*International Journal of Biological Research*, 1 (2) (2013) 41-47
©Science Publishing Corporation
www.sciencepubco.com/index.php/IJBR

# The effects of increased temperature and $CO_2$ on the Amazon fish, tambaqui (*Collosoma macropomum*, Cuvier, 1818)

**Amtyaz *[1], Ramon Barros Baptista [2], Vera Maria Fonseca de Almeida e Val [2 & 3]**

[1] Dept. of Zoology, Sir Syed Govt. Girls College Nazimabad, Karachi, PAKISTAN.
[2] Laboratory of Eco-Physiology and Molecular Evolution (LEEM), National Institute for Research in the Amazon, INPA. Manaus, Brazil
[3] Laboratory for Applied Genetics in Aquaculture (**AGA**), University Nilton Lins (**UNL**), Manaus, AM, Brazil
**Corresponding author E-mail: imtiazsafi76@gmail.com*

**Abstract**

The effects of temperature and carbon dioxide ($CO_2$) changes on different haematological parameters and enzymes activities such as on LDH (Lactate de Hydrogenase), MDH (Malate de Hydrogenase) & GST (Glutathione-S-Transferase) were studied in the liver and skeletal muscles of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) in the Laboratory of Ecophysiology and Molecular Evolution (LEEM) of the National Institute for Amazonian Research (INPA). The experimental procedures involved the exposure of fish to temperature rise from 28Cº to 32Cº and an increased in $CO_2$ concentration up to 10 times higher than the current levels for 24, 48, 72 and 96 hours. The levels of LDH, GST and MDH in both the tissues of Tambaqui (Muscles & Liver) showed a significant decreased at different along the different exposure periods. The levels of plasma glucose in blood decreased and the values of Hb, Ht, RBC, VCM, and HCM & CHCM showed increased after the exposure.

***Keywords***: *Temperature, $CO_2$, Tambaqui fish, Amazon, Brazil.*

## 1 Introduction

Owning one of the greatest biological diversities of the world, the most notable fauna of the Amazon basin is the fish one, whose numbers of species are estimated to be more than 2500. Among the water bodies of the region, the biggest variety of fish is found in Amazon River, presenting a total of 256 species. This number is extremely high compared to the whole Europe hydrographic basins, where only 192 species are registered [1]. The adaptations of fish to the Amazonian flooding areas include several strategies concerning their growth and reproduction. However, they are also characterized for presenting complex morphological, anatomical, physiological, and behavioral adaptations to ensure the survival in an ever-changing environment [2]. A great diversity of species has corresponds to a wide range of biochemical, physiological, phenological and ethological strategies, as well as a range of anatomical and morphological variations to deal with periodical flooding, making the studies in floodable areas very complex. However, an innovative approach may enable the detection of a mechanism for common regulation to all floodable biota components, with benefits for the scientific knowledge, for the productive chain and especially to people in the region.

The Amazon unquestionably presents the largest fresh water resource and fish diversity in the world. In this region, fish is the most important dietary protein source to its population (Per capita consumption of 60 kg/year) and fishing is among the most important economic activity, involving more than 110,000 people[3], [4] .

Tambaqui (*Colossoma macropomum*) is the most important commercial species in the Amazon and the main Brazilian native species for aquaculture. A large number of studies on its culture have been made available in recent years, emphasizing its performance and tolerance to intensive culturing conditions ([5], [6], [7], [8], [9], [3], [10], [11], [12], [13], [14]). However, information on the effect of Temperature and $CO_2$ on Tambaqui is still scarce and required.

The tambaqui (*Colossoma macropomum*) is a freshwater fish of the subfamily; Serrasalminae, family; Characidae and is the largest characin of South America. It is also known by the names pacu, black pacu, black-finned pacu, giant pacu, cachama and gamitana. The tambaqui is found in the Amazon and Orinoco basins in its wild form. It may reach more than 1 meter in total length and 30 kilograms in total weight. It is similar in shape to the Piranha and is

sometimes confused with the carnivorous fish. It is tall and laterally compressed with large eyes and a slightly arched back. Body color is basic black to gray with spots and blemishes in its mid body. All the fins are black and the pectoral fins are small. Around 10 percent of a tambaqui's weight is fat. This species is usually solitary. Adults stay in flooded forests during the first 5 months of flooding and consume fruits and grains. Young and juveniles live in black waters of flood plains until sexual maturity. The tambaqui feeds on zooplankton, insects, snails, and decaying plants. Research has indicated that this species plays an important role in dispersing seeds from fruits. The tambaqui is used in aquaculture because it can live in mineral poor waters and is very resistant to diseases. This species is marketed fresh and frozen. The aim of the present study was to evaluate the effect of water exchange regime as an alternative to minimize aquaculture environmental impact using tambaqui physiological parameters and performance as indicators.

Temperature and $CO_2$ are the major environmental factors, and play a critical role in growth, reproduction, immigration, succession and metabolism and health of ecosystems. A rise in temperature and $CO_2$ changes the physical and chemical properties of water. High water temperature induce increased metabolic rate in the aquatic organisms, resulting for example, in more demand for food in fishes. . In recent years, there was an increase of interventions in the Amazon - such as new hydroelectric plants, changes in the soil, mining and roads - all of which had immediate impacts on the aquatic environment. As this fish is of commercial interest, it is very important for the Amazon, so it is important to know what reactions will be caused by climate changes on Tambaqui. The responses of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) to the stresses associated with acidification and high temperature is still not clearly understood, and a number of recent experiments have addressed this problem. The current work attended to expose tambaqui to higher temperature and higher $CO_2$ to check the main responses to the acute hypercapnia in high temperature.

In the present project we had studied the effect of two major environmental factors on Tambaqui fishes (*Collosoma macropomum)* of Manaus, Amazona's state of Brazil. We have, than, studied the effect of acute increased in temperature and $CO_2$ on LDH (Enzyme for an-Aerobic metabolism), MDH (Enzyme for Oxidative aerobic metabolism, present both in mitochondria and cytoplasm) and GST (an Anti-oxidant Enzyme, also responsive to xenobiotic exposure) from muscles and liver of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818).

# 2 Materials & methods

The experiment was performed in the Laboratory of Ecophysiology and Molecular Evolution (LEEM) of National Institute for Research in the Amazon, INPA of Amazonia state of Brazil, during the period between May and June of 2012. Tambaqui (*Colossoma macropomum)*, were obtained from a fish farming station, located near Manaus, Amazonas State, Brazil, and fish were transported to LEEM Laboratory at INPA, and kept in tanks (1000 L) supplied with continuously water flow and aeration at 28Cº, 12L: 12D photo period and fed for one month. After the acclimatization period, fish were divided into two groups: control (28Cº normocapnia) and experimental (E: 32Cº hypercapnia) groups (n = 6, each group) and assayed after 96h exposure. The control and experimental groups were transferred to the six aquaria (5L, one fish per aquarium) containing water with the same physical and chemical characteristics as those of the acclimatization period. Feeding was suspended 24h before the experiments. Biometry was made in the beginning of the experiment aiming to assess total body length in cm, standard body length in cm, body height in cm and body weight in g. As a result of the experiments we sampled three groups: C (Control 0h); CC (Control 96h) and E (Experimental 96h).

Fishes were killed by medullar section according to Brazilian recommendations of animal care council from the Brazilian Ministry of Science, Technology and Innovation (CONCEA – MCTI). Blood samples were drawn from the caudal vessel using heparinized syringes for determining blood tests before fish death. For the Enzymatic analysis (LDH, MDH and GST) liver and muscle were isolated, immediately frozen in liquid $N_2$, and stored at -80ºC until processing. Tissues were washed in cold 250 mM sucrose solution and homogenized in cold 100 mM phosphate buffer (1:1, w/v), PH 7.0, with a Potter-Elvejhem homogenizer. The homogenates were centrifuged at 18,000g in a Sorvall RC-5B during 30 min at $4^0$C. For the haematological parameters evaluation, hematocrit values (Ht %) were determined after 10 minutes centrifugation in micro-haematocrit tubes. Haemoglobin concentration ((Hb) mg.dL-1) was measured as described by Kampen & Zijlsta (1964). Red blood cell counts (RBC) were obtained with the use of a Neubaur chamber. Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular haemoglobin (MCH) were determined according to Brow (1976). The glucose levels were determined by electronic meter blood glucose, Accu-chek advantage II. For the enzymatic parameters evaluation, the activity of lactate dehydrogenase (LDH; E.C.1.1.1.27), Malate dehydrogenase (MDH; E.C. 1.1.1.37) and GST were estimated in skeletal muscle and liver according to Driedzic & Almeida-Val (1996). For analysis of LDH, MDH & GST activities, samples were homogenized in a buffer containing 150 mM Imidazole, 1mM EDTA, 5mM

dithiothreitol (DTT) and 1 % Triton X-100, PH7.4. To avoid loss of enzyme activity during sample preparation, procedures were performed on ice. Data is presented as Means and Standard Deviation of the Means and were compared.

Table.1: Change in different blood parameters of tambaqui (*Colossoma macropomum*) exposed to different temperature and $CO_2$.

| | Glucose mg/dl | Abs 540 nm | [Hb] g/dl | Ht (%) | RBC ($x10^6$ $mm^3$) | VCM | HCM | CHCM |
|---|---|---|---|---|---|---|---|---|
| C1-0h | 28.00 | 0.112 | 3.27 | 19.00 | 1.39 | 136.69065 | 23.528058 | 17.212632 |
| C2-0h | 38.00 | 0.124 | 3.62 | 23.00 | 1.49 | 154.36242 | 24.300671 | 15.742609 |
| C3-0h | 33.00 | 0.144 | 4.20 | 24.00 | 1.51 | 158.9404 | 27.846358 | 17.52 |
| C4-0h | 44.00 | 0.126 | 3.68 | 23.00 | 1.5 | 153.33333 | 24.528 | 15.996522 |
| C5-0h | 33.00 | 0.151 | 4.41 | 25.50 | 1.55 | 164.51613 | 28.446452 | 17.29098 |
| C6-0h | 41.00 | 0.147 | 4.29 | 25.00 | 1.33 | 187.96992 | 32.273684 | 17.1696 |
| Average | 36.17 | | 3.91 | 23.25 | 1.46 | 159.30 | 26.82 | 16.82 |
| Std. Dev. | 5.40 | | 0.41 | 2.12 | 0.08 | 15.39 | 3.05 | 0.69 |
| | | | | | | | | |
| CC1-96h | 15.00 | 0.162 | 4.73 | 27.00 | 1.21 | 223.1405 | 39.094215 | 17.52 |
| CC2-96h | 28.00 | 0.162 | 4.73 | 23.50 | 1.14 | 206.14035 | 41.494737 | 20.129362 |
| CC3-96h | 29.00 | 0.216 | 6.31 | 27.00 | 2.15 | 125.5814 | 29.335814 | 23.36 |
| CC4-96h | 66.00 | 0.209 | 6.10 | 30.50 | 1.61 | 189.44099 | 37.90559 | 20.00918 |
| CC5-96h | 56.00 | 0.222 | 6.48 | 27.00 | 1.77 | 152.54237 | 36.623729 | 24.008889 |
| CC6-96h | 15.00 | 0.178 | 5.20 | 19.50 | 1.27 | 153.54331 | 40.925984 | 26.654359 |
| Average | 34.83 | | 5.59 | 25.75 | 1.53 | 175.06 | 37.56 | 21.95 |
| Std. Dev. | 7.804912983 | | 0.73 | 3.45 | 0.36 | 33.92 | 4.04 | 3.03 |
| E1-96h | 40.00 | 0.21 | 6.13 | 28.00 | 1.58 | 177.21519 | 38.810127 | 21.9 |
| E2-96h | 30.00 | 0.242 | 7.07 | 28.00 | 2.35 | 119.14894 | 30.069787 | 25.237143 |
| E3-96h | 149.00 | 0.238 | 6.95 | 27.00 | 1.89 | 142.85714 | 36.77037 | 25.739259 |
| E4-96h | 23.00 | 0.219 | 6.39 | 28.50 | 1.83 | 155.7377 | 34.944262 | 22.437895 |
| E5-96h | 30.00 | 0.189 | 5.52 | 27.50 | 1.69 | 162.72 | 32.66 | 20.07 |
| E6-96h | 46.00 | 0.246 | 7.18 | 31.50 | 2.22 | 141.89189 | 32.356757 | 22.80381 |
| Average | 33.80 | | 6.54 | 28.42 | 1.93 | 149.93 | 34.27 | 23.03 |
| Std. Dev. | 9.12 | | 0.59 | 1.46 | 0.27 | 18.28 | 2.92 | 1.94 |

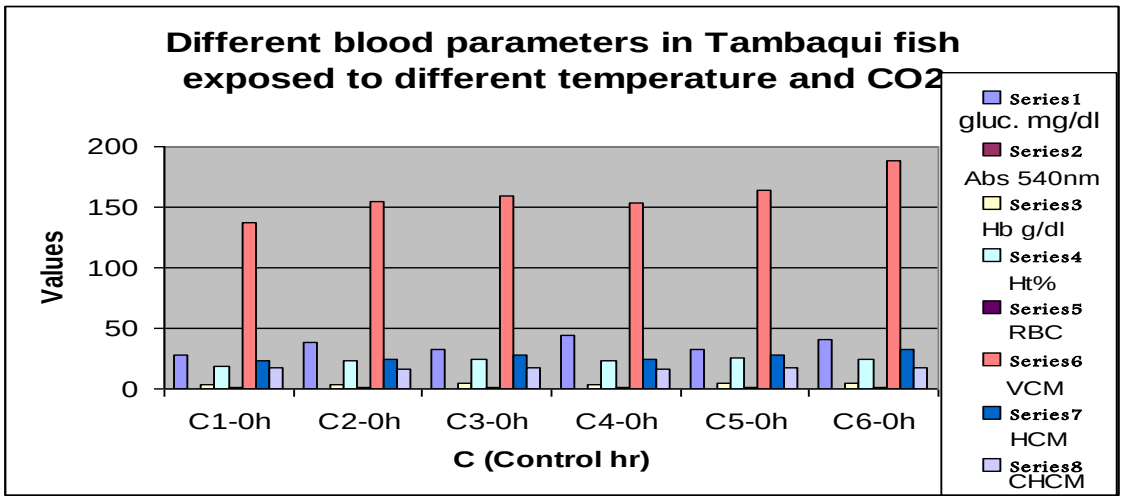


Fig. 1: Different blood parameters in C(Control 0hr)

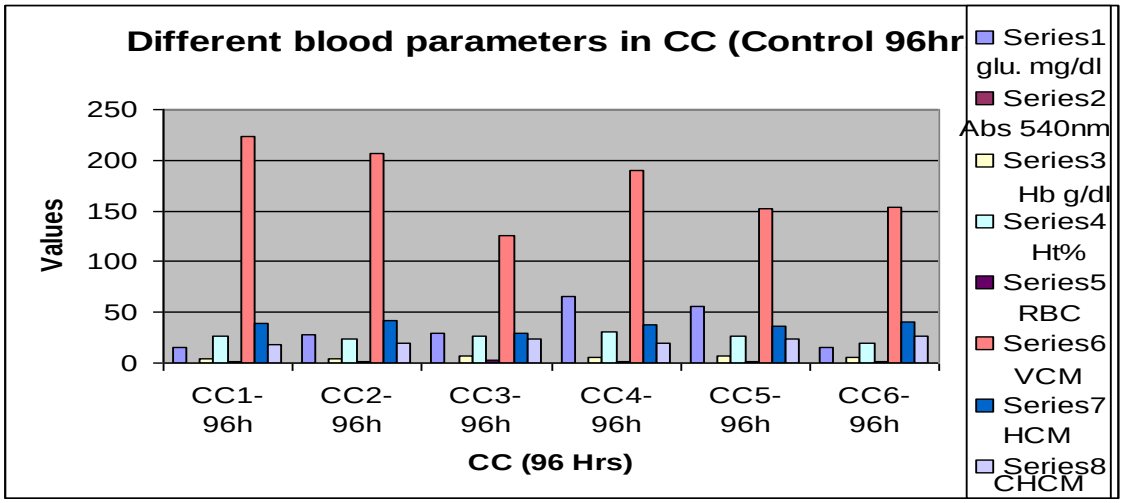


Fig. 2: Different blood parameters in CC(Control 96hrs)

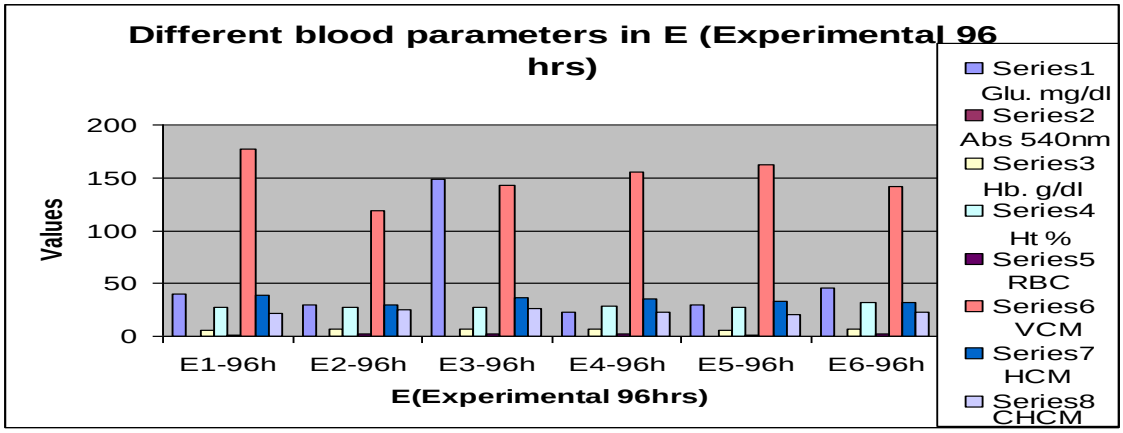


Fig. 3: Different blood parameters in E(Experimental 96hrs)

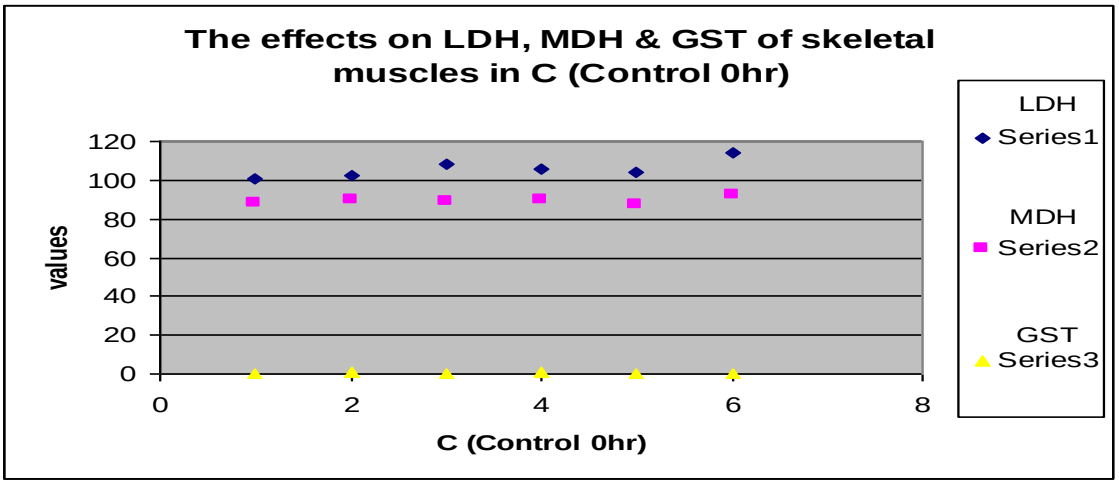


Fig. 4: The effects on LDH, MDH & GST of Skeletal muscles in C (Control 0hr) of Tambaqui fish.

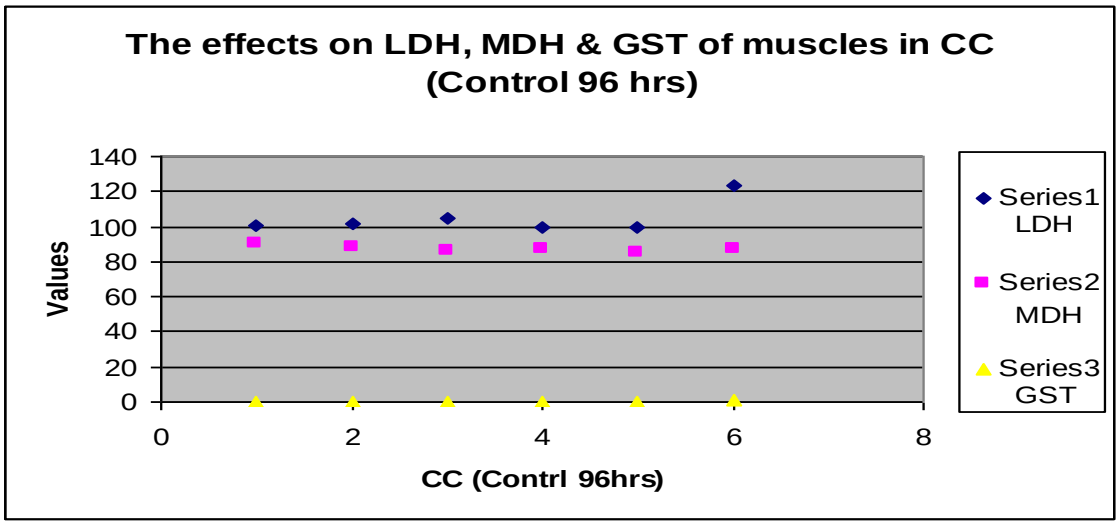


Fig. 5: The effects on LDH, MDH & GST of Skeletal muscles in CC(Control 96hr) of Tambaqui fish.

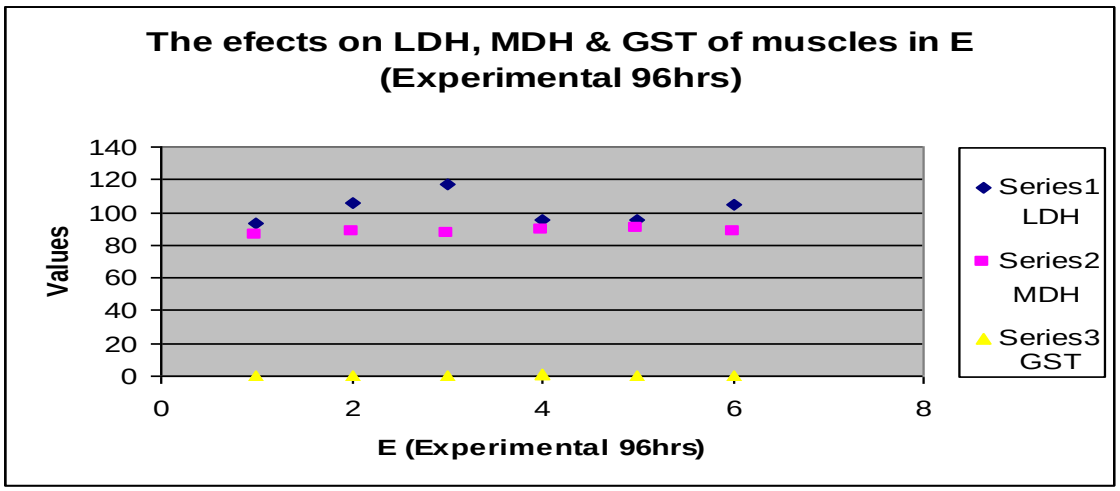


Fig. 6: The effects on LDH, MDH & GST of Skeletal muscles in E(Experimental 96hrs) of Tambaqui fish.

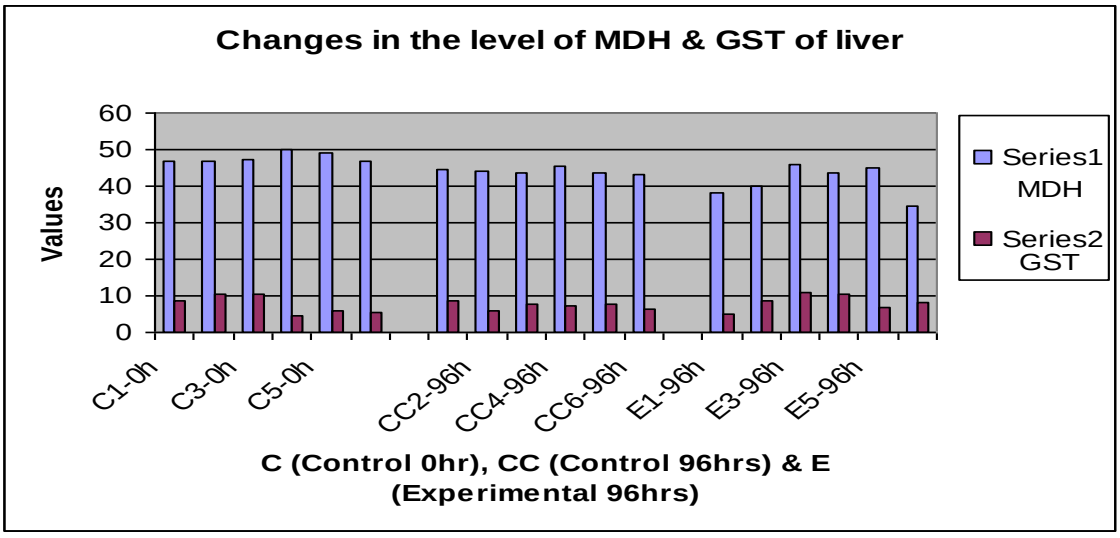


Fig. 7: The effects on MDH & GST of Liver in C(Control 0hr), CC(control 96hrs) and E(Experimental 96hrs) of Tambaqui fish.

Table 2: Change in the level of MDH, LDH & GST (Muscles & Liver) of tambaqui (*Colossoma macropomum*) exposed to different temperature and $CO_2$.

| | LDH Muscle | MDH Muscle | MDH Liver | GST Liver | GST Muscle |
|---|---|---|---|---|---|
| C1-0h | 100.8334033 | 88.38434084 | 46.63384244 | 8.7571288 | 0.298881949 |
| C2-0h | 102.4762862 | 90.20799839 | 46.84485531 | 10.320564 | 0.421265604 |
| C3-0h | 108.4617926 | 88.81784566 | 47.20357717 | 10.370187 | 0.321528375 |
| C4-0h | 105.6525362 | 90.19314309 | 50.0522508 | 4.3390507 | 0.470246574 |
| C5-0h | 104.1009164 | 87.48424437 | 49.06049035 | 5.854989 | 0.334895506 |
| C6-0h | 113.7823553 | 92.26607717 | 46.59163987 | 5.4118852 | 0.369363601 |
| Average | 105.8845483 | 89.55894159 | 47.73110932 | 7.508967 | 0.369363601 |
| Std. Dev. | 4.677243467 | 1.696192676 | 1.464313716 | 2.6393684 | 0.065291828 |
| CC1-96h | 100.3914791 | 90.1278135 | 44.53778135 | 8.7448199 | 0.191517023 |
| CC2-96h | 101.872916 | 88.36446342 | 44.18609325 | 5.793088 | 0.208099824 |
| CC3-96h | 105.1588505 | 86.39474277 | 43.46864952 | 7.8932844 | 0.295753861 |
| CC4-96h | 99.42926045 | 86.67986334 | 45.55767685 | 7.1798188 | 0.138006748 |
| CC5-96h | 99.38390675 | 85.55102894 | 43.67966238 | 7.529292 | 0.473998853 |
| CC6-96h | 123.244172 | 87.32134244 | 43.17323151 | 6.3460231 | 0.665035302 |
| Average | 104.9134308 | 87.4065424 | 44.10051581 | 7.247721 | 0.328735268 |
| Std. Dev. | 9.234942802 | 1.632477829 | 0.866956947 | 1.0649851 | 0.202507343 |
| E1-96h | 93.25502412 | 86.51949357 | 38.36213826 | 5.0098025 | 0.470473018 |
| E2-96h | 106.2529944 | 88.59742162 | 40.02913987 | 8.6427832 | 0.412120001 |
| E3-96h | 116.7984043 | 87.39907958 | 45.68428457 | 11.014187 | 0.403209655 |
| E4-96h | 95.06846865 | 88.73057074 | 43.42644695 | 10.387174 | 0.715626912 |
| E5-96h | 94.89906752 | 90.49818328 | 44.96684084 | 6.9784706 | 0.500 |
| E6-96h | 104.8638545 | 87.74566821 | 34.73271704 | 8.406 | 0.500 |
| Average | 101.8563023 | 88.24840283 | 41.20026125 | 8.406 | 0.500 |
| Std. Dev. | 9.16626939 | 1.369600774 | 4.254529 | | |

# 3 Results & discussion

The physiological responses, known as stress, are grouped as primary, secondary and tertiary responses (Barton [15]). Primary responses are mediated by the neuron-endocrine system with a fast release of stress hormones; catecholamines (adrenaline and noradrenaline) that are released and synthesized through cromafin cells and cortisol, synthesized by the inter-renals cells. Secondary responses are mediated by the stress hormones and involve the various biochemical and physiological effects associated to stress, which includes metabolic, hematological, hydromineral and structural changes that suggest an important change that helps to evaluate the fish health conditions. Tertiary responses affect the animal as a whole, compromising growth, disease resistance and reproductive success. Fish hematological parameters have been widely used as indicator for stress caused by environmental conditions (Affonso et al. [16], Tavares-Dias & Moraes [17], Carvalho & Fernades[18]). In order to overcome stress, hemo-concentration is among the important strategies for increasing blood oxygen carrying capacity under high energy demand situations (carvalho & Fernandes [18], Trenzado et al. [19], Ziskowski et al. [20]). However, stressors can compromise iron absorption, cause erythrocyte malformation or hemolysis, inhibit hemoglobin synthesis, compete for the oxygen bonding site, resulting in hemodilution or anaemia, reducing the blood oxygen carrying capacity(Affonso[21], Tavares-Dias & Moraes [17]). Therefore, the analysis of hematological parameters in fish can contribute to the assessment of animal health.

The hematological parameters: Ht, Hb, RBC, VCM, HCM & CHCM of the fish from the three treatments are represented in Table 1. The similarity of the Tambaqui physiological profile on all three treatments reflects a satisfactory rearing environment. Similar productive performances in pond systems were obtained by Kubitza et al [22] with Micropterus salmoides and Affonso et *al.,* [9] with Tambaqui in pond culture and Good et al. [23] with O. mykiss.

In the present study when the Tambaqui fish, exposed to a temperature rise from 28º to 32Cº and a $CO_2$ concentration 10 times higher than today. However we observed a significant increase in (Hb), (HT), (RBC), (VCM), (HCM) & (CHCM) levels in blood, while a significant decrease was observed in Glucose level in blood as compared to control values (Tab. 1) and (Fig. 1, 2 & 3).

Liver MDH values of were 47.731, 44.100 & 41.200 respectively while the values of GST of liver were 7.508 & 7.247 and 8.406 respectively, as compared to control values (Tab. 2). The MDH values in muscles were 89.558, 87.406, 88.248, while the LDH values of muscles were 105.884, 104.913, 101.856, while the GST Values of Muscles were 0.369 & 0.328 respectively as compared to control values (Tab. 2) and (Fig. 4, 5, 6 & 7). Temperature fluctuations are much underappreciated stressor of fish. Similar finding that the slight change in water temperature

and $CO_2$ can be due to natural heat loading system or thermal pollution in normal or natural water disrupt aquatic ecosystem resulting stress to fish [14].
This study will contribute valuable knowledge needed for fisheries management and aquaculture of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) by increasing the knowledge of ecology and environmental effects on the biology of Tambaqui fish (*Collosoma macropomum*).

## Acknowledgements

The authors wish to thank the entire team of Laboratory of Ecophysiology and Molecular Evolution (LEEM) of INPA. The present work was supported by a grant from Brazilian National Research Council (CNPq), INCT/ADAPTA, FAPEAM and INPA/MCTI.

## References

[1] A. L. Val & V. M. F. Almeida Val, Fishes of the Amazon and their environments. Physiological and biochemical features. Heidelberg: Springer Verlag, 1995.
[2] W. J. Junk & M. T. F. Piedade, Plant life in the floodplain with special reference to herbaceous plants. In The Central Amazon floodplain: Ecological Studies (Junk, W. J., ed.), Berlin: Springer, (1997); 147-185.
[3] E. A. Ono, Cultivar peixes na Amazonica: Possibilidade ou Utopia. Panorama da Aquicultura. (2005), 15: 41 – 48.
[4] G. M. Santos & A. C. M Santos, Sustentabilidade da Pesca na Amazonica. Estudos avancados, Vol. 19 (54), Sao Paulo. 2005.
[5] M. N. Fernandes, J. R. Sanches, M. Matsuzaki, L. Panepucci & F. T. Rantin, Aquatic respiration in facultative air- breathing fish: effects of Temperature & hypoxia. Biology of Tropical fishes, edited by Val, A. l. & almeida-Val, V. M. F., chapter 27,(1999), 341 – 352, INPA, Manaus, Brazil.
[6] A. L. Val & V. M. F. Almeida Val, Crude oil, copper and fish of the Amazon. In Behavior,Physiology and Toxicology Interactions in Fish. VI International Congress on the Biology of Fish. (Sloman, K. A., Wood, C. M. & Mac Kinlay, D., eds.), (2004), 16. Manaus, AM: American Fisheries Society.
[7] A. L. Val, V. M. F. Almeida Val & D. J. Randall, Tropical environment. In The Physiology of Tropical Fishes, (2006), 145. London: Elsevier.
[8] R. Roubach, L. C. Gomes, F. A. L. Fonseca & A. L. Val, Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). Aquaculture Research. (2005), 36(11): 1056–1061.
[9] E. G. Affonso, M. F. Barroncas, E. M. Brasil, M. Pereira-filho, R. Roubach & E. A. Ono, Haematological responses as indicators of the pond culture of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) with and with out water exchange. ADAPTA, adaptation of aquatic Biota. (2012), 1(1): 21 – 27.
[10] L. C. Gomes, E. C. Chagas, J. H. Martins, R. Roubach, E. A. Ono & J. N. P. Lourenco, Cage culture of of Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) in a central Amazon flood plain lake. Aquaculture. (2006), 253: 374 – 384.
[11] C. A. Oishi, L.C. Nwanna & M. Pereira-Filho, Optimum dietary protein requirement for Amazonian Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) fed fish meal free diets. Acta Amazonica (2010), 40 (4): 757 – 762.
[12] M. Q. C. Santos, C. A. Oishi, M. Pereira-Filho, M. A. C. Lima, E. A. Ono & E. G. Affonso, Physiological response and performance of Tambaqui fed with diets supplemented with Amazonian nut. Ciencia Rural (2010), 40 (10): 2181 – 2185.
[13] M. M. Sakuragui, F. E. G. Caterina, K. L. Vasqueuz, D. B. Fagundes, S. R. Nozawa, A. L. Val & V. M. F. Almeida-Val, Heat shock protein expression in Tambaqui fish (*Collosoma macropomum*, Cuvier, 1818) exposed to increased temperature and $CO_2$. ADAPTA, adaptation of aquatic Biota. (2012), 1(1): 34 – 36.
[14] V. T. Tantarpale, S. A. Tantarpale and K. M. Kulkarni, Impact of changed temperature variations on free amino acid in muscle of fresh water fish *Channa striatus*. Science Research Reporter, (2011), 1(3): 143-145.
[15] B. A. Barton, Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology. (2002), 42: 517 – 525.
[16] E. G. Affonso, V. L. P. Polez, C. F. Correa, A. F. Mazon, M. R. R. Araujo, G. Moraes & F. T. Rantin, Physiological responses to sulfide toxicity by the air-breathing catfish, *Hoplosternum littorale* (Siluriformes, Callichthyidae). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. (2004), 39(4): 251–257.
[17] M. Tavares-Dias & F. R. Moraes, Haematologia de peixes teleosteos. Marcos Tavares-Dias (ed.), Ribeirao Preto, SP. (2004), 144pp.
[18] C. S. Carvalho & M. N. Fernandes, Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. Aquaculture. (2006), 251(1): 109 – 117.
[19] C. E. Trenzado, A. E. Morales & M. D. Higuaera, Physiological effects of crowding in rainbow trout, *Oncorhynchus mykiss*, selected for low and high stress responsiveness. Aquaculture. (2006), 258(1–4): 583–593.
[20] J. Ziskowski, R. Mercaldo-Allen, J. J. Pereira, C. Kuropat & R. Goldberg, The effects of fin rot disease and sampling method on blood chemistry and hematocrit measurements of winter flounder, *Pseudopleuronectes americanus* from New Haven Harbor (1987–1990). Marine Pollution Bulletin. (2008), 56(4): 740–750.
[21] E. G. Affonso, Respiratory characteristics of Hoplosternum littorale (Siluriformes, callichthyidae). Acta Amazonica. (2001), 31: 249 – 262.
[22] F. Kubitza, L. L. Lovshin & R. T. Lovell, Identification of feed enhancers for juvenile largemouth bass *Micropterus salmoides*. Aquaculture. (1997), 148(2–3): 191–200.
[23] C., Good, J. Davidson, C. Welsh, B. Brazil, K. Snekvik & S. Summer-Felt, The impact of water exchange rate on the health and performance of rainbow trout *Oncorbynhus mykiss* in water recirculation aquaculture systems. Aquaculture. (2009), 294 (1 – 2): 80 – 85.