

Impact of Acute Anoxia on Stress in Hybrid Catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther)

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ABSTRACT: Dissolved oxygen (DO) depletion was studied by adding 180 ± 0.63 ml of sodium sulfite stock solution, 100,000 mg/l, into 254 L de-chlorinated water without fish. DO dropped from 6 mg/l to 0 mg/l within 20 min. Sodium sulfite, 7.1 mg/l was used for the second experiment. The second experiment was conducted to investigate acute anoxia on stress in hybrid catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther). Catfish, 20.03 ± 7.55 g body weight and 13.87 ± 1.89 cm body length, were exposed to anoxia for 40 min. The results show that blood clotting time (min) and serum cortisol (μ g/dl) were significantly different in experimentals at $p < 0.05$. Fish body weight (g), body length (cm) and % hematocrit (Hct) was statistically non-significant with differences of $p \geq 0.05$. Hybrid catfish exposed to the acute anoxia condition showed significant stress response. Hybrid catfish are well able to live in a variety of DO conditions.

Key words: hybrid catfish, anoxia, stress, dissolved oxygen

INTRODUCTION

Aquatic environments influence the physiology of fishes. Among biological, physical and chemical factors, the most common change is the fluctuation of dissolved oxygen (DO). Drastic changes in oxygen availability are especially observed in eutrophic earth ponds. Usually, DO is present at very low concentrations in the early morning and high concentrations in the late afternoon. An escape behavior is not feasible, and fish respond with compensatory mechanisms to survive in the changing environment. Reductions of the DO level in the water column induce stress in fish.⁽¹⁾ The common response to any stressor is a release of cortisol followed by changes in blood and metabolism.⁽²⁾ Fish need oxygen to aerobically generate energy for locomotion, biosynthetic process, and to maintain their metabolism. Therefore, DO reduction can reduce fish growth rate, feed consumption⁽³⁾ and activities.⁽⁴⁾

Hybrid catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther) is an artificially cross bred fish using female Thai walking catfish, *C. macrocephalus*, and male African catfish, *C. gariepinus*.⁽⁵⁾ This hybrid catfish is an important aquacultural species because of its fast growth rate and high disease resistance. Catfish is an air-breathing species and can survive under anoxic conditions.^(6, 7) Under low DO circumstance, this fish attempts to increase its oxygen extraction capacity. These changes

occur at the behavioral, physiological and tissue levels. However, lack of oxygen can induce both acute and chronic stress in fish.⁽⁶⁾ It is important to understand the fish stress response under fluctuations in DO.

The present study was undertaken to clarify the hybrid catfish response under acute anoxic condition. Primary and secondary indicators of physiological stress response were also investigated.

MATERIALS AND METHODS

This research was comprised of 2 experiments. The first experiment was conducted to control DO without catfish. The second experiment was designed to study fluctuating DO on catfish.

Experiment 1. Sodium sulfite de-oxygenated

Six 1x1x1 m fiberglass tanks were divided into 2 groups. Three fiberglass tanks with and without aeration were control and treatment group, respectively. Each fiberglass tank contained 254 L de-chlorinated water. Throughout the experiment, DO was monitored by oxygen meter (YSI model 57, USA). Stock solution Na_2SO_3 was prepared by dissolving 50g Na_2SO_3 in 500 ml de-ionized water. The 100,000 mg Na_2SO_3 /l stock solutions were gently poured into each treatment fiberglass tank. Air temperature, water temperature, DO and Na_2SO_3 volume were recorded.

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Experiment 2. Effects of fluctuating DO on hybrid catfish

Hybrid catfish

Hybrid catfish, 20.03 ± 7.55 g body weight and 13.87 ± 1.89 cm body length, were purchased from a private fish farm at Chachoengsao province. All catfish were acclimated for 2 weeks in an aquarium for adaptation to the new rearing and feeding condition. Once a day, catfish were fed to satiation with commercial pellet feed. Daily, 30% water change was done. One hundred and fifty hybrid catfish were divided into 2 groups, control and treatment. Each group was comprised of 5 replicates. For each replicate, 300 L fiberglass tanks (1x1x1 m) had 15 fish. All fish were starved for 1 day before starting the experiment.

Acute anoxia

Control groups were air-stone provided. DO was monitored between 5.8-6.2 ppm by oxygen meter (YSI model 57, USA). There was no aeration in the Treatment group. The stock solution, 100,000 mg Na_2SO_3 /l, added into treatment group for DO depletion. DO dropped to 0 ppm within 20 min. This period is termed pre-anoxic condition. Fish were exposed to anoxic condition, 0 ppm DO, for 40 min. Then air-stones were added into the fiberglass tank and DO gradually increased. This condition is referred to as post-anoxia.

Blood parameters

One fish from each fiberglass tank was randomly selected at 0, 5, 10, 15, 20, 30, 40, 50, 60, 65, 70, 75, 80, 85 and 90 min. After tranquilization with 5 ml clove oil/l, blood was taken from the caudal vein by tuberculin syringe (Nipro, Osaka, Japan).

The glass slide method was used to determine blood clotting time. One drop of fish blood was placed on a clean glass slide, and then the needle-tip was

moved up and down to check for fine thread of blood. Timing of fine thread development was recorded.

Whole blood was collected in heparinized capillary tube for haematocrit (Hct, Heamatocrit centrifuge, SR10000, Thailand). All capillary tubes were centrifuged at 11,000 rpm for 5 min and %Hct was recorded.

Blood serum was cortisol investigated (Radio Immunoassay "Coat-A-Count Cortisol", Diagnostic Products Corporation (DPC), USA). Fish serum, 25 μl , was mixed well with 1 ml Iodinated cortisol (^{125}I) by vertical shaking for 1 min. All test tubes were incubated at 37°C for 45 min, and then cortisol concentration ($\mu\text{g}/\text{dl}$) was counted for 1 min in a gamma counter.

RESULTS AND DISCUSSION

As shown in Figure 1, DO dropped from 6 to 0 ppm within 20 min and lasted for 3 hrs. After 3 hrs, air-stones were applied in the water tank and DO was raised. The 180 ± 0.63 ml stock solution, 100 ppm Na_2SO_3 , was added to 300 L de-chlorinated water. Therefore, 7.1 ppm Na_2SO_3 will be used for the second experiment. The reaction is shown as equation 1.⁽⁸⁾



Table 1 shows water quality parameters in the experiment. These parameters were in the normal range for fish growth.⁽⁹⁾ Hybrid catfish averaging weights and lengths of 20.03 ± 7.55 g and 13.87 ± 1.89 cm was not significantly different (Figure 2). The variability of sizes among species is considerable.^(10,11) All fish were tranquilized before taking blood. Hence, there are no other parameters which induce stress condition in fish except the DO level.

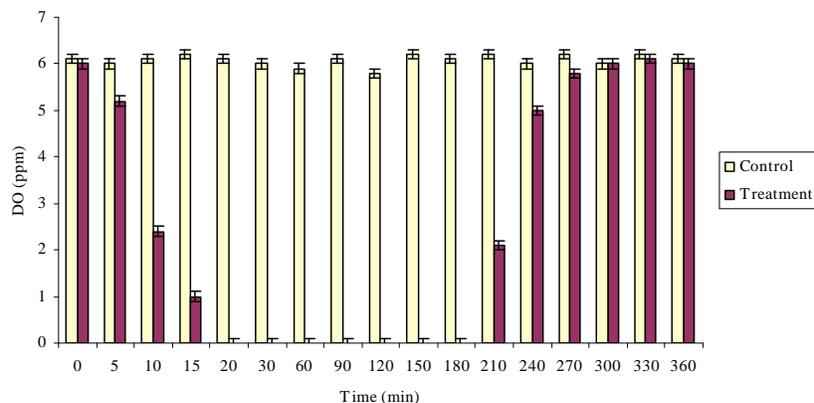


Figure 1. DO depletion by adding 7.1 ppm Na_2SO_3 (mean \pm SD, n=3).

Table 1. General water quality in experiments 1 and 2.

Parameter	Unit	Value (mean ± SD)
Water temperature	°C	28 ± 0
Air temperature	°C	30 ± 0
DO	mg/l	5.60 ± 0.57
pH	-	8.3 ± 0.6
Alkalinity	mg/l	89.5 ± 7.8
Hardness	mg/l	119.00 ± 24.04
Ammonia	mg/l	0.05 ± 0.07
Nitrite	mg/l	0.0395 ± 0.0375
Nitrate	mg/l	1.70 ± 0.14

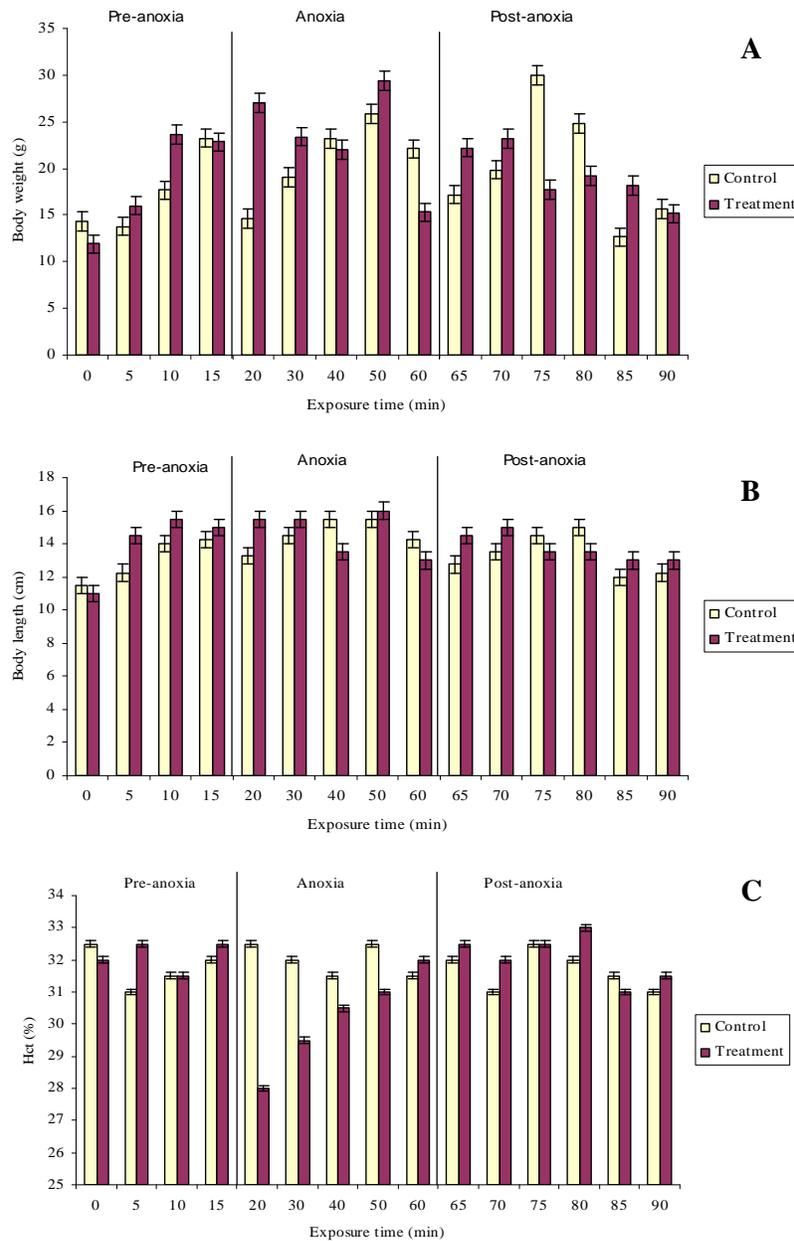


Figure 2. Mean ± SD. of hybrid catfish body weight (A), body length (B) and Hct (C) is shown statistically non significant difference ($p \geq 0.05$, ANOVA, Duncan).

Three stages of DO with 15 hybrid catfish are shown in Figure 3. DO dropped to 0 ppm at 20 min. All fish in treatment groups were exposed to anoxia for 40 min and then brought back to normoxic condition. The observations were recorded for treatment groups. All fish were lethargic and were gasping at the air-water interface. This may indicate the most immediate adjustment to hypoxia and anoxia condition. Catfish can survive in anoxic condition by increasing oxygen uptake from air through an accessory breathing organ, the dendrite. In normoxic conditions, all fish utilize DO from water through gills. Hybrid catfish have a great tolerance to hypoxic and anoxic conditions⁽⁷⁾ similar to Nile tilapia (*Oreochromis niloticus*), goldfish (*Carrasius auratus*), and the common carp (*Cyprinus carpio*). Those morphological and anatomical adaptations are present in air-breathing species to cope with DO constraints.⁽⁶⁾

Blood clotting time and serum cortisol was strikingly increased at statistically significant levels (Figure 4). Both clotting time and cortisol were increased in response to acute oxygen depletion, and then returned

to normal levels after the DO concentration was increased.

Blood clotting time has been claimed to be an indicator of stress in crustacean.⁽¹²⁻¹⁴⁾ However, data for blood clotting time in catfish are scarce. The speed of blood clotting is easy to measure and could be a useful indicator of stress, nutritional diseases, and the presence of bacteria and environmental pollutants.⁽¹⁵⁾ Blood clotting is particularly practical as only a small amount of blood is needed. The determination is rapidly and easily made in field or laboratory.

Cortisol is involved in hydro-mineral balance, energy metabolism,⁽¹¹⁾ and is a physiological index of primary stress.^(16, 17) The primary response of catfish to an anoxic stressor is the secretion of cortisol by the inter-renal cells into the blood stream.^(18, 19) Continuing stress response to acute anoxia condition in hybrid catfish is non-existent. Serum cortisol returned to basal levels. Within 30 min, catfish recovered from the stress induced levels. Cortisol levels in this study indicate that anoxia is not stressful to catfish.

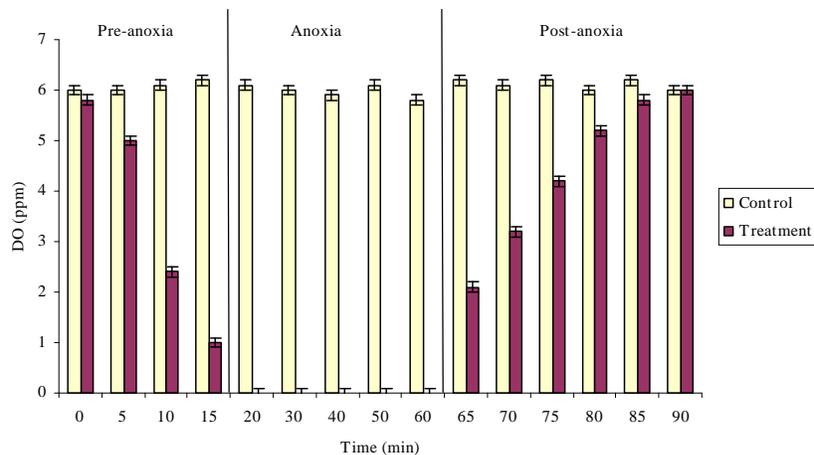


Figure 3. DO presence in experiment with 3 conditions: pre-anoxia, anoxia and post-anoxia.

There was no statistically significant difference in %Hct in hybrid catfish in normoxia and anoxia conditions (Figure 2). The %Hct is an indicator of secondary stress response⁽²⁾ and is typically a response to hypoxic and anoxic exposures.⁽⁴⁾ The hematocrit levels were studied in bonnethead sharks (*Sphyrna tiburo*) and it was found that hypoxic condition (DO = 3 ppm) caused no significant response.⁽²⁰⁾ In contrast, Lykkeboe and Weber⁽²¹⁾ and Wood and Johansen⁽²²⁾ found that hematocrit of carp and eel increased under hypoxic condition. The %Hct in hypoxia and anoxia is higher than %Hct in normoxia and is expressed through increase in ventilation volume,⁽²⁰⁾ increased oxygen

uptake, increase in the blood viscosity and increase in the number of red blood cells.^(4, 23) Hematocrit levels have been suggested to be a response to living in environments with variable oxygen availability as well.

In conclusion, hybrid catfish that were exposed to acute anoxia conditions effectively compensated for reductions in DO. Anoxia was expected to elicit a significant stress response but this was not the case. Catfish are well adapted to and able to compensate for living in a variety of DO levels. Further research is needed to examine gill and dendrite, those structures directly in contact with DO in the water column.

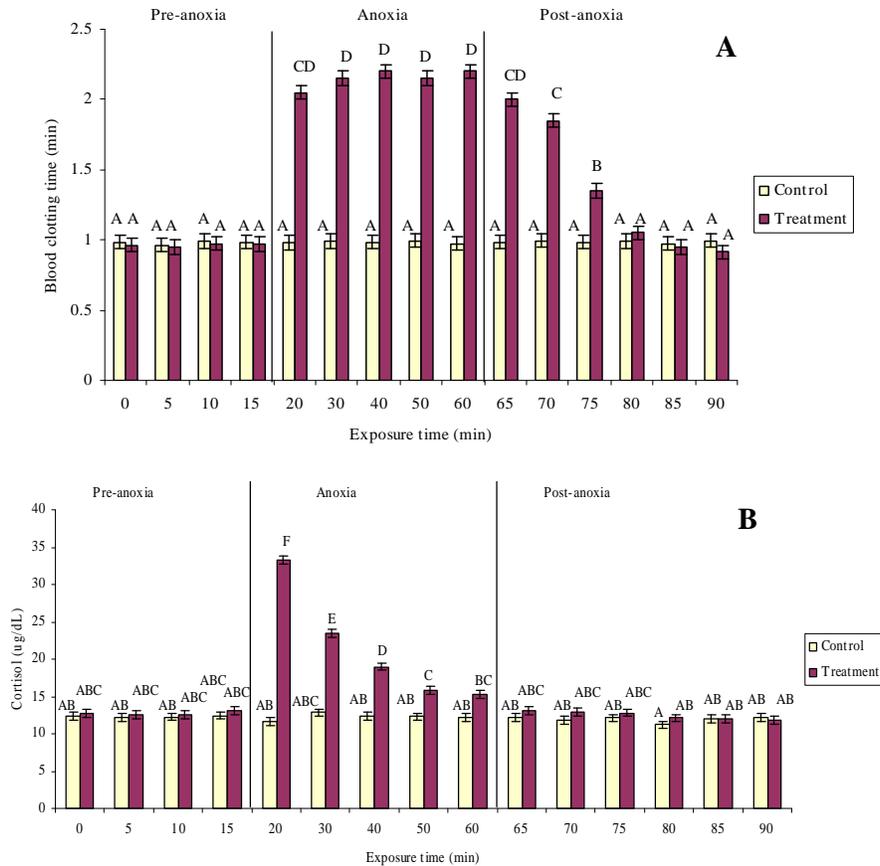


Figure 4. Blood clotting time (A) and serum cortisol (B) are shown as mean ± SD (n = 5). Different upper case letters indicate significant differences at p < 0.05 (ANOVA, Duncan).

ACKNOWLEDGMENTS

This research was financially supported by Chulalongkorn University grants for development of new faculty staff (116/2548).

REFERENCES

- Ishibashi, Y., Ekawa, H., Hirata, H. and Kumai, H. (2002) "Stress response and energy metabolism in various tissues of Nile tilapia, *Oreochromis niloticus*, exposed to hypoxic conditions" *Fisheries Science*. **68**, 1374-1383.
- Morales, A. E., Cardenete, G., Abellan, E. and Garcia-Rejon, L. (2005) "Stress-related physiological responses to handling in common dentex, *Dentex dentex*" *Aquaculture Research*. **36**, 33-40.
- Buentello, J. A., Gatlin, D. M. and Neill, W. H. (2000) "Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish, *Ictalurus punctatus*" *Aquaculture*. **182**, 339-352.
- Taylor, J. C. and Miller, J. M. (2001) "Physiological performance of juvenile southern flounder, *Paralichthys lethostigma*, in chronic and episodic hypoxia" *J. Exp. Mar. Biol. Ecol.* **258**, 195-214.
- Senanan, W., Kapuscinski, R., Na-Nakorn, U. and Miller, L. M. (2004) "Genetic impacts of hybrid catfish farming, *Clarias macrocephalus* x *C. gariepinus*, on native catfish populations in central Thailand" *Aquaculture*. **235**, 167-184.
- Muusze, B., Marcon, J., Thillart, G. and Almeida-val, V. (1998) "Hypoxia tolerance of Amazon fish respirometry and energy metabolism of the cichlid, *Astronotus ocellatus*" *Comparative Biochemistry and Physiology Part A*. **120**, 151-156.
- Delaney, M. A. and Klesius, P. H. (2004) "Hypoxic conditions induce Hsp70 production in blood, brain and head kidney of juvenile Nile tilapia, *Oreochromis niloticus*" *Aquaculture*. **236**, 633-644.
- Boyd, C. E. (1982) "Water quality management for pond fish culture" *ELSEVIER*, Amsterdam, 317p.
- Koeypuksa, W., Yakupitiyage, A. and Tangtrongpiros, J. (2005) "The fate of chlortetracycline residues in a simulated chicken-fish integrated farming systems" *Aquaculture Research*. **36**, 570-577.

10. Perry, S. F., Wood, C. M., Walsh, P. J. and Thomas, S. (1996) "Fish red blood cell carbon dioxide transport *in vitro*: a comparative study" *Comp. Biochem. Physiol.* **113A**, 121-130.
11. Peruzzi, S., Varsamos, S., Chatain, B., Fauvel, C., Menu, B., Falguiere, J., Severe, A. and Flik, G. (2005) "Haematological and physiological characteristics of diploid and triploid sea bass, *Dicentrarchus labrax*" *Aquaculture*. **244**, 359-367.
12. Smith, V. J., Swindlehurst, R. J., Johnson, P. A. and Vethaak, D. A. (1995) "Disturbance of host defense capability in the common shrimp, *Crangon crangon*, by exposure to harbour dredge spoils" *Aquat. Toxicol.* **32**, 43-58.
13. Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, B. and Evans, L.H. (1997) "Total and differential hemocyte counts in western rock lobsters, *Panulirus cygnus*, under post-harvest stress" *Mar. Freshwater Res.* **48**, 863-868.
14. Jussila, J., Paganini, M., Mansfields, S. and Evans, L.H. (1999) "On physiological responses, hemolymph glucose, total hemocyte count and dehydration of marron, *Cherax tenuimanus*, to handling and transportation under simulated conditions, Freshwater Crayfish" *Aquaculture*. **12**, 154-167.
15. Jussila, J., McBride, S., Jago, J. and Evans, L. H. (2001) "Hemolymph clotting time as an indicator of stress in western rock lobster, *Panulirus cygnus*" *Aquaculture*. **199**, 185-193.
16. Ruane, N. M., Huisman, E. A. and Komen, J. (2001) "Plasma cortisol and metabolite level profiles in two isogenic strains of common carp during confinement" *J. Fish Biology*. **59**, 1-12.
17. Gollock, M. J., Kennedy, C. R. and Brown, J. A. (2005) "Physiological responses to acute temperature increase in European eels, *Anguilla anguilla*, infected with *Anguillicola crassus*" *Dis. Aquat. Org.* **64**, 223-228.
18. Reid, S.G., Bernier, N.J. and Perry, S.F. (1998) "The adrenergic stress response in fish: control of catecholamine storage and release" *Comparative Biochemistry and Physiology part C.* **120**, 1-27.
19. Sadler, J., Pankhurst, N. W., Pankhurst, P. M. and King, H. (2000) "Physiological stress responses to confinement in diploid and triploid Atlantic salmon" *J. Fish Biology*. **56**, 506-518.
20. Carlson, J. K. and Parsons, G.R. (2003) "Respiratory and hematological response of the bonnethead shark, *Sphyrna tiburo*, to acute changes in dissolved oxygen" *J. Exp. Mar. Biol. Ecol.* **294**, 15-26.
21. Lykkeboe, G. and Weber, R. E. (1978) "Changes in the respiratory properties of the blood in the carp, *Cypronus carpio*, induced by diurnal variation in the ambient oxygen tension" *J. Comp.Physiol.* **128**, 117-125.
22. Wood, S. C. and Johansen, K. (1973) "Organic phosphate metabolism in nucleated red cells: influence of hypoxia on eel HbO₂ affinity" *Neth. J. Sea Res.* **7**, 328-338.
23. Frey, B. J., Webber, R. E., Aardt, W. J. and Fago, A. (1998) "The haemoglobin system of mudfish, *Labeo capensis*, adaptations to temperature and hypoxia" *Comparative Biochemistry and Physiology Part B.* **120**, 735-742.

Received: September 15, 2006

Accepted: November 28, 2006