ISSN: 0975–833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol.3, Issue, 5, pp.014-017, May, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STRESS INDUCED NON-SPECIFIC IMMUNE RESPONSE MODEL IN THE EXOTIC FISH, *Osphronemus olfax*

***Palanisamy, P., Sasikala, G., Mallikaraj, D., Bhuvaneshwari, N and Natarajan, GM**

*PG and Research Department, Department of Zoology, Government Arts College, Coimbatore, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 15th March, 2011
Received in revised form
11th April, 2011
Accepted 27th April, 2011
Published online 14th May 2011

Key Words:
Air-exposure,
Submergence,
Hypoxic,
Immunoglobulins,
RBC, Hb, Ht, Blood
Glucose, phagocytosis,
Lymphocyte, lysozyme,
Spleen, kidney,
Osphronemus olfax.

ABSTRACT

Results obtained using multifactorial stress model (air-exposure, submergence, hypoxia) showed that elevation of RBC, HB, Ht and blood glucose was linearly correlated with progressive stress uniformly. Submergence caused rapid stress with blood glucose reaching 50% elevation within 60 min. Hypoxic water with access to air had very little effect on blood glucose. Similarly, lowering the temperature significantly affected the blood parameters. The air-exposure model was also used for testing lysozyme activity and phagocytosis assay during stress. Plasma lysozyme was significantly lower in air stressed fish. Submergence increased the number of granulocyte, lymphocytes and activity of phagocytic cells. Spleen and kidney lysozyme activity increased significantly. The titer of total non-specific immunological increased during submergence. But no such effect was observed in air-exposure. These results suggest that submergence stress in stimulating non-specific defense system in the air-breathing fish, *Osphronemus olfax*. This is the first report that submergence stress is playing the role of an immunomodulator in an air-breathing fish.

.

INTRODUCTION

Osphronemus olfax is an exotic air-breathing fish introduced in to Madras in 1865 – 66 (Anonymous, 1962). It is now all established in India in both brackish and freshwater. It is a useful culture and ornamental fish. Generally, air-exposure, submergence, temperature changes are some of the routine stress exposure to ornamental fishes during handling, transportation and marketing. Fish being the inhabitant of closed environment, becomes a useful model in assessing the effects of environmental stressors on physiological, biochemical and immunological parameters (Vashist, Kumari and Jamail, 2000). The effect of various environmental stressors like toxicants (Kumar and Patri, 2002; Khare and Singh, 2002), air-exposure (Natarajan, 1987; Rani, 1994; Vijayalakshmi, 1996; Raveendran, 2000 and Sasikala, 2001) and plant extracts (Mannan, 2002; Parimala, 2002 and Tamil selvi, 2002). Most fish species regulate certain haematological parameters accord in to environmental conditions (Val *et al*., 1990). Emersion produces a significant rise in the RBC count, Hb content and Hct value in the blood of *Macropodus cupanus* (Parthi *et al.,* 2000) and *Channa striata* (Rani, 1944) induced marked variations in the blood cells. Among all the characteristics, haematocrit values can be utilized as a parameter of stress response, as it is rapid, practicable and quantitative (Chavin, 1973). Stress in general influences the blood sugar level (Chavin and Young, 1970), lactic acid content and haematological values (Singh *et al*., 1976). Blood glucose appeared to a be sensitive and reliable indicator of environmental stress in fishes (Nemcsok *et al.,* 1981) and increase in blood glucose of fish is an usual response to most stressors. Recently, enzyme changes (Natarajan, 1985; Caberoy and Quintio, 2000 and Mannan, 2002) and immunological modulations (Melamed *et al*., 1999; Aaltonen *et al.,* 2000 and Sasikala, 2001) are identified as valid biomarkers in stressed conditions in freshwater fishes. Stress is known to be immunosuppressive in fish. Lysozyme activity, which seems to responsive to stressors, in similar between primary and secondary stresses (Maule *et al.,* 1996). Lysozyme is a leucocyte enzyme important in bacterial killing. Increase in lysozyme level increases the protective mechanism. Lysozyme kills some bacterial pathogens (Grende *et al*., 1988) and probably participates in microbe killing. The influence of stress on plasma lysozyme has been widely reported (Yin *et al*., 1995; Mclamed *et al*., 1999; Findlay and Munday, 2000 and Aaltonen *et al*., 2000). Increase in IgM titer suggests activation of some protective or compensatory mechanism. Cadmium exposure initially reduced the IgM titer but after two weeks of exposure the IgM titer increased in *Ictalurus melas* (Albergoni and Viola, 1995). In the present investigation an attempt has been made to stimulate the filed stress conditions in the laboratory and to design a stress induced non-specific immune response model in *Osphronemus olfax.*

Corresponding author: drpalani78@gmail.com

MATERIALS AND METHODS

Osphronemus olfax weighing 11 – 16g were commercially collected from local source and acclimated to the laboratory conditions for 15 days at 28°±1°C. Air-exposed stress was imposed by gradually siphoning the freshwater from the tank without handling fish, thus minimizing disturbances prior to sampling. After 6 hr of air exposure, fishes were collected from the tank using a net. The RH inside the tank was 100%. The air movement over the tank was nil. The temperature of the tank was 28°C. Twelve fishes were also air-exposed for 6 hr to 18°C. For normoxic submergence, fishes were kept in the aquarium for at least 24hr before a wire netting was immersed under the surface of the water to prevent the fishes from coming to the surface to gulp air. The fishes thus submerged had no access to gulp atmospheric air. The O_2 content of the aquaria had a mean O_2 concentration of 6.8mg/l. The water in the aquaria was made hypoxic by vigorously bubbling it though the aeration columns with pure N_2 until the O_2 content was fell to 2.5mg/l. Water PO_2 was continuously monitored by an O_2 electrode. The temperature throughout the submergence tests varied from 28°C ± 0.5°C. suitable controls were maintained without any stress.

After air-exposure and submergence stress fishes were quickly collected without any disturbance and blood was collected from the caudal vein. The blood sample was separated into plasma and red cells by centrifugation at 5500rpm for 5 mins. The plasma was stored frozen at -4°C and remaining blood samples were used for estimations of hematological parameters RBC, Hb and Ht contents were determined following Dacie and Lewis (1969). Blood glucose was determined by the GOD – period method (Noll, 1974) and lactate by a modification of the method of Noll (1974) by using a spectrophotometer. Serum immunoglobulin was measured by an enzyme linked immunoabsorbent assay (ELISA) as described by Aaltonen *et al*. (1974). Lysozyme activity was measured by the turbidometric assay as described by Parry *et al*. (1965). Blood smears were prepared and stained with May-Giemsa and the number of granulocytes and lymphocytes per 10,000 red blood cells were measured. These counts were then recalculated as cells per unit ($1mm^3$) volume of blood. Phagocytic assay was done after Kakuta (1997). All data were statistically analyzed by applying the methods described by Zar (1984).

RESULTS

Six hour air-exposure stress (at 28°C) significantly increased almost all haematological and non-specific immune defense parameters in *O. olfax* (Table – 1). Imposition of cold (18°C) produced significant changes in the RBC, Hb, Ht, blood glucose, lactate and immunological variables (Table – 2). Normoxic submergence stress increased the immunoglobulin, plasma lysozyme and granulocyte counts. Blood glucose and lactate contents were unaffected. Hypoxic submergence stress elevated all the parameters significantly. Kidney played an important role in secreting lysozyme (+222.84%) to combat stress. The increase in the lactate content (+141.94%) definitely indicate the triggering of carbohydrate metabolism towards anaerobic side. Among the stress induced variations, blood glucose seems to be more valuable as its changes are clearly stress dependent. Changes in the blood lactate follows same pattern.

DISCUSSION

The influence of stress on blood glucose has been widely reported by Wedemeyer (1972). It is considered to be an easy and inexpensive indicator of stress (Biron and Benefey, 1994)

Table 1. Air-exposure stress on non-specific immune response of *Osphronemus olfax* at 18°C

Parameters	Control	Air-exposed	% change
RBC ($x10^4/mm^3$)	3.19 ± 0.34	5.91 ± 0.67	+35.27
Hb (g/100ml)	16.32 ± 0.79	20.4 ± 0.80	+25
Ht (%)	47.0 ± 1.10	67.51 ± 3.40	+43.64
Blood glucose (mg/dl)	64.9 ± 30.6	129.5 ± 6.52	+99.54
Lactate (mg/l)	30.8 ± 1.00	8.45 ± 3.40	+174.35
Lysozyme activity (mg/ml)			
Plasma	2150 ± 1215	4500 ± 2100	+109.30
Spleen	0.40 ± 0.20	0.96 ± 0.70	+140
Kidney	68.00 ± 20.6	140.00 ± 28.1	+106.62
Immunoglobulins (IgM) (mg/ml)	20.9 ± 1.52	58.4 ± 1.92	+179.43

Data are given as mean ± S.E. N= 6, All values are significantly different controls $P<0.05$

Various stressors affecting fish, mainly under intensive cultural conditions are reported in the literature. The most important seems to be handling, which can be considered a multi-factorial stress. It includes transfer, exposure to air, temperature shifts, submergence, fish density, water quality etc. Therefore the present purpose of our study was to develop a simple standard procedure that is closely related to handling stress and could easily be used as laboratory model. The multi-factorial stress used in this study involves part of the above described stress components (i.e, air-exposure and submergence) and can be considered as a model. The result indicates that blood glucose changes (Kakuta, 1997) are easily identifiable for any stress induced variations. Among the stressors, hypoxic submergence seems to be more vulnerable to this species. Similarly, cold exposure stimulates adaptive changes in the haematology and immunology. Air-exposure and normoxic submergence alone lowered the plasma lysozyme activity. On the other hand, hypoxic submergence and cold exposure stimulated the enzyme secretion. It can therefore be suggested that the decrease in the plasma lysozyme may be due to its consumption during stress while it is increase in immune organs may indicate a reaction of the

tissues to re-establish homeostasis. Air-exposure and submergence stress increased the RBC count, Hb and Ht level in the eel, *Anguilla bengalensis* (Parthi, 2000). These stressors interfere with the ability to utilize O_2 at the tissue level which results in anoxia and a consequent increase in the accumulation of glucose. The increased accumulation of glucose may be due to increased glycogenolysis or under utilization of glucose at the tissue level. Air-breathing fishes are known to accumulate glucose during stressful situations (Rotland *et al.,* 1997). In general, the hyperglycemia stress response in closely related with internal activation. One of the first responses to a stressor such as air-exposure is the release of stress hormones, adrenaline, noradrenaline and cortisol (Espelid *et al.,* 1996). The release of these catecholamines and cortisol triggers a broad suite of biochemical changes known collectively as secondary stress responses. The type and length of stress we used in our study is entirely new. These studies were intended to mimic conditions that are routinely encountered in aquaculture. Lysozyme is a leukocyte enzyme important in inflammation and bacterial killing. Lysozyme levels were significantly higher in stressed than in unstressed controls, suggesting a protective mechanisms. The accumulation of lactate during stressful situations and its consequent clearance is definitely a hormonally mediated response. The slow accumulation of lactate after normoxic submergence indicate that for *Osphronemus olfax* it is not a stressful situation. Air-exposure and hypoxia submergence alone triggers lactacidosis.

REFERENCE

Aaltonen, T.M., Jokinen, E.I. and Halftone, E.T. 1994. Antibody synthesis in roach (*Rutilus rutilus*): analysis of antibody secreting cells in lymphoid organs with ELISPOT – assay. *Fish. Shell Fish Immunol.,* 4 : 129 – 140.

Aaltonen, T.M., Jokinen, E.I., Lappivaara, J., Markkula, S.E., Salo, H.M., Leppanen, H. and Lammi, R. 2000. Effects of primary and secondary – treated bleached Kraft mill effluents on the immune system and physiological parameters of roach. *Aquat. Toxicol.,* 51: 55 – 67.

Albergoni, V. and Viola, A 1998. Effects of cadmium on catfish, *Ictalurus melas*, humoral immune response. *Fish and Shellfish Immunol.,* 5 : 89 – 95.

Anonymous. 1962. In Wealth of India, Raw Materials, Vol IV, C.S.I.R, New Delhi.

Biron M. and *Benefey,* T.J. 1994. Cortisol, glucose and hematocrit changes during acute stress of *Salvelinus fontinalis*. *Fish Physiol. Biochem.,* 13 : 153 – 160.

Caberoy, N.B. and Quinitio, G.F. 2000. Changes in Na^+ K^+ - ATPase activity and gill chloride cell morphology in the grouper *Epinephelus coioides* larvae and juveniles in response to salinity and temperature. *Fish Physiol. Biochem.,* 23: 83 – 94.

Chavin, W. 1973. Teleostean endocrine and para-endocrine alterations of utility in environmental studies. *In: 'Responses of Fish to Environmental Changes'.* (Ed.), Chawin, W. Thomas, Springfield, IL, pp.199-239.

Chavin, W. and Young, J.E. 1970. Factors in the determination of normal serum glucose levels of goldfish, *Carassius auratus*. *Comp. Biochem. Physiol.,* 33: 629 - 653.

Dacie, J.A. and Lewis, S.M. 1969. Practical Haematology. J and A Churelull Ltd, Londoan.

Espelid, S., Lokken, G.B., Steiro, K. and Bogwald, 1996. *1996.* Effects of cortisol and stress on the immune system in Atlantic salmon (Salmo salar L.). *J.* Fish Shellfish Immunol., 6 : 95 – 110.

Findlay, V.L. and Munday, B.L. 2000. The immunomodulatory effects of levamisole on the non – specific immune system of Atlantic salmon, *Salmo salar. L. J. Fish Disease.,* 23: 369 – 378.

Grinde, B., Lie, O., Poppe, T. and Salte, R. 1988. Species and individual variation in lysozyme activity in fish of interest in aquaculture. *Aquacult.,* 68 : 299 – 304.

Kakuta, I. 1997. Effect of sewage on blood parameters and the resistance against bacterial infection of goldfish, Carassius auratus, *Enviorn. Toxicol*. *Water Qual.,* 12 (1): 43 – 51.

Khare, A. and Singh, S. 2002. Impact of malathion on protein content in the freshwater fish, *Claris batrachus*. *J. Ecotoxicol. Environ. Monit.,* 12(2) : 129-132.

Kumar, K. and Patri, P. 2002. Variations in biochemical composition of climbing perch, *Anabas testudineus* in response to endosulfan toxicity. *J. Environ. Poll.,* 7(2) : 135 –141.

Mannan, M.A.R. 2002. Enzymological responses of a freshwater fish, *Oreochromis mossambicus* to cycas seed extract exposure. M.Phil. Thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.

Maule, A.G., Schrock, R., Slater, C., Fitz Patrick, M.S. and Schreck, C.B. 1966. Immune and endocrine responses of adult Chinook salmon during freshwater immigration and sexual maturation. *Fish. Shell. Immunol.,* 6 : 221 – 233.

Mclamed, O., Timan, B., Avtalion, R.R. and Noga, E.J. 1999. Design of a stress model in the hybrid bass (*Morone saxatilis x Morone Chrysops*). Israeli. *Jour. Aquacult.,* 51(1):10 – 16.

Natarajan, G.M. 1985. Induction of branchial enzymes in snakehead (*Channa striatus*) by oxydemeton. Pest. Biochem. Physiol., 23 : 41 – 46.

Natarajan, G.M. 1987. Studies on some aspects of physiology of South Indian air-breathing fish, *Macropodus cupanus* (Cuv and Val) with special reference to strategies for adapting to changing environmental conditions. Ph.D. Thesis, Bharathiar University, Tamil Nadu, India.

Nemcsok, J., Benedeczky, I., Boross, L., Asztalos, B. and Orban, L. 1981. Subcellular localization of transaminase enzymes in fishes and their significance in the detection of water pollution. *Acta. Biol. Szeged.,* 27(1-4): 9-15.

Noll, F. 1974. Methods of Enzymatic Analysis (Ed. Bergmeyer, H.U), Academic Press, London.

Parimala, K. 2002. Biochemical modulatory effects of cycas seed extract on the freshwater fish, *Oreochromis mossambicus* (Peters). M.Phil. Thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.

Parry, R.M., Chandan, B.C. and Shahani. K. M. 1965. A rapid and sensitive assay of muramidase. Prec. Soc. Exp. Biol. Med., 119: 384 – 386.

Parthi, N. 2000. M.Phil Thesis, Avinashilingam University, Coimbatore, Tamil Nadu, India.

Parthi, N., Natarajan, G.M. and Usha Nandini, R. 2000. Environmentally induced changes in haematology and certain Biochemical parameters of air-breathing fish, *Macropodus cupanus* (Cuv and Val). *J. Ecol. Res. Biocon.,* 1(1): 1 – 8.

Rani, R.P. 1994. Studies on some aspects of bimodal respiration, haematology and nitrogen excretion in the South Indian air-breathing fish, *Channa striata* (Bloch). Ph.D. Thesis, Bharathiar University, Tamil Nadu, India.

Raveendran, S. 2000. Facultative air-breathing, haematology and nitrogen excretion in the South Indian catfish, *Mystus gulio* (Ham). Ph.D. Thesis, Bharathidasan University, Tamil Nadu, India.

Rotland J, Pavlidis M *et al*. (1997). Non-specific immune responses in the red porgy *Pagrus pagrus* after crowding stress. *Aquacult.,* 156: 279 - 290.

Sasikala, G. 2001. Metasystox induced molecular immunological modulations in the South – Indian snake head edible fish, *Channa striata*. M.Phil.Thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.

Tamilselvi, G.2002. Haematological changes in the freshwater fish, *Oreochromis mossambicus* exposed to *Cycas seed* extract. M.Phil. Thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.

Val, A.L., Almedia-Val, V.M.F. and Affonso, E.G. 1990. Adaptive features of Amazon fishes: hemoglobins, hematology, intraerythrocytic phosphates and whole blood Bohr effect of *Pterygoplichthys multiradiatus* (Siluriformes). Comp. Biochem. Physiol., 97B: 435 -440.

Vashist, S., Indukumari, S. and Jamil, K. 2000. The study of alteration in respiratory metabolism of teleost fish *Tilapia mossambica* due to the effect of polluted water. Proc. Nat. Acad. Sci. India. pp. 229 – 236.

Vijayalakshmi, P. 1996. Studies on some aspects of bimodal gas exchange, haematology and nitrogen excretion in the South Indian feather-back, *Notopterus notopterus* (Pallas). Ph.D Thesis, Bharathiar University, Tamil Nadu, India.

Wedemeyer, G. 1972. Some physiological consequences of handling stress in the juvenile coho salmon *(Oncorhynchus kisutch)* and steelhead trout *(Salmo gairdneri)*. *J. Fish. Res. Bol. Can.,* 29 : 1780 – 1783.

Yin, Z., Lam, T.J. and Sin, Y.M. 1995. The effect of crowding stress on the non-specific immune response in carp (*Cyprinus carpio*). *Fish shellfish. Immunol.,* 5 : 519 – 529.

Zar, J.H. 1984. Biostatistical Analysis (II end Edition), Prentice – Hall, New Jersey.

****