ZOOLOGIA 31 (1): 101–104, February, 2014
http://dx.doi.org/10.1590/S1984-46702014000100012

SHORT COMMUNICATION

Stress in *Pimelodus maculatus* (Siluriformes: Pimelodidae) at different densities and times in a simulated transport

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ABSTRACT. The transportation of live fish is a routine procedure in aquaculture, and includes a series of stressful stimuli such as an increase in the stocking density of specimens per volume of water, and abrupt changes in water quality. This study evaluated the water quality and the stress levels on *Pimelodus maculatus* (Lacépède, 1803) fingerlings transported in plastic bags by a mechanical transportation simulator. Fish were stocked at densities 4 (22.88 g/L), 8 fish/L (45.76 g/L) and 12 fish/L (68.64 g/L) and the transportation simulation was performed for 4, 8 or 12 hours. A completely randomized experimental design applied to a 3 x 3 factorial model with three replicates was used. Water quality was evaluated by the analysis of the temperature, pH, conductivity, dissolved oxygen, total ammonia, unionized ammonia and nitrite at the beginning and at the end of the experiment. Stress was assessed by determining tissue cortisol levels by radioimmunoassay, in the beginning and at the end of the study. The densities and the transportation times did not cause mortality, but higher density and times of transport influenced water quality indicators. The simulated transportation of *P. maculatus* showed that all *P. maculatus* fingerlings survived at the maximum density tested, 12 fish/L for 12 hours.

KEY WORDS. Aquaculture; cortisol; mandi-amarelo; simulated transportation; water quality.

The transportation of live fish, which usually consists in removing fishes from ponds to transfer them to another location, is a routine procedure in aquaculture. Larvae, fingerlings, juveniles and small specimens are placed in plastic bags (Carneiro & Urbinati 2001, Conte 2004) filled with 20-25% water and 75-80% dissolved oxygen, and are maintained at temperatures below 28°C. During transportation a series of stimuli stress the fish, for instance capture, the increase in the density of specimens per volume of water, abrupt changes in temperature, handling, the transport itself, unloading, and final storage (Robertson *et al.* 1988). These procedures, contribute to a degradation of the water quality (Conte 2004) by the accumulation of nitrogenous wastes, particularly of ammonia and nitrite, which are stressor agents (Urbinati & Carneiro 2004). The level of toxicity of the total ammonia is related to its concentration in the water, and to other parameters such as pH and temperature (Randall & Tsui 2002). The use of plastic bags to transport fish can promote the accumulation of metabolites in the water, for instance ammonia, and changes in pH, temperature, dissolved oxygen and carbonic gas concentrations (Golombieski *et al.* 2003), which may interfere and alter the metabolism of the fish.

If fish are stressed and underfed at the beginning of the transportation procedure, poor water quality and handling can cause mortality (Waichman *et al.* 2001). When fish is exposed to a stressor, two neuroendocrine axes are activate: the hypothalamus- sympathetic nervous system-chromaffin cells, which results in the release of catecholamines (adrenaline and noroadrenaline) as final products; and the hypothalamic-pituitary-interrenal, which releases corticosteroids (cortisol and cortisone) (Oba *et al.* 2009).

Therefore, cortisol can be used in the analysis of stress, contributing to the determination of the most suitable condition for transportation, and to mitigate adverse effects during and after the procedure.

Pimelodus maculatus (Lacépède, 1803) is a species distributed in many South American countries, for instance Argentina, Bolivia, Brazil, Paraguay and Uruguay (Godoy 1987). This species is relevant to professional fishing in dams and reservoirs (Agostinho *et al.* 1994) and is well accepted by the consumer market. It also has being considered for use in fish culture, and for that reason studies related to spawning and reproduction (Bazzoli *et al.* 1997), larval feeding (Luz & Zaniboni Filho 2001), hatchery (Weingartner & Zaniboni Filho 2004) and growth in cages (Almeida & Nuñer 2009) have been conducted.

In the commercial transportation of fish, the highest possible density of specimens and the smallest possible volume of water are sought after. The goal is to avoid mortality, deterioration of water quality and stress (Wedemeyer 1996, Grøttum 1997). With that focus in mind, we aimed to evaluate the water quality and stress levels on *P. maculatus* fingerlings subjected to simulated transportation at different times and under different densities.

This study was conducted on fingerlings of *P. maculatus* (voucher specimen deposited in the Museu de Zoologia da Univesidade Estadual de Londrina – MZUEL 07909) that were 8.60 ± 0.73 cm long and 5.72 ± 1.55 g, obtained by hormonal induction, and which were kept in acclimatization tanks for a period of 48 h before the beginning of the experiment. In the last 24 hours, feeding was suspended to promote gastrointestinal cleaning (Conte 2004) and also to preserve the water quality by reducing oxygen consumption and ammonia excretion into the water (Carneiro *et al*. 2009).

After this period, the fingerlings were stored in 15 L plastic bags, which constituted the experimental units. Fish were stocked at the following densities: 4 (22.88 g/L), 8 (45.76 g/L) and 12 fish/L (68.64 g/L); the transportation simulation was performed for 4, 8 or 12 hours. A completely randomized experimental design applied to a 3 x 3 factorial model with three replicates was used. Each plastic bag contained 4 L of water and the remaining volume was filled with commercial oxygen. The plastic bags were sealed with rubber rings and packaged in a polystyrene box to avoid mechanical shocks and to promote a stable temperature during the transportation.

The polystyrene boxes with the plastic bags inside were transferred to a shaker table that reproduced the conditions of transportation. The table presented a horizontal movement with a range of 40 cm and 1,080 cycles/hour, so that all experimental units remained in constant agitation during the predetermined time.

The water quality was monitored at the beginning and at the end of each simulated transport cycle (n = 3 per treatment). Water temperature, pH, conductivity and dissolved oxygen concentrations were measured in each experimental unit, using YSI-63 and YSI-55 probes, respectively. Water samples were also collected from each experimental unit for the analysis of total ammonia and nitrite, using the methods described by Koroleff (1983) and Golterman *et al.* (1978), respectively. The fraction of unionized ammonia in water was calculated using the equation described by Johansson & Wedborg (1980).

For the cortisol analysis, five fingerlings were collected from each experimental unit (15 fish per treatment) at the end of each transportation time. At the end fish were euthanized in a container with water and ice, and stored in liquid nitrogen for later cortisol analysis. Cortisol was extracted from a pool of five whole fish, which were ground together prior the analysis and mixed with a phosphate buffered solution (PBSG). This mixture was washed with ether, evaporated on a heated bath, and sonicated after addition of PBSG (de Jesus *et al.* 1991). After extraction, the cortisol concentration was determined by ELISA using the Adaltis® EIAgem-Cortisol kit (intra-assay coefficient of variation = 24.4%; inter-assay coefficient of variation = 26.2%).

The variables water quality and survival, which were recorded at the end of each transportation time, and the amounts of cortisol, were analysed by ANOVA (α = 0.05), followed by Tukey test when necessary (Zar 1996).

The oxygen concentration always exceeded the oxymeter reading capability with values above 20 mg/L, remaining supersaturated in all treatments. The high concentrations of dissolved oxygen at end of the transportation are related to the initial super saturation in the plastic bags, a condition also registered by Oliveira *et al.* (2009) during the transport of *Oreochromis niloticus*.

The increase in the total ammonia concentration and in conductivity indicated that prolonged transportation with fish at the highest density altered the water quality (Table I). The higher ammonia levels observed at higher densities are related to the greater number of fish in each plastic bag. The increase in conductivity is likely a result of ammonia excretion and ion effluxes (Gomes *et al*. 2008).

Table I. Temperature, pH, total ammonia and conductivity (mean ± standard deviation) at the beginning and after simulated transport of fingerlings of *Pimelodus maculatus* for 0, 4, 8, and 12 hours in densities 4 (22.88 g/L), 8 (45.76 g/L) and 12 (68.64 g/L) fish/L. Different letters indicate significant differences among groups in the same day by two-way ANOVA and Tukey test ($p < 0.05$).

Time/Density		Temperature (°C)	pH	Total ammonia (mg/L)	Conductivity (µS/cm)
Hours	0	28.93	6.60	0.00	69.70
		(± 0.72)	(± 0.11)	(± 0.00)	(± 27.70)
	4	20.88 ^{a}	6.27 ^{b}	3.77 ^{a}	98.68 ^{a}
		(± 0.95)	(± 0.11)	(± 3.37)	(± 27.58)
	8	20.68 ^{a}	6.12 ^{a}	4.87 ^{ab}	120.08 ^{ab}
		(± 1.18)	(± 0.09)	(± 3.32)	(± 53.19)
	12	20.58 ^{a}	6.06 ^{a}	8.71 ^{b}	153.70 ^{b}
		(± 0.87)	(± 0.07)	(± 4.20)	(± 59.84)
Fish/L	4	21.68 ^{b}	6.14 ^{a}	4.59 ^{a}	77.67 ^{a}
		(± 0.67)	(± 0.15)	(± 2.62)	(± 20.66)
	8	19.91 ^{a}	6.14 ^{a}	5.35 ^{a}	130.32 ^{b}
		(± 0.46)	(± 0.14)	(± 3.50)	(± 35.54)
	12	20.56 ^{a}	6.17 ^{a}	7.40 ^{a}	164.47 ^{b}
		(± 0.80)	(± 0.09)	(± 5.58)	(± 53.94)

The level of toxicity of total ammonia varies according to water quality. This variation is associated with the concentration of ammonia in the water and with other parameters, such as pH and temperature. The temperature associated with ionic strength and with water pressure interferes with the constant dissociation (pK) of the reaction of ammonia, influencing the concentration of NH_3/NH_4^+ forms in the water (Randall & Tsui 2002, Francis-Floyd *et al*. 2009). In this study, the pH and the temperature did not increase when fish were in higher densities and were transported for longer periods of time, indicating that the densities and the transportation time are suitable for *P. maculatus*.

The toxicity levels of unionized ammonia have not been defined for *P. maculatus*. However, we assumed that the very low concentration of unionized ammonia registered by us is harmless for the species, since levels below 0.02 mg/L are considered safe for most fish species (Foss *et al*. 2003), and no mortality occurred during the simulated transport.

Cortisol release from the interrenal glands is a primary fish response to stressful conditions (Urbinati & Carneiro 2004). In this study, lower concentrations of cortisol when fish densities are higher, and the lack of differences in cortisol concentrations among the transport times (Table II) indicate that the transportation conditions used in this study were appropriate for *P. maculatus*

The transportation of *P. maculatus* without mortality can be conducted at the maximum density analyzed of 12 fish (68.64 g/L), and up to 12 hours.

Table II. Cortisol (mean ± standard deviation) levels and its coefficient of variation (CV) obtained in simulated transport of fingerlings of Pimelodus maculatus for 0, 4, 8 and 12 hours in densities 4 (22.88 g/L), 8 (45.76 g/L) and 12 (68.64 g/L) fish/L. Different letters indicate significant differences among groups in the same day by two-way ANOVA and Tukey test ($p < 0.05$).

Time/Density		Cortisol (ng/g tissue)	CV(%)
Hours	0	155.33 [a] (± 39.85)	25.66
	4	197.56 [a] (± 70.07)	35.47
	8	157.11 [a] (± 37.86)	24.10
	12	153.33 [a] (± 39.24)	25.59
Fish/L	4	190.50 [b] (± 58.68)	30.80
	8	173.50 [ab] (± 39.41)	22.71
	12	133.50 [a] (± 33.57)	25.15

ACKNOWLEDGMENTS

The authors thank Washington de Barros Adamante and Leonardo J.G. Barcellos for their collaboration in the cortisol analysis. Neiva Braun thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico for the post-doctoral fellowship.

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Submitted: 27.VIII.2013; Accepted: 14.XI.2013.
Editorial responsibility: Carolina Arruda Freire