Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

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Course Manual

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ROLE OF STRESS IN DISEASE INDUCTION AND METHODS TO MONITOR STRESS ON AQUATIC ANIMALS

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A. Role of stress in disease induction

A fish's environment is a complex system with varying water quality, temperature, light intensity, current velocity, aquatic vegetation, food availability and social interactions. The response of fishes to these changes assumes greater importance with large scale culture of fish. In case of cultured species, man's interference is a major factor. Fishes are extensively and intensively reared in captivity, often submitted to confinement, handling, transportation and drug treatment. A valuable contribution from the field of biological sciences would be the accumulation of information on the physiological response of fish to these environmental changes.

The two basic concepts of response mechanism are:

i) The Emergency Reaction (ER) described by Cannon (1935) related to activation of sympathetic nervous system and adrenal medulla. The hormonal factors (catecholamines -- adrenaline and noradrenaline) that enables the subject to mobilize its resources quickly for metabolic requirements of flight or fight.

ii) The General Adaptation Syndrome (GAS) described by Selye (1946) characterized by release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which in turn activates the release of corticosteroids, amplify and extends the metabolic effects of catecholamines. The GAS occurs when there is a gradual or prolonged exposure of some of the extremes of physiological or environmental stresses.

Definitions:

1. Stress has been defined as the response of an organism to any demand placed on it such that it causes an extension of a physiological state beyond its normal resting state to the point that chances of survival may be reduced (Selye, 1946).

2. The sum of all the physiological responses by which an animal tries to maintain or reestablish a normal metabolism in the face of physical/chemical force (Selye,1950).

3. Stress is a state produced by any environmental or other factors which extend the adaptive response of an animal beyond the normal range or which disturbs the normal functioning to such an extent that in either case, the chances of survival are significantly reduced (Brett,1958).

The term stressor (stress factor) means the stimulus that inflicts stress on fish and stress refers to the altered state of the fish. The stress response is the physiological or behavioral manifestations that can be measured to indicate the degree of stress. The response to stress is an adaptive mechanism that provides the fish a means to cope up with the stressor in order to maintain a normal homeostatic state. If the stressor is overly severe or long lasting, the continued response can become detrimental to fish's health and

well being or become maladaptive. A stage of exhaustion follows if the organism is incapable of coping up with the disturbance. This stage is associated with the development of pathological conditions or mortality in fish.

Classification of stress

The stress can be classified into acute and chronic type. The acute type are typically physical disturbances such as those caused by handling, transport etc. Chronic stress is due to poor water quality, over crowding, malnutrition etc.

Stress also categorized based on the severity of response as lethal and sub-lethal. Lethal responses usually occur immediately due to spills, exposure to toxicants and cause direct mortality. The sub-lethal is more common than lethal, results from either continuous/periodic exposure to low levels of stressors over a period of time and produce long term effects on production cycle of the fish.

From the source of stress, it can be classified due to (1) Environmental (abiotic) (2) Biological (biotic) and (3) Physical factors. Principal environmental factors are temperature, salinity, pH, dissolved oxygen, suspended particles, pollutants etc. Abundance, aquatic vegetation, food availability, all can contribute to biotic stress. Among the physical factors netting, handling and transportation can be considered stressful.

Stress response

Stress affects the fish at all levels of the organizations from molecular/biochemical level of the individual, to population/the community level. The responses to stress at different levels of organizations are not only inter related functionally but often inter regulated. The organism's response to stress is as much behavioral as physical. How fish behave when challenged by stress provides an outward whole animal manifestation of the complex neurological/physical changes that occur, thus allowing interpretation at an ecological level. Response to stress has been grouped as (a) Primary (b) Secondary and (c) Tertiary responses depending on the sequence in which appears.

When a fish is exposed to stressor the response is initiated by the real or perceived threat by the central nervous system. The hypothalamic-pituitary-interrenal (HPI) axis respond with release of corticotropin releasing hormone (CRH) or factor (CRF) chiefly from the hypothalamus of the brain which in turn stimulates the corticotrophic cells of anterior pituitary or adenohypophysis to secrete adrenocorticotrophic hormone (ACTH). The circulating ACTH further stimulates the interrenal tissue (adrenal cortex homologue) located in the kidney to synthesize and release corticosteroids mainly cortisol into circulation and reach the target cells. Control of cortisol release is through negative feed back of the HPI axis.

Concurrent with the elevation of circulating corticosteroids during stress is the release of catecholamines mainly epinephrine (adrenaline) and norepinephrine (noradrenaline) following sympathetic stimulation of chromaffin tissue (adrenal medulla analogue) of kidney. Other hormones including thyroxine, somatotropin, gonadotropin and reproductive steroids in circulation and serotonin and its derivatives in the brain can become either elevated/suppressed during stress.

As a sequel to the primary response (release of corticosteroids and catecholamine, metabolic (blood glucose, lactate, liver and muscle glycogen) hydro mineral balance (blood ions and molality) and those related to hematology (circulating erythrocytes leucocytes and hematocrit) get altered. Moreover, corticosteroids and catecholamines can directly or indirectly affect all aspects of fish's physiology. The tertiary response refers to aspects of whole animal performance such as growth, reproduction and survival, which are of particular concern to aquaculturists.

Stress and diseases

It has long been recognized that stress and resistance to disease are related but the mechanism of this relationship are extremely complex and little understood. The primary effects of stress occur in the endocrine system which in turn, mediates the secondary effects characterized by metabolic changes in other organs/systems as the animal attempts to maintain homeostasis. One of the major adaptive responses to aggressors is in the immune system. This refers to a variety of tissue reactions ranging from repair of damaged tissues to phagocytosis, inflammation and the multitude of specific and nonspecific responses. It is now recognized that stress can cause derangement of the defense systems which may disturb the balance of other physiological mechanisms and ultimately result in disease.

Many fish carry pathogens under normal conditions and are held in check by the two defensive mechanisms: 1. Nonspecific protection and 2. Specific protection. The line of nonspecific defense are by means of physical guards/barriers such as scales, skin, mucous, lysozymes etc. that impede the entry of pathogens into the system. Once the barriers are broken, the resulting inflammation attracts phagocytic cells, neutrophils and other leukocytes that destroy the invader. The serum and/or mucous of fish contains complement, interferon, lectin etc and some species, substances such as hemolysin, proteinase, chitinase and caeruloplasmin. There is little information concerning these substances, but they may also be involved in nonspecific defense mechanisms of fish against pathogens.

In contrast, specific protection is accorded by specific antibodies against specific antigens. Antigenic exposure may stimulate a humoral or cell mediated response involving production of different classes of immunoglobulins. The physiological stress response and the immune system are under genetic influence. In addition, the fish's past and present rearing environment affect both physiological response to stress and ability to resist pathogens.

Stress in culture system

Some of the common stressors responsible for breaking down of immunity in culture system are:

1. Poor water quality
2. Fluctuations in water temperature
3. Salinity changes
4. Oxygen super saturation in algal bloom
5. Improper medication
6. Overstocking, stocking of incompatible species, netting, handling, over crowding, transport etc.
7. Improper feed and feeding practices

Management practices directed at limiting stress within tolerable limits in culture systems will be the most effective way to prevent outbreak of diseases.

B. Methods to monitor stress

Monitoring and ameliorating stress forms the basis of culture system and fishery environment management. The monitoring of stress can be approached in two ways. The first is by quantifying the immediate physiological responses of an animal to stress and the second through measurement of long term changes in the performance capacity of an animal.

1. Biological indicators

Stress in fish can be monitored by measurement of biological changes (bioindicators) in body fluids, cells, tissues or in other biotic variables that reflects the presence and magnitude of stress. The basic concept of bio-indicators approach is that the effects of stress are manifested at organelle level of biological organization before disturbances are exhibited at individual/population or ecosystem level. No single indicator or even group of indicators can provide all initial information needed to assess long term stress in aquatic animals. The ideal assessment design involves measurement of selected indicators for each major level of organization so that casual linkages between levels can be determined. Once such relationships are established, prediction of long term effects from measurements of the more short term indicators can be made with increased reliability and certainty. Two principal areas of application of bioindicators are in (i) Environmental Regulations (ii) Fishery Management.

(a) Hormonal indicators

Fish respond to stress with characteristic acute increase in plasma levels of the catacholamines (adrenaline and nonadrenaline) and slower but more sustained increases in corticosteroid (cortiso). Increases in catecholamine and corticosteroid levels generally mirrored by increase in plasma levels of glucose generated by glucose mobilizing effects of both classes of hormone. Also, plasma lactate level increase typically in stressed fish, particularly if any aspect of the stressor results increased activity or decrease in oxygen availability. Accurate assessment of the stress response is strongly dependent on establishment of resting or basal levels of physiological parameters used to define stress response. For domestic stock, the effectiveness depends mainly on the quality of husbandry.

(b) Specific biochemical indicators

Some biochemical constituents are affected by certain types of stressors and hence specific biochemical indicators to specific stressors have been proposed. Metallothioneins (cytosolic proteins, heavy metal binding proteins) are synthesized in hepatic, renal and gill tissues when fishes are exposed to heavy metal contaminated environment. Metallothioneins (MT) sequesters most of the free metal ions within the cell and thereby preventing them from binding to the hydryl group of other functionally important proteins and exerting their toxic effects. MT levels 4 - 6 times higher in livers of rainbow trout captured in metal contaminated lake than in livers of fish collected from a relatively clean site have been observed.

Lipid peroxidation, a chemical process resulting in the oxidative deterioration of polyunsaturated lipids in biological membranes, is believed to be one of the primary mechanism of cell injury of xenobiotics (CCl_4, DDT), results in disruption of cell membranes and loss of activity of membrane bound enzymes. The production of melondialdehyde (a breakdown product of lipid endoperoxides, by liver preparation *in vitro* has frequently been measured by colorimetric methods as index of peroxidation.

(c) Heat shock proteins (HSP)

All organisms studied including teleosts response to elevated temperature by synthesizing a group of highly reserved proteins called heat shock proteins. Induction of HSP synthesis is rapid and this maximal 5-10°C above an organism's normal environmental temperature, whereas syntheses of other proteins are suppressed. Synthesis of HSP increases thermo tolerance to subsequent heat exposure. HSP are thought to protect the cells against the pathological effects of heat and other stresses.

(d)Enzymatic indicators

Enzymatic levels are also used as an important indicator of the magnitude of stress. High levels of hepatic monooxygenase activity in fish liver have been reported in areas affected by oil spills compared to normal sites. Monooxygenase activity can be induced in the kidney, gut and gill of fish by certain xenobiotic compounds. Monooxygenase activity could be used as an indicator of pollution.

(e) Immune indicator assays

Immune indicator Assays can be divided into three broad categories.

1. Non specific assays
2. Assays with or without antigenic stimulation
3. Specific indicators of immune response

Non specific indicators of stress give information on gross condition of the defense system. Abnormal hematocrit (volume of RBC per unit of whole blood), gives an indication of stress. A slight reduction in hematocrit value was detected in fish undergoing transportation stress. Similarly abnormal leukocrit (volume of WBC per unit of whole blood) may give a better indication of the status of the immune system and resistance of disease than hematocrit. Variability in leucocrits in fish may be associated with environmental factors such as temperature, seasonality and management factors such as feeding, crowding and holding.

The phagocytic index will be elevated in wild fish undergoing stress or in fish exposed to an antigen. Increased phagocytic index indicates heightened leukocrits for combating stress.

Generally, exposure of the fish to various immunogenic preparations causes a specific action/ effects in the form of elevated cellular responses and final production of antibodies. Various indicators are used to monitor each response of immune system from initial activation to final antibody production. Stress may act on any of these pathways.

2. Histological indicators

No other technique of stress assessment allows many potential sites of injury to be studied so rapidly. Major organs of fish in which response to stress seen include skin, liver, gill, kidney and skeleton. Skin interface between internal tissues and external

environmental stressors, is most characteristic organ for production and secretion of mucus. Physical trauma, exposure to toxic metals or acidic pH will lead to enhanced mucus production. The liver is important in nutrition and toxicology. Hepatocytes in fish liver are arranged as tubules. Alterations of arrangement of hepatocytes can be used as an indicator of stress. Gill perform a variety of critical physiological functions including gas exchange, ion regulation etc. Toxins, pollutants etc. have been shown to cause sufficient disruption of ion regulation to account for death. Ion loss leads to disruption of Donnan equilibrium which in turn leads to hemoconcentration and eventual cardiac failure.

3. Condition related indices

The decline in condition is associated with depletion of energy reserves such as stored liver/muscle glycogen and body fat. This happens due to higher rearing density, acidification or other adverse conditions. A change in feeding pattern or/and increase in metabolic rate may result in decline of condition. Other non-traditional condition indicators are either morphological or physiological, include increase in eye diameter, increased incidence of ,fin and skin damages, skeletal abnormalities tumor growths, infections, parasites and lesions.

4. Organ related indices

The ratio of organ weight to body weight has been used in various stress related studies. The most frequently used of these is the liver somatic index (LSI) or hepatosomatic index (HSI). The HSI drops dramatically during fasting. Other organ weight ratios used in stress 'investigations' include the viscerosomatic index (VSI), the gonadosomatic index (GSI).

5. Growth indicators

Growth is one of the ultimate indicators of health and condition because it integrates all the biotic and abiotic variables acting on the organism and reflects secondary effects of chronic stress. The simplest way to assess growth is to measure absolute weight gains over known periods of time and specific growth rates. Uptake of radioactively labeled aminoacids or ratio of RNA to DNA in muscle also indicates growth.

6. Reproductive indicators

Successful reproduction by teleosts requires several environmental variables within critical ranges. The environmental factors include temperature, photoperiod, salinity, rainfall, turbidity, dissolved oxygen etc. A severe, acute stressor can cause reproductive failure. A moderate chronic stressor, on the other hand may cause reproductive impairment, which leads to gradual reduction to elimination of fish populations.

7. Community/Population/Ecosystem indicators

Relative health of a fish community/population/ecosystem is a sensitive indicator of direct and indirect stresses in the aquatic ecosystem.

Keeping fish healthy in the wild environment and in aquaculture situations should be one of the major goals of our society.

Suggested reading

1. Stress and Fish edited by A.D. Pickering. Academic Press

2. Fish Respiration edited by Steve F. Perry and Bruce Tufts. Academic Press

3. The Fish Immune System edited by George Iwama and Teryuku Nakanishi Academic Press

4. Fish Diseases and Disorders Volume 2. edited by J.F. Leatherland and P.T.K. Woo CABI Publishing

5. Biological Indicators of Stress in Fish edited by S. Marshall Adams American Fisheries Symposium.