Biological indicators of stress in pacu (*Piaractus mesopotamicus*) after capture

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Received August 17, 2007 – Accepted January 30, 2008 – Distributed May 31, 2009

(With 3 figures)

Abstract

The effects of capture (chasing, netting and air exposure) on cortisol, glucose, chloride, sodium, potassium and calcium concentrations, osmolality, hematocrit, hemoglobin concentration, red blood cells count (RBC) and mean corpuscular volume (MCV) were investigated in pacu (*Piaractus mesopotamicus*). A total of 132 fish (49.7 ± 11.7 g) were subjected to capture and 3 minutes air exposure and capture and 5 minutes air exposure. Nine fish at each treatment were sampled at 5, 15, 30, 60 minutes and 24 hours after the procedure. Nine undisturbed fish were sacrificed before the handling and used as controls. Capture resulted in a rise in blood cortisol and glucose 30 and 5 minutes, respectively, after both air exposures. Both indicators returned to resting levels 24 hours after capture. In both fish groups, plasma chloride decreased 60 minutes after capture, not recovering the resting levels within 24 hours after, and serum sodium rose at 15 and 30 minutes and recovered the resting levels 24 hours later. There were no significant changes neither in potassium, calcium and osmolality nor in hematocrit, hemoglobin, RBC and MCV as a consequence of capture. The sequential stressors imposed to pacu during capture activated the brain-pituitary-interrenal axis (cortisol and glucose responses) but the activation of the brain-sympathetic-chromaffin cell axis was apparently moderate (ionic and hematological responses).

Keywords: capture, air exposure, stress, *Piaractus mesopotamicus.*

Indicadores biológicos de estresse em pacu (*Piaractus mesopotamicus*) após captura

Resumo

Os efeitos da captura (perseguição, contenção em puçá e exposição aérea) no perfil sanguíneo do cortisol, glicose, cloreto, sódio, potássio, cálcio e na osmolaridade, hematócrito, hemoglobina, número de células vermelhas (CV) e volume corpuscular médio (VCM) foram investigados no pacu (*Piaractus mesopotamicus*). Um total de 132 peixes (49,7 ± 11,7 g) foi submetido à captura com 3 ou 5 minutos de exposição aérea. Nove peixes de cada tratamento foram amostrados 5, 15, 30, 60 minutos e 24 horas depois e outros nove peixes foram amostrados antes da captura e considerados controle. A captura resultou em aumento do cortisol e glicose no sangue 30 e 5 minutos depois da captura, respectivamente, independente do tempo de exposição aérea. Ambos os indicadores recuperaram os valores controle em 24 horas. Nos dois grupos de peixes, o cloreto plasmático diminuiu 60 minutos após captura e não recuperou os valores controle, enquanto o sódio sérico aumentou entre 15 e 30 minutos recuperando a condição controle em 24 horas. Não houve alteração significativa nos valores de potássio, cálcio, osmolaridade ou no hematócrito, hemoglobina, CV e VCM como consequência da captura. Os estressores sequenciais aplicados no pacu durante a captura ativaram o eixo cérebro-pituitária-interrenal (respostas do cortisol e glicose), mas a ativação do eixo cérebro-sistema simpático-células cromafins foi aparentemente moderada (respostas iônicas e hematológicas).

Palavras-chave: captura, exposição aérea, estresse, *Piaractus mesopotamicus*.

1. Introduction

There are many potential applications of the stress response. Experimental biologists need to know the baseline from which to assess whatever response they are studying. It is also important to know whether fish under intensive aquaculture are in or out of a stressed state. Aquaculture development depends on the establishment of appropriate management practices. Thus the features of the physiological stress responses can serve this purpose.

Various stressors, including fish capture (Mugnier et al., 1998; Arends et al., 1999; Barcellos et al., 2001; Rocha et al., 2004; Morales et al., 2005), are necessary components of modern intensive aquaculture (Wendelaar Bonga, 1997). The fish response to such stressors involves a series of physiological changes in an attempt to compensate for the challenge imposed upon it and, thereby cope with the stress. Such changes have been broadly categorized into primary, secondary and tertiary responses including hormonal, metabolic, osmoionic and hematological disturbances and have been used to characterize the degree of stress fish experienced (McDonald and Milligan, 1997; Wendelaar Bonga, 1997; Wojtaszek et al., 2002).

The majority of international research effort has gone into the stress responses of salmonids (McCormick et al., 1998; Ackerman et al., 2000; Barton, 2000), but other species such as carp (*Cyprinus carpio* L.) (Vianen et al., 2001), tilapia (*Oreochromis niloticus* L.) (Van der Salm et al., 2005), turbot (*Scophthalmus maximus* L.) (Van Ham et al., 2003) and sea bream (*Sparus aurata* L.) (Arends et al., 1999) have gained attention due to their potential for aquaculture in many countries. However, little research has been done on native South American fishes. Among the Brazilian farmed fish, research on stress has been carried out on matrinxã (*Brycon cephalus*) (Günther, 1869) (Carneiro and Urbinati, 2001; Ide et al., 2003; Rocha et al., 2004; Urbinati et al., 2004), tambaqui (*Colossoma macropomum*) (Cuvier, 1818) (Gomes et al., 2003a), pirarucu (*Arapaimas gigas*) (Schinz, 1822) (Gomes et al., 2003b) and jundiá (*Randia quelen*) (Quoy and Gaimard, 1824) (Barcellos et al., 2001). Despite pacu (*Piaractus mesopotamicus*) (Holmberg, 1887) being considered one of the most important native species (Queiroz et al., 2005) and studies on its reproduction (Romagosa et al., 1990), larviculture (Jomori et al., 2003), feeding and nutrition (Souza et al., 2000; Bechara et al., 2005; Takahashi et al., 2006; Abimorad et al., 2007) are available, there is sparse knowledge on the stress response of the species (Krieger et al., 1989; Martins et al., 2000; Takahashi et al., 2006). In the present study, the physiological responses (hormonal, metabolic, ionic and hematological) to capture (chasing, netting and air exposure) were investigated in the pacu.

2. Material and Methods

2.1. Fish and experimental procedure

Juveniles of pacu (132 fish, 49.7 ± 11.7 g) were randomly distributed in thirty three 100 L boxes (4 fish per box), with constant water and air flow, where they were kept for 15 days to acclimate to the experimental conditions. Feeding was stopped 24 hours before the capture that consisted in chasing, netting and exposed the fish to the air. Fish were submitted to the conditions: T1: undisturbed fish (control) (3 boxes), T2: chasing and 3 minutes of air exposure (15 boxes), T3: chasing and 5 minutes of air exposure (15 boxes). Nine fish of T2 and T3 (3 fish/3 boxes) were sampled at each sampling time (5, 15, 30, 60 minutes and 24 hours after handling). The control fish (n = 9) were sampled before handling. Fish were anesthetized (benzocaine, 66 $mg.L^{-1}$) and bled by caudal vessels puncture and serum and plasma were separated. The four fish of each box were simultaneously anesthetized but only 3 were sampled.

2.2. Analyses

Glucose (King and Garner, 1947), hematocrit, red blood cell count (RBC), mean corpuscular volume (MCV) and hemoglobin were determined in total blood (Celm DA-500), chloride (kit Labtest) and osmolality (Osmometer Wescor Mod 505) in plasma and cortisol (RIA, kit Diagnostics Products Corporation), sodium, potassium and calcium (ion selector Iselab Drake) in serum.

Water temperature (29.1 ± 0.5 °C) and dissolved oxygen (4.4 ± 0.4 $mg.L^{-1}$) (Yellow Springs Instruments, Yellow Springs, OH, USA, YSI 55), pH (10.2 ± 0.4) (YSI 63) and total ammonia (0.0102 ± 0.0054 $mg.L^{-1}$, Nessler Reactive) were monitored throughout the experiment.

2.3. Statistical analysis

A completely randomized design (CRD) was employed and results were analyzed by a two-way analysis of variance (ANOVA), with 2 treatments (capture with 3 or 5 minutes air exposure) and 5 sampling times (5, 15, 30 and 60 minutes and 24 hours after capture) as the factors plus the resting condition (control fish). Data were expressed in means ± standard deviation of the mean. Means were compared by Tukey test ($p < 0.05$).

3. Results

No mortality was observed in any group during the experiment. There were hormonal and metabolic changes after the handling imposed on the fish. Serum cortisol concentrations in air-exposed fish either for 3 minutes (T2) or 5 minutes (T3) rose within 30 minutes ($p = 0.0109$) and then dropped until 24 hours later. The rise was higher in fish of treatment 3 ($p = 0.0074$) regardless of the sampling (Figure 1). Blood glucose in both air-exposed fish (T2 and T3) increased within 5 minutes ($p = 0.0001$) compared to control (undisturbed fish) and recovered the resting values within 24 hours (Figure 1). Capture elicited mild changes in ionic balance of pacu. Plasma chloride levels dropped in fish of treatments 2 and 3 within 60 minutes, not recovering the resting levels within 24 hours ($p = 0.0291$) (Figure 2). Serum sodium increased ($p = 0.0026$) at

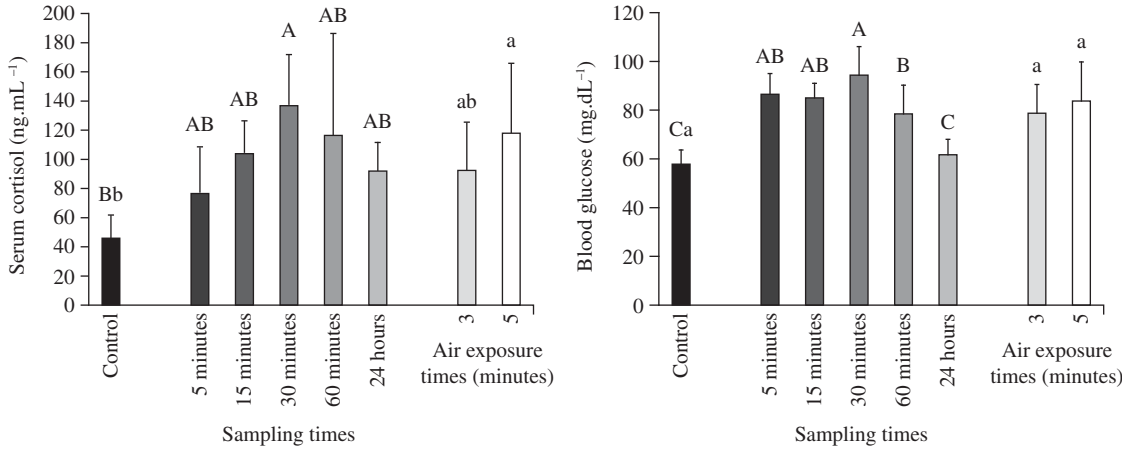

Figure 1. Blood cortisol and glucose of pacu undisturbed and air exposed for 3 and 5 minutes. Different capital letters indicate differences among sampling times (all air exposure times are represented in the same bar) and small letters between treatments (all samplings are represented in the same bar). Bars represent means of treatments or samplings. Vertical bars represent SEM.

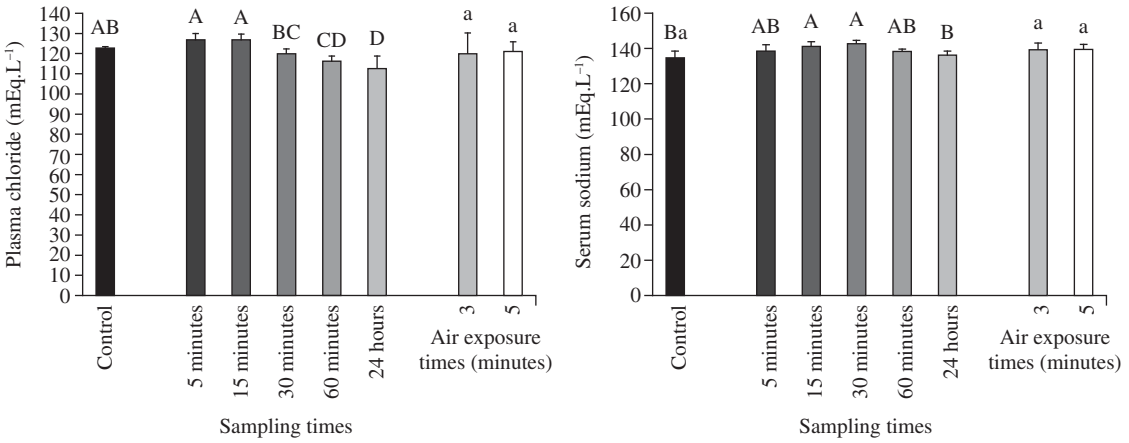

Figure 2. Plasma chloride and serum sodium of pacu undisturbed and air exposed for 3 and 5 minutes. Different capital letters indicate differences among sampling times (all air exposure times are represented in the same bar) and small letters between treatments (all samplings are represented in the same bar). Bars represent means of treatments or samplings. Vertical bars represent SEM.

15 and 30 minutes in both air-exposed fish (T2 and T3) and returned to resting levels 24 hours later (Figure 2). Potassium concentration ($p = 0.2901$) and osmolality ($p = 0.5332$) did not change after air exposure (Table 1). In fish of treatments 2 and 3, calcium levels did not differ significantly until 60 minutes after handling and decreased after 24 hours ($p = 0.0001$) compared to the other samplings but not to the control condition (Table 1). Excepting for MCV, which rose 30 minutes after capture and returned to resting levels 24 hours later ($p = 0.0440$), the hematocrit (Table 1), red blood cells count (RBC) and hemoglobin (Figure 3) were not affected by capture ($p = 0.8382$; $p = 0.2980$ and $p = 0.5463$, respectively).

4. Discussion

Stress is a biological response of adaptation to adverse conditions and fish respond by activating responses such as increased circulating cortisol and glucose (Wendelaar Bonga, 1997), changes in blood ionic balance (McDonald and Milligan, 1997) and hematological profile (Wojtaszek et al., 2002). Although laboratory studies can be criticized for lack of realism, they allow for a systematic determination of general behavioral and physiological principles of stress response that is not possible in the field.

Interactions of sequential stressors, as those to which the pacu was exposed in the present study, often cause increased stress in fish. Capture, for instance, includes

Table 1. Hematocrit, serum potassium and calcium and osmolality of pacu undisturbed and air exposed for 3 and 5 minutes. Different capital letters indicate differences among samplings and small letters between treatments.

		Sampling times					
	Air exposure	**5 minutes**	**15 minutes**	**30 minutes**	**60 minutes**	**24 hours**	**Means**
Hematocrit (%)	Control						34.5 ± 3.21^{Aa}
	3 minutes	31.4 ± 2.22	$33,2 \pm 2.22$	34.7 ± 1.35	33.3 ± 1.10	37.1 ± 0.77	34.0 ± 2.40^{a}
	5 minutes	34.9 ± 1.68	34.2 ± 2.42	32.8 ± 1.76	33.8 ± 3.39	36.6 ± 1.30	34.5 ± 2.31^{a}
	Means	33.2 ± 2.58^{A}	33.7 ± 2.16^{A}	33.7 ± 1.76^{A}	33.5 ± 2.27^{A}	36.9 ± 0.99^{A}	
Serum potassium ($mEq.L^{-1}$)	Control						2.34 ± 0.49^{Aa}
	3 minutes	3.03 ± 0.74	2.33 ± 0.32	2.61 ± 0.48	2.35 ± 0.42	2.95 ± 0.13	2.66 ± 0.49^{a}
	5 minutes	2.43 ± 0.35	2.34 ± 0.44	2.25 ± 0.55	2.64 ± 0.40	2.77 ± 0.51	2.48 ± 0.43^{a}
	Means	2.73 ± 0.61^{A}	2.34 ± 0.34^{A}	2.43 ± 0.50^{A}	2.50 ± 0.40^{A}	2.86 ± 0.35^{A}	
Serum calcium ($mEq.L^{-1}$)	Control						1.30 ± 0.06^{ABa}
	3 minutes	1.48 ± 0.22	1.54 ± 0.16	1.88 ± 0.24	1.74 ± 0.47	1.01 ± 0.19	1.53 ± 0.39^{a}
	5 minutes	1.45 ± 0.03	1.51 ± 0.08	1.60 ± 0.22	1.79 ± 0.40	1.03 ± 0.08	1.48 ± 0.31^{a}
	Means	1.46 ± 0.14^{A}	1.53 ± 0.11^{A}	1.74 ± 0.26^{A}	1.77 ± 0.39^{A}	1.02 ± 0.13^{B}	
Osmolality ($mOsmol.L^{-1}$)	Control						306.0 ± 5.8^{Aa}
	3 minutes	310.9 ± 6.2	312.0 ± 3.9	307.5 ± 1.8	309.7 ± 7.1	298.1 ± 16.3	307.6 ± 8.9^{a}
	5 minutes	314.5 ± 4.5	314.8 ± 1.7	314.8 ± 7.8	306.3 ± 8.4	317.0 ± 4.6	313.5 ± 6.3^{a}
	Means	312.7 ± 5.2^{A}	313.4 ± 3.1^{A}	311.2 ± 6.4^{A}	308.0 ± 7.2^{A}	307.5 ± 14.9^{A}	

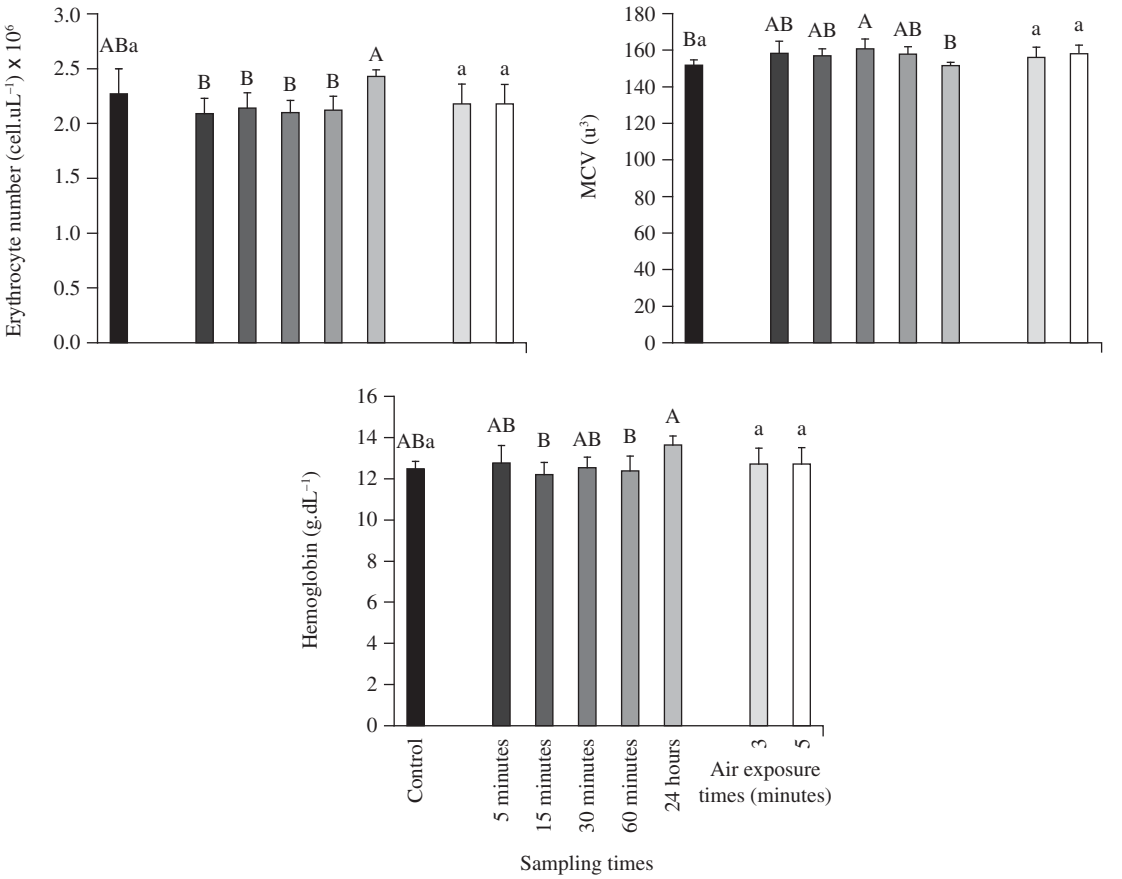

Figure 3. Number of erythrocytes, mean corpuscular volume (MCV) and hemoglobin concentration of pacu undisturbed and air exposed for 3 and 5 minutes. Different capital letters indicate differences among sampling times (all air exposure times are represented in the same bar) and small letters between treatments (all samplings are represented in the same bar). Bars represent means of treatments or samplings. Vertical bars represent SEM.

the chasing and swimming of fish, physical injury provoked by the contact among fish and with the net and anoxia by air exposure (Mugnier et al., 1998; Arends et al., 1999; Ross and Ross, 1999; Barcellos et al., 2001; Morales et al., 2005). Confirming the literature regarding fish stress, elevated circulating cortisol and glucose of pacu were short-term responses in both air-exposed fish after capture and the recovery occurred within 24 hours. However, the magnitude of glucose responses was low (from 60 to 94 $mg.dL^{-1}$) compared to values found previously in pacu in stressed state (Krieger et al., 1989; Martins et al., 2000). Air exposure of *Sparus aurata* for 3 minutes resulted, within 30 minutes, in an increase in plasma concentrations of cortisol and glucose. After 2 hours, plasma cortisol and after 12 hours plasma glucose had returned to control concentrations (Arends et al., 1999). On the other hand, netting and exposure of juvenile turbot to air for 1-4 minutes had no immediate effect on plasma cortisol concentrations (Mugnier et al., 1998). The stress response of pacu involved activation of the brain-pituitary-interrenal axis, as indicated by the cortisol and glucose elevations and it was probably elicited by the combined stimuli of fish swimming when chased, netting and the hypoxic condition provoked by air exposure.

Many stressors affect the ionic balance in fish (Wendelaar Bonga, 1997) facilitated by cathecolamine-induced increase of gill permeability responsible for chloride and sodium exchange with the environment (McDonald and Milligan, 1997). Decrease in plasma concentration of chloride and sodium were found in other stressed freshwater fish as in *Brycon cephalus* after transport (Carneiro and Urbinati, 2001; Urbinati et al., 2004). In our experiment, a partial and moderate disturbance of the ionic balance was found in both air exposed fish. In stressed pacu, chloride decreased 60 minutes after capture whereas sodium concentration increased transiently between 15 and 60 minutes after capture. Additionally, calcium and potassium levels and osmolality were not affected by the sequential stressors during capture. Apparently only a moderate and rather specific loss of permeability control occurred as consequence of capture.

The low magnitude of glucose responses, mild chloride and sodium disturbances, and the lacking potassium, calcium and osmolality responses indicated low activation of the brain-sympathetic-chromaffin cell axis, and hence a low release of catecholamines, which seemed though to occur to a higher extent at severe stressors (Mazeaud and Mazeaud, 1981). Several studies are known to initiate catecholamine secretion in fish including physical disturbance (Ristori and Laurent, 1985) and hypoxia (Ristori and Laurent, 1989). However, the degree of hypoxia required to initiate the responses is highly variable. Studies have shown that there exist hypoxia-tolerant species such as carp (Vianen et al., 2001) in contrast to hypoxia-intolerant species such as rainbow trout (Boutilier et al., 1988). A recent study that provided the first data on plasma catecholamines level in tropical fish (Perry et al., 2004) has shown that plasma catecholamines levels remained constant in pacu exposed to acute hypoxia, suggesting an inoperative or absent humoral adrenergic stress response in this species.

The results of the hematological assessement of stressed pacu confirmed the low degree of activation of the brain-sympathetic-chromaffin cell axis. Peripherical blood analysis has been used as a diagnosis to assess healthy state in fish and the effect of several stressors on them (Wojtaszek et al., 2002). The most significant effect of catechoalmines release during stress is to enhance blood O_2 transport by increasing the carrying capacity (Wells and Weber, 1990) and by enhancing Hb-O_2 binding affinity (Cossins and Richardson, 1985). No significant changes were found in the hematological parameters tested in pacu after the sequence of stressors during the capture. Concluding, the results of this work indicate that the submission of sequential stressors on pacu during capture activated the brain-pituitary-interrenal axis (cortisol and glucose responses) but the activation of the brain-sympathetic-chromaffin cell axis was apparently moderate (ionic and hematological responses).

Acknowledgements — We would like to thank Mrs. Damares Perecim Roviero for the technical support, to Piscicultura Águas Claras (Mococa, SP) for the fish donation and to the Aquaculture Centre of Sao Paulo State University for the facilities.

References

ABIMORAD, EG., CARNEIRO, DJ. and URBINATI, EC., 2007. Growth and metabolism of pacu (*Piaractus mesopotamicus*) juveniles fed diets containing different protein, lipid, and carbohydrate levels. *Aquaculture Research*, vol. 38, no. 1, p. 36-44.

ACKERMAN, PA., FORSYTH, RB., MAZUR, CF. and IWAMA, GK., 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry,* vol. 23, no. 4, p. 327-336.

ARENDS, RJ., MANCERA, JM., MUNOZ, JL., WENDELAAR BONGA, SE. and FLIK, G., 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. *Journal of Endocrinology*, vol. 163, no. 1, p. 149-157.

BARCELLOS, LJG., WOEHL, VM., WASSERMANN, GF., QUEVEDO, RM., ITTZÉS, I. and KRIEGER, MH., 2001. Plasma levels of cortisol and glucose in response to capture and tank transference in *Rhamdia quelen* (Quoy and Gaimard), a South American catfish. *Aquaculture Research*, vol. 32, no. 2, p. 121-123.

BARTON, BA., 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *North American Journal of Aquaculture*, vol. 62, no. 1, p. 12-18.

BECHARA, JA., ROUX, JP., DIAS, FJR., QUINTANA, CIF. and MEABE, CAL., 2005. The effect of dietary protein level on pond water quality and feed utilization efficiency of pacu *Piaractus mesopotamicus* (Holmberg, 1887). *Aquaculture Research*, vol. 36, no. 6, p. 546-553.

BOUTILIER, RG., DOBSON, GP., HOEGER, U. and RANDALL, DJ., 1988. Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. *Respiratory Physiol*ogy, vol. 71, no. 1, p. 69-82.

CARNEIRO, PCF. and URBINATI, EC., 2001. Salt as a stress response mitigator of matrinxã *Brycon cephalus* (Teleostei: Characidae) during transport. *Aquaculture Research*, vol. 32, no. 4, p. 297-304.

COSSINS, AR. and RICHARDSON, PA., 1985. Adrenaline-induced Na^+/H^+ exchange in trout erythrocytes and its effects upon oxygen-carrying capacity. *The Journal of Experimental Biology*, vol. 188, no. 1, p. 229-246.

GOMES, LC., CHIPPARI-GOMES, AR., LOPES, NP., ROUBACH, R., ARAUJO-LIMA, CARM. and URBINATI, EC., 2003a. Effect of fish density on the stress physiological responses and mortality of juvenile tambaqui *Colossoma macropomum* during transportation. *Journal of the World Aquaculture Society,* vol. 34, no. 1, p. 76-84.

GOMES, LC., ROUBACH, R., CAVERO, BAS., PEREIRA-FILHO, M. and URBINATI, EC., 2003b. Transport of Pirarucu *Arapaima gigas* in a closed system. *Acta Amazonica,* vol. 33, no. 4, p. 637-642.

IDE, LM., URBINATI, EC. and HOFFMANN, A., 2003. The role of olfaction in the behavioral and physiological responses to conspecific skin extract in a teleost fish, *Brycon cephalus*. *Journal of Fish Biology*, vol. 63, no. 2, p. 332-343.

JOMORI, RK., CARNEIRO, DJ., MALHEIROS, EB. and PORTELLA, MC., 2003. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. *Aquaculture*, vol. 221, no. 2, p. 277-287.

KING, EJ. and GARNER, RJ., 1947. Colorimetric determination of glucose. *Journal of Clinical Pathology*, vol. 1, no. 1, p. 30-33.

KRIEGER, MHA., DELATTRE, E., CAROLSFELD, J., CECCARELLI, P. and MENEZES, FV., 1989. A time-course study of physiological indicators of handling stress in the tropical fish *Piaractus mesopotamicus* (pacu*). Brazilian Journal of Medical and Biological Research*, vol. 22, no. 9, p. 1019-1022.

MARTINS, ML., MORAES, FR., MORAES, JRE. and MALHEIROS, EB., 2000. Falha da resposta do cortisol ao estresse por captura e por carragenina em *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae). *Acta Scientiarum*, vol. 22, no. 4, p. 545-552.

MAZEAUD, MM. and MAZEAUD, F., 1981. Adrenergic responses to stress in fish. In PICKERING, AD. (Ed.). *Stress and fish*. London: Academic Press Inc. p. 49-76.

McCORMICK, SD., SHRIMPTON, JM., CAREY, JB., O'DEA, MF., SLOAN, KE., MORIYAMA, S. and BJORNSSON, BTH., 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture*, vol. 168, no. 2, p. 221-235.

McDONALD, G. and MILLIGAN, L., 1997. Ionic, osmotic and acid-base regulation in stress. In IWAMA, GW., PICKERING, AD., SUMPTER, JP. and SCHRECK, CB. (Eds.). *Fish stress and health in aquaculture*. Cambridge: Cambridge University Press. p. 119-144.

MORALES, AE., CARDENETE, G., ABELLÁN, E. and REJÓN-GARCÍA, L., 2005. Stress-related physiological responses to handling in common dentex (*Dentex dentex* Linnaeus, 1758). *Aquaculture Research*, vol. 36, no. 1, p. 33-40.

MUGNIER, C., FOSTIER, A., GUEZOU, S., GAIGNON, JL. and QUEMENER, L., 1998. Effect of some repetitive factors on turbot stress response. *Aquaculture International*, vol. 6, no. 1, p. 33-45.

PERRY, SF., REID, SG., GILMOUR, KM., BOIJINK, CL., LOPES, JM., MILSOM, WK. and RANTIN, FT., 2004. A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, vol. 287, no. 1, p. 188-197.

QUEIROZ, JF., LOURENÇO, JNP., KITAMURA, PC., SCORVO FILHO, JD., CYRINO, JEP., CASTAGNOLLI, N., VALENTI, WC. and BERNARDINO, G., 2005. Aquaculture in Brazil: research priorities and potential for further international collaboration. *World Aquaculture Magazine*, vol. 36, p. 45-50.

RISTORI, MT. and LAURENT, P., 1985. Plasma catecholamines and glucose during moderate exercise in the trout: comparisons with bursts of violent activity. *Experimental Biology*, vol. 44, no. 4, p. 247-253.

_____, 1989. Plasma catecholamines in rainbow trout (*Salmo gairdneri*) during hypoxia. *Experimental Biology*, vol. 48, no. 5, p. 285-290.

ROCHA, RM., CARVALHO, EG. and URBINATI, EC., 2004. Physiological responses associated with capture and crowding stress in matrinxã *Brycon cephalus* (Gunther, 1869). *Aquaculture Research*, vol. 35, no. 3, p. 245-249.

ROMAGOSA, E., PAIVA, P. and GODINHO, HM., 1990. Pattern of oocyte diameter frequency distribution in females of the Pacu, *Piaractus mesopotamicus* (Holmberg 1887) (*Colossoma mitrei* Berg 1895), induced to spawn. *Aquaculture*, vol. 86, no. 1, p. 105-110.

ROSS, LG. and ROSS, B., 1999. *Anesthetic and sedative techniques for aquatic animals*. Oxford: Blackwell Science. 176p.

SOUZA, VL., OLIVEIRA, EG. and URBINATI, EC., 2000. Effects of food restriction and refeeding on energy stores and growth of pacu, *Piaractus mesopotamicus*. *Journal of Aquaculture in the Tropics*, vol. 15, no. 3, p. 371-379.

TAKAHASHI, LS., ABREU, JS., BILLER, JD. and URBINATI, EC., 2006. Efeito do ambiente pós-transporte na recuperação dos indicadores de estresse de pacus juvenis, *Piaractus mesopotamicus*. *Acta Scientiarum Anim. Science*, vol. 28, no. 4, p. 469-475.

TAKAHASHI, LS., BALDAN, AP. and URBINATI, EC., 2006. Growth performance and energetic metabolism of pacu, *Piaractus mesopotamicus* (Holmberg, 1887) fed diets supplemented with ammonium metavanadate. *Aquaculture Research*, vol. 37, no. 13, p. 1372-1377.

URBINATI, EC., ABREU, JS., CAMARGO, ACS. and LANDINES, MAP., 2004. Loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities. *Aquaculture*, vol. 229, no. 4, p. 389-400.

VAN DER SALM, AL., SPANINGS, FAT., GRESNIGT. R., WENDELAAR BONGA, SE. and FLIK, G., 2005. Background

adaptation and water acidification affect pigmentation and stress physiology of tilapia, *Oreochromis mossambicus*. *General and Comparative Endocrinology*, vol. 144, no. 1, p. 51-59.

VAN HAM, EH., VAN ANHOLT, RD., KRUITWAGEN, G., IMSLAND, AK., FOSS, A., SVEINSBØ, BO., FITZGERALD, R., PARPOURA, AC., STEFANSSON, SO. and WENDELAAR BONGA, SE., 2003. Environment affects stress in exercised turbot. *Comparative Biochemistry and Physiology A. Molecular & Integrative Physiology*, vol. 136, no. 3, p. 525-538.

VIANEN, GJ., VAN DEN THILLART, GEEJ., VAN KAMPEN, M., VAN HEEL, TI. and STEFFENS, AB., 2001. Plasma lactate and stress hormones in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) during stepwise decreasing oxygen levels. *Netherlands Journal of Zoology*, vol. 51, no. 1, p. 33-50.

WELLS, RMG. and WEBER, RE., 1990. The spleen in hypoxic and exercised rainbow trout. *Journal of Experimental Biology*, vol. 150, no. 1, p. 461-466.

WENDELAAR BONGA, SE., 1997. The stress response in fish. *Physiological Reviews*, vol. 77, no. 4, p. 591-625.

WOJTASZEK, J., DZIEWULSKA-SZWAJKOWSKA, D., LOZINSKA-GABSKA, M., ADAMOWICZ, A. and DZUGAJ, A., 2002. Hematological effects of high dose of cortisol on the carp (*Cyprinus carpio* L.): cortisol effect on the carp blood. *General and Comparative Endocrinol*ogy, vol. 125, no. 2, p. 176-183.