METABOLIC IMPACT OF HANDLING

ON *Pseudoplatystoma coruscans,*

A WIDESPREAD TELEOST FISH

Gilberto Moraes
Department of Genetic and Evolution. Federal University of Sao Carlos.
Sao Carlos. Sao Paulo. Brazil.
gil@power.ufscar.br

Paulo Mauricio Bidinotto
Department of Genetic and Evolution. Federal University of Sao Carlos.
paulobidinotto@hotmail.com

Introduction

The aquatic environment is physically more restrictive than the terrestrial. Water bodies characteristics fluctuate and are dependent on many surrounding factors. Variables such as temperature, pH, and oxygen concentration, among others, are important factors for fish culture. In addition to water quality factors, fish culture procedures such as handling and transportation can be important sources of stress in intensive aquaculture. Handling, followed by transportation of the largest number of fish into the smallest possible volumes of water necessitates correctly designed systems to prevent adverse water quality changes. Basic physiological needs require continuous circulation of freshly aerated water to all parts of hauling tanks. Another adverse situation that must be minimized is the accumulation of ammonia during fish transport operations. This is usually the main shock imposed on the fish during crowding and transportation.

Different techniques of handling are more or less stressing, sometimes simulating disturbances resulted from chasing (Schwalme and Mackay, 1990; Waring et al.,1995). In view of this, several metabolic responses may proceed from such stressful conditions and different adjustments are expected. Metabolic costs associated with stress resulting from physical disturbances have been described (Saunders, 1963; Barton and Schreck, 1987; Fletcher, 1992). If the fish has a metabolic demand to supply the stress cost, this means less available energy for other performance components (Barton and Iwama, 1991). Cortisol seems to have an important role in metabolic responses from stress (Barton and Schreck, 1987).

Activity of certain metabolic enzymes should be affected by cortisol (Davis et al., 1985; Vijaian et al., 1991). As well, an increase in metabolic intermediates, like glucose, is ascribed to cortisol (Leach and Taylor, 1982; van der Boon et al., 1991) but other investigations are needed to clarify this effect.

The kinds of responses to stress are different among fishes and are displayed at different levels (Davis and Parker, 1983; 1986; Sumpter et al., 1986; Pickering and Pottinger, 1989). However, differences among species seem to be, in part, attributed to strains (Barton et al., 1986; Pottinger and Moran 1993; Noga et al., 1994), discrete stocks (Iwama et al., 1992) or between wild and hatchery fish, suggesting a "domestication" effect. Moreover, the stress response seems to have a genetic component (Heath et al., 1993) and some fish may be genetically predisposed to exhibit different responses to cortisol (Pottinger et al., 1992) albeit heritability estimates to date are low (Fevolden et al., 1993). In light of this fact, selection of the best strains of fish for aquaculture systems is one point to be considered.

This work reports preliminary data concerning metabolic and hematological responses of a single population of the tropical catfish (pintado) *Pseudoplatystoma coruscans* after stress caused by handling, a common practice in tropical aquaculture systems.

Material and Methods

Fish

Young specimens of *P. coruscans* weighting 8.0 ± 2.0 g were purchased from Projeto Pacu – Campo Grade – MS, Brazil and transported in hauling boxes to the laboratory. The animals were stocked in 2,000L plastic tanks at 25 ± 3^{o}C under natural photoperiod with filtered, well aerated water and nourished with carnivorous fish ration pellets. After 90 days under these new environmental conditions, the animals reached 38.48 ± 7.30 g and were submitted to the following experimental design.

Experimental design

Forty animals were carefully divided into five groups of eight fish in the same environment. After 15 days, four groups were captured and transferred to plastic 50L boxes, staying there for three minutes. After that, three groups were placed back into the tanks to recovery and one group, after withdrawing blood samples, was immediately killed to collect liver, white muscle and kidney. The three groups under recovery were sampled at different time intervals (12, 24, and 48 hours). The fifth group remained in the tank and was used as a control and the animals were sampled right after the 48 hours group.

Analytical procedures

Blood was withdrawn from the caudal vein in heparinized syringes and used for hematological and plasma analyses. Tissues were excised, promptly frozen in nitrogen and kept at –20^o C for subsequent analysis. The whole blood was used for haematocrit, red cell count and hemoglobin determination (Drabkin, 1948). The whole plasma was used for triglyceride (Doles' Colorimetric Kit) and total protein (Deutscher, 1990) colorimetric determination. Free protein trichloroacetic acid extracts from plasma and tissues were used to estimate glucose (Dubois et al., 1956), lactate (Harrower and Brown, 1972) and pyruvate (Lu, 1939) by colorimetric methods. Glycogen was estimated by acid hydrolitic method (Dubois et al., 1956) in liver, kidney and white muscle after alkaline digestion and alcohol precipitation (Bidinotto et al., 1997).

Statistical analysis

The means ± standard deviation were compared with the control using an ANOVA test and the Kolmogorov-Smirniv test was fitted to a normal distribution. If any difference was detected among samples, the Dunnet test was used to group the means. Samples of non-homogeneous variances were applied by non-parametric ANOVA (Kruskal-Wallis) to the row data and the same Dunnet test was used to group the means. The confidence interval used was $p<0.05$.

Results and Discussion

The effect of handling was reflected in the blood by the increase in hemoglobin/red blood cell content of the (Table 1). This effect may be caused by a decrease in blood cell volume, noticeable after only 24 hours. Immediate increase in haematocrit after handling, followed by similar increases of hemoglobin and red blood cells, suggests the release of erythrocytes into the circulatory system by hematopoietic structures. This hematological response may be to compensate for the metabolic demand resulting from physical disturbance.

The concentration of plasma triglycerides, lactate and glucose were significantly decreased after handling (Figures 1, 2 and 3). The continuous decrease in plasma triglycerides over 48 hours suggests a persistent use of this energy-rich molecule to sustain the metabolic demand resulting from the stress. Plasma glucose concentration decreased during the first 12 hours after stress but, afterwards, it tended to fluctuate around normal values. The energetic cost from this stress was very high and particularly aerobic. The amount of lipids metabolized over 48 hours after stress was very large (70µmols of triglycerides) as compared to glucose (3.3 µmols). This is characteristic of aerobic preference. The liver glycogen decreased throughout the experimental recovery (Fig. 4). However, considering that glucose and lactate remained constant and plasma glucose decreased, it is plausible to suggest the use of the glycogen within the liver. In view of the large amount of glycogen hydrolyzed (183 µmols of glucosyl-glucose per gram of wet tissue) it is improbable that its use was as an energy source. Therefore, transformation of glucose into other kinds of metabolites is suggested. It is interesting to observe that white muscle lactate decreased 12 hours after the stress, returning slightly to normal values. White muscle glycogen were also slightly decreased (1 µmols per gram of wet tissue) during 12 hours from the stress, returning back to normal values after 48 hours. Glucose was consumed at higher rates (4 µmols per gram of tissue) than glycogen, but not statistically significantly. However, considering the usual anaerobic preference of white muscles, this metabolic behavior suggests an inconspicuous use of anaerobic metabolism to supply the energetic demand in such tissue after handling. The kidney consumed about 7.00µmols of glucose/mg of wet tissue after 24 hours of handling. However, the bulk of glycogen remained almost constant (13.00µmols of glucosyl-glucose/mg of wet tissue). The glucose decrease was very smooth and after 24 hours, the recovery had begun. The kidney glycogen stock was 36.00µmols of glucosyl-glucose/mg of wet tissue, and its consumption was not significant. However, the use of glucose was very expressive, decreasing from the stress until 24 hours later (27 μmols per gram of tissue). Considering that after handling lactate remained constant, it is possible to assume an oxidative metabolism in this tissue.

The hematological response observed to handling, as well as the organismal metabolic changes, are both very suggestive that *P. coruscans* presents an oxidative preference to supply the metabolic cost from physical disturbance. The large plasma triglyceride decrease compared to the slight glucose consume is a strong indication that lipids are the preferential fuel supply for handling demands from stress.

Table 1. Blood values after 3 minutes of handling. in *Pseudoplastystoma coruscans.*

	Control	0 h	12 h	24 h	38 h
Hemoglobin (g/100 mL)	5.92 ± 0.76	9.22 ± 1.28 (*)	7.49 ± 0.98	7.98 ± 1.22 (*)	6.51 ± 0.96
RBC (10^6 cels/mL)	1.26 ± 0.83	1.67 ± 0.33 (*)	1.59 ± 0.28 (*)	1.25 ± 0.14	1.12 ± 0.16
Hematocrit (%)	17.58 ± 2.67	24.20 ± 1.68 (*)	19.58 ± 2.15	14.43 ± 1.93	16.00 ± 2.98
CMV (nm^3)	14.04 ± 2.15	15.13 ± 3.69	12.63 ± 2.31	11.69 ± 1.48 (*)	14.52 ± 3.12
MCH (μg/cell)	4.73 ± 0.63	5.77 ± 1.55 (*)	4.83 ± 0.94	6.46 ± 1.11 (*)	5.91 ± 1.14 (*)

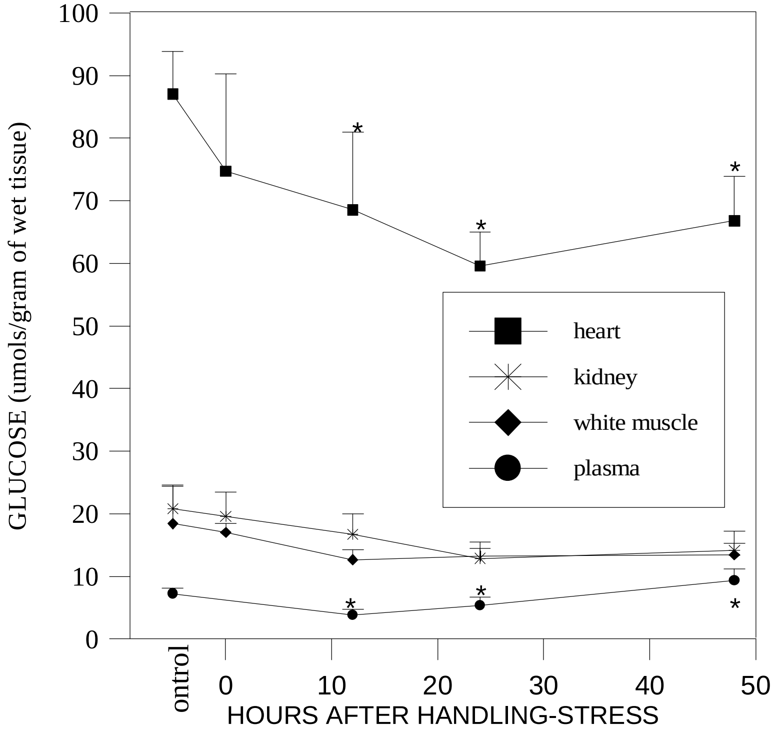

Figure 1. Tissue glucose concentration after 3 minutes of handling in *Pseudoplastystoma coruscans*.

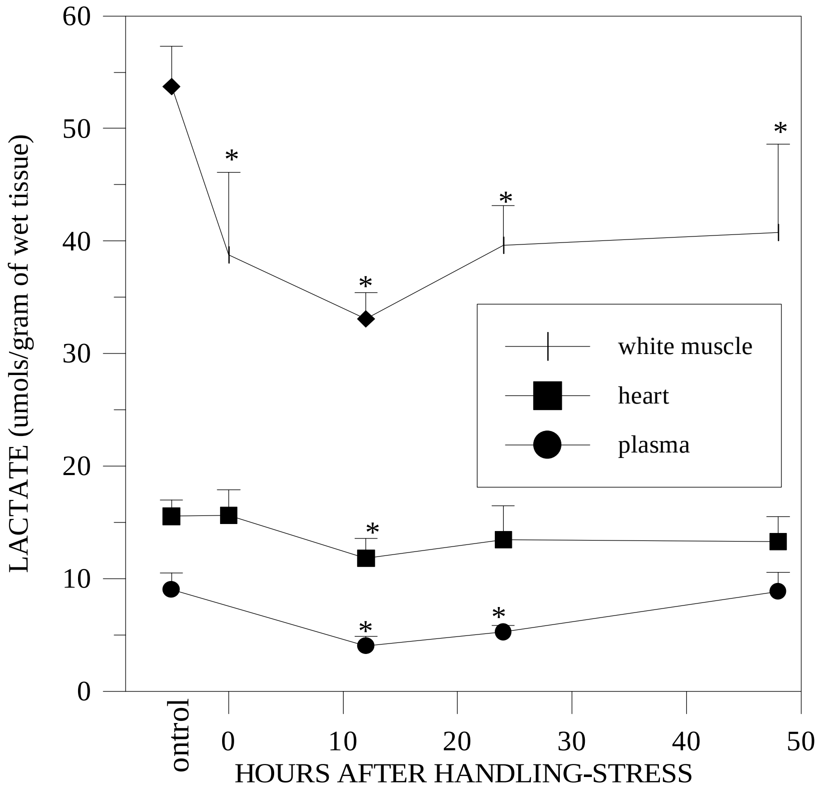

Figure 2. Tissue lactate concentration after 3 minutes of handling in *Pseudoplastystoma coruscans*.

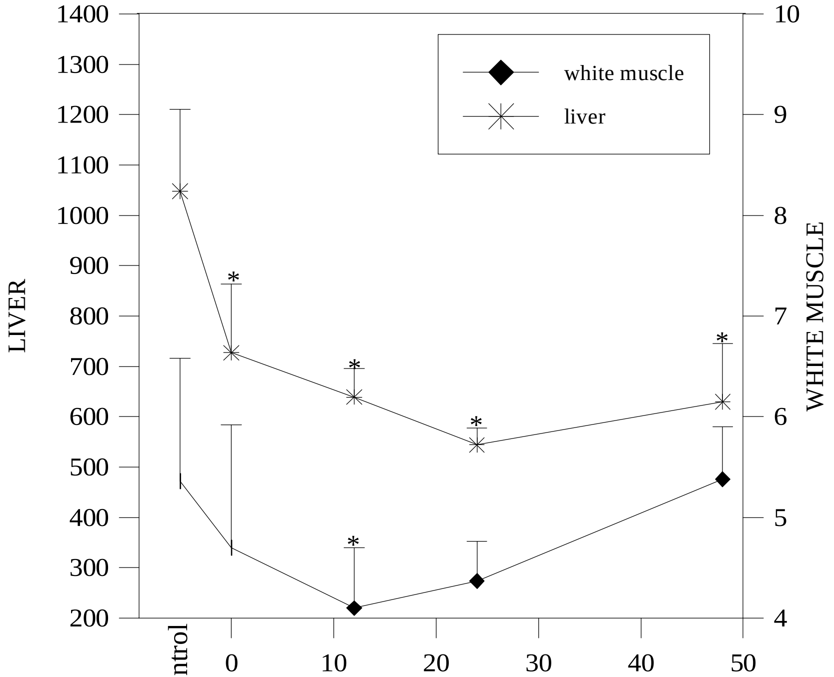

Figure 3. Tissue glycogen concentration (μmols glucosyl-glucose/gram of wet tissue) after 3 minutes of handling in *Pseudoplastystoma coruscans*.

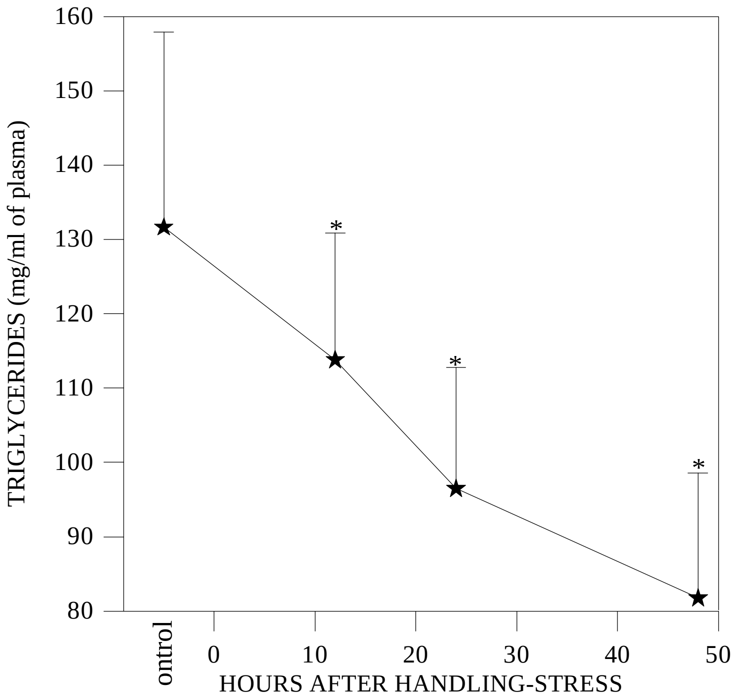

Figure 4. Plasma triglyceride concentration after 3 minutes of handling in *Pseudoplastystoma coruscans*.

References

Barton B.A. and C.B. Schreck. 1987. Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. 62: 299-310

Barton, B.A. and G.K Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effect of corticosteroids. Annual Review of Fish Diseases. 1: 3-26

Barton, B.A., Schereck, C.B. and L.A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transaction of the American Fisheries Society. 115: 245-51

Bidinotto, P.M., Moraes, G. and R.H.S. Souza. 1997. Hepatic glycogen and glucose in eight tropical fresh water teleost fish: A procedure for field determinations of micro samples. Bol. Tec. do CEPTA. 10: 53-60

Davis, K.B. and N.C. Parker. 1983. Plasma corticosteroid and chloride dynamics in rainbow trout, Atlantic salmon, and lake trout during and after stress. Aquaculture. 32: 189-94

Davis, K.B. and N.C. Parker. 1986. Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. Transaction of the American Fisheries Society. 115: 495-99

Davis, K.B., Torrance, P., Parker, N.C. and M.A. Suttle. 1985. Growth, body composition, and hepatic tyrosine aminotransferase activity in cortisol-fed channel catfish, *Ictalurus punctatus*, Rafinesque. Journal of Fish Biology. 27: 177-84

Deutscher, M.P. 1990. Guide to protein purification. *In:* Methods in Enzymology 182. pp894

Drabkin, D.L. 1948. The standadization of hemoglobin measurment. Am. J. of the Med. Sci. 215(1): 110-111

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and F. Smith. 1956. Colorimetric methods for determination of sugars and related substances. Anal. Chem. 28: 350-358.

Fevolden, S.E., Refstie, T. and B. Gjerde. 1993. Genetic and phenotypic parameters for cortisol and glucose stress response in Atlantic and rainbow trout. *Aquaculture*, 118, 205-16

Fletcher, C.R. 1992. Stress and water balance in the plaine *Pleuronectes platessa.* Journal of Comparative Physiology. 162B: 513-519

Harrower, J.R. and C.H. Brown. 1972. Blood lactic acid. A micromethod adapted to field collection of microliter samples. J. Appl. Physiol. 32(5): 224-228.

Heath, D.D., Bernier, N.J., Heath, J.W. and G.K. Iwama. 1993. Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry. Canadian Journal of Fisheries and Aquatic Sciences. 50: 435-42.

Iwama, G.K. McGeer, J.C. and N.J. Bernier. 1992. The effect of stock and rearing history on the stress response in juvenile coho salmon (*Oncorhynchus kisutch*). ICES Marines Science Symposium. 194: 67-83

Leach, G.J. and M.H. Taylor. 1982. The effect of cortisol treatment and protein metabolism in *Fundulus heteroclitus*. General and Comparative Endocrinology. 48: 76-83

Lu, G.D. 1939. The metabolism of pyruvic acid in normal and vitamin B-deficient state. I. A rapid specific and sensitive method for the estimation of blood pyruvate. Biochem. J. 33: 249-254

Noga, E.J., Kerby, J.H., King, W., Aucoin, D.P. and F. Giesbrecht. 1994. Quantitative comparison of the stress response of striped bass (*Morone saxatilis*) and hybrid striped bass (*M. saxatilis X M. chrysops* and *M. saxatilis X M. americana*). American Journal of Veterinary Research. 55: 405-409

Pickering, A.D. and T.G. Pottinger. 1989. Stress response and disease resistance in salmonid fish: effect ofchronic elevation of plasma cortisol. Fish Physiology and Biochemistry. 7: 523-78

Pottinger, A.D. Pickering, T.G. and M.A. Hurley. 1992. Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 103: 257-89

Pottinger. T.G. and T.A. Moran. 1993. Differences in plasma cortisol and cortisone dynamics during stress in two strains of rainbow trout (*Oncorhynchus mykiss*). Journal of Fish Biology. 43: 121-30

Saunders, R.L. 1963. Respiration of the Atlantic cod. Journal of the Fisheries Research Board of Canada. 20: 373-86

Schwalme, K. and W.C. Mackay. 1990. Mechanisms that elevate the glucose concentration of muscle and liver in yellow perch (*Perca flavens* Mitchill) after exercice-handling stress. Can. J. Zool. 69: 456-461

Sumpter, J.P., Dye, H.M. and T.J. Benfey. 1986. The effect of stress on plasma ACTH, αMSH, and cortisol levels in salmonid fishes. General and Comparative Endocrinology. 62: 377-85

van der Boon, J., van den Thillart, G.E.E.J.M. and A.D.F. Addink. 1991. The effect of cortisol administration on intermediary metabolism in telost fish. Comparative Biochemistry and Physiology. 100A: 47-53

Vijaian, M.M., Ballantyne, J.S. and J.F. Leatherland. 1991. Cortisol induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. General and Comparative Endocrinology. 82: 476-86

Waring, C.P., Stagg R.M. and M.G. Poxton. 1996. Physiological responses to handling in the turbot. Journal of Fish Biology. 48: 161-17