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Concept of Stress and Its Mitigation in Aquaculture

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Introduction

Fishes are cold-blooded animals (ectothermic/poikilothermic) that cannot regulate their body temperature; hence, their physiological mechanisms are directly or indirectly temperature dependent. Some degree of thermoregulation is present in some fishes like tunas and *Lamnidae* 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 Neil 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 Mcleay 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: the first one is non-chemical (biological

method) and the other one is the chemical method. The non-chemical (biological) method includes the entire environment management which includes water quality management such as temperature, dissolved oxygen, ammonia, nitrogen, nitrite and salinity and stocking density, uniform-size stocking, stocking ratio in polyculture, etc. The chemical method includes dietary supplementation of vitamin C, vitamin E, tryptophan, immunostimulants, etc.

Concept of Stress and Stressors

Stress

Stress was defined by 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.

Stressors

The factor that causes stress is called stressors. Rearing of aquatic organisms in a man-made environment has resulted in exposure to a number of stressors, which may not be experienced to the same degree in natural environments. Stressors in aquaculture systems may be categorised as shown in Table 1 (Wedemeyer et al. 1999).

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Table 1 Type of stressors 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 nonpathogenic), macro-organisms (parasites), lateral swimming space requirements
4.	Procedural	Handling, hauling, stocking, disease treatment, feeding methods (manual and automated)

Stress Responses

Fishes are ectothermic organisms and therefore are unable to control their body temperature within narrow temperature limits (Hazel 1993). As a result, their physiology is dependent on ambient temperature. The environmental temperature determines the metabolic rate, physiology and growth of fishes (Morgan et al. 1999). Water temperature influences O₂ concentration, metabolism and growth of fishes (Langston et al. 2002).

Stressor is the causative factor and stress is a response. The stress response of fishes follows the general vertebrate pattern. A key element in the stress response is a switch from anabolism to catabolism. The quantum of response may vary with the nature of stress and its duration, and it also depends on factors like age, sex, maturation stage, species and strain of the fish.

When fishes and other aquatic organisms experience environmental disturbances that lie outside the normal range, the effect may be dramatic. At the organism level, a series of physiological changes occur following stressful challenges, which are adaptive in nature. These physiological responses are termed as “general adaptation syndrome” (GAS), Seyle 1950. It consists of:

1. An *alarm reaction* in which “stress hormones” (catecholamine and corticosteroids) 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 severe or long lasting

Stress response has been classified into primary (neural and neuroendocrine responses), secondary (physiological consequence of such primary response) and tertiary response (changes

in behaviour, growth rate, increased susceptibility to diseases and change in population) (Wedemeyer and Mcleay (1981)). Primary stress response results in the activation of the neuroendocrine system, which brings about changes in metabolism, osmoregulation and haematology (Barton 2002). The primary stress response is the physiological alarm, during which there is an increase in the levels of “stress hormones”, i.e. corticosteroids and catecholamines. These primary responses result in a suite of biochemical and physiological changes leading to secondary stress response, characterised by elevated glucose levels, haemato-immunological changes and change in other metabolic enzymes. Secondary stress responses are believed to be adaptive mechanisms and are particularly important for fishes to recover from stress. However, during chronic conditions the fish loses its capacity to adapt. This results in pathological changes, reduction in reproductive success, decrease in growth rate and decreased disease resistance that leads to death of the organism thereby causing change in the population and biodiversity of that species, which is termed as tertiary stress response.

Primary Stress Responses

1. Release of adrenocorticotrophic hormones (ACTH) from the adenohypophysis
2. Release of “stress hormones” (catecholamines, i.e. adrenalin, noradrenalin and dopamine and corticosteroids especially cortisol) from the head kidney

Sensory perception of stress is a prerequisite for stress response in animals. In fishes, an adverse condition stimulates the afferent neural pathway that runs in the sympathetic nervous system from the hypothalamus to the chromaffin

tissue of the head kidney and stimulates the chromaffin tissue, which 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. 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. The release of catecholamines is extremely rapid compared to the release of cortisol.

Corticotrophin-releasing hormone (CRH) or factor (CRF) is released from the hypothalamus of the brain, which stimulates corticotrophic cells of anterior pituitary (adenohypophysis) to secrete adrenocorticotrophic hormone (ACTH), which stimulates interrenal cells (adrenal cortex homologue) to synthesise and release corticosteroids particularly cortisol which is the principal corticosteroid in fish. The release of cortisol in teleost is delayed relative to catecholamine release. Resting and unstressed levels of circulating corticosteroids in fishes are less than 30–40 ng/ml (Wedemeyer et al. 1990). Characteristic cortisol elevation of fishes in response to acute stressors tends to range within about 30–300 ng/ml (Wedemeyer et al. 1990; Barton and Iwama 1991). Elevation of plasma catecholamines and cortisol due to primary stress leads to secondary stress responses.

Secondary Stress Responses

In fishes, cortisol enters the 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 hyperglycaemia, hyperlacticaemia, 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 and change in haematology (reduction of white blood cells, leucopenia) (Barton and Iwama 1991). Stress is an energy demanding process and the animal mobilises energy substrate to cope with stress

metabolically. The production of glucose under stress assists animals to cope with the increased energy demand. The stress hormones adrenalin and cortisol have been shown to increase plasma glucose production in fishes by both gluconeogenesis and glycogenolysis.

Catecholamines, in particular, have marked influence on cardiovascular functions leading to change of blood circulation, gill perfusion and oxygen carrying capacity of blood. Corticosteroids on the other hand are known to stimulate the ion-transport mechanism in the gill and kidney.

These secondary stress responses are believed to be adaptive mechanisms and are particularly important for fishes to recover from stress by maintaining oxygen supply to the tissues, to regain osmotic and ionic equilibrium and to meet the increased energy demands imposed by exposure to environmental stressor. Typically these changes persists only for few hours or days following acute exposure to the stressor and therefore do not result in any deleterious effect on the animal.

Intracellular stress response is characterised by the production of a family of proteins known 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 (Palmisano et al. 2000; Ming et al. 2003), which play an important role in homeostasis. HSPs are a family of highly conserved cellular proteins that have been observed in all organisms (Feder and Hofmann 1999) including fishes (Iwama et al. 1998). 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 stress. HSP has an ability to mediate misfolded or denatured functional proteins caused by various stressors in the cell; hence, this protein is also known as molecular chaperone.

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 associated with stress.

1. Whole animal:

- (a) Impaired growth, parr-smolt transformation (smelting), spawning success and migration behaviour and spawning
- (b) Increased disease incidence (infectious and noninfectious)

2. Population parameters:

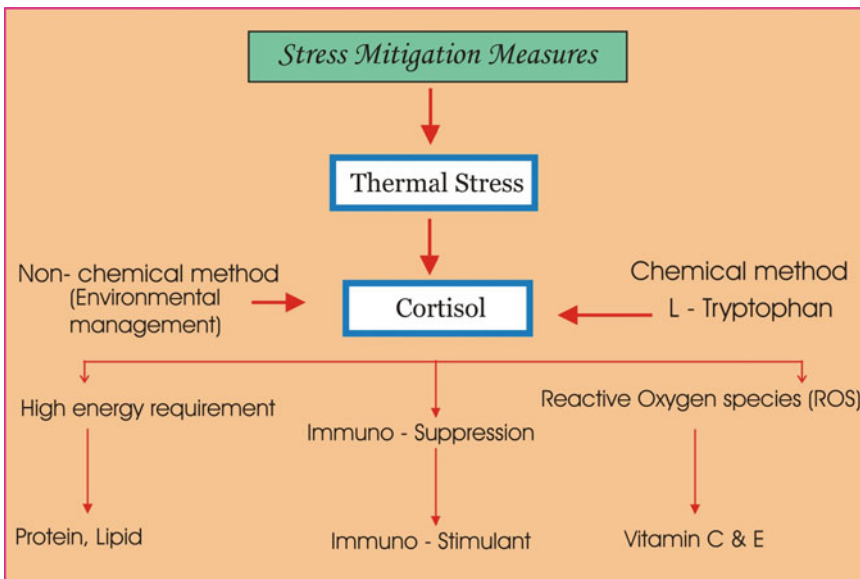
- (a) Reduced intrinsic growth rate, recruitment, compensatory reserve and productivity
- (b) Altered community species abundance and diversity

Thus, when fishes 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 get

affected. This signifies that the primary responses are the changes at the endocrine level, whereas the tertiary responses refer to those changes that can be easily seen by observing the animal.

Stress Mitigation Methods

One of the most promising areas of research is the development of strategies to reduce the stress during various aquaculture practices. There are two approaches to mitigate stress, the first one is non-chemical (biological method) and the other is chemical method. The non-chemical (biological) method includes the entire environmental management, which includes water quality management such as temperature, dissolved oxygen, ammonia, nitrogen, nitrite, salinity, etc., and optimum stocking density, uniform-size stocking, stocking ratio in polyculture, etc. The chemical method includes dietary supplementation of vitamin C, vitamin E and tryptophan and immunostimulants, etc.



Flow diagram: stress mitigation measures

Table 2 Showing some of the important water quality parameters and their optimum range for freshwater fish culture

Sl. no.	Water quality parameters	Optimum range
1	Dissolved oxygen	>5 ppm
2	Temperature	28–31 °C
3	Turbidity	20–60 cm
4	Total hardness	40–100 ppm
5	Total alkalinity	60–300 ppm
6	Ph	7.0–7.5
7.	NH ₃ –N	<0.05

Non-chemical Methods (Environmental Management)

Water quality is defined as the suitability of water for the survival and growth of fishes, whereas water quality management is a technique to bring the water quality in desirable level for the growth and survival of fishes. Environment management in aquaculture means water quality management such as dissolved oxygen (DO), water temperature, alkalinity, water Ph, turbidity, hardness, etc. (Table 2).

Chemical Methods

Dietary Supplementation of Protein, Vitamin C and Vitamin E

Oxidative stress is caused by the production of reactive oxygen species and nitrogen species during stress (Gordon 2001). Oxygen radicals is generated due to respiratory burst activity of phagocytes, present in cells under normal conditions but its production increases during pathophysiological conditions and stress. The use of immunostimulants and antioxidants to ameliorate the damage to immune system by stress has been studied by many workers. Fishes fed with immunostimulant glucan prior to transportation have increased specific and nonspecific immune response as is evident by higher number of lymphocytes and enhanced phagocytosis (Jeney et al. 1997).

It is reported that supplementation of high protein and vitamin C reduced the bioaccumulation and stress responses in *Channa*

punctatus (Sarma 2004). Dietary high protein and vitamin C were supplemented for ameliorating stress (Manush et al. 2005) in *Macrobrachium rosenbergii*. Vitamin C is considered to play an important role in animal health as antioxidants inactivate damage of free radicals produced during normal cellular activity from various stressors that have been reported confirming the protective role of vitamin C. Vitamin C can interact with other antioxidants such as carotenoids and vitamin E. A high concentration of vitamin C at the cell membrane regenerates the reduced vitamin E created during oxidation and reduction process. Vitamin C is highly interactive and may fortify antioxidant defences and enhance immune response indirectly by maintaining optimal vitamin E levels. Vitamin C and vitamin E act as membranes protecting agents and give stability to lipid bilayer.

Dietary Supplementation of L-Tryptophan

Tryptophan is one of the eight essential amino acids, necessary for the development of the vitamin niacin/nicotinic acid and serotonin. It cannot be synthesised in the body and thus must be obtained from food or supplements. L-tryptophan acts as cortisol blocker hence reduces stress-induced production of plasma cortisol.

Dietary Supplementation of Lactoferrin

Lactoferrin (LF) is a family of iron-binding glycoproteins having molecular weight of 80 KDa that originated from some secretion of mammals. It is a kind of immunostimulant and has a lot of biological functions, e.g. iron absorption and transportation, bacteriostatic effects and enhancement of mucosal immunity system in mammals. Orally administration of LF enhances nonspecific defence system and phagocytic activity in rainbow trout and decreases plasma cortisol level in goldfish by LF administration. Dietary LF also enhances tolerance to physiological stressors such as air-exposed stress in juvenile Japanese flounder against high stocking density stress in rainbow trout and common carp and low salinity stress in shrimp (Koshio et al. 2000).

Conclusion

During aquaculture practices animals come across many different types of stressors such as physical, chemical and biological. To cope up with these stressors physico-biochemical process of biomolecules, cells, organelles and organisms vary from species to species. Exposure of fishes to extreme environmental conditions elicits a cascade of physiological and biochemical changes characterised as primary, secondary and tertiary stress responses. Stress can be mitigated by biological and chemical methods.

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