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Short Communication

Stress response in transport of juvenile cobia *Rachycentron canadum* using the anesthetic benzocaine

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ABSTRACT. This experiment evaluated the efficacy of benzocaine to reduce stress response during transport of juvenile cobia. Fish (30 g) were packed in bags and transported for 8 h (stocking density = 10 g L^{-1}). Three concentrations of benzocaine were evaluated: 0, 2, and 6 mg L^{-1}. Blood samples were taken for glucose and hematocrit before transportation, and then at 0, 2, 24, and 48 h after. Water quality parameters were verified. No mortality was observed. Total ammonia nitrogen was higher (2.46 mg L^{-1}) and pH was lower (6.92) at 2 mg benzocaine L^{-1}. There was an increase in blood glucose for all treatments on arrival, and it was higher for those exposed to benzocaine at 6 mg L^{-1}, although at 48 h they were all similar. The hematocrit did not differ among treatments. The results suggest: 1) the density 10 g L^{-1} is considered safe for juvenile cobia transport; 2) benzocaine did not mitigate stress response on cobia during transport, therefore its use is not recommended for this purpose.

Keywords: *Rachycentron canadum*, Rachycentridae, anesthesia, physiology, hematology, water quality, marine fish.

Respuesta al estrés del transporte en juveniles de cobia *Rachycentron canadum* anestesiados con benzocaína

RESUMEN. En este experimento se evaluó la eficiencia de la benzocaína para reducir la respuesta al estrés durante el transporte de juveniles de cobia. Los peces de 30 g se almacenaron en bolsas y se transportaron durante 8 h (densidad de almacenaje = 10 g L^{-1}). Se evaluaron tres concentraciones de benzocaína: 0, 2 y 6 mg L^{-1}. Se tomaron muestras de sangre para glucosa y hematocrito antes del transporte y después de 0, 2, 24 y 48 h. Se verificaron los parámetros de calidad de agua. No se observó mortalidades. La concentración de amoníaco total fue alta (2,46 mL^{-1}) y el pH fue bajo (6,92) a 2 mg de benzocaína L^{-1}. Al término del transporte se determinó un aumento de glucosa en la sangre en todos los tratamientos, siendo mayor en los peces expuestos a 6 mg de benzocaína L^{-1}, aunque a las 48 h todos fueron similares. El hematocrito no mostró diferencias entre los tratamientos. Los resultados sugieren que: 1) la densidad de10 g L^{-1} es segura para el transporte de juveniles de cobia; y 2) la benzocaína no redujo la respuesta de estrés en cobia durante el transporte, por lo tanto no se recomienda su uso para este propósito.

Palabras clave: *Rachycentron canadum***,** Rachycentridae, anestesia, fisiología, hematología, calidad del agua, peces marinos.

Fish transport is a common procedure that exposes the animals to adverse stimuli, being recognized as a potential physiological stressor (Benovit *et al*., 2012). It has been reported that benzocaine may minimize the stress caused by transport in some species such as *Brycon amazonicus* (5 to 20 mg L^{-1}) and *Puntius filamentosus* (20 mg L^{-1}) (Carneiro *et al*., 2002; Pramod *et al*., 2010).

Cobia, *Rachycentron canadum* (L.), is an important species for aquaculture, due to its fast growth, high feed efficiency, and high quality flesh (Webb Jr. *et al*., 2007). Cobia weighting 1-3 g have been transported in

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closed systems at densities between 5 and 25 g L^{-1} (Colburn *et al*., 2008; Stieglitz *et al*., 2012). However, Liao *et al*. (2004) recommended cobia should weigh at least 30 g prior to stocking in cages. Considering benzocaine at 150 mg L^{-1} is an effective anesthetic for juvenile cobia (298 g) (Trushensky *et al*., 2012), the aim of this study was to evaluate the effect of benzocaine on the stress response of juvenile cobia (30 g) transported in a closed system.

Juvenile cobia used in this study was reared for seven weeks in a recirculating aquaculture system (RAS) maintained at 26.5°C and salinity of 30. Fishes were fed three times daily with a commercial diet containing 57% crude protein and 14.5% lipid (NRD, INVE, USA) and fasted for 24 h before transport.

In order to evaluate the stress mitigating effectiveness of benzocaine, the concentrations of 0, 2 and 6 mg L^{-1} were tested. Stock solutions were prepared from the dilution of benzocaine (Henrifarma Produtos Químicos e Farmacêuticos Ltda., Brazil) in commercial alcohol (96%) in a proportion of 1:9 (w:v). All treatments were run in triplicate.

Fish (30.9 ± 9.3 g; 17.3 ± 2.0 cm) were placed in 60 L polyethylene bags filled with 10 L of seawater and 20 L of pure oxygen. The bags, with stocking density of 10 g L^{-1}, were packed in styrofoam boxes to keep the temperature stable (22.4 ± 0.2°C). The fish were then transported by truck for 8 h. Three bags filled only with water (Without Fish) were also transported, and served as a control for water quality.

Before the transport, nine fish were sampled for blood collection as a control group. Fish were also sampled at 0, 2, 24, and 48 h after transport. The blood was collected from the caudal vein with a heparinized syringe (1 mL). Glucose was measured with a portable glucometer (Accu Cheek Advantage, Roche Diagnostics®, Germany) and hematocrit was determined centrifuging blood for 10 min at 16,128 xg (Hematocrit Centrifuge H-240, Hsiang Tai Machinery Industry Co., Taiwan). Fish survival was observed throughout the experimental period.

Water samples were collected at the end of transportation to verify temperature, dissolved oxygen (DO), carbon dioxide (CO_2), alkalinity, total ammonia nitrogen (TAN), gaseous ammonia (NH_3-N) and pH. Temperature and DO were measured with YSI Model 550A m (Yellow Springs Instruments, USA) and the pH with pH meter FE20-FiveEasy™ (Mettler Toledo, Switzerland). Alkalinity was measured following APHA (1998) and CO_2 was calculated with the software CO_2 Analysis Salt® (Timmons & Ebeling, 2010). TAN was verified accordingly to Solorzano (1969) and NH_3-N was calculated using the equations of Ostrensky *et al*. (1992).

The statistical analysis of water quality parameters and hematological parameters were done by one-way ANOVA Tukey test was used when significant differences were detected. All analyzes were performed with a significance level of 5% (Sokal & Rohlf, 1995).

There was no mortality during or after transport. The water quality parameters after 8 h of transport are shown in Table 1. The final oxygen concentration at 2 mg L^{-1} was lower than those transported with benzocaine at 6 mg L^{-1} ($P < 0.05$). (Table 1).

Alkalinity did not differ among treatments ($P > 0.05$), but pH reached the lowest level at 2 mg L^{-1} ($P < 0.05$), differing from Without Fish and 0 mg L^{-1}. Therefore, CO_2 concentrations were higher at 2 mg L^{-1} when compared to 0 mg L^{-1} ($P < 0.05$). However, pH and CO_2 did not differ ($P > 0.05$) between 0 and 6 mg L^{-1}. All treatments showed higher CO_2 levels than the treatment Without Fish ($P < 0.05$). Regarding the concentrations of TAN, cobia transported at 2 mg L^{-1} produced more ammonia than in the other treatments ($P < 0.05$). However, for NH_3-N, no significant difference was found among treatments ($P > 0.05$).

The values observed for glucose up to 48 h after transport are shown in Fig. 1. Immediately after transport (0 h), glucose concentrations were all higher than control. Glucose was higher ($P > 0.05$) for cobia transported with 6 mg L^{-1}, while there was no significant difference ($P < 0.05$) between the treatments 0 and 2 mg L^{-1}. There was a reduction of glucose concentration after 24 h in all treatments, and there was no significant difference among them ($P > 0.05$), but fish transported with 0 and 2 mg L^{-1} had glucose levels still higher than the control ($P < 0.05$). After 48 h, the situation remained unaltered and only fish on treatments 0 and 6 mg L^{-1} showed the same glucose level to the control (Fig. 1). There were no significant differences ($P > 0.05$) for the hematocrit values among times or treatments, varying from 20 to 40%.

During transport, water deterioration process is initiated within the first hour after packing the bags (Paterson *et al*., 2003). The increase of the CO_2 concentration in the water can result in hypercapnia, decreasing the carrying capacity of oxygen by hemoglobin due to the Bohr effect (Souza & Bonila-Rodriguez, 2007). The oxygen transport to tissues is reduced in environments where CO_2 levels are higher than 40 mg L^{-1} (Wedemeyer, 1996). In the present study, the CO_2 concentrations were higher at the treatment with 2 mg L^{-1}, reaching 13 mg L^{-1}, but still lower than the upper limit (40 mg L^{-1}) recommended by Wedemeyer (1996).

Accordingly to Rodrigues *et al*. (2015), pH down to 6.5 is considered safe for cobia. Therefore, pH levels probably did not cause blood and histopathology distur-

Table 1. Water quality parameters (mean ± SD) measured immediately after 8 h of transport of juvenile cobia *Rachycentron canadum* using benzocaine. Different letters on the same line indicate significant differences ($P < 0.05$) among treatments (Without Fish, 0, 2 and 6 mg benzocaine L^{-1}), determined by one-way ANOVA and Tukey test. DO: dissolved oxygen (O_2 L^{-1}); alkalinity (mg L^{-}) as $CaCO_3$; CO_2 (mg L^{-1}); TAN: total ammonia nitrogen (mg L^{-1} NH_4^+ + NH_3^-); NH_3-N (mg L^{-1}).

Parameters	Benzocaine (mg L^{-1})			
	Without Fish	0	2	6
DO	24.40 ± 0.43a	17.57 ± 3.15bc	17.33 ± 3c	20.37 ± 1.96b
pH	8.31 ± 0.06a	7.19 ± 0.19a	6.92 ± 0.08b	7.03 ± 0.08ab
Alkalinity	126.66 ± 2.88a	125.91 ± 3.02a	123.75 ± 3.11a	125.00 ± 0.00a
CO_2	0.00 ± 0.58a	7.00 ± 3.77b	13.00 ± 2.44c	10.50 ± 1.98bc
TAN	0.08 ± 0.02a	0.96 ± 0.8 a	2.46 ± 0.42b	1.28 ± 0.63ab
NH_3-N	0.006 ± 0.001a	0.007 ± 0.005a	0.008 ± 0.001a	0.006 ± 0.002a

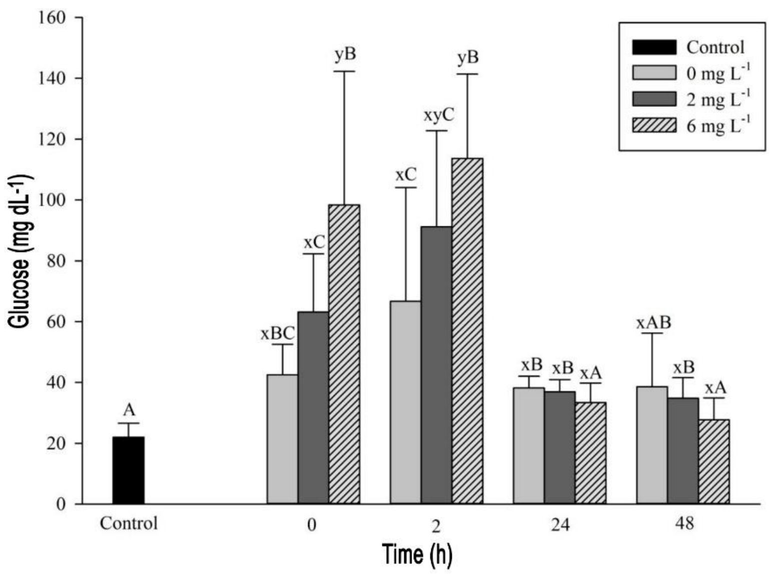

Figure 1. Glucose levels (mg dL^{-1}) for juvenile cobia *Rachycentron canadum* transported in a closed system with different concentrations of benzocaine (0, 2, and 6 mg L^{-1}) along different times: control (before transport), and 0, 2, 24, and 48 h after transport. Different lowercase letters indicate significant differences ($P < 0.05$) among different treatments at each time interval, and different capital letters indicate significant differences ($P < 0.05$) for the same treatment along time, both determined by one-way ANOVA and Tukey test.

bances in this study, where the lowest pH was 6.9. Although TAN concentrations were higher at 2 mg L^{-1}, no differences were found to NH_3-N, since pH was lower at the highest TAN levels and the equilibrium between gaseous and ionic ammonia is regulated mainly by the pH level (Ip, 2010). Hence, NH_3-N remained within safe concentrations for cobia (Rodrigues *et al.*, 2007).

It was observed for Atlantic salmon (*Salmo salar*) that the use of anesthetics reduced activity and stress during transport (Iversen *et al.*, 2009). However, studies indicate that some anesthetics can itself induce stress response (Zahl *et al.*, 2010). Brazilian flounder *Paralichthys orbignyanus* (Valenciennes) showed mortality when transported with the essential oil of *Aloysia gratissima* (Benovit *et al.*, 2012).

The sedative effect of anesthetics could reduce metabolic rate, general activity and stress response during transport (Husen & Sharma, 2014). Trushenski *et al*. (2010) exposed cobia to stress challenges and one hour after air exposure, glucose concentration reached its peak at 189 mg dL^{-1}. In the present study, fish exposed to 6 mg L^{-1} had elevated blood glucose (up to 120 mg dL^{-1}) until 2 h after transport, but after 48 h, their blood glucose was similar to control. The ornamental fish *P. filamentosus* transported for 48 h with benzocaine at 20 mg L^{-1} presented lower glucose levels (165 mg dL^{-1}) and no mortality when compared to unsedated fish (180 mg dL^{-1}; 30% of mortality) (Pramod *et al.*, 2010). However, these authors verified that glucose levels did not reach the baseline levels at the end of 48 h, showing the magnitude and duration of the stress response can be different for a given species, or among species.

The catecholamine release promotes an increase in the number of red blood cells, increasing the hemoglobin concentration in the blood (Wendelaar-Bonga, 1997). However, in the present study, there were no differences for the values of hematocrit among times or treatments. Cobia (298 g) anesthetized with benzocaine at 150 mg L^{-1} also did not present differences in its hematocrit percentage between recovery times, varying from 27 to 41% (Trushenski *et al*., 2012). For adults of *B. amazonicus*, the hematocrit did not differ for fish transported at 5, 10, and 20 mg benzocaine L^{-1} either (Carneiro *et al.*, 2002).

Colburn *et al*. (2008) and Stieglitz *et al.* (2012) studied the transport of juvenile cobia weighting 1 to 3 g, while Liao *et al*. (2004) recommended the size of 30 g for cobia cage stocking. Although benzocaine has not been an effective anesthetic to reduce the stress response for cobia transport, this study is the first to disclosure transport of 30 g cobia in a closed system.

According to the results shown in this work, transporting juvenile cobia (30 g) at the density 10 g L^{-1} is considered safe. The use of benzocaine did not mitigate the stress response. Actually, blood glucose was higher for fish transported with benzocaine up to 2 h after transport. However, it is important to notice that fish transported with benzocaine at 6 mg L^{-1} had glucose levels similar to the control 24 h after the transport, and fish transported without benzocaine recovered only at 48 h. Nevertheless, future investigations should focus on transport with different anesthetics at higher stocking densities in order to optimize the transport of juvenile cobia to cage sites.

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