IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 1 Issue 8, October 2014.
www.ijiset.com
ISSN 2348 – 7968

Serum Cortisol and Glucose: Reliable Bioindicators of stress in the Fish Labeo rohita

S.N.C. Ray[1] and R.C. Sinha[2*]
[1]Centre for Environment & Nature Conservation, Department of Zoology, Patna University, Patna-05
[2]Centre for Environment & Nature Conservation, Department of Zoology, Patna University, Patna-05

Abstract

The fish as a bioindicator species plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The endocrine response to pesticidal stress in the fish *Labeo rohita* was investigated in the present study. Exposure of the fresh water fish, *Labeo rohita* to sub- lethal concentration (5.6 ppm) of methyl parathion over a period of six hours has revealed that the pesticide has a profound effect on the cortisol, T_3, glucose and glycogen contents of the liver and muscle of the fish. The level of cortisol increased significantly ($p<0.06$) after two hours and after six hours of exposure (increased approximately twice to that of the control) but on the contrary T_3 decreased significantly ($p<0.002$) till six hours showing an inverse relationship. It was interesting to note that the glucose content increase was highly significant ($p< 0.001$) till six hours (approximately 3.5 times) as compared to control in contrast to liver glycogen suggesting glycolysis during stress. But the muscle glycogen responded differently because the muscle glycogen increased significantly ($p<0.006$) during 4th hour, 5th ($p<0.002$) and 6th ($p<0.009$) hours. The results suggest that the methyl parathion extended potent effects on the endocrine system through activating their hypothalamo-pituitary- internal axis and disturbing the thyroid function. The physiological significance of these observations is discussed in the present paper.

Key Words: Bioindicator, Labeo rohita, Cortisol, T_3, T_4, TSH, Methyl parathion, Glucose

Introduction

The response of stress in fish is characterized by the stimulation of the hypothalamus which results in the activation of the neuro-endocrine system and a subsequent cascade of metabolic and physiological changes [1, 2]. These changes enhance the tolerance of an organism to face an environmental variation or an adverse situation while maintaining a homeostasis [3, 4].

Under the condition of stress, the body of the fish immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNS) and the release of stress hormones, cortisol and catecholamines (adrenalin and nor-adrenalin) into the blood stream [5]. Secondary responses occur as a consequence of the released stress hormone [6], causing changes in the blood and tissue chemistry e.g. an increase in plasma glucose [7, 8]. This entire metabolic pathway produces a burst of energy to prepare the fish for an emergency situation [9].

Some plasma chemicals may be useful tools to evaluate the heath and or stress condition of the fishes [10, 11, 12]. Since stress has been reported to elevate plasma cortisol [13, 14, 15, 16] and glucose levels [17, 18], many researchers consider as a 'rule of thumb' that fishes undergoing stressful situation exhibit

ISSN 2348 – 7968

plasmatic increases of cortisol and glucose [19, 20, 21]. The secretion of cortisol is slower than catecholamines but its effects are more prolonged [22, 23], combining mineral and glucocorticoid actions to restore homoeostasis [14, 24, 25]. Cortisol activates glycolysis and gluconeogenesis processes in fish and chromaffin cells that release catecholamines which further increase glycogenolysis and modulate cardiovascular and respiratory functions [26]. This whole process increases the substrate level (glucose) to produce enough energy according to the demand of the animal.

Thyroid hormones affect various aspects of metabolism. Thyroid hormones play a role in a stress related mobilization of glucose in fish. Thyroxin treatment caused hyperglycemic effect in some cases [27]. However, other studies observed hypoglycemic responses in thyroxin treatment [28]. Closely related significant elevations of plasma T_4 and glucose induced by disturbance stress were reported [29]. Thyroid hormone administration has been reported to accelerate fish growth [30, 31, 32] and support of this concept, elevated concentrations of T_3 were found to in periods of rapid growth [33, 34, 35]. The apparent inconsistencies in these experiments may partially reflect interaction of the thyroid hormones with the other hormones such as growth hormones and cortisol. Thyroidal treatment was found to promote the anabolic effects of growth hormone (lower liver glycogen and high serum cortisol) in rainbow trout [36, 37] on the other hand, growth hormone treatment significantly elevated plasma T_3 concentration of rainbow trout [37, 38] indicating an increased peripheral conversion of T_4 to T_3. Sub- lethal concentration of arsenic decreased thyroxin concentration [39].

Many teleost fish rely primarily on protein and lipid sources for energy and they are considered to possess enzyme system for the utilization of the carbohydrates [40, 41]. In these species, amino acids such as arginine and lysine have been to be more effective than glucose in simulating insulin release [42, 43]. However, carbohydrate metabolism increases in high energy demand such as stress. In stress blood glucose is elevated as a result of both glycogenolysis and gluconeogenesis [14]. Stress has been described as an energy drain of energy that might be utilized in growth diverted to catabolic utilization [44, 4, 45]. Mobilization of readily available energy in the form of glucose is suggested to enhance the survival of fish [46, 47]. It is perhaps, not surprising, therefore, that elevation of plasma glucose has been recognized as a part of generalized stress response in fish [3, 48, 49, 50] and fish exposed to pollutant [51, 52, 53, 54].

It is apparent from the literature cited above that there are apparent inconsistencies in these experiments which may partially reflect the interaction of thyroid hormones with other hormones and cortisol. As such, in the present study, this effect of acute toxicity of methyl parathion on the endocrine responses to the pesticidal stress in the fish *Labeo rohita* has been studied.

Materials & Methods

Labeo rohita, a common carp were obtained from the local hatchery. Fishes were acclimated to the laboratory conditions for about 5 days. They were kept in aquarium tank (250L) and the water was constantly aerated by static system. During the acclimation period, they were given artificial (commercial) feed and grounded shrimps available in the local market to avoid the possible effects of starvation on any parameters under study. The feeding and maintenance of the fishes and physico-chemical characteristics of the aquarium were measured. Short term test of acute toxicity over a period of 6hrs were performed on these fishes following the renewal bioassay. Fishes were exposed

ISSN 2348 – 7968

intraceolomatically with 1/3rd of 16.8 ppm of methyl parathion (LC_{50}) for 6 hours to evaluate *in vitro* concentration of serum cortisol and T_3 by Chemiluminescence Immuno Assay Reader Neo- Lumax, model-4901 because it has measuring ranges over many other immunoassay methods and excellent for detection and quantification. Serum glucose was determined spectrophotometrically by GOD- POD method, serum SGPT and SGOT were determined by Reitman and Frankel method [55]. Glycogen was estimated by Kemp and Andrinne method [56] as modified by Kanungo and Sinha [57].

Blood Collection:

The fishes were taken out of the aquarium individually through fish net with a minimum possible disturbance. After preliminary investigations, the blood samples were collected from caudal fin as described by many authors. In the present study, the blood collection from caudal fin had to be abandoned because there was an unusual increase in the enzymatic activities were recorded, which might have leaked from the surrounding muscle tissues. Thus, cardiac sampling was the only suitable method available as an alternative to obtain blood under the present study. After the blood sampling the liver and muscle tissues were taken for different assays.

Liver and Muscle:

Liver and muscle were taken out and soaked with filter paper and subsequently analyzed for glycogen. Prior to the assay, the tissues were properly homogenized in an electrical homogenizer and centrifuged at 4^0C in a cooling centrifuge (Remi, Model: C-30).

Results & Discussion

Fig. 1 & Table 1 show that there is a significant increase in cortisol when the fish, *Labeo rohita*, is exposed to sub- lethal concentration of methyl parathion till 5 hours and during 6th hour of exposure the concentration of cortisol remain the same as that of 5th hour. Similarly, there is a parallel significant increase in serum glucose ($p< 0.001$) after 1 hour of exposure till 6 hours in contrast to the liver glycogen wherein the decrease is significant ($p<0.003$) during 2nd hour and thereafter till 6 hours ($p< 0.001$). The results also reveal that T_3 concentration decreases significantly during 3rd, 4th, 5th and 6th hours of exposure (Fig. 1 & 2), whereas muscle glycogen increases significantly during 4th, 5th and 6th hours of exposure.

Cortisol has been widely used to assess the state of health of the fish exposed to stress [58, 59]. Changes in the concentration of plasma cortisol however, depend upon the nature of stress stimuli and the duration of the stress as well as also the magnitude and severity of stress [60, 61, 51] and species under investigation [62]. In the present study, since there is a significant increase in the cortisol concentration immediately after exposure and continuous increase till 6th hour (Tab. 1, Fig. 1 & 2), it could be concluded that fishes exposed to the sub- lethal concentration of methyl parathion undergo an immediate stress and continue to be under stress till 6th hour with a parallel increase of glucose concentration in contrast to liver glycogen (Tab. 1), suggesting thereby glycolysis.

Many authors have reported that cortisol increases glycolysis resulting in the rise of blood glucose whereas some authors have reported that it is the catecholamines which is known as ‘fight and flight’ hormone which increases the blood glucose initially and thereafter the cortisol further increases the

ISSN 2348 – 7968

blood glucose. As such, it was considered of interest to investigate whether the rise of glucose in stressed condition was due to glycogenolysis or gluconeogenesis? To prove this hypothesis, SGPT and SGOT activities were also assayed. It was found that SGPT & SGOT activities increased after 2 hours and continued to increase till 6th hour of exposure suggesting that gluconeogenesis took place after 2 hours and as such the initial rise of blood glucose was due to the catecholamines and not due to the cortisol (Tab. 1) as reported by Martinez- Porchas *et al* [63].

In gluconeogenesis, free amino acids are precursors of glucose synthesis and regulation of the process in the liver is suggested to be mediated by cortisol. The gluconeogenesis effect of cortisol has also been reported by Vijayan *et al* [64]. The hyperglycemic effect of cortisol is due to the inhibitory effect of the hormone on glucose oxidation and utilization in peripheral tissues. This is corroborated by our result of T_3 which decreased significantly during 6th hour of exposure. The decreased liver glycogen in response to elevated cortisol has also been reported [65, 66, 67, 36]. Therefore, the present study shows that cortisol action is inversely related to T_3 action (Fig. 3).

Conclusion

1. The secretion of cortisol is slower than the catecholamines but its effects are more prolonged and its action is to restore homeostasis.
2. An adverse role of cortisol is suggested to be linked to mobilization of energy reserves through catabolic functions. After stress, the cortisol level returns to basal level to avoid tissue damage because it is known that high level of cortisol cause death in fishes by tissue degeneration and damage of homeostatic mechanism.
3. Thus, cortisol and glucose are good indicators for acute stress. As such, cortisol and glucose could be regarded as reliable biomarkers of stress.

Acknowledgement:

The authors are grateful to DBT, Govt. of India for financial assistance for the project (Sanction Letter no. BT/PR4762/BCE/8/903/2012; dated: 11/03/2013). The authors also thank the Head of the Department of Zoology for extending all logistic support.

ISSN 2348 – 7968

Table: 1

Parameter (Serum)	Control	Time Duration of Treatment					
		1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
Cortisol (µg/dl)	13.5±4.2	16.8±2.3 (p<0.003)	18.7±2.1 (p<0.066)	19.8±2.9 (p<0.026)	21.2±2.9 (p<0.002)	23.5±2.3 (p<0.006)	23.7±4.9 (p<0.016)
T_3 (ng/ml)	1.2±0.4	1.2±0.17 (p<0.709)	0.9±0.04 (p<0.120)	0.6±0.1 (p<0.021)	0.4±0.09 (p<0.012)	0.4±0.1 (p<0.006)	0.3±0.1 (p<0.002)
Glucose (mg/dl)	51.6±4.9	54.5±8.04 (p<0.467)	80.3±9.3 (p<0.001)	124.2±14.4 (p<0.001)	139.0±41.3 (p<0.001)	172.4±6.0 (p<0.001)	171.8±18.7 (p<0.001)
SGOT (IU/l)	7.6±0.7	6.7±0.2 (p<0.2)	6.8±1.1 (p<0.7)	7.0±0.2 (p<0.6)	7.0±0.2 (p<0.5)	7.2±2.2 (p<0.9)	8.8±0.7 (p<0.2)
SGPT (IU/l)	5.1±1.0	8.3±0.6 (p<0.06)	8.6±1.4 (p<0.09)	9.0±0.6 (p<0.07)	7.5±0.8 (p<0.05)	8.1±0.9 (p<0.08)	8.6±0.9 (p<0.06)
Parameter (Tissue)							
Liver Glycogen (mg/g 0f tissue)	10.4±0.5	10.1±1.0 (p<0.444)	7.1±1.2 (p<0.003)	7.0±0.6 (p<0.001)	5.6±0.6 (p<0.001)	5.2±0.5 (p<0.001)	5.0±0.9 (p<0.001)
Muscle Glycogen (mg/g 0f tissue)	1.7±0.2	1.4±0.1 (p<0.07)	1.2±0.2 (p<0.022)	1.92±0.3 (p<0.1)	2.4±0.3 (p<0.011)	2.4±0.4 (p<0.033)	2.2±0.3 (p<0.032)

ISSN 2348 – 7968

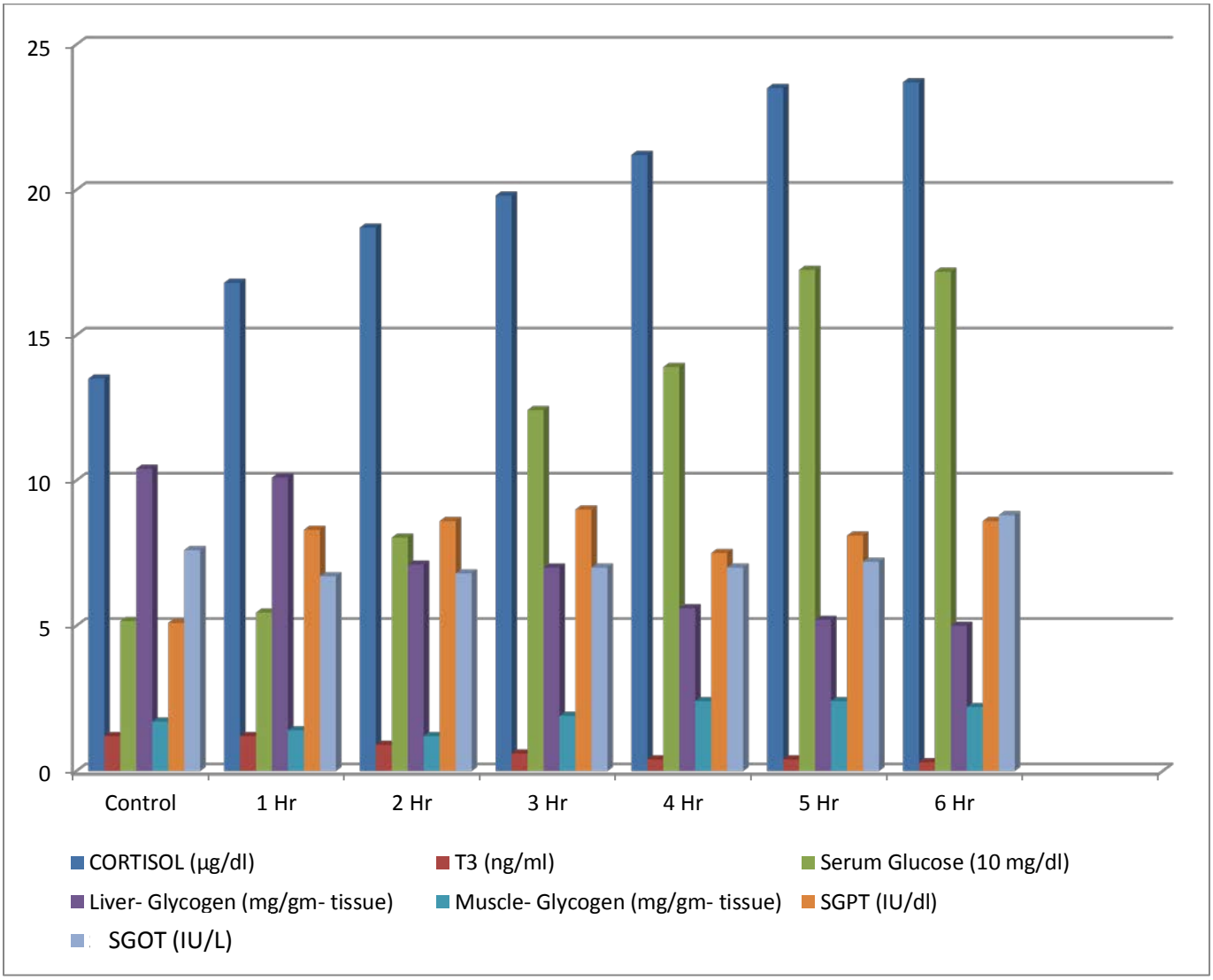

Fig: 1 Effect of Methyl parathion stress on the level of Cortisol, Thyroxin (T_3), Serum Glucose, Liver Glycogen, Muscle Glycogen, SGPT and SGOT

ISSN 2348 – 7968

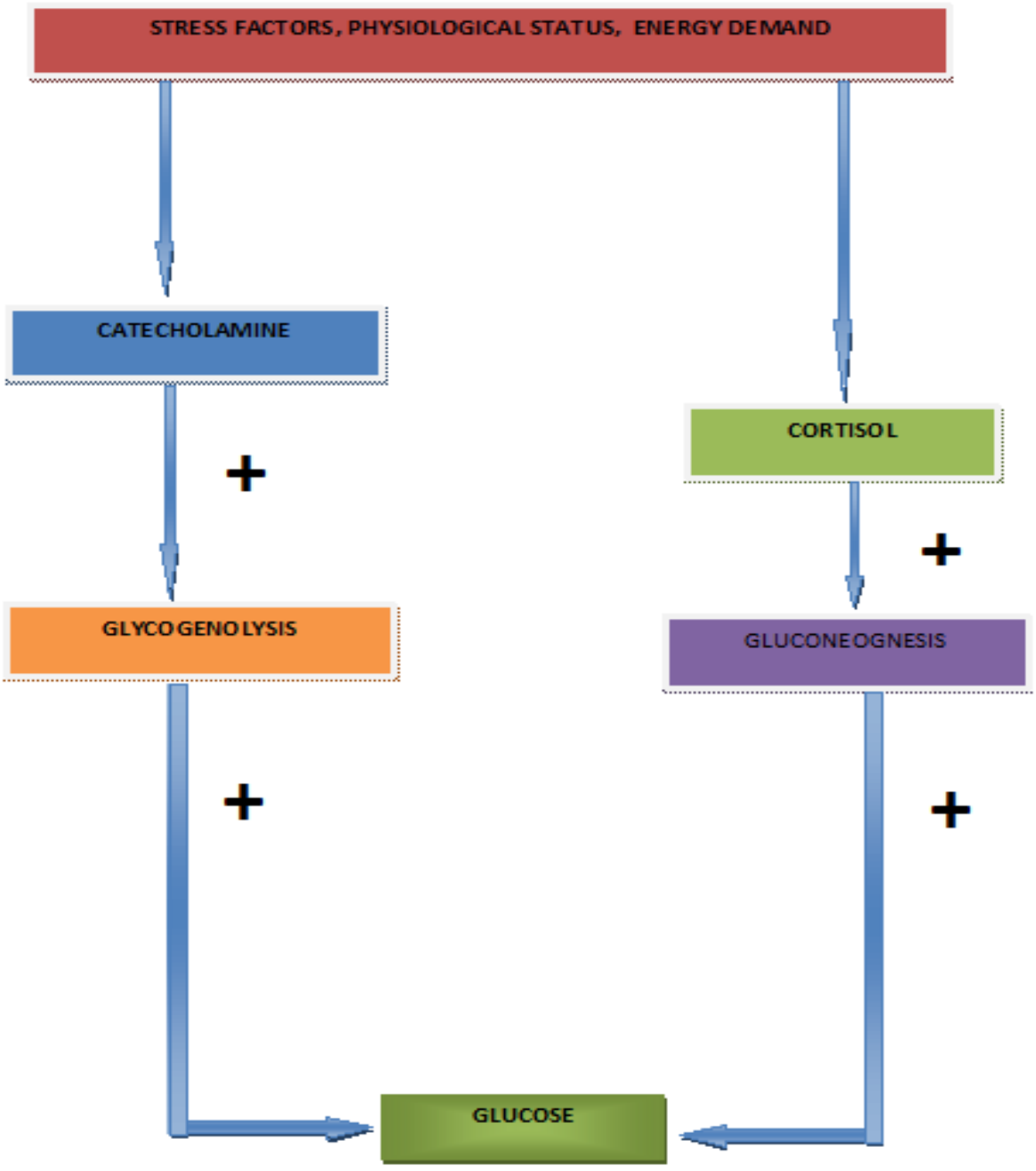

Fig 2: Systematic Representation of the Dynamics of Cortisol and Catecholamines in the Production of Glucose during the Pesticidal Stress

ISSN 2348 – 7968

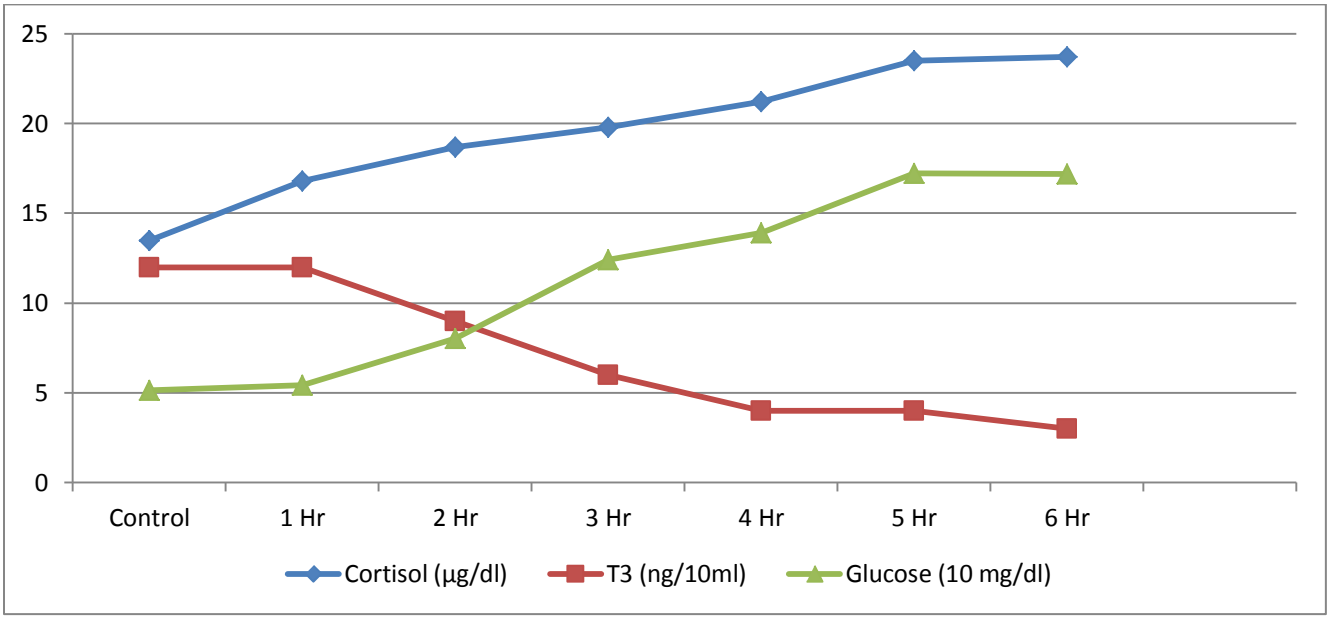

Fig. 3: Showing Antagonistic Relationship between Cortisol and Thyroid Hormone T_3 as a Function of Methyl Parathion

Reference

[1] Wedemeyer, G.A. and Yasutake, W.T. (1997). Clinical methods for the assessment of the effect of environmental stress on fish health. *Tech. Pap. USFWS. n*o. 89.

[2] Lowe, C.J. and Davidson, W. (2005). Plasma osmolarity, glucose concentration and erythrocyte responses of two Antarctic fishes to acute and chronic thermal change. *J. of Fish Biol.* 67: 752-766.

[3] Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. (1977). Primary and secondary effects of stress in fish; some new data with a general review. *Trans. American Fish Soc.* 106: 201-212.

[4] Pickering, A.D. (1990). Stress and suppression of somatic growth in teleost fish. In: *Progress in Comparative Endocrino. (Epple, A., Scanes, C.G. and Stetson, E.D., eds.) Wiley- Liss, New York,* pp. 473-479.

[5] Randall, D.J. and Perry, S.F. (1992). Catacholamines. In: *Fish Physiol. (Randell, D.J. and Hoar, W.S. eds.) Vol. XIIB. The Cardiovascular System, Academic Press, New York*, pp. 255-300.

[6] Barton, B.A. and Iwana, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on response and effects of corticosteroids. *Annual Rev. Fish Diseases*, 1: 3-26.

[7] Barton, B.A., Schreck, C.B., and Fowler, L.G. (1988). Fasting and diet content affect stress- induced changes in plasma glucose and cortisol in juvenile Chinook salmon. *The Progressive Fish Culturist,* 50: 16-22.

[8] Begg, K. and Pankhurst, N.W. (2004). Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus. J. of Fish Biol.*, 64: 133-145.

[9] Rottman, R.W., Fransis- Floyd, R. and Durborow, R. (1992). The role of stress in fish disease. *SRAC Publication* no. 474, 4p.

[10] Sadler, J., Pankhurst, N.W., Pankhurst, P. M. and King, H. (2000). Physiological stress responses to confinement in diploid and triploid Atlantic salmon. *J. of Fish Biol.,* 56: 506-518.

[11] Campbell, T.W. (2004). Blood biochemistry in lower vertebrates. In:*55th Annual Meeting of the American College of Veterinary Pathologists (ACVP) and 39th Annual Meeting of American Society of Clinical Pathology (ASVCP), ACVP and ASVPC (eds.).*

[12] Wagner, T. and Congleton, J.L. (2004). Blood chemistry correlates of nutritional condition, tissue damage and stress in migrating juvenile Chinook salmon (*Oncorhynchus tshawaytscha*). *Canadian J. of Fisheries and Aqu. Sce.,* 61: 1066- 1074.

[13] Pottinger, T.G. and Mosuwe, E. (1994). The corticosteroidogenic response of brown and rainbow trout alevins and fry to environmental stress during a critical period. *General and Comp. Endocrinol.,* 95: 350-362.

[14] Wendelaar- Bonga, S.E. (1997). The stress response in fish. *Physiological Reviews,* 77: 591-625.

[15] Pottinger, T.G., Rand- Weaver, M. and Stumper, J.P. (2003). Over- winter fasting and refeeding in rainbow trout: plasma growth hormones and cortisol levels in relation to energy mobilization. *Comparative Biochem. and Physiol,* 95: 313- 317.

[16] Haukenes, A. H., Barton, B.A. and Bollings, H. (2008). Cortisol response of pallid sturgeon and yellow perch following challenge with lipopolysaccharide. *J. of Fish Biol..,* 780- 784.

[17] Wedemeyer, G.A. and Yasutake, W.T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. *Tech. Pap. USFWS.,* no. 89.

[18] David, M., Shivakumar, R., Mushigeri , S.B. and Kuri, R,C. (2005). Blood glucose and glycogen levels of stress in the fresh water fish, *Labeo rohita* under fenvalerate intoxication. *J. of Ecotoxicol. & Env. Monitoring,* 15: 1-5.

[19] Hattingh, J. (1997). Blood sugar as an indicator of stress in the freshwater fish, *Labeo capinsis* (Smith). *J. of Fiosh Biol.* 10: 191-195.

[20] Balm, P.H.M., Lambert, J.D.G. and Wendelaar- Bonga, S.E. (1999). Corticosteroid biosynthesis in the inerrenal cells of the teleost fish, *Onchyromis mossambicus. Gen. and Compar. Endocrinol.,* 76: 53-62.

[21] Barcellos, L.J.G., Nicolaiewsky, S. de Souza, S.M.G. and Lulhier, F. (1999). Plasmatic levels of cortisol in response to acute stress in Nile tilapia, previously exposed to chonic stress. *Aquacul. Reser.,* 30: 437-444.

[22] Gamperl, A.K., Vijayan, M.M and Boutilier, R.G. (1994). Experimental control of stress hormone levels in fishes: techniques and applications. *Reviews on Fish Biol. and Fisheries,* 4: 215-255.

[23] Waring, C.P., Stagg, R.M. and Poxton, M.G. (1996). Physiological responses to handling in the turbot. *J. of Fish Biol.,* 48: 161- 173.

ISSN 2348 – 7968

[24] Maule, A.G., Shreck, C.B. and Sharp, C. (1993). Seasonal changes in cortisol sensitivity and glucocorticoid receptor affinity and number in leukocytes of Coho salmon. *Fish Physiol. And Biochem.,* 10: 497-506.

[25] Colombe, L., Fostier, A., Bury, N, Pakdel, F. and Guiguen, Y. (2000). A mineralocorticoid like receptor in the rainbow trout, *Oncorhynchus mykiss:* cloning and characterization of its steroid binding domain. *Steroids,* 65: 319-328.

[26] Reid, S.G., Vijayan, M.M., and Perry, S.F. (1996). Modulation of catecholamine storage and release by pituitary- interregnal axis in the rainbow trout, *Oncorhynchus mykiss. J. Comp. Physiol.* B 165: 665-676.

[27] Chan, D.K.O. and Woo, N.Y.S. (1978). Effect of cortisol on the metabolism of the eel, *Anguillia japonica. Gen. Comp. Endocrinol.* 35: 205-2015.

[28] Murat, J.C. and Serfaty, A. (1970). Au subject d'un effect hypoglycemiant de la thyroxione chez la carpe, *Cyprinus carpio L.C. Séance Sac. Biol.* 164: 1842-1845.

[29] Himic, B.A. and Eales, J.G. (1990). Acute correlated changes in plasma T_4 and glucose in physically disturbed cannulated rainbow trout, *Oncorhynchus mykiss. Comp. Biochem. Physiol.* 97: 165-167.

[30] Higgs, D.A. , Fagerlund, U.H.M. and Mcbride, J.R. (1979). Influence of orally administered L-Thyroxine of 3, 5, 3'- triiodo- L-thyronine on growth, food consumption and food conversion of underyearlying coho salmon, *Oncohynchus kisutch. Can. J. of Zoo*. 57: 1974-1979.

[31] Saunders, R.L., Mccormick, S.D.,Henderson, E.B., Eales, J.G. and Jhonson, C.E. (1985). The effect of orally administered 3, 5, 3'-triiodo-thyronine on growth and salinity tolerance of the Atlantic salmon, *Salmo* solar. *Aquaculture,* 45: 143-156.

[32] Woo, N.Y.S., Chung, A.s.B. and NG, T.B. (1991). Influence of oral administration of 3, 5, 3'-triiodo-thyronine on growth, digestion, food conversion and metabolism in the underlying red sea bream, *Chrysophryus major* (Temmink and Schlegel). *J. Fish Biol.* 39: 450-468.

[33] Leatherland, J.F., Hilton, J.W. and Singer, S.J. (1987). Effects of thyroid hormones supplementation of canola meal- based diets on growth and interenal and thyroid gland physiology of rainbow trout, *Salmo gairdneri. Fish Physiol. Biochem.* 3: 730-782.

[34] Gannam, A.L. and Lovell, R.T. (1991). Effects of feeding 17α- methyltestosterone, 11-ketotestosterone, 17β- estradiol and 3, 5, 3'-triiodo-thyronine to channel catfish, *Ictalurus punctatu., Aquaculture*, 92: 377-388.

[35] Soengas, J.L., Rey, P., Rozas, G., Andres, M.D. and Aldegunde, M. (1992). Effect of cortisol and thyroid hormone treatment on the glycogen metabolism of selected tissues of domesticated rainbow trout, *Oncorhynchus mykiss. Acquaculture,* 101: 317-328.

[36] Fagerlund, U.H.M., Huggs, D.A., Mcbride, J.R., Plotnickoff, M.D. and Dosanjh, B.S. (1980). Thje potential for using the anaerobic hormone17α- methyltestosterone and/ or 3, 5, 3'-triiodo-thyronine in the freshwater rearing of coho salmon, *Oncorhynchus kisutch* and the effects of subsequent seawater performance. *Can. J. Zoo.* 58: 1424-1432.

ISSN 2348 – 7968

[37] Farbridge, K.J. and Leatherland, J.F. (1988). Interaction between ovaine growth hormone and triiodo-L-thyronine on metabolic reserves of rainbow trout, *Salmo gairdneri. Fish Physiol. Biochem.* 5: 141-151.

[38] Maclatchy, D.L. and Eales, J.G. (1990). Growth hormone stimulates thyroxine 5' monodeiodinase activity and 3, 5, 3'-triiodo-thyronine levels in rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.*. 78: 164-172.

[39] Nichols, J.W., Wedemeyer, G.A., Mayer, F.L., Dickhoff, W.W., Gregorgy, S.V., Yasutake, W.T. and Smith, S.D. (1984). Effects of fresh water exposure to arsenic trioxide on the par- smolt transformation of coho salmon, *Oncorhynchus kisutch. J. Environ. Toxicol. Chem.* 3: 143.

[40] Cowey, C.B. and Sargent, J.R. (1979). Nutrition. In: *Fish Physiol. Vol. 8 (Hoar, W.S., Randall, D.J. and Brett. J.R. eds.)*, *Academia Press, New York.* pp. 1-69.

[41] Walton, M.J. and Cowey, C.B. (1992). Aspects of intermediary metabolism in salmonid fish. *Comp. Biochem. Physiol.* 73B: 59-79.

[42] Higuera, M. and Cardenas, O. (1986). Hormonal effects of gluconeogenesis from (U ^{14}C) glutamate in rainbow trout, *Salmo gairdneri. Comp. Biochem. Physiol.* 58B: 517-521.

[43] Petersen, T.D.P., Hochavhka, P.W. and Saurez, R.K. (1987). Hormonal control of gluconeogenesis in rainboe trout hepatocytes: regulatory role of pyruvate kinase. *J. Exp. Zool.* 243: 173-180.

[44] Barton, B.A. (1988). Endocrine and metabolic responses of fish to stress. In: *Proc.* 19th Annual Conference of *International Association of Aquatic Animal Medicine, Orlando, Florida.* 19: 41-55.

[45] Mc Donald, D.G., Goldstein, M.D. and Mitton, C. (1993). Responses of hatchery- reared brook trout, lake trout and splake to transport stress. *Trans. American Fish Soc.*, 122: 1127-1138.

[46] Barton, B.A. and Iwama, G.K. (1991). Physiological changes in the fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Rev. Fish Diseases,* 1: 3-26.

[47] Pickering, A.D. (1993). Endocrine- induced pathology in stresses salmoid fish. *Fish. Res.* 17: 35-50.

[48] Davis, K.B. and Parker, N.C. (1990). Physiological stress in striped bass: effect of acclimation temperature. *Aquaculture,* 91: 349-358.

[49] Melotti, P., Roncarati, A., Garella, E., Garnerval, O., Mosconi, G. and Polzonetti- Magni, A. (1992). Effects of handling and capture stress on plasma glucose, cortisol and androgen levels in brown trout, *Salmo trutta morpha fario. J. Appl. Icthyol.* 8: 234-239.

[50] Barry, T.P., Lapp. A.F., Kayes, T.B. and Malison, J.A. (1993). Validation of microtitre plate ELISA for measuring cortisolin fish and comparision of stress response of rainbow rout, *Oncorhynchus mykiss* and lake trout, *Salvelinus namaycush. Aquaculture,* 117: 351-363.

[51] Gluth, G. and Hanke, W. (1984). A comparision of physiological changes in carp, *Cyprinus caprio,* induced by several pollutants at sublethal concentration-II. The dependence of temperature. *Comp. Biochem. Physiol.* 79C: 39-45.

[52] Goss, G.G. and Wood, C.M. (1988). The effects of acid and acid/ aluminium exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdnei. J. Fish Biol.* 32: 63-76.

[53] Whitehead, C. and Brown, J.A. (1989). Endocrine responses of brown trout, *Salmo trutta L.,* to acid, aluminium and lime dosing in Welsh hill stream. *J. Fish Biol.* 35: 59-71.

[54] Alkindi, A.Y.A., Brown, J.A., Waring, C.P. and Collins, J.E. (1996). Endocrine, osmoregulatory, respiratoty and hematological parameters in flounder exposed to water solublefraction of crude oil. *J. Fish Biol.* 49: 1291-1305.

[55] Reitman, S. and Frankel, S. (1957). *Am. J. Clin. Path*. 28: 56.

[56] Kemp, A. and Andrienne, J.M.K.V.H. (1954). A colorimetric micro-method for the determination of glycogen in tissues. *Biochem. J.,* 56: 646-648.

[57] Sinha, R.C. and Kanungo, M.S. (1967). Effect of starvation on the scorpion, *Palamneus bengalensis. Phtsiol. Zool.* 40: 386-390.

[58] Mazeaud, M.M and Mazeaud, F. (1981). Adrenergic responses to stress in fish. In: *Stress & Fish. (Pickering, A.D., ed.,), Academic Press, London, NewYok.* Pp. 49-75.

[59] Pickering, A.D. (1993). Growth and stress in fish production. *Aquaculture,* 111: 51-63.

[60] Barton, B.A. and Toth, L.T. (1980). Physiological stress in fish: a literature review with emphasis on blood cortisol dynamics. *Fisheries Research Report No.* 21, *Fisheries Research Section, Fish and Wild Life Division, Albetra Department of Energy and Natural Resources, Albetra.*

[61] Pickering, A.D., Pottinger, T.G. and Christle, P. (1982). Recovery of brown trout, *Salmo trutta* L., from acute handling stress: a trime- course study. *J. Fish. Biol.* 20: 229-244.

[62] Waring, C.P. Stagg, R.M. and Poxtom, M.G. (1992). The effect of handling of flounder, *Platichythys flesus* L. and Atlantic salmon *Salmo salar* L. *J. Fish Biol.,* 41: 131-144.

[63] Marcel Martinez-P., Luios, R.M. and Rogelio, R. (2009). Cortisol and glucose: Reliable biomarkers of fish stress. *Pan. Am. J. Aquatic Sce.,* 4(2): 158-178.

[64] Vijayan, M.M., Reddy, P.K., Leatherland, J.F. and Moon, T.W. (1994). The effect of cortisol on hepatocyte mrtabolism in rainbow trout. A study using the steroid analogue RU- 486. *Gen. Comp. Endocrinol.* 96: 75-84.

[65] Barton, B.A., Schreck, C.B. and Fowler, L.G. (1988). Fasting and diet content affect stress- induced changes in plasma glucose cortisol in juvenile Chinook salmon. *The Progressive Fish Culturist.* 50: 16-22.

[66] Foster, G.D. and Moon, T.W. (1986). Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (Le Sueur). *Fish Physiol. Biochem.* 1: 113-124.

[67] Vijayan, M.M. and Leatherland, J.F. (1989). Cortisol- induced changes in plasma glucose, protein and thyroid hormone levels and liver glycogen content of coco salmon, *Oncorhynchus kisutch* Walbaun. *Can. J. Zool.* 67: 2746-2750.