

Original article

An external tag for fish: tagging effects in different fish size classes and its influence on growth performance and hematology of *Lophiosilurus alexandri* (Siluriformes: Pseudopimelodidae)

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This study tested an external tag for juveniles of *Lophiosilurus alexandri* and measured the effects of tagging in different size classes of fishes. Experiment 1 evaluated the retention rate and influence of the tag on survival and growth of three hundred fishes divided Small, Medium and Large size classes. After 90 days of experiment the fishes had 100% survival and the tag had a higher retention rate for animals of the Large size class. Experiment 2 evaluated the tag's influence on hematology parameters of forty-two fishes at 5, 10 and 30 days post-tagging. In this experiment both tagged and untagged animals experienced 9.4% mortality. The hematocrit was higher on the 30th day for tagged animals than for untagged animals. No difference was observed for leukocytes, plasma protein, erythrocytes and mean corpuscular volume. It was concluded from Experiment 1 that the tag reduced growth performance when applied to small sized juvenile *L. alexandri*, and that the tag retention rate increased with increasing animal size. Among the hematological parameters studied for juveniles on the Experiment 2 the tag only influenced the hematocrit parameters, which shows that the tag can be used without considerable influence on the hematological parameters of juvenile *L. alexandri*.

Keywords: Catfish, Identification, Pacamã, Physiology, Repopulation, Retention.

Esse estudo testou a utilização de marcadores externos em juvenis de *Lophiosilurus alexandri*, assim como o tamanho mínimo ideal do peixe para marcação. No experimento 1 foi avaliado a taxa de retenção e a influência do marcador sobre a sobrevivência e crescimento dos peixes divididos por tamanho nas classes: Pequeno, Médio e Grande. Após 90 dias, os peixes apresentaram 100% de sobrevivência e uma taxa de retenção mais elevada para os animais classificados como grandes. O segundo experimento avaliou a influência do marcador sobre os parâmetros hematológicos dos animais após 5, 10 e 30 dias da marcação. Neste experimento, foram observados valores mais elevados para o hematócrito no 30^o dia para os animais marcados, enquanto nenhuma diferença foi observada para leucócitos, proteína plasmática, eritrócitos e volume corpuscular médio. Concluiu-se o que o marcador reduziu a taxa de crescimento dos animais menores, e que a taxa de retenção aumentou com o aumento do tamanho do animal. Dos parâmetros hematológicos avaliados, o marcador influenciou apenas no valor do hematócrito, o que mostra que o marcador pode ser usado sem considerável influência nos parâmetros hematológicos do *L. alexandri*.

Palavras-chave: Bagre, Identificação, Fisiologia, Pacamã, Repovoamento, Retenção.

Introduction

Increasing commercial (FAO, 2016) and recreational (Johnston *et al.*, 2007) fishing, along with pollution and alterations of river courses through the construction of large reservoirs for hydroelectric plants, have placed natural fish-

eries resources under strong pressure (Auer, 1996; Agostinho *et al.*, 2010; Mcdougall *et al.*, 2014). As a result, several countries have developed interests in promoting stocking programs to restore fish stock and to prevent the extinction of endangered species (Svâsand *et al.*, 2000; Steffensen *et al.*, 2010; Kawamura *et al.*, 2012).

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Despite the efforts of repopulation programs, few studies have been carried out to assess their effectiveness (Agostinho *et al.*, 2010; Barroca *et al.*, 2015). One of the tools used for this type of verification and evaluation is the use of tags (Svåsand *et al.*, 2000) that facilitate the identification of individual fish after their release (Specziár, Turcsányi, 2014).

A number of fish tags have been described in the literature (Griffiths, 2002; Gibbons, Andrews, 2004; Woods, Martin-Smith, 2004; Brennan *et al.*, 2007; Zeeh, Wood, 2009), and they can be classified as internal or external (Navarro *et al.*, 2006; Brewer, Norcross, 2012). Internal tags can have high retention rates for some species of fish (Ward *et al.*, 2008), however, they require the use of special equipment to read the information contained in the tags (Smith *et al.*, 2017). External tags, on the other hand, have advantages as low cost, facility of application and excellent visibility (Griffiths, 2002). Depending on the species of fish evaluated, external tags may have a low retention rate, clogging and grabbing risks, swimming influences, reduced growth and insults at the site of application, factors that contribute to the low recapture rate (Jepsen *et al.*, 2015). The information contained in these tags can allow collaboration between fishers and researchers, in which some kind of reward (Alves, 2007; Rudershausen *et al.*, 2014) can be offered to the fishers when catching a tagged fish to encourage the relay of information to researchers (Sumpton *et al.*, 2008).

Ideal tags should not influence fish performance, which can be assessed through growth performance and survival (Woods, Martin-Smith, 2004). However, each type of tag can influence fish performance differentially (Navarro *et al.*, 2006). Therefore, it is necessary the evaluation of the tag's effects on the fish performance in a controlled environment before being used in natural environment (Frederick, 1997; Winner *et al.*, 1999; Booth, Weyl, 2008).

Hematology is a tool of great importance for assessing animal welfare, physiological status, disease and stress (Krystan, Grant, 2015). Under conditions of stress and increased physical effort, fish may increase the concentration of erythrocytes and hematocrits to improve blood oxygenation (Acerete *et al.*, 2004). Leukocytes are defense cells responsible for monitoring possible infections and tissue damage (Tavares-Dias, de Moraes, 2007) and may present changes due to stress (Adeyemo, 2007). Plasma protein plays an important role in the osmotic regulation of blood and can be used as an indication of homeostatic disturbance (Verdegem *et al.*, 1997). Thus, it is evident the need to evaluate the hematology of animals submitted to handling conditions such as marking.

Pacamã, *Lophiosilurus alexandri* Steindachner, 1876, family Pseudopimelodidae, order Siluriformes (Barros *et al.*, 2007), is a sedentary piscivorous fish species endemic to the Rio São Francisco Basin in Brazil (Cardoso *et al.*, 1996). This species is nocturnally active (Kitagawa *et al.*, 2015) and is listed in the Brazil Red Book of Threatened Species of Fauna as "vulnerable to extinction" (ICMBio, 2016). Thus, *L. alexandri* has been the subject of repopulation programs (Sato, 2014).

The objective of this work was to evaluate a new visually observable external tag for fish measuring the tagging effects on different size classes of fishes and the influence on zootechnical and hematological parameters of *L. alexandri*.

Material and Methods

The study was carried out in the Laboratório de Aquicultura (LAQUA) of the Universidade Federal de Minas Gerais (UFMG), through two experiments. During the experiments, fish were fed commercial extruded feed containing 38% crude protein as 3-4 mm diameter pellets delivered to apparent satiation twice daily (08:00 am and 04:00 pm). After 30 minutes of feeding, feces and unconsumed food were siphoned. Water quality parameters were measured every three days: temperature and pH were measured using a COMBO (HANNA brand) portable pH meter; dissolved oxygen (DO) with a digital oximeter (HANNA brand); and ammonia with a colorimetric kit (LabconTest).

For tagging fish in both experiments, the harpoon tags that are supposed to fix the tag to a spine(fin) inside the fish, applicator and application methodology used followed the methodology deposited with the patent: BR1020170075770 titled MARCADOR VISUAL EXTERNO PARA IDENTIFICAÇÃO DE PEIXES E APLICADOR, which is shown in Fig. 1. The tag was manufactured from polyethylene terephthalate modified with glycol (PETG), weighed 0.16 grams, and was applied using a 30 mm long and 1.5 mm diameter needle coupled to a metal rod. For application of the tag the needle was inserted approximately 5 mm into the right side of the musculature near the dorsal fin.

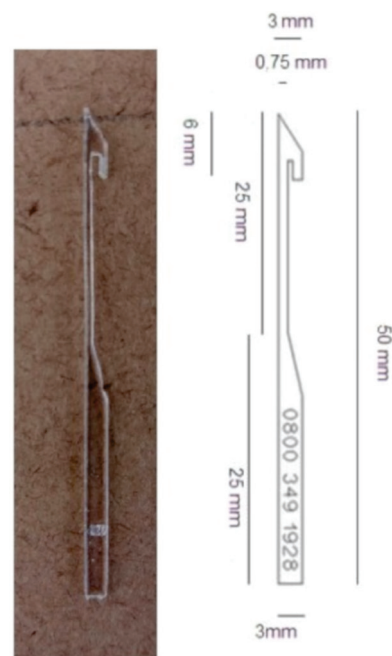


Fig. 1. Visual tag for fish described in the patent BR1020170075770.

The species used in this study were offspring from broodstock wild-caught from the São Francisco River. The broodstock were adapted to conditions of captivity in the Laboratory of Aquaculture (Laqua) of the Universidade Federal de Minas Gerais (UFMG) for nine years. The representative material of the species used in this study was deposited in the collection of the Coleção Ictiológica at the Museu de Ciências Naturais da PUC Minas (MCNIP 3217).

Experiment 1. Ideal minimum size for tag application, retention rate and influence on performance of juvenile of *L. alexandri*. This experiment was carried out according to protocols approved by the Comissão de Ética no Uso de Animais (CEUA; Ethics Committee on Animal Use) of UFMG (Protocol 243/2017).

Hatchery reared juvenile *L. alexandri*, previously produced in LAQUA, were classified into three size classes after weighing with an analytical scale (Marte AD 2000 0.01 g precision) and measuring length with a digital caliper (Gama - precision 0.01 mm) (Tab. 1). After classification, the juveniles were acclimatized for seven days and then distributed among 12 200 L tanks with a useful volume of 180 L of water each such that there were four replicates of each size class. Each tank received 25 animals. For retention rate and performance evaluations, 15 animals from each tank were tagged with the external tag while the remaining 10 (control) remained untagged. A greater number of tagged animals was used to account for the possibility of tag loss and the subsequent reduction in the number of tagged animals.

Tab. 1. Mean and standard deviation of the weight and length of the *Lophiosilurus alexandri* used in the different size classes.

Size classes	Weight (g)	Length (cm)	Animals tagged by treatment	Animals untagged by treatment (control)
Small	14.50 ± 0.63	10.58 ± 0.63	60	40
Medium	23.95 ± 1.76	11.71 ± 0.52	60	40
Large	31.96 ± 3.50	13.50 ± 0.60	60	40

The tanks were maintained in a water recirculation system with controlled water temperature of 27.66 ± 0.40 °C, DO of 6.38 ± 0.46 mg L⁻¹, pH of 8.34 ± 0.04 and total ammonia < 0.5 mg L⁻¹. The photoperiod was maintained at 12 hours of light. The animals were observed over a period of 90 days. The tag retention rate was determined by Equation 1 based on daily observations of the animals. When a loose tag was noted in the tank, both the tag and the fish, indicated by the presence of the implant injury, were removed from the experiment to avoid distortions in the score of tagged *versus* untagged fish.

$$RR = (Tf / Ti) * 100$$

Equation 1. Equation used to measure the retention rate of tags, where: RR is the retention rate, Ti is number of animals initially tagged and Tf is the number of tagged animals that remained.

After 90 days, the final weight (Wf) and the final length (Lf) of the fishes were measured. The weight gain (WG) and survivorship rate (SR) were calculated by the following equations:

$$WG = Wf - Wi$$

Equation 2. Equation used to calculate weight gain (WG), where: Wf and Wi are the final and initial fish weight, respectively.

$$SR = (Nf / Ni) * 100$$

Equation 3. Equation used to calculate the survivorship rate (SR), where: Nf is the final number of fish survivors, and Ni is the initial number of individuals.

Experiment 2. Influence of tag on hematology of juvenile *L. alexandri*. This experiment was carried out according to protocols approved by CEUA of UFMG (Protocol 25/2017 CEUA of UFMG). Forty-two juvenile *L. alexandri* (17.09 ± 1.02 cm and 64.28 ± 11.80 g) were acclimated for 30 days in a 1000 L tank kept in a recirculation system with controlled water temperature of 27.64 ± 0.41 °C, DO of 6.45 ± 0.47 mg L⁻¹, pH 8.35 ± 0.03 and total ammonia < 0.5 mg L⁻¹. The photoperiod was 12 hours of light. After acclimatization, 10 animals were randomly selected for blood analysis as an initial baseline. Of the remaining 32 animals, 16 received a tag and 16 remained untagged. These fish were divided into two 1000 L tanks under the previously described conditions, with eight tagged fish and eight untagged fish in each, in an experimental design with two treatments and 16 replicates, with each fish being considered a replicate. After five days, blood was collected from all tagged (n = 8) and untagged (n = 8) fish from one of the two tanks. The same procedure was performed after 10 days for fish from the second tank (n = 8 tagged and n = 8 untagged). After 30 days, all of the fish from both tanks were again sampled for blood. The retention rate of the tags and the mortality rate of the fish were monitored during the 30 days of the experiment.

For blood collection, each fish was restrained with a moist cloth and 1 mL of blood was acquired by venous puncture of the vertebral artery using 1 mL syringe containing 10% heparin. Aliquots of blood were used to determine total plasmatic protein (TPP), hematocrit (Htc), erythrocytes (Er) and leukocytes (Leuk). The hematocrit was determined by the microhematocrit method (Goldenfarb *et al.*, 1971) using capillary tubes. After the determination of the hematocrit, the capillaries were broken above the plasma line, which was then placed in a refractometer (RTS-101ATC) to determine total plasmatic protein.

Counts of erythrocytes and leukocytes were performed according to the direct method of counting in a Neubauer chamber, after staining with Natt-Herrick dye in a hemocytometer and mean corpuscular volume (MCV) was calculated by Equation 4.

$$MCV = (Er / Htc) * 10$$

Equation 4. Equation used to calculate mean corpuscular volume (MVC), where: Er is the number of erythrocytes and Htc is the hematocrit.

Statistical analysis. All data were tested for normality (Shapiro Wilk test) and homoscedasticity (Levene test). For normality, the hematocrit results were log (10) transformed. Hematological results between days were analyzed by ANOVA followed by the Tukey test at 5% probability. Data comparing the results between treatments for both hematological and performance were analyzed by Student's t-test. Tag retention rate throughout the days of Experiment 1 was analyzed by two-way ANOVA followed by Tukey test at 5% probability. The software Infostat was used for all statistical analyses.

Results

Experiment 1. Comparing RR for the same size class over days (Fig. 2) revealed that the RR of the tags in the three fish classes decreased significantly ($P < 0.05$) 30 days post-tagging and remained similar until 60 days ($P > 0.05$). At 90 days post-tagging, the Medium size class had a RR similar ($P > 0.05$) to those at 30 and 60 days. The Large size class had RR like that at 60 days but different from that at 30 days, while the Small class had a lower RR at 90 days ($P < 0.05$) than at 30 and 60 days.

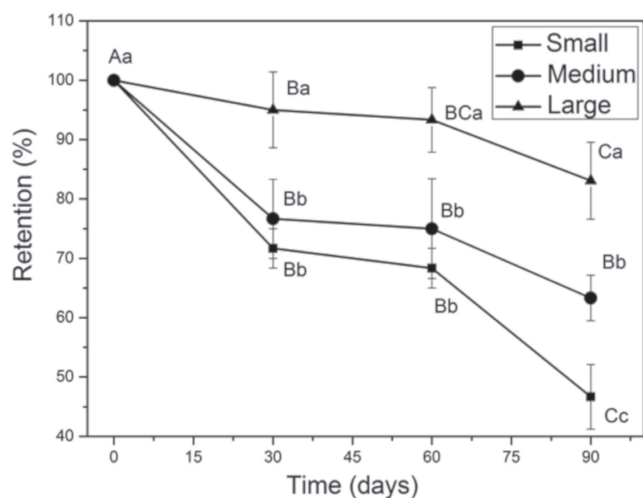


Fig. 2. Retention rate of tags for the different size classes of *Lophiosilurus alexandri* evaluated every 30 days for a period of 90 days. Means followed by different letters (uppercase between days and lowercase between sizes) differ significantly ($p < 0.05$) by two-way ANOVA followed by Tukey test.

Comparing RR of the different size classes for the same day revealed that the RR for the Large size class was higher ($P < 0.05$) than that of the Small and Medium classes for

all the days evaluated. The RR for the Small and Medium size classes were similar ($P > 0.05$) at 30 and 60 days post-tagging, while at 90 days the Medium size exhibited a higher RR ($P < 0.05$) than the Small size class.

Fig. 3a shows a photograph of a tagged fish of the Large size class at the end of 90 days, and in Fig. 3b the scar caused by the detachment of the tag from a fish of the Small size class can be seen. Animals that were withdrawn from the experiment due to tag loss recovered and showed 100% survivorship during the period of the experiment. Comparing the performances of the tagged and untagged fish (Tab. 2) of the same size class revealed that fish of the Small size class had lower WG and Wf ($P < 0.05$) than the untagged animals, while Lf was similar ($P > 0.05$). There were no differences ($P > 0.05$) in performance for the fish of the Medium and Large size classes.

Tab. 2. Weight gain and final weight and length of the *Lophiosilurus alexandri* in the different size classes after 90 days. Weight gain (WG), final weight (Wf) and final length (Lf). Means for tagged and untagged fish of the same size class followed different letters differ significantly ($p < 0.05$) by the Student t-test.

Size classes	WG (g)		Wf (g)		Lf total (cm)	
	Untagged	Tagged	Untagged	Tagged	Untagged	Tagged
Small	23.2±0.6a	21.0±0.4b	38.5±0.6a	36.4±0.4b	13.0±0.2a	12.6±0.4a
Medium	27.4±3.6a	26.4±2.4a	51.8±3.6a	50.8±2.4a	15.82±2.1a	14.4±0.2a
Large	42.4±5.5a	45.1±3.3a	76.0±5.5a	78.8±3.2a	17.55±0.3a	17.7±0.2a

Experiment 2. Deaths of one tagged and one untagged fish were recorded on the 10th and the 12th day of the experiment, respectively. In addition, tag loss of one fish was observed on the 14th day, thus yielding a RR of 93.33%. Both, fish mortality and tag detachment lead to a total of 15 untagged and 14 tagged fishes after 30 days of experiment.

Fig. 4a shows that the erythrocyte count exhibited no difference ($P > 0.05$) between tagged and untagged fish. However, there was variation among the different days analyzed with higher values ($P < 0.05$) at 5 and 10 days and lower values at 30 days, whether fish were tagged or not. At the beginning of the experiment the fish had intermediate levels.

Leukocytes (Fig. 4b), total plasmatic protein (Fig. 4c) and MCV (Fig. 4d) did not differ between tagged and untagged fish over time ($P > 0.05$).

Regarding hematocrit (Fig. 4e), there was an increase for both the tagged and untagged fish on the fifth day post-tagging ($P < 0.05$). On the 10th day, the hematocrit of the untagged fish remained higher ($P < 0.05$) than the basal level, while the values for tagged fish were similar to the base line ($P > 0.05$). On the 30th day, the untagged fish had lower levels ($P < 0.05$) than those recorded previously, whereas tagged fish had hematocrit levels similar to the basal treatment and different ($P < 0.05$) from the tagged fish on days 5 and 10. The hematocrit only differed ($P < 0.05$) between tagged and untagged fish on the thirtieth day, when tagged fish had higher values.



Fig. 3. Photograph of tagged *Lophiosilurus alexandri*. **a.** Large-size class animal after 90 days post-tagging. **b.** Small-size class animal with wound caused after tag detachment during the experiment. Scale bar = 30 mm.

Discussion

There was no mortality of tagged and untagged fishes in Experiment 1, however, there were deaths of one tagged and one untagged individual in Experiment 2. These results indicate that the external tag can be used with juvenile *L. alexandri*, and that the observed mortality may just be due to an effect of the conditions of cultivation. Gil *et al.* (2017) compared the survival rate of juvenile *Argyrosomus regius* (83.7 ± 24.5 g and 19.5 ± 1.9 cm) and found lower survivorship (60%) for animals tagged with an abdominal T-bar, and higher survivorship (83.3%) for fish tagged with an anchor T-bar, which were similar to the untagged animals. Studying *Lutjanus campechanus* (43.7 ± 5.6 g), Phelps, Rodrigues (2011) found that fish tagged or not with an abdominal T-bar, PIT tag and mandibular tag for 150 days did not differ in survivorship.

The highest retention rate of the tags recorded in the present study was for fish of the Large size class during the entirety of Experiment 1. This finding may be due to the fact that larger fish have a greater amount of muscle at the application site. The RR obtained was expected since it was not found in the literature, studies with high retention rates for external tags applied in small animals. Based on this we suggest the application of the tag in juveniles of *L. alexandri* larger than 13.50 ± 0.60 cm and 31.96 ± 3.50 g to ensure better retention rates.

Smith *et al.* (2017) compared the retention rates of PIT and T-bar tags in *Coregonus sardinella* (13.6-15.8 cm and 18.3-31.6 g) using different tagging protocols (with and without anesthesia followed by baths of salt for recuperation

from handling at concentrations of 10 and 3 mgL⁻¹). These authors recorded mean retention rate for the T-bar treatment of 76% after 30 days post-tagging and variation among protocols of 25 to 45% after 60 days, while the PIT tag treatment had 100% retention in all the treatments. With blue catfish (*Ictalurus furcatus*) of 36.4 ± 0.1 cm kept in excavated ponds for six months, Bodine, Fleming (2014) documented a retention rate of 94% for T-bar tags at two months and 76% at six months, while for PIT tags they recorded 100% retention. Thus, it is evident that the efficiency of a tag is related to the type of tag, the species tagged and the size of the fish, as well as the management methods employed.

In the present study, the lower growth performance found only for the Small size class of tagged fish compared to untagged fish of the same size may be related to the greater proportion of tag weight relative to body weight, which represented on average: 1.15%, 0.67% and 0.5% of dry weight for Small, Medium and Large size classes, respectively. There is no well-defined relationship between tag weight and animal body mass, so this variation may be due to the type of tagging and the species studied (Jepsen *et al.*, 2015). Some studies recommend the use of taggers weighing less than 2% of an animal's weight in the air (Jepsen *et al.*, 2003), others recommend an even lower ratio (1.25%) (Counihan, Frost, 1999; Sutton, Benson, 2003), while other authors challenge this rule and suggest higher values (Brown *et al.*, 1999; Smircich, Kelly, 2014). Even using smaller tags than those recommended in the literature, the present study found fish of the Small class to have lower growth performance, indicating that this tag should be avoided for small fish and that it may need to be adjusted or a new model developed for this size class of fish. The external tag in a fish can cause imbalance, wounds (Halls, Azim, 1998) and infections due to the friction of the tag with water (Thorsteinsson *et al.*, 2002). It can result in low rates of tag retention, fish survival and growth (Rikardsen, 2000; Rikardsen *et al.*, 2002; Strand *et al.*, 2002). *Lophiosilurus alexandri* is a lentic and bottom-dwelling species, for which swimming was recorded only at the time of feeding. However, friction generated between the tag and water during swimming was minimal and only affected growth performance of smaller fish. Greenstreet, Morgan (1989) studied the use of external ultrasonic tags with three sizes of Atlantic salmon (*Salmo salar*) and observed a loss of body mass for the smaller animals (> 16.0 cm), lower growth performance for the average animals (between 16.0 and 18.0 cm), and similar the control growth performance for larger animals (> 18.0 cm). Rikardsen *et al.* (2002) compared the retention rate and influence on performance of two visual tags with the Arctic trout (*Salvelinus alpinus*) of 17.0-20.9 cm, and documented a retention rate of 94% for Floy tags and 78% for VI alpha tags 160 days post-tagging. However, the authors found lower growth rate for animals tagged with Floy tags.

Animals that experienced tag detachment and were transferred to another tank during the period of the experimental recovered from the injury caused by the tag and showed no

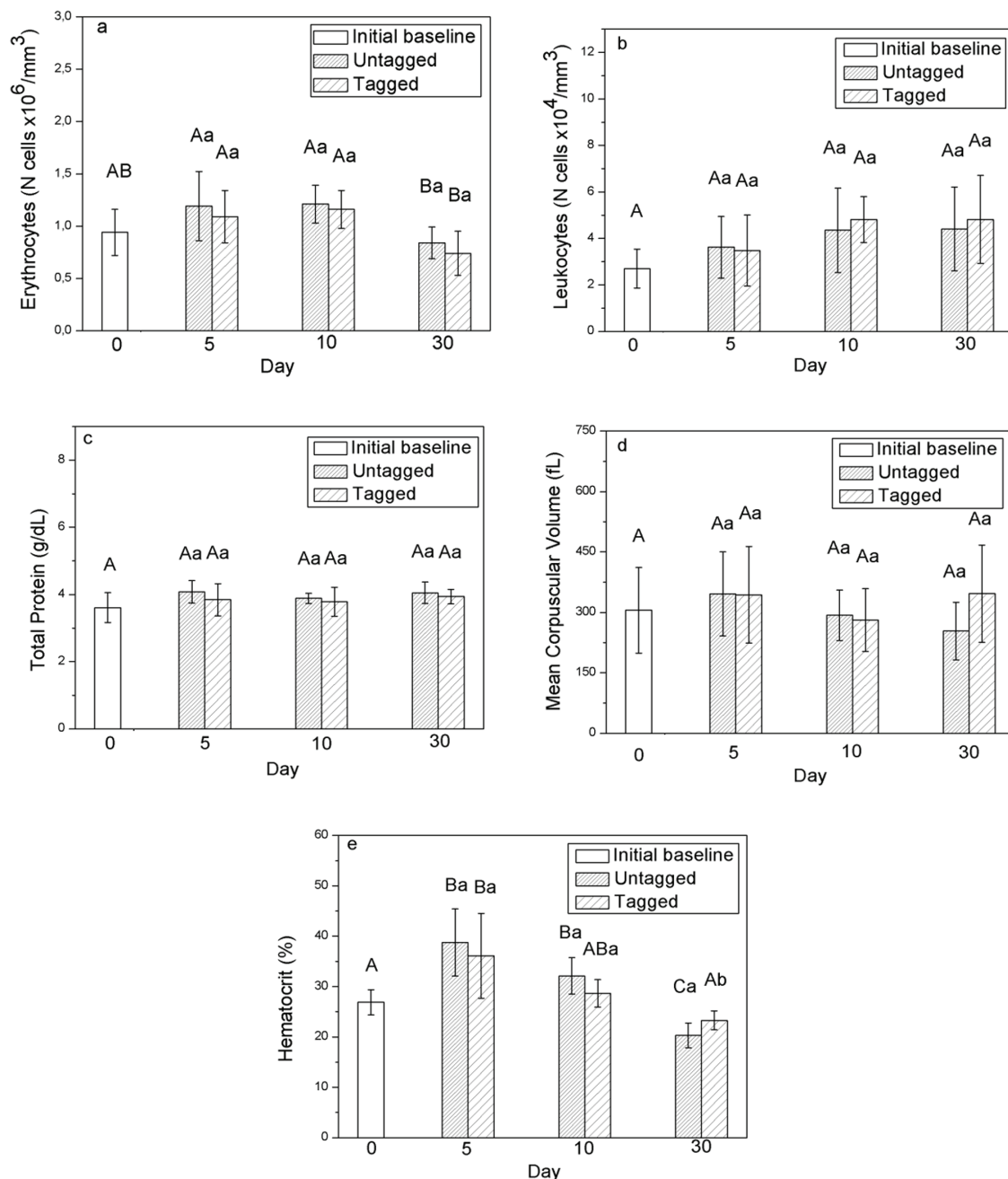


Fig. 4. Hematological parameters of tagged and untagged *Lophosilurus alexandri* on the different days analyzed: **a.** Er. **b.** Leuk. **c.** TPP. **d.** MCV. **e.** Htc. Means followed by different capital letters differ significantly ($p < 0.05$) between days by two-way ANOVA followed by Tukey test. Means with different lowercase letters differ significantly ($p < 0.05$) between tagged and untagged fish by Student's t-test.

mortality. This suggests that, even if in another environment, if the tag is detached the animals have the capacity to recover.

The hematological values of the present study are consistent with those described previously for the same species, including values reported for erythrocytes and leukocytes by Mattioli *et al.* (2017), hematocrit by Kitagawa *et al.* (2015) and plasmatic protein by Costa *et al.* (2016). The increase in the number of erythrocytes and hematocrit on the fifth and tenth day after tagging may be related to stress caused dur-

ing the management of the fish, since they took eight days to start feeding. However, on the thirtieth day the values returned to basal levels, indicating adaptation to the tags. The increase in hematocrit concentration may also be indicative of greater physical effort during a stressful situation (Ace-rete *et al.*, 2004). Similar results were described by Lewis, Muntz (1984) and Steinhause *et al.* (2006), that evaluated the influence of external tags containing batteries and signal transmitters on fish parameters. In both experiments was

observed that even using heavy tags, that interfere in the fish behavior (opercular movement and swimming), and no effects were observed on the hematological parameters.

The tag used in the study for the period of 90 days reduced growth performance when applied to juvenile *L. alexandri* of the Small size class but did not interfere in the growth performance of juvenile fish in the Medium and Large size classes.

The retention rate of the tag increased with increasing size of the animals, reaching 84.83% at the end of 90 days for the Large size class. The hematocrit of animals of 64.28 ± 11.80 g was higher in the first days, indicating a situation of stress, but it recovered by the end of 30 days. On the other hand, no changes were found for total plasma protein, leukocytes, erythrocytes and mean corpuscular volume. This finding shows that the tag can be used without causing major influences on hematocrit, plasma protein, erythrocytes, leukocytes and mean corpuscular volume of *L. alexandri*.

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