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PROGRAMA DE PÓS-GRADUAÇÃO EM SUSTENTABILIDADE DE ECOSSISTEMAS COSTEIROS E MARINHOS

LAURA SIMÕES ANDRADE

CATCH-AND-RELEASE EFFECTS ON BEHAVIORAL AND PHYSIOLOGICAL PARAMETERS OF THE HIGH RESILIENT TROPICAL WOLF FISH *HOPLIAS MALABARICUS*

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Dissertação apresentada a Universidade Santa Cecília como parte dos requisitos para obtenção de título de mestre no Programa de Pós-Graduação em Sustentabilidade de Ecossistemas Costeiros e Marinhos, sob a orientação da Profa. Dra Helen Sadauskas-Henrique e coorientação do Prof. Dr. Domingos Garrone Neto.

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DEDICATÓRIA

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Dedico este trabalho aos meus pais, ao meu companheiro e a meus familiares que me apoiaram de diversas maneiras durante esta importante etapa de minha vida

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ABSTRACT

Catch-and-release (CandR) activity is rapidly increasing in many countries. Among the fish species appreciated by sport fishing, *Hoplias malabaricus* (trahira) are known for the aggressiveness and sportiness when attacking the baits, making it very appreciated in this modality. However, until our knowledge, no study has been performed evaluating the CandR effects on this fish species. The present study aimed to evaluate the injuries caused by the hook (hook location, bleeding, and facility of hook removal), the mortality predictor (RAMP), measure the physiological parameters (cortisol, glucose, lactate, ionic composition (Na+, K+ and Cl-) and osmolality) in plasma, and evaluate the short-term post-release mortality, of the trahira after fishing simulation with air exposure for 0 s (control), 30 and 60s in two campaigns (September 2018: austral Spring, and April 2019: austral Autumn). The use of the barbless J-hook together with low period of air exposure proved to be efficient for CandR fishing activity of the trahira, once the short-term mortality was low (6.66%) which indicates the fish integrity during the CandR simulation. Also, fish presented low rate of injuries and bleeding, low number of impaired reflexes (low RAMP score), and low effects on fish physiology. No alterations on stress responses (cortisol, lactate and glucose) was observed, only Na+ plasma concentrations increased in relation to air exposure, while differences in RAMP score and K+ plasma concentration were related with the fish weight and the water temperature, but not with the air exposure. Our results increase the knowledge of the CandR activity effects on the trahira and can be useful for the management of fishing tourism, contributing with the dissemination of the best-practices and the sustainability of the activity.

Keywords: sport angling. fishing tourism. mortality. stress. conservation physiology. Erythrinidae.

RESUMO

A modalidade pesque-e-solte, do inglês catch-and-release (CandR) vêm crescendo rapidamente em vários países. Entre as espécies apreciadas por pescadores esportivos, a *Hoplias malabaricus* (traíra) é conhecida por sua agressividade e esportividade quando fisgada, sendo apreciada nesta modalidade. Entretanto, até o nosso conhecimento, nenhum estudo foi realizado para avaliar os efeitos do CandR nesta espécie. O presente estudo buscou avaliar as injúrias causadas pelo anzol (localização do anzol, sangramento e facilidade de remoção do anzol), o preditor de mortalidade (RAMP), medindo parâmetros fisiológicos (cortisol, glicose, lactato, composição iônica (Na^+, K^+ e Cl^-) e osmolalidade) plasmáticos, e avaliar a mortalidade imediata pós soltura, na traíra após a simulação de pesca esportiva com exposição ao ar de 0 s (controle), 30 e 60 s em dois momentos (Primavera e Outono). A utilização do anzol sem farpa do tipo J associada ao pequeno período de exposição ao ar provou ser eficiente para a pesca esportiva da traíra, uma vez que a mortalidade imediata foi baixa (6.66%) o que indica a integridade do peixe durante o CandR. Os peixes ainda apresentaram baixa taxa de injúrias e sangramentos, baixo número de reflexos comprometidos (baixo RAMP score) e baixo efeito na fisiologia dos peixes. Não houve alteração nas respostas ao stress (cortisol, lactato e glicose), apenas a concentração de Na^+ plasmático aumentou em relação a exposição ao ar, enquanto diferenças no RAMP score e na concentração de K^+ plasmático foram relacionados ao peso dos peixes e a temperature da água, mas não com exposição ao ar. Nossos resultados aumentam o conhecimento dos efeitos do CandR na traíra e pose ser útil na gestão do turismo de pesca, contribuindo com a disseminação de boas práticas e a sustentabilidade dessa atividade.

Palavras-chave: pesca esportiva. Turismo de pesca. mortalidade. stress. Fisiologia da conservação. Erythrinidae

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LISTA DE ABREVIATURAS E SIGLAS

A.	-	*Astyanax*
ANOVA	-	Análise de variância
C	-	N obtido
CandR	-	*Cacth* and *release*
cm	-	Centímetro
CT	-	Comprimento total
°C	-	Graus Celsius
FAO	-	Food and Agriculture Organization of the United Nations
g	-	grama
h	-	hora
H.	-	*Hoplias*
IBAMA	-	Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
ICMBIO		Instituto Chico Mendes de Conservação da Biodiversidade
MDIC	-	Ministério da Industria, Comércio Exterior e Serviços
mg/dL	-	Miligrama por decilitro
mg/L	-	Miligrama por litro
Min.	-	minuto
ml	-	mililitro
mm	-	milímetro
mmol/L	-	Milimolar por litro
ms/cm	-	milisiemens por centímetro
N	-	Número de indivíduos por tratamento
N°	-	Número
nm	-	nanômetro
NPCFR	-	*National Parks of Canada Fishing Regulations*
p	-	peso
r	-	Coeficiente de determinação (r^2)
RAMP	-	*Reflex Action Mortality Predictors*
RPM	-	Rotação por minuto

s	-	segundos
SEM	-	Erro padrão da média
SISBIO	-	Sistema de Autorização e Informação em Biodiversidade
ssp	-	Espécies
VOR	-	*vestibular-ocular response*
WDFW	-	*Washington Department of Fish* and *Wildlife*
µL	-	microlitro
x^2	-	Chi-quadrado

LISTA DE SÍMBOLOS

%	-	Porcentagem
º	-	Graus
±	-	Mais ou menos
-	-	Menos
=	-	Igual
x	-	multiplicação
/	-	divisão
X^2	-	Chi-quadrado
α	-	alfa
$<$	-	Menor que

SUMÁRIO

INTRODUCTION

In recreational fishing, catch-and-release (CandR) is a practice that occurs when the fish is caught by hook and line, rods and reels and then released back to the water, presumably to survive and promote a sustainable practice of the activity (Arlinghaus *et al.*, 2013). With the increasing interest in CandR around the world, there was a need to evaluate the integrity of the post-release effects on fish, since recreational fishing could affect fish stocks, alter food chains through the selective collection of predators, contribute to habitat and wildlife disturbance (Cooke and Cowx, 2004, 2006; Post *et al.*, 2011; Thompson *et al.*, 2018). It is also known that CandR effects can be related to biotic (life-history stages, sex, sensibility to stress responses, robustness of a target species etc.) and abiotic (e.g. temperature; dissolved oxygen) factors (McLean *et al.*, 2016; Twardek *et al.*, 2018; Roth *et al.*, 2018), which requires species-specific evaluations to the development of best practices and to the contribution to resources protection and long-term sustainability.

Several studies have already been carried out evaluating the effects of CandR on fish, including: hook location, type of hook and rate of hook injury (Cooke *et al.*, 2003; Kerr *et al.*, 2016; Thompson *et al.*, 2018); fisher's experience (Meka, 2004; Dunmall *et al.*, 2016); fish size (Meka, 2004; Lennox *et al.*, 2016); fight time (Meka, 2004; Lennox *et al.*, 2018); post-release behavior (Schreer *et al.*, 2005; Lennox *et al.*, 2018); observation of mortality predictors (RAMP), and physiological measures such as cortisol, glucose and lactate (Campbell *et al.*, 2010; Lennox *et al.*, 2015; Bower *et al.*, 2016); assisted ventilation techniques to facilitate physiological and behavioral recovery (Brownscombe *et al.*, 2016); effects of post-release mortality (Cooke *et al.*, 2003; Campbell *et al.*, 2010; Thomé-Souza *et al.*, 2014; Lennox *et al.*, 2015; Lennox *et al.*, 2017); risk of post-release predation (Campbell *et al.*, 2010); effects of air exposure (Lennox *et al.*, 2015; McLean *et al.*, 2016; Roth *et al.*, 2018a, 2018 b; Twardek *et al.*, 2018) and temperature (Gingerich *et al.*, 2007; Thompson *et al.*, 2008; Mclean *et al.*, 2016; Roth *et al.*, 2018a). However, although the literature about CandR is vast, most studies are focused on popular fish of Australia, Canada, the United States and Europe (Freire, 2012). For developing countries such as Brazil, studies on CandR practices are still scarce, corresponding to an important gap to be filled (Thomé-

Souza *et al.*, 2014; Lennox *et al.*, 2015; Barroco *et al.*, 2017; Lennox *et al.*, 2018).

The use of reflex action mortality predictors (RAMP) is becoming an approach very used for evaluating the CandR effects on fish (Davis, 2010; Campbell *et al.*, 2010; Lennox *et al.*, 2015; Bower *et al.*, 2016). The RAMP is an indicator of the fish vitality (i.e. capacity for survival), measured through the presence/absence of some specific reflexes, normally present on fish under non-stress conditions. A final RAMP score is calculated and used as a proxy for compromised fish wellness (Davis, 2007 and 2010). For some fish species, such as red snapper (*Lutjanus campechanus*), golden dorado (*Salminus brasiliensis*) and white sturgeon (*Acipenser transmontanus*), RAMP score successfully predicted post-release mortality (Campbell *et al.*, 2009; Gagne *et al.*, 2016; McLean *et al.*, 2016). On the other hand, RAMP score was unsuccessful when predicting post-release mortality for fat snook (*Centropomus parallelus*) and wild steelhead fish (Lennox *et al.*, 2015; Twardek *et al.*, 2018). Physiological assessment of fish species can also be considered a useful tool to evaluate the stress, which together with the RAMP score, could bring to light a scheme regarding the post-release wellness and mortality. For example, for some target fish species, alterations on the physiological parameters (e.g. glucose, lactate and cortisol) was observed and correlated with the RAMP score and post-release mortality (McLean *et al.*, 2016). All these information supports the need for species-specific studies, with context-specific assessment of the techniques.

Fish exposed to some CandR stressful situations (e.g. air exposure; handling stress) can experience increases in the primary and secondary stress responses (Barton, 2002; Thompson *et al.*, 2008; Brownscombe *et al.*, 2015). The primary physiological responses to stress, consists on the release of adrenaline or catecholamines (Gingerick and Drottar, 1989) to blood stream, which will increase the plasma cortisol levels (Wendelaar Bonga, 1997; Barton *et al.*, 2002; Schreek and Tort, 2016). The secondary response to stress consists of the release of glucose from the liver into the blood stream, resulting in hyperglycemia (Wendelaar Bonga, 1997; Barton *et al.*, 2002; Schreek and Tort, 2016). This glucose can be synthesized through lactate (Momnsen *et al.*, 1999), due to the increasing energy demand during the stress. Stress also directly affects the hydromineral balance in fish deregulating levels of potassium (K^+), sodium (Na^+) and chloride (Cl^-) (Wendelaar Bonga, 1997), which can impair the osmo-ionic functions.

Moreover, exposure of fish to air associated with elevated water temperatures can lead to increased physiological and behavioral disturbances, such as an increase in the metabolic rate (Somero and Hofmann, 1996; Twardek *et al.*, 2018), increased heart rate (Anderson *et al.*, 1998; Prystay *et al.*, 2017), biochemical changes in blood and muscle (Brownscombe *et al.*, 2015; Lennox *et al.*, 2015; Prystay *et al.*, 2017) and lack of movement or loss of balance (Danylchuk *et al.*, 2007), which can increase the probability of immediate or late mortality during the CandR events (Cooke and Suski, 2005; Arlinghaus *et al.*, 2007; Gingerich *et al.*, 2007; Raby *et al.*, 2015). Thus, the interaction between air exposure and high temperatures is relevant when trying to understand the effects of CandR (Cooke and Schramm, 2007).

The tropical wolf fish (also named trahira), *Hoplias malabaricus* (Characiformes, Erythrinidae) (Bloch, 1794), is a medium-sized fish (up to 50-60 cm) widely distributed through the freshwater systems of South America, from Costa Rica to Argentina, with the exception of the west of the Andes and in rivers of Patagonia (Oyakawa, 2003, 2006; Buckup *et al.*, 2007). It is a species with a high physiological plasticity, occupying from small lakes with low dissolved oxygen levels to brackish water environments (Cameron and Wood, 1978; Fernandes *et al.*, 1994; Oyakawa, 2003; Menezes *et al.*, 2007). *Hoplias malabaricus* is part of a group of freshwater predators economically important for local fishers in many Neotropical countries (Balboni *et al.*, 2011; Santos *et al.*, 2018). The trahira is also targeted by recreational fishing through South America, especially in Brazil, where *H. malabaricus* is cited as one of the main native species caught by anglers in inland waters (Freire *et al.*, 2012). However, assessments of the CandR effects on *H. malabaricus* are not available in the literature.

Within this background, the objectives of this study were to (i) evaluate the injuries caused by the hook (hook location, bleeding, and facility of hook removal); (ii) evaluate the mortality predictor (RAMP); (iii) measure the physiological parameters (cortisol, glucose, lactate, ionic composition (Na^+, K^+ and Cl^-) and osmolality) in plasma, and (vi) evaluate the short-term post-release mortality, of the trahira (*Hoplias malabaricus*) after sport fishing simulation with air exposure for 0s (control), 30 and 60s in two campaigns (Spring and Autumn).

MATERIAL AND METHODS

ANGLING EXPERIMENTS

Individuals of *Hoplias malabaricus* (weight: 507.47 g ± 52.90 g; total length: 33.67 cm ± 1.56 cm) mean ± std; N= 60) were collected during day, in two campaigns (September 2018: austral Spring, and April 2019: austral Autumn), in three lakes located in Registro, São Paulo, Southeastern Brazil (24°30’48.62”S, 47°52’03.87”W) (Figure 1). Fish were caught with simple bamboo rods equipped with line (nylon 40 mm) and hook (“J” barbless hook nº 3/0), using live baits (tetras of the genus Astyanax), in an attempt to simulate one of the most traditional fisheries that is directed to *H. malabaricus* in Brazil.

Field work submitted fish to three different treatments: control, without air exposure (control, treatment 1), and air exposure handling experiments for 30 (treatment 2) and 60 seconds (treatment 3) (Table 1). Air exposure periods were selected following Lennox *et al.* (2015) and adapted to the real handling time usually spent by anglers (Roth *et al.*, 2018b), aiming to provide best practices for CandR. For all treatments, immediately after capture, fish were evaluated for hooking location (critical: eyes; gills; and esophagus, and non-critical: upper jaw and lower jaw), hooking removal (easy, hard and impossible) and presence of injuries (bleeding and/or other indicator of tissue damage).

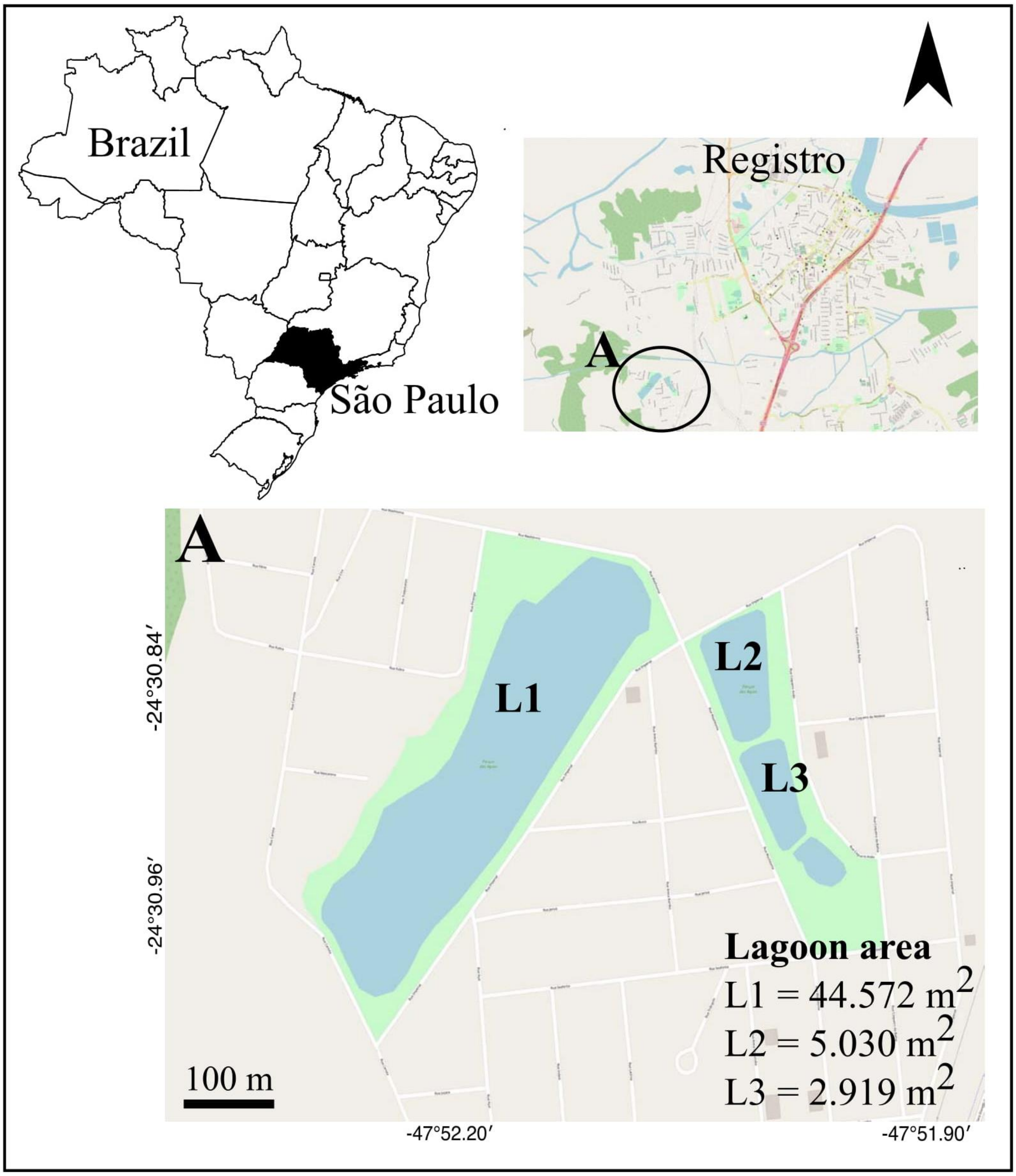

Figure 1. Map of the lagoons (L1, L2 and L3) where the angling experiments take place. m^2= square meters of each lagoon.

After treatment, fish were measured (total length, cm) and weighted (g) using ichtiometers and wet bags, respectively, filled with water to minimize stress and mucus removal. Fish were then identified with an external T-bar anchor tag (Floy Tag and Mfg. Inc., Washington, USA), had a blood sample collected through caudal vein puncture using heparinized needles and syringes, and evaluated through a reflection action prediction mortality test (RAMP) (Figure 2 and 3). RAMP is a fast and low-cost tool, easily applied in the field, which measures the vitality and indicate the possibility of a fish survival after CandR (Davis, 2010).


A
B
C
D
E
F
G

Figure 2. Step-by-step of all procedures performed on the traira (*Hoplias malabaricus*) during the angling experiments. A: capture of the fish and transference to the ichthyometer; B: blood collection; C: allocation of the external *T-bar* anchor tag in the dorsal musculature; D: total length measurement into an ichtiometer filled with lagoon water; E: weighing into wet bags; F: RAMP evaluation into livewell placed in the lagoon; G: releasing of the fish.

For *H. malabaricus*, a modified protocol for RAMP was used, excluding one of the five reflexes usually tested (Tail-grab; Body-complex; Head-complex; Orientation/Equilibrium and Vestibular ocular response – VOR). The VOR was absent in all fish used for treatment 1 (control), which demonstrated low applicability for *H. malabaricus*. Thus, fish were evaluated for the four remaining reflexes, being classified as unimpaired (0) and impaired (1) (Figure 3). A RAMP score was then calculated, based on a simple proportion of the four measured reflexes that were impaired in an individual (i.e., 0 = no reflexes impaired; 0.25 = one reflex impaired; 0.5 = two reflexes impaired; 0.75 = tree reflexes impaired; and 1= four reflexes impaired). In some cases (and in all treatments), fish were too vigorous to allow the complete testing of reflexes, being considered as unimpaired. However, in cases in which fish presented high RAMP score, animals were kept into a livewell for recovery before release. The short-term mortality was evaluated during the fishing experiments.

In addition, water temperature (°C) was measure three times a day during the angling experiments (morning, noon and twilight), using a multiparameter probe (Ysi® Pro 2030), to test its eventually influence in the RAMP results in relation to the two campaigns (i.e. Spring and Autumn).

Figure 3. Four reflexes tested on the traira from control and air exposure handling experiments for 30 and 60 s: A: *Tail-grab*, B: *Head-Complex*, C: *Body-complex*, D: *Orientation*.

For the physiological analysis, after collection, blood samples were kept on ice and immediately centrifuged for plasma separation (5000 rpm, 10 min, room temperature). Plasma were store in -20°C until physiological analysis in laboratory (cortisol; glucose; lactate; Na+, Cl-, K+, and osmolality). Cortisol

(Diagnostics Biochem Canada Inc.), glucose, lactate and chloride (Labtest®, Brazil) were determined through colorimetric commercial kits in undiluted plasma samples. The Na+ and K+ were determined in plasma samples diluted (1:200) using a flame photometer (Quimis Q498M). Plasma osmolality was determined in samples without dilution, using a vapor pressure micro-osmometer (Wescor 5600, VAPRO®). The choice of using laboratory commercial kits for measuring glucose instead of using a portable equipment (Accutrend Plus Roche® monitor), which is widely used in studies evaluating the CandR effects on non-neotropical (McLean *et al.*, 2016; Twardek *et al.*, 2018), and neotropical fish species (Danylchuk *et al.*, 2014; Lennox *et al.*, 2015; Bower *et al.*, 2016) was due the device's detection limit be much higher (3.88 mmol/L) than the glucose in trahira's blood.

The experimental procedures followed Brazilian Animal Care guidelines and was previously approved by UNISANTA's Animal Care Committee (Registration number 07/2018) and by the Brazilian National Institute for Biodiversity Conservation (Registration number ICMBio/SISBIO 63102-1/2018).

STATISTICAL ANALYSES

All data were reported as means ± std (n = 10). Statistical significance was accepted at $P < 0.05$. Significant differences among treatments in RAMP score were determined through two-way repeated measures ANOVA (factors: campaigns x treatments), followed by a posteriori Holm-Sidak multiple comparison test. The effect of the treatments on the physiological and behavioral parameters responses (cortisol, glucose, lactate, Na+, K+, Cl-, osmolality and RAMP score) was evaluated using a mixed model analysis of covariance (ANCOVA), temperature and weight was treated as covariables. Also, in the same analysis was possible to verify the influence of the fish weight and water temperature on the physiological and behavioral responses between the treatments. When differences between treatments were found, a posteriori Dunnet test was applied. To attempt the test premises, the residuals were tested for homoscedasticity and normality. To correct patterns in the residuals the physiological and behavioral parameters as well as the fish weight and water temperature were log transformed. T-test was performed between the two campaigns for the RAMP score and temperature. Qui-square (X2) test was used to verify the relation between RAMP score and the presence of injuries and bleeding. All analysis was performed employing the R statistical software (R version 3.1.3; R Core Team, 2015). All graphs employed Sigma Plot 11.0 software (Jandel Scientific; www.systatsoftware.com).

RESULTS

ANGLING EXPERIMENTS

During the Spring, the water temperature ranged from 22.3 to 28.8°C (mean of 24.58°C ± 0.44), and during the Autumn, the water temperature ranged from 25°C to 32°C (mean of 27.71°C ± 0.64), with difference between the campaigns (P = 0.001) (Table 1). Fish weight for Spring and Autumn, respectively, ranged from 180 to 1225 g (628.0 g ± 119.2) and 70 to 635 g (321 g ± 66.0) for the control group; from 80 to 1110 g (439.0 g ± 107.24) and 110 a 2005 g (774 g ± 186.3) for the group exposed to air for 30s; and from 115 to 1395 (432.5 g ± 118.2) and 58 to 1215 g (449 g ± 128.16) for the group exposed to air for 60s (Figure 4B). Besides these numerical differences between the treatments and campaigns for the fish weight, no statistical differences were found (P= 0.388 for treatments, and P= 0.883 for campaigns). Once fish were capture with the same fishing equipment in all treatments (control and air exposure handling experiment for 30 and 60s) and during the two campaigns, the anatomical location of the hook; hook injuries and bleeding were presented together instead per treatment per campaign.

Table 1. Water temperature (mean ± std) and number of fish (n) *per* treatment 1 (control, without air exposure), 2 (air exposure for 30s) and 3 (air exposure for 60s) *per* campaign (Spring and Autumn). *represents statistical differences between campaigns.

	Water temperature (°C)	Treatment 1 (without air exposure)	Treatments with air exposure (seconds)	
			Treatment 2 (30s)	Treatment 3 (60s)
Spring	24.58 ± 0.44	n = 10	n = 10	n = 10
Autumn	27.71 ± 0.64*	n = 10	n = 10	n = 10

For the anatomical location of the hook, 46 (76.6%) fish were hooked in non-critical locations (upper jaw and lower jaw), and 14 fish (23.3%) were hooked at critical locations (nine fish hooked in the eye, two in the gills, another two in the esophagus and one in the tongue). Two fish hooked in the esophagus were classified as deeply hooked which one hook was classified as impossible to remove and the other as difficult, requiring the use of pliers for removal. The removal of the hook was classified as easy for 53 (88.3%) fish, as difficult for six (10%), and as impossible for one (1.6%). The fish classified as impossible removal died during the angling experiments. In relation to injuries and bleeding, three (5%) fish had injuries without bleeding, while 13 (21.6%) had injuries with bleeding at hook location.

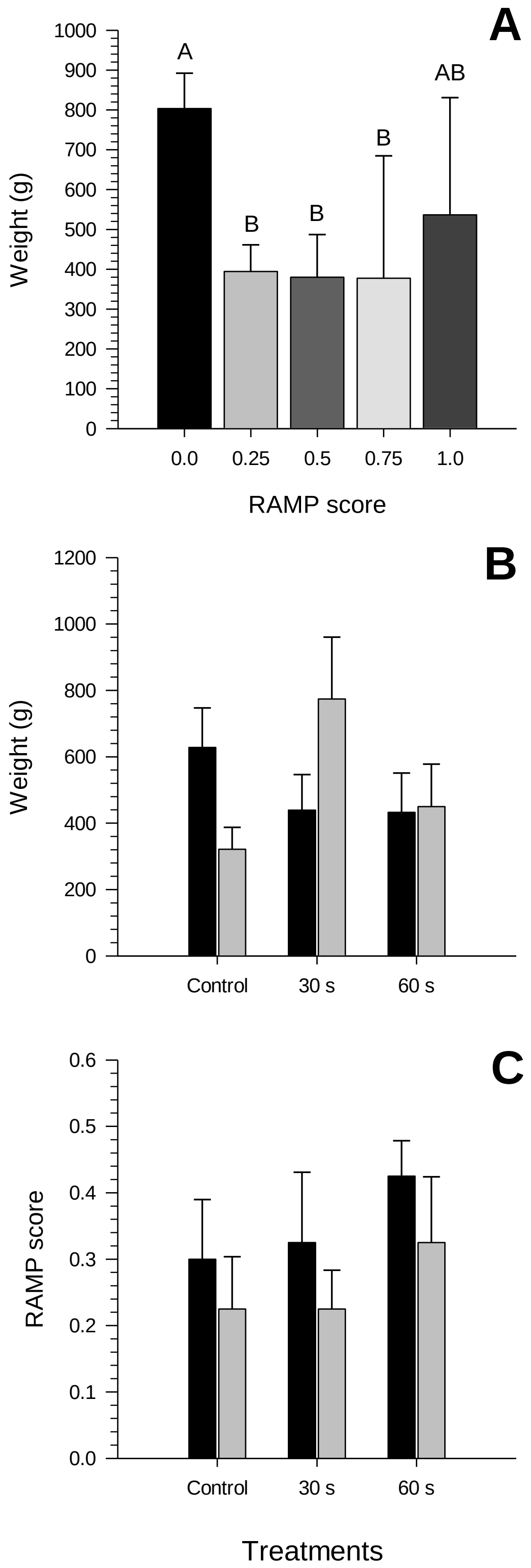

Figure 4. RAMP results. (A) Fish weight (mean ± std) per RAMP score category (0= no reflex impaired; 0.25= one reflex impaired; 0.5= two reflexes impaired; 0.75= tree reflexes impaired; and 1.0= four reflexes impaired). (B) Fish weight per treatment. (C) RAMP score per treatment: 1 (control), 2 (air exposure for 30s) and 3 (air exposure for 60s). Spring (black) and Autumn (gray). Uppercase letters represent statistical differences between treatments.

REFLEX ACTION MORTALITY PREDICTORS (RAMP)

Forty-three (71.6%) of the 60 fish caught exhibited reflexes impairment (Table 2). Head-complex and body-flex were the most commonly impaired reflexes. When considering only the influence of the water temperature (Spring x Autumn), of the 30 fish caught in the Spring, seven fish (23.3%) had no reflexes compromised, while 23 fish (76.6%) had compromised reflexes (mainly body-flex and head-complex). In the Autumn, ten fish (33.3%) had no reflexes compromised, while 20 (66.6%) had compromised reflexes (mainly body-flex and head-complex). The RAMP score for the Spring was 0.35 ± 0.04 and for the Autumn was 0.26 ± 0.04 (N=30 per campaign), with no differences between campaigns (P=0.164) (Table 2).

Table 2. Number of fish (n) in each RAMP score category that presented their reflexes impaired *per* campaign, and RAMP score *per* campaign. 0 = no reflex impaired; 0.25 = one reflex impaired; 0.5 = two reflexes impaired; 0.75 = three reflexes impaired; 1 = four reflexes impaired.

Campaign/ number of fish	RAMP score categories/number of fish in each RAMP category	Reflexes				RAMP score
		Tail-grab	*Body-flex*	*Head-complex*	*Orientation/ Equilibrium*	
Spring/(n=30)	0/(n=7)	0	0	0	0	0.35 ± 0.04
	0.25/(n=8)	0	2	6	0	
	0.5/(n=13)	0	12	13	1	
	0.75/(n=0)	0	0	0	0	
	1.0/(n=2)	2	2	2	2	
Total of fish	30	2	16	21	3	
Autumn/(n=30)	0/(n=10)	0	0	0	0	0.26 ± 0.04
	0.25/(n=10)	0	5	5	0	
	0.5/(n=7)	4	5	4	1	
	0.75/(n=2)	2	2	1	1	
	1.0/(n=1)	1	1	1	1	
Total of fish	30	7	13	11	3	

When considering the influence of the treatments in relation to water temperature, for the Spring, the RAMP score were 0.30 ± 0.08; 0.32 ± 0.10; 0.42 ± 0.05, and for the Autumn were 0.25 ± 0.07; 0.25 ± 0.05; 0.32 ± 0.09 in the control and air exposure handling experiment for 30 and 60s, respectively (n=10) (Figure 4C). Despite the numerical increases in the RAMP score, no statistical differences were found between the treatments (F= 0.08; P= 0.91). However, the fish weigh (F= 10.53; P= 0.002) and the water temperature (F= 9.72; P= 0.002) had significant contributions for RAMP scores which was not related with the treatments, as highlighted by the ANCOVA (Table 3). This relation was clear when we plotted the fish weight against the RAMP score (Figure 4A), where decreases in weight were found between 0 and 0.25; 0.5; and 0.75 RAMP categories (Figure 4A). On the other hand, no differences were found between the RAMP score and the presence of bleeding (X^2 = 1.57, df= 4, P= 0.813) or injuries (X^2= 0.48, df= 4, P= 0.975). The Linear Regression was positive for RAMP score and temperature (Log(Ramp) = -3,851 + (2,486 * logtemp, N = 60, R = 0,336, P= 0.009).

Table 3. Values of F and P for the covariables (weight and temperature) and treatment for each dependent variable (cortisol, glucose, lactate, Na^+, K^+, Cl^-, osmolality and RAMP score) obtained through the multiple regression model analysis. Bold values represent significant correlations.

	Cortisol	Glucose	Lactate	Na^+	K^+	Cl^-	Osmolality	RAMP score
Weight	F= 1.71 P= 0.19	F= 3.51 P= 0.06	F= 0.98 P= 0.93	F= 0.25 P= 0.62	F= 15.03 **P= 0.0002**	F= 2.49 P= 0.12	F= 0.02 P= 0.088	F= 10.53 **P= 0.002**
Temperature	F= 1.21 P= 0.27	F= 2.78 P= 0.10	F= 0.09 P= 0.75	F= 0.23 P= 0.62	F= 17.15 **P= 0.0001**	F= 0.66 P= 0.42	F= 0.53 P= 0.47	F= 9.72 **P= 0.002**
Treatments	F= 0.02 P= 0.97	F= 0.48 P= 0.62	F= 0.12 P= 0.89	F= 3.63 **P= 0.03**	F= 0.47 P= 0.62	F= 0.42 P= 0.66	F= 1.17 P= 0.31	F= 0.08 P= 0.91

EFFECTS OF THE TEMPERATURE AND AIR EXPOSURE ON PHYSIOLOGICAL PARAMETERS

No differences in physiological parameters (cortisol, glucose, lactate, K+, Cl-, osmolality) were found between the treatments (Table 3). Only Na+ plasma concentrations (mmol/L) had significant increases in fish air exposed for 30 and 60s (Figure 5). Potassium plasma concentrations (mmol/L) were influenced by the water temperature (F= 17.15; P= 0.0001) and fish weight (F= 15.03; P= 0.0002), but not by the treatments (Table 3).

Table 4. Mean values (mean ± std) of cortisol (ng/ml), glucose (mmol/L), lactate (mmol/L), Na^+ (mmol/L), K^+ (mmol/L), Cl^- (mmol/L) and osmolality (mOsm/KgH_2O) of *Hoplias malabaricus* individuals *per* campaign (Spring and Autumn) *per* Treatment: 1 (control), 2 (air exposure for 30s) and 3 (air exposure for 60s).

	Spring			**Autumn**		
	Control	30s	60s	Control	30s	60s
Cortisol (ng/ml)	63.27 ± 24.34	43.90 ± 14.86	62.88 ± 21.35	53.79 ± 15.80	66.23 ± 19.60	52.24 ± 17.99
Glucose (mmol/L)	2.47 ± 0.15	2.36 ± 0.06	2.36 ± 0.14	2.59 ± 0.08	2.69 ± 0.10A	2.53 ± 0.09
Lactate (mmol/L)	1.76 ± 0.37	2.69 ± 0.70	2.99 ± 0.68	1.63 ± 0.34	1.45 ± 0.35	1.42 ± 0.32
Na^+ (mmol/L)	146.81 ± 2.05	151.38 ± 2.99	156 ± 2.11	148 ± 1.96	156.58 ± 2.41	150.81 ± 1.93
K^+ (mmol/L)	4.35 ± 0.42	5.08 ± 0.42	4.63 ± 0.18	5.66 ± 0.23	5.36 ± 0.33	5.89 ± 0.49
Cl^- (mmol/L)	93.22 ± 6.15	98.80 ± 3.79	96.92 ± 2.81	93.54 ± 3.53	97.77 ± 7.24	97.77 ± 4.46
Osmolality (mOsm/KgH_2O)	283.2 ± 4.26	294.3 ± 8.40	309.9 ± 9.22	307.3 ± 3.13	301.3 ± 6.29	303.3 ± 4.28

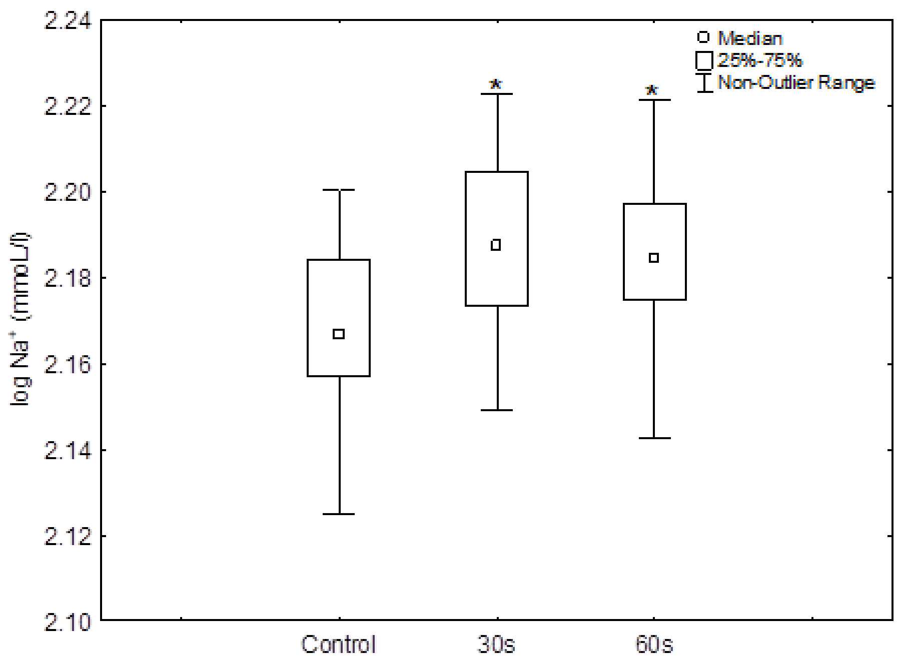

Figure 5. Plasma Na^+ concentration in *Hoplias malabaricus per* treatment: 1 (control), 2 (air exposure for 30s) and 3 (air exposure for 60s). *represents statistical differences between treatment 1 and treatments 2 and 3.

SHORT-TERM MORTALITY

Of the sixty fish caught, only four died during the angling experiments, two in the Spring and two in the Autumn. The two fish from the Spring, one was from control group, which was hooked by the gills, leading to serious injury without bleeding (weighted 285 g, with a RAMP score= 0.5), followed by death after 20 hours. The other fish was from air exposure handling experiment for 30s, were fish was deeply hooked, with the impossible removal of the hook (weighted 460 g, with a RAMP score= 1). Despite this, this fish already presented a serious injury in the side of the body when it was hooked, that together with the injury in the esophagus, caused its death after, approximately, thirty minutes of its capture. During the Autumn, the two death fish were from the air exposure handling experiment for 60s. One fish was hooked by the jaw (weighted 70 g, with a RAMP score= 1), causing immediate mortality, and the other hooked by the gill with injury and bleeding at the site of the hook (weighted 685, with a RAMP score= 0.75), causing immediate mortality. The water temperature at the time of catching the fish that died in the autumn was 29.6°C. Therefore, within 6 days of angling experiment in the two campaigns, 93.33% of the trahira survived. The average RAMP scores were higher for these fish (RAMP score mean= 0.87 ± 0.12) than in the fish that survived (RAMP score mean= 0.30 ± 0.03).

DISCUSSION

In the present work, the use of the barbless J-hook together with low period of air exposure proved to be efficient for CandR fishing activity of the trahira, once the short-term mortality was low (6.66%) which indicates the fish integrity during the CandR simulation. Also, fish presented low rate of injuries and bleeding, low number of impaired reflexes (low RAMP score), and low effects on fish physiology. No alterations on stress responses (cortisol, lactate and glucose) was observed, only Na+ plasma concentrations increased in relation to air exposure, while differences in RAMP score and K+ plasma concentration were related with the fish weight and the water temperature, but not with the air exposure. Interesting to mention a recapture of one trahira after seven months of its marking. The fish was health and had an increment of 100 g in weight. Even though this fish was excluded from the sampling, once the multiple recaptures was not proposed, this is an important finding that highlights the importance of best practices during CandR activity for fish conservation.

The survival of about 93% of the post-released trahira is in accordance with other studies that evaluated the CandR fish mortality related to air exposure. Survival of about 90% was reported by other studies with temperate (Muoneke and Childress, 1994; Bartholomew and Bohnsack, 2005) and neotropical fish species (Thomé-Souza et al, 2014; Barroco et al, 2018; Lennox *et al.*, 2018). According to Bartholomew and Bohnsack (2005), the main cause of post-release mortality is related with the anatomical position of the hook, type of the hook, presence of injuries and bleeding, time of air exposure, and environmental factors such as water temperature. Of the four fish that died during this study, three were caught in vital organs (gills and deeply hooked) with presence of injuries, corroborating with several authors (Muoneke and Childress, 1994; Cooke *et al.*, 2003; Thomé-Souza et al, 2014; Barroco *et al.*, 2018). It is interesting to note that from the four fish that died, only one was hooked in non-critical location (lower jaw, without injury). This was a small fish, measuring 20 cm. Mortality related to size was also reported for striped bass (19.2% for fish lower than 61.5 cm) (Millard *et al.*, 2005), and for three species of the neotropical peacock bass (3.5% for *Cichla temensis*; 2.3% for *C. orinocensis*; and 5.2% for *C. monoculus* for fishes lower than 42 cm) (Thomé-Souza *et al.*, 2014). These results highlight the importance of having normative instructions for a minimal capture size during CandR activity. Normally, this is based on the size that 50% of the fish species population reach the maturation (L50). For some studies performed in South America lakes and rivers, the L50 for the trahira can varies from 15-33 cm (Marques *et al.*, 2007; Lima *et al.*, 2017). Anyway, the L50 can change between fish genotype and environment (Wootton, 1992). In Brazil, where this study took place, the minimal capture size for CandR follows regional regimentations. For example, Minas Gerais; Goiás and Paraná States, have minimal capture size of 30 cm for H. malabaricus (Portaria IEF N° 111, 2003; Lei n° 19.337, 2016; Portaria IAP N° 135, 2018), while São Paulo state has no size restrictions for this fish species.

The RAMP proved to be efficient for the rapid evaluation of neurological and/or muscular impairments caused by the CandR on the trahira, once this endpoint had never been performed on this fish species before. From the five reflexes (Tail-grab; Body-complex; Head-complex; Orientation and Vestibular ocular response (VOR)), only VOR was not tested, because this parameter was no viable due to the behavior of the species. Similar fact was reported by Bower *et al.*, (2016), when evaluating the effects of amateur fisheries on tucunaré (*Cichla ocellaris*) in Puerto Rico. Moreover, in the present study, we observe that the head-complex may not be representative of the trahira vitality, once this reflex was absent in fish from all treatments, even in control. Moreover, the fish from control that had only this reflex absent, looks health and vigorous to allow for complete testing of reflexes. This way, the head-complex did not looks to be a good reflex mortality predictor for the trahira, highlighting the importance of adopting a species-specific approach when studding the CandR effects. The trahira of the control for both campaigns also showed alterations of other reflexes, such as body flex, demonstrating that, since the hooking moment, these fish suffered commitment of their reflexes. It is important to mention that from the four fish that died during the angling experiment, one was from control group in the Spring, hooked by the gills with injurie and high impaired reflexes (RAMP scores = 0.5). The same way, Lennox *et al.*, (2015) and Bower *et al.*, (2016) found compromised reflexes in control fish (mostly body-flex and tail grab and body-flex), while McLean *et al.*, (2016) did not find any compromised reflex in withe sturgeon (Acipenser transmontanus) from fish not air exposed. Although the RAMP score was not different between the treatments and between the campaigns, the fish air exposed for 60s showed the highest RAMP scores in both campaigns. Other studies also found increases in the number of compromised reflexes in fish air exposed (Humborstad *et al.*, 2009; Raby *et al.*, 2012; Bower *et al.*, 2016; Twardek *et al.*, 2018), which indicates muscular exhaustion and cognitive impairment (Raby *et al.*, 2012). On the other hand, the positive and significant relation between RAMP score and temperature (Log(Ramp) = -3,851 + (2,486 * logtemp, N = 60, R = 0,336, P= 0.009) showed that fish caught at higher temperatures presented more compromised reflexes, which was not related with the air exposure (Table 4). The same way, Gingerich *et al.*, (2007) and McLean *et al.*, (2016) found more compromised reflexes in fish caught at higher water temperatures. The water temperature is one of the most prominent factors affecting fish survival and stress associated with CandR events (Bartholomew and Bonhsack, 2005; Arlinghaus *et al.*, 2007). Fish caught at elevated water temperatures could present high percentage of injuries (Arlinghaus *et al.*, 2007), physiological disturbances (Gustaveson *et al.*, 1991; Cooke and Suski, 2005), like alterations in blood biochemistry (Wilkie *et al.*, 1997); behavior changes, like lack of movement or equilibrium (Danylchuk *et al.*, 2007); and increased mortality (Wilkie *et al.*, 1997; Kraak *et al.*, 2018).

The same way for the temperature, the fish weight seemed to play an important role during the CandR angling experiments, but they had counter effects. While a positive relation between water temperature and RAMP score was observed, a negative relation for fish weight and RAMP score took place. In other words, smaller fish had greater commitment of their reflexes than larger fish. According to Davis (2010), impaired performance, measured through RAMP score, may be associated with factors not related to health, but with size. Anyway, this relationship was not found for other fish species. For example, some studies evaluated the consequences of CandR on physiological and behavioral responses of rainbow trout (*Oncorhynchus mikiss*) (Twardek *et al.*, 2018), white sturgeon (*Acipenser transmontanus*) (McLean *et al.*, 2016), and shortnose sturgeon (*Acipenser breviostrum*) (Struthers *et al.*, 2018) and no relationship between RAMP score and weight was observed. Although the relationship between the RAMP score and weight was not found by other authors, this result highlights the importance of establishing additional care with smaller fish, contributing to best practices and sustainability of this fishing modality, since smaller fish are more sensitive to capture stress (Davis, 2002), as already discussed.

Stressful situations, like air exposure; handling; alterations of the physical and chemicals water variables, can activate mechanisms of the general stress responses in fish. The primary and secondary stress responses consist in the rapid liberation of hormones in blood stream, such as catecholamines and cortisol, which is linked with increases in glucose and lactate levels. These responses are related with the increasing ability to cope with the stress and keep the homeostasis during challenging situations (Wendellar Bonga and Lock, 1992). In the present work, no increases in cortisol, glucose and lactate were observed in traira air exposed for 30 and 60s, anyway, the levels of these parameters were similar with found in the literature for other neotropical fish species (Bower *et al.*, 2016; Gagne *et al.*, 2016; McLean *et al.*, 2016), except for glucose. For the trahira, glucose values from control fish at two campaign was low (~ 2.5 mmol/L) compared to the basal values of other neotropical fish species (around 3.8 mmol/L for *Brycon cephalus*, *Centropomus parallelus* and *Colossoma macropomum*) (Tavares-Dias *et al.*, 1999; Gomes *et al.*, 2003; Lennox *et al.*, 2015), but were higher than the basal values of a fish species from the same genus, *Hoplias unitaeniatus*, (~ 0.646 mmol/L) (Mariano *et al.* 2009) and similar with values found for trahira by Sakuragui (2006) (~ 1.8 mmol/L). Even though cortisol, glucose and lactate levels be effective indicator of stress in events such as sport fishing (Thompson *et al.*, 2008; Campbell *et al.*, 2009; Gagne *et al.*, 2016; McLean *et al.*, 2016; Barton, 2002; Twardek *et al.*, 2018), other authors also did not find increases on these parameters on fish under CandR events (Bower *et al.*, 2016; Roth *et al.*, 2018a). Normally, these physiological effects took place when fish species were submitted to air exposure for prolonged periods. Anyway, besides these high air exposure periods be more believable to affecting the fish physiology, they are not representative. Recent works (Cook *et al.*, 2015; Roth *et al.*, 2018a; 2018b) demonstrated that majority of the works on CandR effects on fish uses air exposure periods far greater than those experienced in CandR fisheries, which according to Roth *et al.* (2018b) can be considered lower than 1 min (Thompson *et al.*, 2008; Arlinghaus *et al.*, 2009; Gagne *et al.*, 2016; McLean *et al.*, 2016;). The air exposure periods on CandR literatures range from 30s to 19 min (Gingerich *et al.*, 2007; Thompson *et al.*, 2008; Arlinghaus *et al.*, 2009; McLean *et al.*, 2016; Brownscombe *et al.*, 2017; Twardek *et al.*, 2018).

In the present work, the trahira air exposed for 30 and 60s had no alterations in the cortisol, glucose, lactate, ions (K^+, Cl^-) and osmolality which indicates that lower periods of handling the fish out of the water was feasible to keep their homeostasis. On the other hand, Na^+ plasma concentrations increased in fish under air exposure (30 and 60s) in relation to control fish. The plasma Na^+ concentration in control (146.8 ± 2 mmol/L) was similar with values found in the literature for trahira (110 ± 4 mmol/L, Cameron and Wood, 1978); and for others neotropical freshwater fish species like tambaqui (*Colossoma macropomum*, 159 ± 4 mmol/L) (Sadauskas-Henrique *et al.*, 2019) and jeju (*Hoplerythrinus unitaeniatus*, 152 ± 2 mmol/L) (Mariano *et al.*, 2009). Nevertheless, we hypothesized that this increase could be due a hypercapnia acidosis. For example, Rainbow trout (*Oncorhynchus mykiss*) air exposed for 60s after exhaustive exercise had larger extracellular acidosis than trout which were only exercised, where a retention of CO_2 in the blood were observed together with decreases of 80% of the oxygen tension (Ferguson and Tufts, 1992). For freshwater fishes, during conditions that lead to respiratory acidosis, as hypoxia, the H^+ ions are excreted by the gills through apical membrane electroneutral

Na+/H+ exchanger (NHE) (Krogh,1938), and/or via a Na+ channel linked to a V-type H+ ATPase (reviewed by Perry *et al.*, 2006) in attempt to excrete the H+ and recovery the homeostasis. However, in fish under air exposure conditions, these mechanisms at gills cannot be recruited. In this sense, the teleost kidneys can have an important role in acid-base regulation whereas an enhanced acid excretion accompanies a systemic acidosis (Wood and Caldwell, 1978). The CO2 in renal tubule cell is hydrated by the type II carbonic anhydrase (Schwartz, 2002), the generated H+ as excreted into the lumen by the Na+/H+ (NHE) exchanger and/or the V-type H+ ATPase while the HCO3- ions are removed across the basolateral membrane by the Na+-HCO3- cotransporter from the lumen to blood (reviewed by Perry *et al.*, 2006). This way, a reabsorption of Na+ by the trahira kidney could took place to attempt the extrusion of H+ and compensate hypercapnic acidosis during the air exposure. Unfortunately, blood pH of the trahira in the present study was not analyzed to confirm this hypothesis. However, hypercapnia acidosis during air exposure is a common response of the organism to keep the homeostasis. Like, Ferguson and Tufts (1992) found significant increases in blood pH of Rainbow trout during 60s of air exposure after exhaustive exercise, they justify this increases due the high PCO2 in blood, but, the authors did not measure plasma ions concentration. Few works of CandR effects on fish physiology measured blood ions (Gale *et al.*, 2011; McLean *et al.*, 2016; Twardek *et al.*, 2018) and found differences in ions concentrations between the air exposure times. Anyway, once the exhaustive exercise and the air exposure can cause metabolic acidosis, it is necessary to consider these physiological parameters when evaluating the CandR effects on fish even when fish are air exposed for short time. Besides the K+ plasma concentration did not differ in the trahira air exposed for 30 and 60s in relation to control, it was influenced by the water temperature and fish weight (Table 4). Lake of alterations in K+ plasma concentration was already reported by other authors when evaluating the effects of sport fishing on fish (Suski *et al.*, 2007; Thompson *et al.*, 2008; Danylchuck *et al.*, 2014). Until our knowledge, this is the first work that found an influence of the fish weight when evaluating the physiological effects CandR on fish, while the influence of water temperature was already evaluated for other fish species (Thompson *et al.*, 2008; Campbell *et al.*, 2009; Gagne *et al.*, 2016; Twardek *et al.*, 2018). Alterations in plasma K+ levels could be related with increases in metabolism (Rogers *et al.*, 2003) that is known to increases with fish weight and water temperature. Also, the metabolic acidosis could not only decrease the Na+ renal excretion, as already discussed, but also decreases the K+ renal excretion (Brown, 1986), increasing the K+ plasma concentration. This way, not only the air exposure influenced the physiological responses of the trahira, but the fish weight and the water temperature also played an important role in fish responses as already discussed.

CONCLUSION

In conclusion, the use of best-practices (use of barbless hook; low periods of air exposure; the use of a wet bag, and an ichtiometer filled with water (to avoid air exposure), during the fish handling) proved to be efficient for CandR fishing activity of the trahira. The RAMP score, firstly assessed for this fish species, was efficient to evaluate the fish integrity and demonstrated the importance of having normative instructions for a minimal capture size during CandR activity. The lake of alterations on some physiological responses (cortisol, lactate and glucose) was in accordance with low fish short-term mortality due the best-practices adopted, but highlighted the importance of another endpoints, as Na+ and K+ plasma concentration, that can indicates metabolic acidosis, a normal response of fish to air exposure and exercises, which can causes long-term mortality. Finally, the fish weight and water temperature had big contributions on RAMP score and K+ plasma concentration, which highlights the importance of considering its influences while evaluating the effects of the CandR activity on fish species, avoiding biases of the results interpretation.

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