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Hematological parameters of Mugil liza mullet submitted to different concentrations of formalin and freshwater

Parâmetros hematológicos da tainha (Mugil Liza) submetida a diferentes concentrações de formalina e água doce

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ABSTRACT: Lebranche mullet, *Mugil liza* is a species of high economic value for fishing. Despite the importance of the species, little is known about the effects of therapeutic baths on the hematological parameters of mullet. Such parameters are important tools for facilitating the early detection of diseases and illnesses that may impair the performance of the animals. This study evaluated the hematological parameters of mullet submitted to therapeutic baths with formalin and freshwater. For the experiment a total of 108 mullets were used, divided in groups of six individuals per tank. In this experiment, immersion baths were tested for 1 hour in freshwater (zero salinity), formalin at concentrations: 150, 200, 250, 300 and 350 mg L^{-1} in seawater and a control (without formalin and parasitized), in triplicate. After the immersion bath, fish blood was collected from the caudal vein for evaluation of hematological parameters. The blood was destined to erythrogram, leukogram, thrombogram and glucose determinations. The hematological analyses found significant difference in the number of erythrocytes, lymphocytes, hematocrit, hemoglobin, MCV, MCH, MCHC and glucose. However, there was no significant difference in thrombocyte, total leukocyte, monocyte and neutrophil values. It is concluded that 150 mg L^{-1} formalin concentration and freshwater bath for one hour can be used for mullet as it causes mild hematological changes.

KEYWORDS: Aquaculture; hematology; chemotherapy; treatment.

RESUMO: A tainha, *Mugil liza*, é uma espécie de elevado valor econômico para a pesca. Apesar da importância da espécie, pouco se sabe sobre os efeitos dos banhos terapêuticos nos parâmetros hematológicos da tainha. Tais parâmetros são importantes ferramentas para facilitar a detecção precoce de doenças e enfermidades que possam prejudicar o desempenho dos animais. Este estudo avaliou os parâmetros hematológicos da tainha submetida a banhos terapêuticos com formolina e água doce. Para o experimento foi utilizado um total de 108 tainhas, divididas em grupos de seis indivíduos por tanque. Neste experimento, foram testados banhos de imersão por 1 hora em água doce (salinidade zero), formolina nas concentrações: 150, 200, 250, 300 e 350 mg L^{-1} em água do mar e um controle (sem formalina e parasitado), em triplicata. Após o banho de imersão, o sangue dos peixes foi coletado da veia caudal para avaliação dos parâmetros hematológicos. O sangue foi destinado às determinações de eritrograma, leucograma, trombograma e glicose. As análises hematológicas encontraram diferença significativa no número de eritrócitos, linfócitos, hematócrito, hemoglobina, VCM, HCM, CHCM e glicose. No entanto, não houve diferença significativa nos valores de trombócitos, leucócitos totais, monócitos e neutrófilos. Conclui-se que a concentração de 150 mg L^{-1} de formalina e o banho em água doce durante uma hora podem ser utilizados para a tainha, uma vez que provocam alterações hematológicas leves.

PALAVRAS-CHAVE: aquicultura; hematologia; quimioterápico; tratamento.

INTRODUCTION

The Mugilidae family is marine and coastal, with 17 genera and 66 species (Monteiro-ribas; Bonecker, 2001). All genera of the family, Mugilidae, the only genus found in Brazil is *Mugil* (Menezes, 1985). Seven species of this genus are found in Brazil, with *Mugil liza* and *M. platanus* found in larger quantities (Ramirez, 2011). On the Atlantic coast of South America, *M. liza* is the main species of great importance in artisanal and industrial fisheries (Reis *et al.*, 1994).

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In 2020, fish from the Mugilidae family were among the most produced in coastal and marine fish farming, with a production of 291.2 thousand tonnes. This production was surpassed only by atlantic salmon and milkfish (FAO, 2020). The species, *M. liza,* has been considered promising for culture because it presents desired characteristics for fish farming, such as versatility and adaptability to large variations in salinity, temperature, turbidity, sedimentation, and dissolved oxygen (Cerqueira *et al*., 2017; Scheuer, 2017; Holanda *et al*., 2020). Mullets are also easily adaptable to the captive environment, and feeding is omnivorous, which facilitates their acceptance of an artificial diet and reduces production costs (Baldisserotto, 2013). In Brazil, research has been conducted in the areas of captive reproduction, hormonal induction, spawning, larviculture, zootechnical performance, technology for commercial production (Cerqueira *et al*., 2017) and the diagnosis and treatment of diseases, especially Monogenea parasites (Pahor-filho *et al*., 2012). Despite the importance of mullet for fisheries and its potential for cultivation, there are no studies evaluating the effects of formalin and fresh water on *M. liza* hematology.

Because of these advantages, incentives are now offered for the cultivation of *M. liza*, which are cultivated in intensive systems. When poorly managed at high stocking densities, the animals are exposed to different types of environmental stressors, leading to a greater susceptibility to certain pathogens and consequently to diseases, which leads to irrecoverable economic losses for the producer. Of the main pathogens present in cultivation, monogenoid metazoans and trichodinid protozoa are the most prominent (Jerônimo *et al*., 2016; Maciel *et al*., 2018).

When such parasitic infestation occurs, without rigorous and appropriate control measures, the economic impact on the producer can be significant and even irrecoverable, owing to the high mortality of fish (Shinn *et al*., 2015). Therefore, it is important to identify these pathogens in each fish species so that prophylactic and therapeutic measures can be adopted for the control and treatment of the disease, as a way to prevent the appearance of these pathogens in culture systems.

Parasite control in fish can be achieved by the administration of chemotherapeutic agents in bath form. Of the chemotherapeutic agents, formaldehyde has been shown to be effective in combating ectoparasites (Buchmann, 2022) and its use in aquaculture is permitted (Costello *et al*., 2001). Sanches *et al*. (2008) recommended freshwater bathing as a therapeutic treatment for marine fish when infested with monogenoid ectoparasites. Dorneles *et al*. (2019) also reported that freshwater bathing was effective in controlling *Neobenedenia melleni* ectoparasites and recommended it as an alternative treatment causing no harm to the animal or the environment, and at a low cost to the fish farmer.

In aquaculture, formalin is a chemotherapeutic agent used to treat of protozoan ectoparasite of the genus *Trichodina* spp. in tilapia *Oreochromis niloticus* (Xu *et al*., 2015); to control ectoparasitic protozoa of the species *Ichthyophthirius multifiliis*, *Ichithyophthirius necator* and the genus *Trichodina* spp. in trout of the species (Balta *et al*, 2008); as an antiparasitic of *Neobenedenia melleni* in the green grouper, *Epinephelus marginatus* (Sanches, 2008); in the control of the monogenoid, *Dactylogyrus minutus,* in carp *Cyprinus carpio* (Tancredo *et al*., 2019); and as a treatment of trichodiniasis in tilapia (García-Magaña *et al*., 2019).

Although formalin has been shown to be an excellent antiparasitic, it is necessary to study each case to verify its efficiency in different fish species and ectoparasites. Formalin is harmful to fish, humans, and the environment due to its toxicity (Akpoilih; Adebayo, 2010; Chmelova *et al*., 2016; Leal *et al*., 2018). Tavares-Dias (2020) reported that formalin can impair the physiology and adversely affect the health of exposed fish. They also stated that hematological parameters were useful for identifying the chronic toxicity of formalin and its effects on animals.

Studying the hematological characteristics of cultured fish is crucial for the development of aquaculture, especially in the identification of healthy, stressed or infected fish (Oliveira *et al*., 2021). Hematological parameters can facilitate the early detection of diseases and/or illnesses that impair animal performance (Rehulka *et al*., 2004). This will ensure that the treatment of diseases is more specific, rapid, and effective (Tavares Dias; Moraes, 2006). The objective of this study was to evaluate hematological parameters of *M. liza*, all parasitized with Neobedenea sp., after bathing with formalin and freshwater, to contribute to the knowledge of the effects of this treatment on its hematology.

MATERIALS AND METHODS

The experiment was carried out at the Marine Pisciculture Laboratory - LAPMAR of the Federal University of Santa Catarina, located at the Professor Elpídio Beltrame Mariculture Station, Barra da Lagoa, Florianópolis, Santa Catarina. The mullets, Mugil liza used in the experiment were F1 from reproduction in the laboratory itself. Before starting the experiment, the fish were in a 6000 L tank with natural photoperiod, aeration and continuous water flow. For the experiment, 500 L tanks with interrupted aeration were used. The experiment was approved by the Ethics in Animal Use Committee (CEUA/UFSC PP00862).

In the experiment, freshwater and formalin concentrations of 150, 200, 250, 300, and 350 mg L^{-1} and a control were tested (salt water without formalin). Formalin concentrations and formalin immersion time were determined by Pahor-Filho *et al*. 2012. In this study, 108 mullets were divided into six treatments with six fish per tank in triplicate, with continuous aerationthroughout the trial period. The fish had a mean weight and total length of 202.9 ± 69.2 g and 20.9 ± 5.8 cm, respectively. All fish were parasitized with platyhelminth

ectoparasites of the Monogenea class, *Neobenedenia* sp.. After 48 hours of acclimatization, formalin was added to the tanks with the fish for the immersion bath for 1 hour. In the treatment with immersion bath with freshwater, the fish were transferred to tanks containing freshwater (zero salinity), where they remained for 1 hour. Immediately afterwards all six fish per tank were anesthetized with 75 mg L^{-1} Benzocaine® for the evaluation of hematological parameters. All water parameters were evaluated during the 48h of acclimatization and during the experimental period. The parameters were kept within the recommended range for the species: temperature, 21.2 ± 0.19 °C; dissolved oxygen, 5.47 mg L^{-1} ± 0.23; pH, 8.37 ± 0.03; and salinity, 35%; measured with a multiparameter water quality probe YSI® 85 (Xylem, Washington, D.C., United States).

For hematological analyses, blood samples were collected from six fish per experimental unit after the fish were captured and anesthetized using 75 mg L^{-1} Benzocaine®. Blood was collected by puncturing the tail vessel using 3 mL syringes containing an anticoagulant solution, ethylenediaminetetraacetic acid® (EDTA 10%) (Ranzani-Paiva *et al.*, 2013). Blood was used to determine the hematocrit (%), hemoglobin concentration (Hb), total erythrocyte count (RBC) in a Neubauer chamber, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), according to the methods of Wintrobe (1934). Blood extractions were performed for total thrombocyte and leukocyte counts and for differential leukocyte counts using rapid staining with MGGW (May–Grünwald–Giemsa–Wright) (Tavares-Dias; Moraes, 2003). Immediately after blood collection, plasma glucose levels were assessed using an Accu-Chek® Advantage portable glucometer (Roche Diagnostics, Basel, Switzerland).

The study design was randomized. The data were submitted to the Shapiro–Wilk and Levene tests to assess data normality and homoscedasticity of variances, respectively. Subsequently, the data were subjected to one-way analysis of variance (ANOVA). When differences were found between treatments, the values were subjected to Tukey's test to verify the differences between treatments. Statistica® 10.0 software was used to perform all tests with a significance level of 95% ($P < 0.05$).

RESULTS

Hematological analyses revealed statistically significant differences in erythrocyte, lymphocyte, hematocrit, hemoglobin, MVC, MCH, MCHC, and glucose values (Table 1). Erythrocytes and lymphocytes increased markedly at 300 mg L^{-1} formalin, compared to the control treatment. The hematocrit percentage was higher in the freshwater group than in the group treated with 350 mg L^{-1} formalin. The hemoglobin concentration was higher in the fish in the 150 mg L^{-1} formalin bath than in the freshwater bath. The fish in the 300 mg L^{-1} formalin bath had a lower MCV than in the freshwater bath. For MCH, 300 and 350 mg L^{-1} formalin baths showed markedly lower values than other treatments. The freshwater bath showed the lowest value of MCHC of all treatments. Treatment with 150 mg L^{-1} formalin produced the highest MCHC compared to freshwater and 200, 250, and 350 mg L^{-1} formalin. Plasma glucose levels were higher in the fresh water and formalin baths of 200 mg L^{-1} and lower at 150 and 250 mg L^{-1} formalin (Table 1).

Table 1. Hematological parameters (mean ± standard deviation) of *M. liza* after the use of 37% formalin at concentrations: 150, 200, 250, 300 and 350 mg L^{-1}, freshwater in the form of immersion bath for one hour and control

Parameters	Treatments							
	Control	Freshwater	150 mg L^{-1}	200 mg L^{-1}	250 mg L^{-1}	300 mg L^{-1}	350 mg L^{-1}	*p*-value
RBC (x 10^6 μL^{-1})	3.93 ± 0.24^{b}	3.92 ± 0.22^{b}	4.11 ± 0.17^{ab}	4.01 ± 0.29^{ab}	3.95 ± 0.34^{b}	4.36 ± 0.44^{a}	4.05 ± 0.54^{ab}	0.008
T (x 10^3 μL^{-1})	6.71 ± 5.37	8.16 ± 3.45	5.07 ± 3.69	4.82± 2.51	4.64 ± 2.44	5.72 ± 3.57	6.47 ± 5.50	0.141
LT (x 10^3 μL^{-1})	392.23 ± 24.38	392.88 ± 22.45	410.78 ± 17.13	400.81 ± 28.67	394.92 ± 33.91	435.76 ± 42.77	410.38 ± 55.41	0.005
L (x 10^3 μL^{-1})	373.72 ± 14.77^{b}	378.76 ± 31.98^{b}	395.97 ± 15.93^{ab}	387.42 ± 30.56^{b}	385.32 ± 33.00^{b}	425.79 ± 42.04^{a}	397.63 ± 52.84^{ab}	0.000
M (x 10^3 μL^{-1})	9.58 ± 8.27	5.40 ± 2.94	8.81 ± 6.94	8.33 ± 6.33	5.96 ± 3.92	3.77 ± 1.66	5.44 ± 2.90	0.084
N (x 10^3 μL^{-1})	12.01 ± 9.28	8.84 ± 6.42	7.45 ± 5.34	6.88 ± 3.89	6.49 ± 3.68	5.16 ± 1.47	7.87 ± 3.93	0.098
Ht (%)	36.6 ± 3.70^{ab}	39.60 ± 4.41^{a}	36.40 ± 4.10^{ab}	37.00 ± 2.74^{ab}	37.20 ± 3.29^{ab}	37.40 ± 2.16^{ab}	35.40 ± 3.90^{b}	0.048
Hb (g dL^{-1})	12.27 ± 0.89^{ab}	11.70 ± 0.85^{b}	12.71 ± 1.11^{a}	12.04 ± 0.83^{ab}	12.20 ± 1.04^{ab}	11.95 ± 0.59^{ab}	11.75 ± 1.35^{ab}	0.048
MCV (fL)	94.51 ± 9.08^{ab}	99.70 ± 10.83^{a}	90.66 ± 8.50^{ab}	92.71 ± 7.28^{ab}	92.84 ±5.23^{ab}	81.85 ± 23.31^{b}	91.94 ± 10.00^{ab}	0.007
MCH (g dL^{-1})	31.54 ± 2.08^{a}	30.12 ± 1.54^{a}	31.48 ± 2.14^{a}	30.03 ± 1.57^{a}	30.90 ± 1.15^{a}	26.15 ± 7.69^{b}	29.13 ± 2.05^{b}	0.000
MCHC (g dL^{-1})	34.10 ± 2.04^{ab}	29.28 ± 1.48^{c}	35.02 ± 1.90^{a}	32.62 ± 1.94^{b}	32.84 ± 1.13^{b}	32.37 ± 0.18^{ab}	32.63 ± 1.42^{b}	0.000
G (mg dL^{-1})	52 ± 16.14^{ab}	71 ± 19.31^{a}	41 ± 21.04^{b}	68 ± 31.21^{a}	42 ± 18.84^{b}	51 ± 6.91^{ab}	51 ± 17.29^{ab}	0.000

Parameter legend: (RBC) Red blood cell; (T) Thrombocyte; (TL) Total leukocyte; (L) Lymphocyte; (M) Monocyte; (N) Neutrophil; (Ht) Hematocrit; (Hb) Hemoglobin; (MCV) Mean corpuscular volume; (MCH) Mean corpuscular hemoglobin; (MCHC) Mean corpuscular hemoglobin concentration; (G) Glucose.

*Different letters indicate significant statistical differences by Tukey's analysis ($p<0.05$).

DISCUSSION

In the present study, mullet were exposed to five different concentrations of formalin and freshwater that caused changes in blood parameters.

Erythrocytes are the most numerous cells in the blood, and the transport of oxygen and carbon dioxide is carried out by hemoglobin. (Tavares-Dias; Moraes 2004). In fish, the number of erythrocytes may increase due to the presence of stressors. A stress-induced increase in erythrocyte indices has been reported by Dobsikova *et al.* (2009), Fazio *et al.* (2015), and Aguirre-Guzman *et al.* (2016). In the current study, there was an increase in the number of erythrocytes after a formalin bath at a concentration of 300 mg L^{-1}, indicating that the bath at this concentration caused stress to the animal. Similar to the present study, Jung *et al.* (2003) reported an increase in the total number of erythrocytes at a concentration of 300 mg L^{-1} formalin for flounder *Paralichthys olivaceus* treated for 3 h and attributed this increase to the formalin, which may have inhibited oxygen transfer in the fish.

Short-term stress can result in an increase in the number of white blood cells (WBCs) (leukocytosis); however, acute stress usually causes leukopenia (Tort, 2011). Stress can also affect differential WBC counts, causing changes in lymphocytes, neutrophils, and monocytes (Aguirre-Guzman *et al.*, 2016; Grzelak *et al.*, 2017). In the current study, there was an increase in the number of lymphocytes after a 300 mg L^{-1} formalin bath when compared to the control, freshwater bath, and 200 and 250 mg L^{-1} formalin baths. According to Şahan (2020), carp *Cyprinus carpio* weighing 51.13 ± 8.18 g and treated for 30 minutes with formalin at 150 mg L^{-1} showed an increase in the number of lymphocytes. Şahan (2020) attributed this increase in lymphocytes to a reaction of the body to therapeutic doses of formalin. In the present study, a difference in this parameter may have occurred above this concentration, caused by the stress that the formalin bath induced in the treated animals.

The hematocrit percentage depends on the number and size of red blood cells and can be affected by several factors, such as water quality, chemical compounds, and infectious diseases (Mcbeath *et al.*, 2015; Witeska *et al.*, 2015). According to Tavares and Moraes (2004), the increase or decrease in hematocrit through stress causes hemoconcentration, which can occur as a result of the release of erythrocytes by the spleen; or hemodilution, which is a decrease in hematocrit values. In the current study, the freshwater bath caused an increase in the hematocrit value compared to the 350 mg L^{-1} formalin bath. In contrast, Prakoso *et al.* (2016) found that hematocrit percentage in the barred knifejaw, *Oplegnathus fasciatus*, exposed to 5 ppm salinity for 24 h, was substantially reduced compared to fish reared at 15, 25, and 35 ppm, indicating that low salinity causes stress to the animals.

In the current study, the freshwater bath did not cause stress, as the hematocrit values showed no statistical difference from the control; however, the hematocrit values of the 350 mg L^{-1} formalin bath were reduced compared to the freshwater bath. In tilapia, *Oreochromis niloticus*, treated with 150 and 250 mg L^{-1} formalin for 1 h, there was a decrease in hematocrit values (Perera; Pathiratne, 2005).

Hemoglobin is often used as an indicator of fish health and is linked to the regulation of immune function (Ali *et al.*, 2017). The increase or decrease of this parameter is related to the type of stressor to which the fish are submitted (Tavares-Dias; Moraes, 2004). In the current study, a 150 mg L^{-1} formalin bath caused an increase in the hemoglobin content compared to freshwater and also showed similar values to the control and other concentrations of formalin. Beevi and Radhakrishnan (1987) found an increase in this parameter in Mozambique tilapia *Sarotherodon massambicus* after a 80 mg L^{-1} formalin bath for 1 h, which may have occurred to compensate for respiratory efficiency impaired by formalin in the treated fish, since this chemotherapeutic drug affects gill function. In contrast to the present study, Perera and Pathiratne (2005) observed that tilapia treated with 150 and 250 mg L^{-1} formalin baths for 1 h had a decrease in this parameter. In the current study, a 150 mg L^{-1} formalin bath caused mild stress compared to a freshwater bath, which had a lower hemoglobin rate and was similar to the control and other treatments. Prakoso *et al.* (2016) reported that levels of this parameter in the barred knifejaw exposed to salinity of 5 ppm for 24 h were substantially reduced compared to values in fish reared at 15, 25, and 35 ppm. Hemoglobin is essential for the transport of oxygen, and exposure to low salinity affects energy demand and oxygen consumption (Morgan; Iwama, 1991). Thus, reduced hemoglobin levels tend to decrease the rate of oxygen consumption and cause stress in fish (Prakoso *et al.*, 2016). In the current study, the freshwater bath did not show a statistically significant difference from the control but may have caused slight stress in treated animals compared to the 150 mg L^{-1} formalin bath.

The MCV, MCH, and MCHC are hematometric indices that can be used for the evaluation and classification of anemia and stress detection. These indices are related to hematocrit and red blood cell number, hemoglobin rate and red blood cell number, and hemoglobin and hematocrit percentages (Wintrobe, 1934).

In the current study, these indices were variable, and after a formalin bath at 300 mg L^{-1}, there was a decrease in MCV, compared to fresh water. Şahan (2020) reported a decrease in this parameter in carp treated with 150 mg L^{-1} formalin for 30 minutes, which might have occurred because formalin causes stress to the animals. Okomoda *et al.* (2010) also reported a decrease in MCV in catfish, *Clarias gariepinus*, treated with 370 and 480 mg L^{-1} formalin for 96 h, which may be indicative of anemia in the animals after treatment. Jung *et al.* (2003), on the other hand, found that there was no marked difference in MCV in sole (*P. olivaceus*) treated with

100, 212, and 300 mg L^{-1} formalin for 3 h. The 300 mg L^{-1} formalin bath showed no statistical difference from the control but may have caused stress in the animals compared to the freshwater bath. There was an increase in MCV in the freshwater bath, relative to the 300 mg L^{-1} formalin bath. No values related to an increase in MCV after freshwater baths have been reported in the literature.

The 300 and 350 mg L^{-1} formalin baths showed lower MCH values than the control and other treatments. In contrast, Chmelova *et al*. (2016) reported no difference in 0.17 ml L^{-1} formalin (38%) in carp *C. carpio* treated for 1 h. The 150 mg L^{-1} formalin caused an increase in MCHC compared to fresh water and 200, 250, and 350 mg L^{-1} formalin. In contrast, Şahan (2020) observed a decrease in MCHC in carp treated with 150 mg L^{-1} formalin for 30 minutes. The freshwater baths indicated a lower MCHC compared to the control and other treatments. In the literature, no value related to a decrease in this parameter after a freshwater bath was found.

Glucose is the main energy source used by fish during periods of acute stress (Mariano *et al*., 2011). According to Bonga (1997), an increase in blood glucose levels is a result of stress in fish, and can be used as a parameter to determine stress levels in animals. In this study, a freshwater bath and formalin at a concentration of 200 mg L^{-1} caused an increase in glucose compared to 150 and 250 mg L^{-1} formalin. Prakoso *et al*. (2016) reported that glucose levels in barred knifejaw exposed to 5 ppm salinity for 24 h were higher than those in fish reared at 15, 25, or 35 ppm. The same authors also reported that glucose levels tended to increase with decreasing salinity. This behavior was associated with low activity in fish exposed to low salinity as a result of increased stress. Tsuzuki *et al*. (2001) and Choi *et al*. (2007) reported that fish experience increased stress when exposed to low salinity. According to Kakuta (1991), there was an increase in glucose levels in carp *C. carpio* after 2 h of exposure to 280 mg L^{-1} formalin. Williams and Wootten (1981) reported that glucose levels in trout *Oncorhynchus mykiss* increased after a 200 mg L^{-1} formalin bath for 1 h because this chemotherapeutic agent causes stress to the fish. In the present study, although no significant difference was found between the freshwater bath and 200 mg L^{-1} formalin, compared to the control, these two treatments caused mild stress compared to 150 and 250 mg L^{-1} formalin.

CONCLUSION

The present study revealed that formalin can be used at the concentration of 150 mg L^{-1} for one hour for therapeutic purposes in fish of the species *M. liza*, because the hematological changes caused were mild due to low glucose concentration, higher hemoglobin concentration and (MCHC). However, greater changes were observed at higher concentrations of formalin. Thus, it is recommended to avoid its use in high concentrations due to its toxicity. Regarding the freshwater bath, the present study found that there were mild hematological alterations in mullet, and its use is recommended.

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