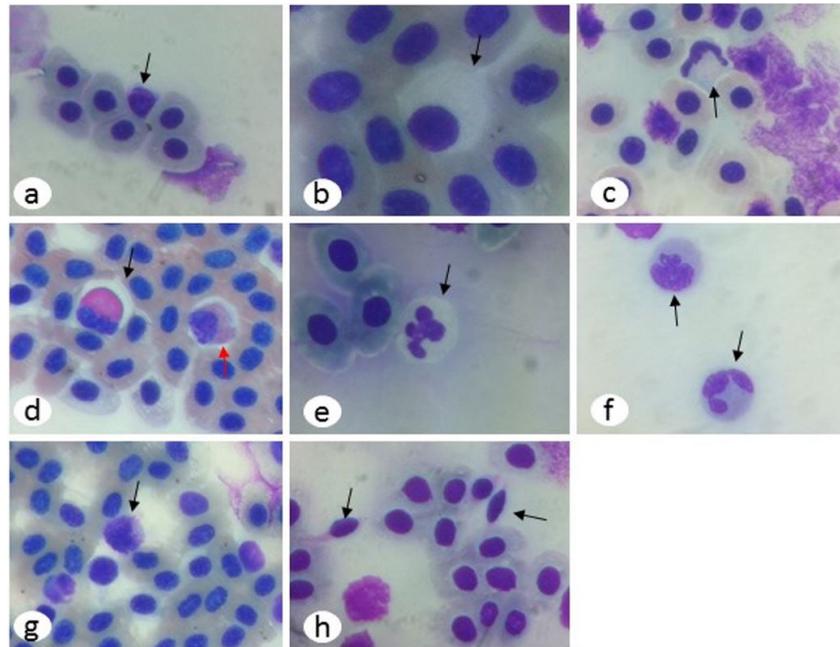


Figure 1. Cells observed in blood smears from shortnose guitarfish (*Zapteryx brevirostris*), stained using the rapid panoptic kit at a magnification of 400x. (a) lymphocyte; (b) special granulocytic cell (SGC); (c) monocyte; (d) eosinophil (black arrow) and neutrophil (red arrow); (e) neutrophil; (f) neutrophil; (g) basophil; (h) thrombocyte.

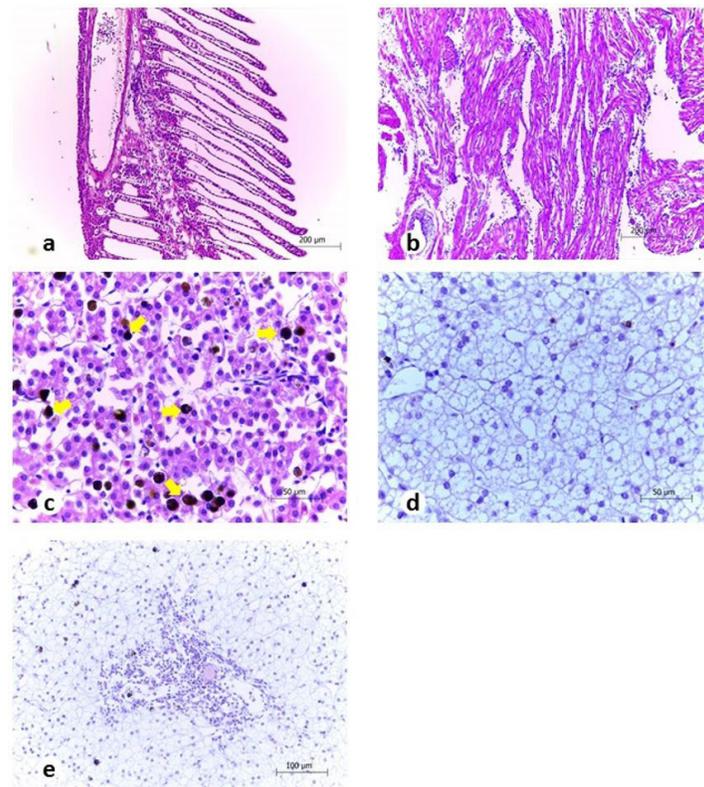


Figure 2. Histopathological analysis on organs from shortnose guitarfish (*Zapteryx brevirostris*): (a) gill without microscopic alteration in $T_{Control}$ group (10x); (b) striated cardiac muscle without microscopic alteration in $T_{Control}$ group (10x); (c) liver presenting slight hepatic vacuolar degeneration in the treatments that involved some period of fasting ($T_{7 \times 14}$ and $T_{14 \times 14}$), with presence of melanomacrophages (yellow arrow) (40x); (d) liver presenting severe hepatic vacuolar degeneration in the control treatment ($T_{Control}$) (40x); and (e) liver presenting periportal infiltrate in the treatment with fasting of greater severity ($T_{14 \times 14}$) (20x).

that went through a period of fasting ($T_{7 \times 14}$ and $T_{14 \times 14}$) presented slight hepatic vacuolar degeneration, with slight thrombosis and hepatic atrophy (Figure 2c).

DISCUSSION

Zootechnical performance

Complete compensatory gain was achieved through the treatment $T_{7 \times 14}$, since the zootechnical performance and health data of this treatment group were similar to those of the control group ($T_{Control}$). However, the treatment $T_{14 \times 14}$ only gave rise to partial compensatory gain. Comparison with carnivorous species of marine teleosts that present complete compensatory gain showed that there was similarity regarding age group (all individuals were adults) (Foss et al., 2009) and that there was no hyperphagia, i.e. situations of increased food intake (Cho et al., 2006).

The physiological performance that is expected when complete compensatory gain occurs consists of presence of hyperphagia in association with an increased SGR. These adaptations physiologically explain the recovery of the weight that was not gained during the fasting period (Sogard and Olla, 2002; Won and Borski, 2013; Barbieri et al., 2016). Through greater food consumption, lipid reserves can be recovered and growth (as quantified via the SGR) can be accelerated (Won and Borski, 2013). However, the treatment $T_{7 \times 14}$ did not alter the daily food consumption (49.69 g) in relation to the control (53.31 g), while the treatment $T_{14 \times 14}$ reduced food consumption by more than 44% (29.73 g). Similar results were found for the olive flounder (“Japanese halibut”) *Paralichthys olivaceus*, in which the groups that presented complete compensatory gain did not present hyperphagia and the groups that presented partial compensatory gain presented reduced daily food consumption (Cho et al., 2006; Rezende et al., 2018).

It has been observed among teleosts that food consumption is dependent on the quantity offered and frequency of feeding. These factors influence the rate of stomach emptying and the return of appetite, and they may result in changes to stomach weight (Riche et al., 2004). In the present study, stomach weight (gastrosomatic index) and intestinal weight (enterosomatic index) decreased through the treatment that involved the longer period of food deprivation ($T_{14 \times 14}$). This suggests that the tissue responsible for enzyme production and nutrient absorption became reduced. This response has the aim of returning the organism to physiological equilibrium, given that there was a drastic reduction in the quantity of food consumed. Thus, the organism reduces its numbers of digestive cells, or their size, because maintaining the baseline metabolism of the remaining cells involves energy expenditure (Cho et al., 2006).

The growth in the control group can be considered to have been low (Pedersen et al., 2009). One factor that contributed towards this was that all the fish used in this experiment were adults, with total lengths greater than 380 mm (Marion et al., 2011). This low growth in the control group contributed towards the lack of significant difference between the fish that were fed every day and those that were subjected to a period of food deprivation. Thus, it can be stated that the complete compensatory gain achieved

in the $T_{7 \times 14}$ group was due to increased growth among these fish during the refeeding period but, rather, to the low growth among the $T_{Control}$ fish, in that a period without food would not interfere significantly in their growth.

The AFC of the $T_{7 \times 14}$ group was lower than that of the $T_{Control}$ fish, i.e. for the $T_{7 \times 14}$ group, a smaller quantity of food produced a greater quantity of body weight. Thus, after the fasting period, these fish made better use of the food, such that they replaced their energy reserves and used the nutrients for growth (Won and Borski, 2013). This was the only zootechnical parameter that behaved as expected, since the food conversion decreased and the specific growth rate increased (Cho et al., 2006; Foss et al., 2009).

Among the performance indices, the hepatosomatic index is given by the relationship between the weight of the liver and the total weight of the viscera. Thus, the heavier the liver is, the greater the index is and the greater the lipid reserves of the fish are. The liver is the main location for storage of triglycerides and, in some species of elasmobranchs, 80% of the weight of this organ may be composed of lipids. This reserve is the main source of energy during fasting periods and, in some cases, it may assist in enabling floatation (Grant et al., 2012). In the present study, the fish that were subjected to the treatment $T_{14 \times 14}$ presented lower hepatosomatic indices than those of the other groups. This meant that these fish were consuming their lipid reserves to supply the requirements for their baseline metabolism basal, as would be expected. The levels of the enzyme alkaline phosphatase, which would reflect any presence of severe lesions in hepatocytes, did not vary. Hence, even in this situation of prolonged and recurrent fasting, hepatic gluconeogenesis was functioning.

Hematological analysis

Table 2 demonstrates that the $T_{14 \times 14}$ group presented hypochromic microcytic anemia, with hypoproteinemia and low creatinine. Because of the reductions in the hematological parameters (MCV, MCH and hematocrit), the fish in the $T_{14 \times 14}$ group exhibited anemia that was classified as microcytic and hypochromic, i.e. the erythrocytes were smaller (lower MCV) and contained less hemoglobin (lower MCH). This is a severe condition that leads to reduction in hematocrit, as seen in the present study. The most common cause of this type of anemia is iron deficiency in the diet over a prolonged period. This anemia is initially classified as microcytic and normochromic, which denotes a situation in which the quantity of hemoglobin inside cells of smaller size is considered normal. If the deficiency persists, the production of hemoglobin will no longer be sufficient, and this will result in microcytic hypochromic anemia, i.e. a situation in which the erythrocytes are smaller and contain little hemoglobin (Eivazi-Ziaei et al., 2008). This compromises gas transportation because of the low levels of the main gas transportation agent. This anemic condition was shown to be a severe clinical sign, since there was high mortality in the $T_{14 \times 14}$ group (42.90%).

The majority of the proteins that are accounted as total plasma proteins (TPP) consists of albumin, while the remainder comprises globulins. Albumin is synthesized in the liver (Dallagnol et al., 2014). During the treatment $T_{14 \times 14}$, gluconeogenesis was prioritized in the

liver in order to obtain energy and supply baseline metabolism. This resulted in a significant reduction in TPP, such that a condition of hypoproteinemia was exhibited.

Also during the treatment $T_{14 \times 14}$, there was a reduction in the plasma creatinine levels. Plasma creatinine is a marker of renal function that can be determined more reliably than can urea. Creatinine levels are directly influenced by diet, such that changes to this parameter may be associated with reductions of the diet, rather than to renal lesions that would impair filtration. Moreover, baseline serum creatinine is directly correlated with the body mass of fish, i.e. those that present good body condition (as in the $T_{Control}$ group of the present study) have normal creatinine levels that are higher than those of fish in a poor body condition (as in the $T_{14 \times 14}$ group) (Heysmsfield et al., 1982).

The low number of monocytes found in the present study (Table 3), compared with what was found in other hematological studies on rays, shows that the water quality and management conditions under which these fish were kept were adequate (Ferreira et al., 2010). Monocytes (Figure 1) are the largest leukocytes present in the circulation in fish. In tissues, they are named macrophages and they act mainly on inflammatory foci, with the function of phagocytizing cell debris and pathogenic microorganisms. The low abundance observed reflects the low presence of pathogens challenging the immune system of rays (Ranzani-Paiva et al., 2013; Campos-Garcia et al., 2016). One of the advantages of the water recirculation system in which the fish of the present study were kept was the lower risk of introduction of pathogenic microorganisms into the system. The water used in the present study came from a trustworthy source and had been passed through mechanical filtration before it entered the tanks.

Histological analysis

The liver is the main organ of accumulation of lipid reserve (Grant et al., 2012). The fish in the $T_{Control}$ group presented moderate to severe hepatic degeneration, which reflected the greater accumulation of lipids in the liver. These ended up occupying the space of the hepatocytes, thus leading to vacuolar degeneration. Because these fish were fed every day, there was greater deposition of natural fat in this organ. On the other hand, in the treatments that involved some period of fasting ($T_{7 \times 14}$ and $T_{14 \times 14}$), there was slight hepatic vacuolar degeneration, i.e. smaller quantities of lipids were accumulated. The lipid reserves were consumed during the periods of fasting to maintain the baseline metabolism of these fish. These microscopic lesions corroborate the results relating to the hepatosomatic index, shown above, i.e. a higher value for the index in the control group.

CONCLUSIONS

The species exhibited complete compensatory growth, showing resilience to cyclic fasting periods of one week. However, it showed partial compensatory gain when subjected to more severe periods of food deprivation (two weeks) that could compromise the survival of the species due to mortality. Hematocrit, total plasma protein, creatinine and hematimetric indexes were hematological

parameters that signaled the fasting in the species, as well as histopathological analyzes in the liver.

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