Transport with different benzocaine concentrations and its consequences on hematological parameters and gill parasite population of matrinxã *Brycon cephalus* (Günther, 1869) (Osteichthyes, Characidae)

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ABSTRACT. The objective of this study was to evaluate the transport stressing effects and the different benzocaine concentrations on hematological parameters and parasite population of matrinxã *Brycon cephalus* (Osteichthyes, Characidae). Thirty fish (1.0 kg mean weight) were hauled in four 200-L plastic tanks for four hours, at different benzocaine concentrations (B0 = 0.0 g/L, B1 = 5.0 g/L, B2 = 10.0 g/L and B3 = 20 g/L). Before loading, blood and gills of 5 fish were collected. Other three samplings were performed after transport, and 24 and 96 hours later (recovery period). Blood cortisol, glucose, hematocrit, white cell differential count and the number of the gill parasite *Piscinoodinium* sp. were determined. Plasma cortisol increased in all treatments after transport, returning to initial levels 24 hours later. Blood glucose was also elevated after transport in all treatments, but only B0 and B1 fish recovered initial levels in 24 hours. B2 and B3 fish did not recover the initial levels until the end of the experimental period. After transport, the lymphocytes relative percentage decreased and the neuthrophils relative percentage increased, in all treatments. Ninety-six hours later, only B0 and B1 fish had recovered the initial levels of these parameters. The number of *Piscinoodinium* sp. on the gills of B3 fish was higher than in the other treatments 96 hours after transportation. The use of benzocaine did not mitigate stress responses and some negative effects were observed in fish transported with the highest concentrations.

Key words: transport, stress, benzocaine, matrinxã, *Brycon cephalus.*

RESUMO. Estresse devido ao transporte e à ação da benzocaína em parâmetros hematológicos e população de parasitas em matrinxã, *Brycon cephalus* (Günther, 1869) (Osteichthyes, Characidae). O objetivo deste trabalho foi avaliar os efeitos do transporte, sob diferentes concentrações de benzocaína, em parâmetros hematológicos e na população de parasitas de brânquias do matrinxã *Brycon cephalus* (Osteichthyes, Characidae). Trinta peixes (peso médio 1,0 kg) foram transportados em tambores plásticos de 200 L por quatro horas. Cada tambor foi preparado com uma concentração de benzocaína (B0 = 0 mg/L; B1 = 5 mg/L; B2 = 10 mg/L e B3 = 20 mg/L). Anteriormente ao carregamento dos tambores, cinco peixes foram amostrados para determinar a condição inicial. Outras três amostragens foram feitas posteriormente: na chegada, 24 e 96 horas após o transporte. Os peixes transportados sob efeito da benzocaína apresentaram menor resposta de fuga durante a captura comparado aos peixes do B0, sendo que os peixes do B3 mostraram dificuldade de manter o equilíbrio durante a viagem. Após o transporte, registraram-se os níveis mais elevados de cortisol plasmático, em todos os tratamentos, com retorno aos níveis iniciais após 24 horas. A glicemia elevou-se na chegada, em todos os tratamentos, e após 24 horas apenas os peixes transportados nas duas concentrações mais altas ainda não haviam recuperado os valores iniciais. Na chegada, a porcentagem de linfócitos decresceu, permanecendo neste patamar após 24 horas, sendo que os peixes do B2 e B3 não retornaram à condição inicial até o final do período experimental. Foi observado aumento da porcentagem de neutrófilos, desde a chegada até 24 horas após o transporte, em todos os

tratamentos. Os peixes do B2 e B3 mantiveram elevadas as porcentagens de neutrófilos até 96 horas após o transporte. Na última coleta, constatou-se que o número do parasita branquial *Piscinoodinium* sp. havia aumentado nos peixes do B3. Foi possível observar que o uso da benzocaína contribuiu com a elevação da glicemia e dos níveis plasmáticos do cortisol após o transporte, sendo registrados efeitos negativos das duas concentrações mais altas em vários parâmetros hematológicos e no número de *Piscinoodinium* sp. aderido às brânquias. Concluiu-se, portanto que o uso da benzocaína não reduziu o estresse causado pelo transporte em matrinxãs, atuando inclusive como agente estressor adicional.

Palavras-chave: transporte, estresse, benzocaína, matrinxã, *Brycon cephalus.*

Introduction

The fast growing of the Brazilian aquaculture emphasizes the crescent demand for scientific studies on fish transport. One of the most important markets for fish in Brazil is the fee fishing operation, which require fish in good physiological status and ready to be caught.

Several studies have shown the positive effect of anesthetics during transport of many fish species (Tomasso *et al.*, 1980; Mishra *et al.*, 1983; Carmichael and Tomasso, 1988). The reduction of fish metabolism, visual stimulus, oxygen consumption and ammonia excretion are cited as some of the advantages of using these chemicals (Schreck, 1982; Wurts, 1995). On the other hand, several other studies point out the negative effects, or the inefficiency, of using anesthetics during transport of some fish species (Strange and Schreck, 1978; Robertson *et al.*, 1988). These effects include the reduction on fish protective capacity against opportunistic parasites (Schreck, 1996).

The objective of this study was to evaluate the effects of transport stress and different benzocaine concentrations on hematological parameters and gill parasite population of matrinxã, *Brycon cephalus*, a very important Amazon species in the Brazilian aquaculture industry.

Material and methods

Experimental protocol

Matrinxã, *Brycon cephalus* (mean weight 1.0 kg), were submitted to food restriction for 48 hours in earthen ponds before transport. A hundred and twenty fish were loaded in four 200-L plastic tanks (30 fish in each) placed on a pick-up truck and hauled for four hours. During transport, pH, temperature, un-ionized ammonia, CO_2 and dissolved oxygen were measured every 30 minutes. Dissolved oxygen was maintained above 6 mg/L, using pure bottled oxygen. Each tank contained different benzocaine concentrations: B0 = 0.0, B1 = 5.0, B2 = 10.0 and B3 = 20.0 mg/L.

Samplings and laboratory analysis

Five fish were sampled to characterize the initial status, immediately after seining from an 140-m^2 earthen pond. Another three samplings were performed after transport (arrival) and 24 and 96 hours later (recovery period). During the recovery period the fish were stocked in four 40-m^2 earthen ponds.

Fish were anesthetized with benzocaine at 50 mg/L during samplings. Blood was collected by punction of the caudal vein with heparinized syringes and needles. After blood glucose measurement (King and Garner, 1947), blood was centrifuged and plasma frozen at -20 °C for cortisol analysis (Radioimunoessay, DPC® Kit). Hematocrit was determined with microcapillary tubes centrifuged for 5 minutes at 5,000 rpm. Blood smears were air dried, fixed with ethanol, and later stained (Rosenfeld, 1947) for differential cell counting under a microscope (magnification of 450x). Counts were made randomly throughout the smear and relative percentages of lymphocytes, neutrophils and monocytes were calculated.

All branchial arches were removed during the first and the last samplings, placed in plastic flasks with 1:4000 formalin solution, shaken and allowed to rest for 2 hours. After that, a new formalin solution was added to the flasks, until reaching a final 10% concentration. The arches were removed from the solution and the opportunistic parasite, *Piscinoodinium sp.*, present in the formalin, were counted in Mac Master chamber (Martins, 1995).

Statistics

A split-plot plus control (initial level) design was employed and data were analyzed by two-way analysis of variance, with treatments (B0, B1, B2 and B3) and samplings (arrival, 24, and 96 h after transport) as factors, for cortisol, blood glucose, hematocrit and differential cell counting. For data concerning gill parasite numbers, experimental design was entirely randomized and data analyzed by one-way ANOVA. Tukey's multiple range test was employed when statistic differences were detected ($P<0.05$).

Results

Water quality during transport

Water un-ionized ammonia continually increased during *en rout* transport, reaching mean levels as high as 12.5 mg/L, after 4 hours. Mean CO_2 also increased, ranging from 18.7 to 124.3 mg/L. Mean pH decreased by the first hour, from 7.0 to 6.2, and remained as low as 6.1 till the end of transport (Figure 1). Increment of 4ºC was observed in the water temperature during the same period, reaching 29.3ºC at the end.

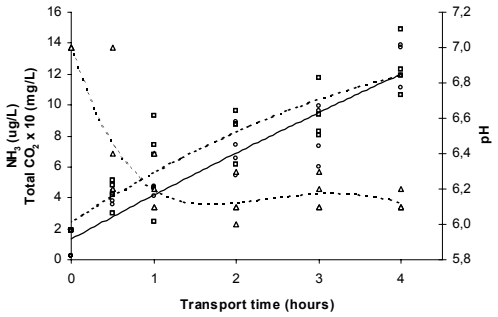

Figure 1. Un-ionized ammonia (º), CO_2 (□) and pH (Δ) in the transport water. The continuous, dotted and dashed lines follow total ammonia, CO_2 and pH averages, respectively

Fish behavior after transportation

Fish from B1, B2 and B3 treatments showed less escape reaction when captured from the transport tanks than B0 fish. B3 fish presented some body injuries and difficulty to maintain swimming balance. Only B3 group presented mortality (50%) during the experimental period.

Physiological parameters

Plasma cortisol levels did not differ (P>0.05) among treatments, within each sampling time. Nevertheless, plasma cortisol titer was higher at arrival in fish of all treatments, returning to initial levels 24 hours later (Table 1).

Table 1. Plasma cortisol (ng/dL), hematocrit (%), and relative percentage of monocytes of matrinxã *Brycon cephalus* submitted to transport stress. SEM are presented within parenthesis (N=5, initial levels; N=20, other sampling times). Different letters in the same row indicate significant difference (P<0.05)

	Sampling times			
	Initial levels	Arrival	24 hours	96 hours
Plasma cortisol (ng/dL)	125.9 (10.13) b	255.7 (12.12) a	113.7 (6.64) b	102.5 (7.58) b
Hematocrit (%)	47.0 (1.60) ab	46.0 (0.59) b	47.8 (1.06) ab	50.4 (0.72) a
Monocytes (%)	7.00 (0.45) a	5.44 (0.89) a	8.68 (0.89) a	5.60 (1.03) a

Blood glucose elevated (P<0.001) at arrival in all treatments, and B0 and B1 fish recovered the initial level 24 hours later while B2 fish took 96 hours and B3 fish did not recover it (P=0.007) until the end of the experimental period (Figure 2). No statistical differences were detected on hematocrit among the treatments and between the initial level and the average of the treatments in each sampling time (Table 1).

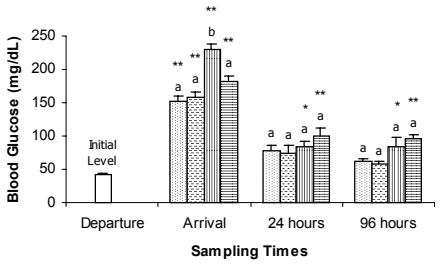

Figure 2. Blood glucose (mg/dL) of matrinxã submitted to transport stress in the presence of benzocaine at the concentrations 0.0 (dotted bars), 5.0 (cashed bars), 10.0 (vertical lined bars), and 20.0 mg/L (horizontal lines bars). Different letters indicate differences (P<0.05) among treatments at the same sampling time. Single and double asterisks indicate differences (P<0.05 and P<0.01, respectively) between treatments and initial level (open bar); all treatments share the same initial level. Vertical bars represent SEM (N=5)

Differential count of white cells

The relative percentage of lymphocyte decreased (P<0.01) at arrival in all treatments, remaining low for 24 hours. Only B0 and B1 fish recovered the initial value after 96 hours (Figure 3).

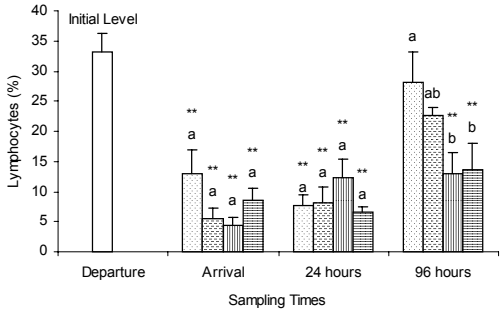

Figure 3. Relative percentage of matrinxã lymphocytes submitted to transport stress in the presence of benzocaine at the concentrations 0.0 (dotted bars), 5.0 (cashed bars), 10.0 (vertical lined bars), and 20.0 mg/L (horizontal lines bars). See Figure 2 for explanation of letters and asterisks accompanying the value bars

Contrarily, the relative percentage of neutrophils increased (P<0.01) at arrival, remaining high until 24 hours. B2 and B3 fish did not recovered the initial value (P<0.003) until the end of the experimental period (Figure 4). Monocyte relative percentage ranged from 5 to 9% during the

experiment in all treatments, presenting no statistical differences (P>0.05; Table 1).

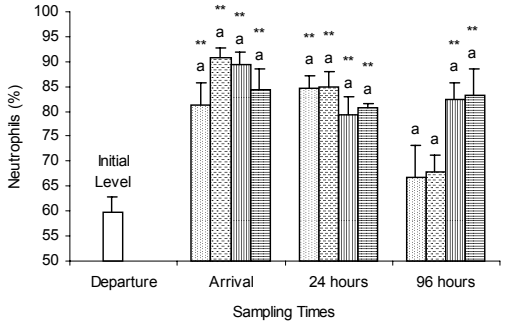

Figure 4. Relative percentage of matrinxã neutrophils, submitted to transport stress in the presence of benzocaine at the concentrations 0.0 (dotted bars), 5.0 (cashed bars), 10.0 (vertical lined bars), and 20.0 mg/L (horizontal lines bars). See Figure 2 for explanation of letters and asterisks accompanying the value bars

Gill parasite

At the end of the experiment, the number of the gill parasite *Piscinoodinium* sp. was higher (P<0.003) in B3 fish when compared to the initial value. B3 fish also presented more parasites (P<0.02) than fish from the other treatments (Figure 5).

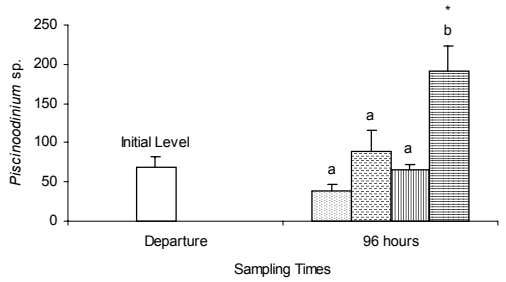

Figure 5. Number of *Piscinoodinium* sp. ($x10^3$) on the matrinxã gills, submitted to transport stress in the presence of benzocaine at the concentrations 0.0 (dotted bars), 5.0 (cashed bars), 10.0 (vertical lined bars), and 20.0 mg/L (horizontal lines bars). See Figure 2 for explanation of letters and asterisks accompanying the value bars

Discussion

There are several kinds of anesthetics used during fish transport (Strange and Schreck, 1978; Mishra *et al.,* 1983; Carmichael and Tomasso, 1988). However, many studies have demonstrated these chemicals contradictory effects, concerning the anesthetic utilized, the fish species, the transport equipment employed among others (Strange and Schreck, 1978; Tomasso *et al*., 1980; Mishra *et al.,* 1983; Robertson *et al.,* 1988).

Cortisol is a steroid hormone secreted by neuro-endocrine events related to the hipotalamo-hipofise-interenal axis, activated by several types of stimuli (Donaldson, 1981). Transport is an important procedure in fish production and very important source of stress to the animals (Tomasso *et al*., 1980; Carmichael *et al*., 1984; Iversen *et al.,* 1998; Carneiro and Urbinati, 2001). MS-222 (tricaine metanosulfate) avoided elevations on plasma cortisol of chinook salmon, *O. tshawytscha*, when used during handling (Strange and Schreck, 1978). On the other hand, the same authors did not observed benefic effect of MS-222 when the fish were exposed for long periods at low concentrations, similar conditions to those of a transport. Robertson *et al.* (1988) reported higher plasma cortisol levels in red drum transported for 5.5 hours with MS-222. The present study also showed no advantage of using benzocaine during transport of matrinxã with respect to the high cortisol levels after the disturbance.

The blood glucose increase in a fish submitted to a stressor is due to the elevated energetic demand by the organism (Morgan and Iwama, 1997). The increased blood glucose levels of matrinxã after transportation agrees with the results obtained in studies with most of the teleost species (Mazeaud and Mazeaud, 1981; Carmichael *et al.,* 1983; Carmichael, 1984; Robertson *et al.,* 1987; Barton, 2000).

B0 and B1 fish recovered the initial blood glucose levels within 24 hours after transportation, similar to that observed in red drum (Robertson *et al.,* 1987). Largemouth bass, *M. salmonides*, needed longer recovery periods (from 47 to 63 hours) to reestablish this parameter (Carmichael, 1984). Otherwise, the matrinxã transported with the highest benzocaine concentrations (B2 and B3 fish) did not recover blood glucose levels, suggesting an additional stressful effect of these concentrations.

According to McDonald and Milligan (1997), stress provokes hemoconcentration in many freshwater fishes, observed by the increasing in the hematocrit value. Otherwise, mudfish *Labeo umbratus* showed hematocrit reduction after capture and transport stress (Hattingh and Van Pletzen, 1974). Despite the absence of significant differences in this parameter, between the initial levels and the levels obtained in the following sampling, slight variations were observed during the recovery period, which may be resultant of adaptive mechanisms presence (Wedemeyer, 1997).

Several hematological alterations are observed in fish under stressful condition. Decrease in

lymphocyte relative percentage and increase in lymphocyte relative percentage are alterations usually observed in fish after transportation (Sopinska, 1984). Other stress sources, such as the presence of toxic elements in water and crowding, can even provoke lymphopenia in fish (Dick and Dixon, 1985; Pickering and Pottinger, 1987). European eel, *Anguilla anguilla*, showed these alterations after receiving a diet with cortisol (Johansson-Sjobeck *et al*., 1978; Pickering, 1984). The results obtained with matrinxã reinforce observations with other species, despite the fact that the relative percentage of white cell itself does not prove the occurrence of lymphopenia or neuthrophilia. Chinook salmon, *O. tshawytscha*, that had been stressed by handling, were more vulnerable to *Vibrio anguillarum* than unstressed controls.

This debilitation in disease resistance was correlated with a depression in the ability of pronephric and splenic lymphocytes to produce antibody (Maule *et al*., 1987). The immediate environment of any fish is teeming with microorganisms. A wide variety of bacteria and parasites normally live in harmony with their host, inhabiting the skin, gills or the inside of the gastro-intestinal tract. Under normal conditions, such commensal organisms do not harm the host and, indeed, have a vested interest in its continuing existence. However, under stressful conditions, such as transportation, the fish may become stressed and the normal balance between host and parasite may be upset. A commensal organism on the fish, or other microorganisms in the surrounding water, may then become involved in the resulting disease process as secondary pathogens. The majority of fish infectious diseases come about due to opportunistic invasion of stressed fish by secondary pathogens and can be avoided, or greatly reduced, by simple ways (Schreck, 1996).

Any restructuring of energy sources may have deleterious effects on fish health because the process of cellular and humoral disease resistance is energy demanding (Schreck, 1996). B3 fish presented more *Piscinoodinium* sp. attached to the gills than the fish from other treatments 96 hours after transportation. The mortality rate observed in this treatment during the recovery period might also have been influenced by the presence of parasites, which carry fixation structures that cause severe injuries to the epithelial tissues. The parasite damages fish gill by attaching to the epidermis, and many parasite species will actively feed on the underlying tissues and blood, leading to respiratory distress (Willians, 1972; Lom, 1977, 1981). Shaharon-Harrisson *et al*. (1990) reported mortality rate up to 100% in many fish species caused by *Piscinoodinium pillulare*. Martins (1998) also observed high mortality rate caused by this parasite in hybrid tambacu *Colossoma macropomum* x *Piaractus mesopotamicus*.

There have been different hormonal responses to stressors and different types of anesthetics among fish species. However, this study showed that benzocaine did not avoid elevations in plasma cortisol levels in matrinxã after transport stress. Besides, high benzocaine concentrations provoked negative effects in most of the hematological parameters analyzed, as well as in the number of attached gill parasite, concluding that benzocaine does not seem to reduce transport stress and may also act as an additional stressor.

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