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STRESS AND STRESS RESPONSES IN TELEOST

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INTRODUCTION

Fishes are cold blooded animals (ectothermic/ poikilothermic) that cannot regulate their body temperature, hence their physiological mechanism are directly or indirectly temperature dependent. Some degree of thermoregulation is present in some fishes like Tunas and Lamnid sharks, in which the brain, eye and muscle temperature have been reported to be 2-12°C higher than the ambient water temperature (Steven and Neill, 1978). Hence the temperature is one of the most important factors affecting the poikilothermic organisms.

Different stressors such as physical, chemical, biological and procedural exist in different stages of aquaculture practices. The physiological responses (stress response) of the animals exposed to stressor are of three types i.e. primary response, secondary response and tertiary response (Wedemeyer and Mc Leay, 1981).

It is generally accepted that the animal responds to a variety of stressors, which is an adaptive mechanism and called as general adaptation syndrome (GAS) (Barton and Iwama, 1991).

There are two approaches to mitigate the stress first one is non-chemical (biological method) and the other one is the chemical method. Non-chemical (biological) method includes the entire environment management which includes water quality management such as temperature, dissolved oxygen, ammonia, nitrogen, nitrite, salinity etc. and stocking density, uniform size stocking, stocking ratio in polyculture etc. Chemical method includes dietary supplementation of vitamin C, Vitamin E, tryptophan, immunostimulants etc.

STRESS AND STRESSORS

Seyle (1950): Stress means the sum of all the physiological response by which an animal tries to maintain or re-establish a normal metabolism on the face of physical or chemical force.

Stress in aquaculture systems may be categorized as below: (Wedemeyer, 1999)

Table 1: Type of Stress and its examples

Sr. No.	Type of Stress	Examples
1.	Physical	Temperature, Light, Dissolved oxygen, Sound
2.	Chemical	Water quality, Pollution, Diet, Metabolic waste
3.	Biological	Stocking density, Microorganisms (pathogenic and non-pathogenic), Macro organisms (parasites), Lateral swimming space requirements
4.	Procedural	Handling, Hauling, Stocking, Disease treatment, Feeding methods (manual and automated)

Stressors are physical, biological and chemical factors that cause stress. Stressor is causative factor and stress is a response. Stress can also be classified as:

Acute Stress

An acute stress is short duration stress in which the time course of response outlasts that of stress such as netting, hauling, grading, handling, prophylactic treatments are some of the acute forms of stress encountered in aquaculture systems. Chasing fish to exhaustion or holding them in a net out of water for 30- 60 sec have been common protocols utilized to study acute stress responses in fish (Iwama *et al.,* 1999, 2003).

Chronic Stress

Chronic stress is a continuous form of stress. In aquaculture systems, overcrowding, variable water quality and problems of social dominance of some members of the population over others (e.g. Prawn, Sea bass) results in chronic stress (Pickering, 1981).

STRESS RESPONSES

Stressor is causative factor and stress is a response. The stress response of fish follows the general vertebrate pattern (Weytes *et al.*, 1999). A key element in the response is a switch from anabolism to catabolism, the animal uses up the energy as it attempts to avoid or overcome the immediate threat (Pickering, 1992). The quantum and nature of stress response may vary with nature and factors such as age, sex, developmental stages, maturation stage, environmental temperature, species and strain of fish (Vijayan and Moon, 1994; Iwama *et al.*, 1999). Changes in stress responses also depend on the type of stress and stress duration (Iwama *et al.*, 1992).

At the organism level, a series of physiological changes occur following stresses, which are adaptive in nature. These physiological changes/ responses are termed as **'General Adaptation Syndrome'** (GAS), Selye, 1950. It is consist of (1) an alarm reaction in which catecholamine and corticosteroids "stress hormones" are released (2) a stage of resistance during which adaptation occurs (3) a stage of exhaustion in which adaptation is lost because the stress was too sever or long lasting (Wedemeyer and Mcleay, 1981).

Stress produces effects that threaten or disturb the homeostatic equilibrium and elicit a coordinated set of behavioural and physiological responses thought to be compensatory and or adaptive, enabling the animal to overcome the threats. However, during chronic stress, the stress response may lose its adaptive value and become dysfunctional, which may result in inhibition of growth, reproductive failure and reduced resistance to pathogens (Wendelaar Bonga, 1997; Jobling, 1995).

The stress response in fish has been broadly categorized into the primary (neural and neuro-endocrine responses), secondary (physiological consequence of such primary response) and tertiary responses (inhibition of growth, reproductive failure and reduced resistance to pathogens (Barton, 1997; Wendealaar Bonga, 1997).

Primary Stress Responses

I. Release of adrenocorticotropic hormones (ACTH) from the adenohypophysis.

II. Release of "stress hormones" (catecholemines i.e. adrenalin, nor-adrenalin and dopamine, and corticosteroids especially cortisol) from the head kidney.

Sensory perception of stress is a prerequisite for stress response in animals. In fish, an adverse condition stimulates the afferent neural pathway that run in the sympathetic nervous system from the hypothalamus to the chromaffin tissue of the head kidney. Direct stimulation of the chromaffin tissue leads to the release of catecholamines. Catecholamine (Adrenalin/ Epinephrine) is released from the chromaffin tissue in the head kidney of teleosts, and also from the ending of adrenergic nerves (Randall and Perry, 1992). Because catecholamines, predominantly epinephrine in teleostean fishes are stored in chromaffin cells, their release is rapid and the circulating levels of these hormones increase immediately with stress (Mazeaud *et al.,* 1977; Randall and Perry, 1992; Reid *et al.*, 1996). The release of catecholamines is extremely rapid compared to the release of cortisol (Pickering, 1992).

Corticotropin-releasing hormone (CRH) or factor (CRF), released from hypothalamus of brain, which stimulates corticotrophic cells of anterior pituitary (adenohypophysis) to secrete adrenocorticotropic hormone (ACTH), which stimulates interrenal cells (adrenal cortex homologue) to synthesize and release corticosteroids particularly cortisol (Balm *et al.*, 1994), which is the principal corticosteroid in fish (Pickering, 1993; Schreck, 1996). ACTH is thought to regulate cortisol production in acutely stressed fish where as, MSH (Melanocyte Stimulating Hormone) appears to take over those functions in chronic stress. Both ACTH and a MSH are under the control of hypothalamic factors. ACTH is under the control of corticotrpin releasing hormone (CRH) and a MSH under the control of both

CRH and thyroxine releasing hormone (TRH) (Lamers *et al.*, 1992; Lamers *et al.*, 1994). As both the chromaffin tissue and the interrenal tissue lie in close proximity in fish, there is a possibility that a paracrine control for the stress hormone regulation exist in fish (Reid *et al.*, 1996). The release of cortisol in teleost is delayed relative to catecholamine release.

The resting and stress levels of adrenaline and cortisol concentration in the plasma of salmonids are: adrenaline < 3 and 20-70 μ moles / L and cortisol < 10 and 40- 200 μg / ml respectively (Barton, 2002). Resting and unstressed levels of circulating corticosteroids in fish are less than 30-40 μg / ml (Wedemeyer *et al.*, 1990). Characteristic cortisol elevation of fishes in response to acute stressors tends to range within about 30 to 300 μg / ml (Wedemeyer *et al.*, 1990; Barton and Iwama, 1991).

Table: The following table gives the effect on cortisol and level due to some commonly encountered stress in aquaculture practices.

Sr. No.	Stressor	Cortisol	Species	References
1.	Handling	Increases	Rainbow trout and cohosalmon, Goldfish; Walleyes; Atlantic Salmon	Wedemeyer (1972); Umminger and Gist (1973); Mazaeud *et al.*, (1977); Barton and Zitzow (1995); Vijayan *et al.*, (1997a); Davis and Schreck (1997); Carey and Mc Cormick (1998); Mazur C.F., and Iwama (1993).
2.	Transportation	Increases	Atlantic salmon, Rainbow trout	Iverson *et al.*, (1998) Barton and Peter (1982)
3.	Crowding, Confinement and high stocking density	Increases	Red Porgy, Tilapia, Gill head sea bream, Mrigal	Rotlant and Tort (1997); Vijayan *et al.*, (1997b); Montero *et al.*, 1999); Tejpal *et al.*,(2008).
4.	NH_3 and poor water quality	Increases	Channel catfish	Tommasso *et al.* (1981)
5.	Disease infection	Increases	Rainbow trout	Ruane *et al.* (2000)

Secondary Stress Responses

Secondary stress responses are adaptative mechanisms and are important to recover from stress by maintaining oxygen supply to the tissues, regaining osmotic and ionic equilibrium and meeting the increased energy demands.

During the stress response, cortisol enters liver cells where it binds to nuclear receptor, resulting in activation of genes that produce a series of enzymes that have a range of metabolic effects. This results in a suite of biochemical and physiological changes which

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During the stress response, cortisol enters liver cells where it binds to nuclear receptor, resulting in activation of genes that produce a series of enzymes that have a range of metabolic effects. This results in a suite of biochemical and physiological changes which may include hyperglycemia, hyperlacticaemia, and depletion of tissue glycogen reserves, lipolysis and inhibition of protein synthesis. Other changes may include the osmotic and ionic disturbances due to diuresis and loss of electrolyte from the blood, change in haematology such as erythrocytic changes and reduction of white blood cells (leucopenia).

In nut-sell secondary response to stress includes:

- Change in osmoregulatory and ionic balance
- Metabolic changes
- Immuno-heamatological changes
- Cellular response

Osmoregulation and Ionic Balance

The fish blood being less concentrated than the surrounding environment in seawater results in exosmosis which is compensated by drinking water. In freshwater, the opposite mechanism takes place as the water enters the body from external medium and is passed out as urine. Catecholamines cause rapid changes in vascular and respiratory system, making the gills permeable resulting in the net loss of Na^+ and K^+ ions in freshwater and gain in seawater. On the contrary cortisol plays an osmoregulatory role by maintaining the levels of Na^+ /K^+ ATPase (Pickering, 1992 and Eddy, 1981).

Metabolic Changes

Catecholamine and cortisol induce glycogenolysis and gluconeogenesis respectively. Both processes together cause a rise in blood glucose level. Blood glucose and hepatic glycogen are therefore commonly measured parameters of stress response (Manush *et al.,* 2005). Glycogenolysis starts before gluconeogenesis due to the early secretion of catecholamines (Vijayan *et al.,* 1997). The use of carbohydrate as energy substrate is only for short-term responses to acute stress and / or as a last resort as the fish has limited ability to utilize carbohydrate source. It lacks proper transporters for glucose and lactate from blood to tissue. The enzyme hexokinase needed for the phosphorylation of glucose is also less active in fish as compared to mammals. However stress increases the importance of carbohydrate metabolism in the whole animal budget and blood glucose and lactate level generally rises during stress.

Besides lactate, amino acids are the preferred substrate for gluconeogenesis in fish. Fish utilize protein and lipid sources rather than carbohydrate for energy fulfilment (Demeal, 1978). Proteins represent a very important source of energy in fishes (Belinski, 1974) and teleost have developed capacity for converting amino acid to glucose (Bever and Dunn, 1981). Amino acid is converted to glucose by gluconeogenesis or is used for energy production through conversion to TCA (Tricarboxylic Acid) cycle intermediates by transaminases i.e.

alanine amino transferase (ALT) and aspartate amino transferase (AST) by transamination (Suarez. and Mommosen T.P, 1987; Moon and Foster 1995). The utilization of protein during stress depends on the type of stressors. During acute stress such as hypoxia or exhausting exercise carbohydrate sources are utilized and protein is not used, as the flux through TCA cycle is diminished (Thillart and Raaij, 1995; Moyes and West, 1995). During chronic stress, the amino acid is utilized as energy source.

In addition to amino acids, cortisol mobilizes lipids for energy purposes in teleosts (Dave *et al.*, 1979; Lidman *et al.,* 1979; Sheridan, 1986; Vijayan *et al.*, 1991). Most of the longer chain unsaturated fatty acids are utilized for membrane phospholipids and eicasonoid compound synthesis where as the shorter chain fatty acid is used primarily for energy metabolism (Weber and Zwingelstein, 1995).

All the aerobic organisms have a well developed antioxidant system which includes low molecular mass free radical scavengers such as glutathione and other thiols, ascorbate, vitamin E, and enzymes such as superoxide dismutase (SOD), catalase (CAT) and array of glutathione- dependent enzymes (Benzie, 2000). Superoxide dismutase (SOD), a cytosolic enzyme that is specific for scavenging superoxide radical, is involved in protective mechanisms within tissue injury following oxidative process and phagocytosis. Catalase (CAT) is another major primary antioxidant defense component that works primarily to catalyze the decomposition of H_2O_2 to H_2O, sharing this function with glutathione peroxidase (GPX). Therefore, both these enzymes detoxify H_2O_2 derived from SOD activity. In the presence of low H_2O_2 levels, organic peroxides are the preferred substrate for GPX. However at high H_2O_2 concentrations, they are metabolized by CAT (Yu, 1994).

Hematological Changes

Stress hormone increases the oxygen carrying capacity of RBC (Randall and Perry, 1992), and induces the release of RBC from the spleen into general circulation, causes the swelling of RBC and increases the affinity of haemoglobin for oxygen (Primmett *et al.*, 1986, Tiihonen *et al.,* 1995). The number of erythrocytes, haemoglobin concentration and hematocrit were increased after the stress (Vosyyliene *et al.,* 1996, Svobodova *et al.*, 1995, Mc Kim *et al.,* 1970), however these value decreases after starvation (Kawatsu, 1996). This increase may result from the release of immature red cells by the spleen and could be an immediate response to the acute stress mediated by catecholamines (Tort *et al.*, 2002,). Langston *et al.,* (2002) and Burrows (1987) reported that during stress condition contraction of spleen increases, which causes an increase in volume of circulating blood and thereby releasing large numbers of thrombocytes into the circulation.

Immunological Changes

Various kinds of environmental changes that are stressful lower the resistance of the animals to infection and disease. It is seen that a variety of bacterial, fungal and parasitic diseases become a problem if animals are held at unfavourable conditions that is under stress (Wedmeyer *et al.,* 1999). Stressor- induced immunomodulation has mainly been attributed to cortisol (Ellis, 1981; Barton., 1991), which is produced by the cells in the interregnal tissue and enhanced during acute and chronic stress.

Cortisol has an immunoregulatory function rather than as immunosuppressive role. Cortisol acts as a regulator of the immune system, stimulating non-specific defences and suppressing the specific immune response (Weyts *et al*., 1998). The general effect of cortisol on the immune system is the reduction of lymphocytes (lymphocytopenia or lymphopenia) and increase in the neutrophils (neutrophilia) (Ellasasser and Clem, 1987; Sunyer *et al*., 1995), but number of granulocytes after stress often remains constant or may rise (Ellsaesser *et al.,* 1987; Ainsworth., 1991; Morgan *et al.,* 1993., Narnaware *et al.,* 1996. Ellis (1981) and Pickering *et al*., 1987 suggested that stress induces reduction in disease resistance is due to suppression of number and function of circulating lymphocytes. The fraction of B-lymphocytes in circulation decreased after exposure to single or multiple temperature shock (Mare Engelsma *et al*., 2003). In vitro studies already revealed that cortisol induces a decrease in both relative and absolute number of B-lymphocytes in PBL (Peripheral Blood Leucocytes) (Weyts *et al.,* 1998a). Similar observation is also made for other stressors for example, transport stress in channel catfish *Ictalurus punctatus* (Ellsaesser *et al*., 1987), confinement of brown trout *Salmo trutta* (Morgan *et al*.,1993) resulted in diminution of circulating B-lymphocytes and 30-60 sec. air-exposure of juvenile Coho salmon (*Oncorhynchus kisutch)* reduced circulating leucocytes (Maule *et al*., 1990). This again confirms lymphopenia to be one of the classical stress-induced phenomenons that occur during stressful conditions of different nature or intensity. The immunosuppressive action of those steroids (cortisol, sex steroid) are receptor mediated process (Maule *et al.,* 1990) that may either result in cell death (Slater *et al*., 1995) or effect cell signaling function (Tripp *et al.,* 1987).

Cellular Responses to Stress

A wide variety of stressful stimuli including environmental (UV radiation, heat shock, heavy metals, and amino acids), pathological (viral, bacterial, parasitic infections or fever, inflammation, malignancy, or autoimmunity) or physiological stimuli (growth factors, cell differentiation, hormonal stimulation, or tissue development induce a marked increase in intracellular stress response characterized by a family of proteins referred to as heat shock proteins (HSP). Exposure of cells or whole organisms to heat shock results in a reversible increase in the synthesis of some acute phase proteins against subsequent shock known as HSP (Iwama *et al*., 1998; 1999; Currie *et al*., 2000; Palmisano *et al*., 2000 Ming *et al*., 2003) which play an important role in maintaining homeostasis. They were first discovered in the chromosomal puffs of drosophila salivary glands after thermal shock (Ritossa, 1962).

In the normal unstressed cells heat shock proteins are essential for folding and translocation of newly formed proteins and renaturation of denatured proteins. The expression of these proteins increases manifolds in the cells during various forms of stress where there is an accumulation of malfolded proteins (Gething and Sambrook, 1992). HSP has an ability to mediate misfolded or denatured functional proteins caused by various stressors in the cell (Iwama *et al.*, 1999). This protein is also known as molecular chaperone (Elliss, 1999) and plays a role in novel protein synthesis. HSP-70 plays an important role in protein biogenesis under normal cellular conditions, which is significant during the growth of an organism. Thus enhanced protein synthesis requires increased amount of HSP (Pal and Mukherjee, 2003).

TERTIARY STRESS RESPONSES

Chronic exposure to stressors provokes tertiary stress responses that result in a number of pathological changes and reduction in reproductive success, depression of growth rate and decreased disease resistance. Tertiary stress response represents whole animal and population level changes (Barton, 1997) associated with stress.

i. Whole animal:

   a. Impaired growth, Parr-smolt transformation (smelting), spawning success and migration behaviour and spawning.

   b. Increased disease incidence (infectious and noninfectious)

ii. Population parameters:

   a. Reduced intrinsic growth rate, recruitment, compensatory reserve and productivity.

   b. Altered community species abundance and diversity.

Thus, when fish are exposed to environmental stressor, a hierarchy of responses is initiated and if the stress is severe or long lasting, successively higher levels of biological organisation gets affected. This signifies that the primary responses are the changes at the endocrine level; where as the tertiary responses refer to those changes that can be easily seen by observing the animal (Jobling, 1995). A fish's or fish population's tolerance to environmental alterations thus depends at least in part, upon the individual fish's ability to regulate stabilizing processes so as to accomplish the required physiological or behavioral adaptation (Elliott, 1981).

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