STRESS IN AQUACULTURE

M. M. Ghughuskar,
Ph.D.Scholar,
Aquaculture Division,
ICAR-Central Institute of Fisheries Education,
Panch Marg,Off Yari Road, Versova, Andheri (W), Mumbai – 400 061.

Abstract

Stress is an important aspect in aquaculture which has many repercussions on growth and disease out breaks .This stress management in aquaculture venture is key to harvest sustainable production. In aquaculture practices fishes are confronted with environmental factors like chemical, biological, physical and procedural which causes stress. This review is an attempt to elucidate the stress and its effect on physiology, biochemical and morphological of aquaculture candidate species.

Introduction

Stress is an important aspect in successful aquaculture venture is now becoming common term among aqua culturist. Culture practices like handling , sorting, grading, transport band poor water quality impose stress on fishes which results in poor growth, increase in incidence of diseases and mortality which leads to loss to aqua culturist. Environmental changes are stressful and lower the resistance of both hatchery and husbandry fishes to infections and other diseases. It is widely known that variety of bacterial, parasitic and other diseases become havoc only when fish are being held under unfavourable environment conditions for that concerned are species or strain. Unfavourable environment conditions include crowding, temperature fluctuation , inadequate dissolved oxygen ,excessive or rough handling,sub- lethal level of toxic material *etc.* Fish disease do not occur as a single caused event but are the end result of etiologic agent ,the fish and environment. Example: Facultative bacterial fish pathogen such as *Aeromonas ,Pseudomonas and myxobacterial spp.* are present in hatchery water supplies, epizootic will usually not occur unless environmental quality and host defence system of fish also got deteriorated.

Fish in culture system continuously get affected by environmental fluctuation and management practices such as handling, crowding, hauling, drug treatment and (particularly during stocking) unfavourable or fluctuating temperature and water chemistry. All these

together lead to fright that can impose a considerable stress on limited homeostatic mechanism of fishes.

Environmental factors which can adversely impact fish in culture system and cause stress.

Sl. No.	Environmental factor causing stress	Example
1.	Chemical	Water chemistry, pollution, diet composition, nitrogenous and other metabolic wastes.
2.	Biological	Population density, other fishes, lateral swimming space requirement. Microorganisms (Pathogenic and non pathogenic). Macroorganisms (ecto and endo parasitic).
3.	Physical	Temperature, light, sound,dissolved gases.
4.	Procedural	Handling ,hauling, stocking, feeding methods (manual and automated), disease treatment.

*Note: Wedemeyer and Scheraga, 1999 (Environmental stress and Fish Diseases)

Stress requiring an adjustment exceeds a fish ability to accommodate will be lethal. Less severe stress will predispose to physiological disease or to infectious disease if fish pathogens are present (Wedemeyer,1976).

Concept of stress

The term stress is frequently used interchangeably with the stressing agent (stressor) itself. Stress is taken to mean the metabolic response of animal to stressor.

As per Selye (1950), " the sum of all physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of physical or chemical force".

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

Stress and Adaptation

A series of morphological, biochemical and physiological changes occur as a result of stress ,which in higher animals collectively constitute the general adapatation syndrome (GAS). It is usually divided into three stages (Selye,1973).

- An alarm reaction.
- A stage of resistance (that is adaptation to the stress has occurred).

- A stage of exhaustion (that is adaptation has been lost because the stress was too severe or long lasting).

Selye (1950) has given actual sequence of physiological and biochemical alterations in an animal as it attempts to maintain homeostasis in the face of stressful environmental change.

ACTH (Adrenocorticotrophic Hormone) is released from the adenohypophysis following neurotropic stimuli mediated through the hypothalamus, " stress hormone" (Cortisone, corticosterone, epinephrine), which is released from the adrenal (interregnal tissue)

- Na^+ and Cl^- retention get increased (renal tubular epithelium).
- K^+ excretion gets increased (renal tubular epithelium).
- Blood glucose level gets increased (hyperglycemia).
- Nitrogen metabolism gets increase and the animal goes into a negative nitrogen balance.
- Thyroid output gets increased.

Sypathetic nervous system over reacts.

a. Spleen contracts, additional erythrocytes enter the circulation.

b. Respiration rate and cardiac output gets increased and in turn the systolic blood pressure also.

Stress response

It is conventional to classify stress responses as per Chavin,1973; Schreck, 1976; Wedemeyer, 1976; Mazeaud *et al.*, 1977.

Primary stress response

Primary stress response is the activation of two main components of neuroendocrine system;the first to be activated in chromaffin system of the head kidney which is under the control of sympathetic nervous system. This results in the secretion of catecholamine,adrenaline noradrenaline and to lesser extent dopamine. The other neuroendocrine system is the hypothalamus pituitary interrenal axis . This system results in the secretion of cortisol,the principal corticosteroid in fish (Henderson and Garland,1980; Pickering,1993; Schreck,1996).

Effect of Catecholamine

Catecholamine promotes rapid change in the vascular and respiratory system of fish, the net result being an increase in oxygen uptake and utilization (Pickering ,1992). The

consequences of some of the respiratory and vascular adjustment is a net loss of ions in freshwater and net increase in seawater. Stressors induce elevated epinephirine and norepinephrine level enhances blood oxygen transport directly by elevating or maintaining haemoglobin oxygen affinity via increasing the number and affinity of beta adreno receptor in erythrocytes (Reddy and Leatherland,1998). Catecholamine affects carbohydrate metabolism by stimulating glycogenolysis (Mommsen *et al*,1988)

Effect of Cortisol

Cortisol is a major corticosteroid in fish which is produced by the cells of interrenal gland. It is under endocrine control of pituitary. The main mediators are adrenocorticotropic hormone (ACTH) and alpha melanocytes stimulating hormone (Alpha MSH). The ACTH is thought to regulate cortisol production in acutely stressed situation whereas MSH appear to take over this function in chronic stress. Both ACTH and alpha MSH are under control of hypothalamic factors. The ACTH is under the corticotrophin releasing hormone (CRH) and alpha MSH secretion both CRH and Thyroxine releasing hormone (TRH) (Chester Jones *et al.,* 1980; Lamers *et al.*,1992; Olivereau and Olivereau,1991). Cortisol stimulates glucogenesis ,the production of carbohydrate from non- carbohydrate sources (Janssen and Waterman,1988). The release of catecholamine is extremely rapid compared to the release of cortisol (Pickering,1992).

The level of cortisol shoots up during stressor but in some cases of chronic stress, plasma cortisol level may come down to basal level despite the presence of stressor. Work is needed to know whether cortisol levels are reduced as consequences of decrease in secretion rate or faster metabolic clearance rate (Pickering,1992).

Pottinger(1990) reported that not only the circulating cortisol level but also the number of cortisol receptor in target tissues come down. This may be the mechanism by which the damaging effect of prolonged activation of HPI axis is reduced (Pickering,1992).The cortisol is the most extensively studied primary response although epinephrine and norepinephrine levels have also been determined on application of various stressors. Cortisol can be used as a marker to study stress responsiveness of rainbow trout in a selection programme (Pottinger *et al.*, 1989).

Table 1 The effects of some commonly encountered stress in aquaculture on cortisol and epinephrine /norepinephrine level are as follows:

Sl. No.	Stressor	Cortisol	Fish Species	Source of reference
1	Handling	↑	Rainbow trout ,Coho salmon Goldfish, Walleyes, Atlantic salmons	Wedemeyer (1972), Umminger and Gist (1973), Barton and Zitzow (1995), Vijayan *et al* 1997, Davis and Schreck (1997), Iverson (1998), Carey and Mc Cormick (1998)
2	Transportation	↑	Atlantic salmon, Coho salmon ,Rainbow trout	Iverson (1998), Schreck (1981) Barton and Peter(1982)
3	Crowding and confinement	↑	Red porgy, Tilapia	Rotllant and Tort (1997) , Vijayan *et al.*, (1991)
4.	NH_3 and poor water quality	↑	Channel catfish	Tommasso *et al.*, (1981)
5.	Disease infection	↑	Rainbow trout	Ruane *et al.*, (2000)

Note ↑indicate rise in cortisol levels.

Pituitary Thyroid axis

The level of Thyroxine has been observed to increase or decrease with various forms of stress. Thyroxine has an anabolic role and helps in growth. Stunted growth or reduction in growth may be due to reduction in secretion of thyroxine (Pickering,1993). It is speculated that reduction in food intake during stress may result in reduced level of thyroxin as the pituitary thyroid axis is very sensitive to nutritional status (Leatherland *et al.*,1993)

Many workers have studied the effect of various forms of stressors on the thyroxine level but results are very conflicting . High stocking density reduces thyroxine level in rainbow trout (*Oncorhynchus mykiss*) and brook charr (*Salvenilus fontinalis*) (*Leatherland*, 1988). Transportation is said to reduce the thyroxine level in Coho salmon (*Oncorhynchus tshawytsche*) (Specker and Schereck,1980) where as it returns to the basal level after an initial decrease during transport of Atlantic salmon (Carey and Mc Cormick,1998). The level of T_3 and T^4 reduces in rainbow trout after transfer from farm to laboratory conditions (Osborn and Simpson,1974) The effect of cortisol on thyroxine level is not uniform. Cortisol injection decreases level of T_3 and T_4 in *Anguilla anguilla* (Redding *et al.* ,1986), whereas it suppressed T_3 level and not T_4 in salmon (Redding *et al.* ,1984). Growth hormone stimulates the conversion of T_4 to T_3 and suppressing of GH level also contributes to the reduction of thyroidal activity in stress fish (Pickering ,1993).

Pituitary Gonadal Axis

Reproductive steroids are anabolic during early stages of sexual maturation in fish (Pickering ,1993). Cortisol affects at the level of pituitary gland by reducing in number of hepatic oestrodial receptor (Pottinger and Pickering,1993). Thus cortisol mediated gonadal steroid suppression in the early stages of reproduction cycle may be another pathway responsible for stress induced growth suppression (Pickering,1993). Cortisol administration or stressful aquaculture practices have been reported to bring various changes in reproductive physiology. These responses have been interpreted as indicative of an effect of cortisol on gonadotropin secretion (Reddy and Leatherland ,1998).

Secondary Stress Response

According to Barton (2000) the secondary stress responses includes

- Change in osmoregulatory capacity and ionic balance.
- Haematological changes.
- Metabolic changes.

Changes in osmoregulatory capacity and ionic balance.

The salt contents of fish blood and body fluids correspond to approximately 1/3 of concentration of seawater in both marine and freshwater. The fish blood being less concentrated than the surrounding environment in seawater, results in exosmosis, which is compensated by drinking of water. In freshwater opposite mechanism takes places, the water enters the body from the external medium and is passed out in urine. Changes in external environment such as variation in salinity, temperature,Co_2 and presence of specific pollutants bring alterations changes in body fluid composition.(Eddy,1981). The hormonal changes associated with stress bring about variable differences in osmoregulation and ionic balance.

Catecholamine causes rapid changes in vascular and respiratory systems mainly the gill permeability, resulting in net loss of Na^+ and K^+ ions in freshwater and gain in seawater. On the contrary cortisol plays an osmoregulatory role by maintaining the level of Na^+/ K^+ ATPase (Pickering,1992 and Eddy,1981).Stress affects the fish weight , a weight loss occurring in seawater and weight gain in freshwater. This change is caused due to the change in permeability of gill membranes induced by adrenaline. In sea water, the loss of water due to increased permeability is accompanied by the loss of ability to drink water probably due to gastric muscular contraction induced by the catecholamine . The loss of water makes the blood

more concentrated whereas in freshwater the water intake increases and the blood becomes diluted (Mazeaud *et al.*,1977)

Handling caused hypochloremia in coho salmon and steel head (Wedemeyer,1972), whereas handling and crowding didnot reduce chloride content in juvenile coho salmon (Wedemeyer,1976). Similarly exercise in rainbow trout, transport of large mouth bass and Atlantic salmon reduces the chloride content (Randell *et al.*,1972; Carmicheal ,1984; Iverson *et al.*,1998)

Acute multiple handling in Chinook salmon reduced the sodium ions while increased the potassium ions ,as observed at hourly intervals (Barton *et al.*,1986). Handling stress in four freshwater fish species, *Carassius carrasius ,Perca fluviatilis ,Rutilus rutilus and salmon trutta* causes significant discharge of sodium ions and slightly of potassium (Vinogradov and Klermon,1985) Normally chloride ions in urine of *Lophius piscatarius* was nil but increased after handling (Foster and Berglud,1953). Rainbow trout smolts are more sensitive to stress than rainbow trout parr, as handling reduced chloride level in smolts but not in parr (Carey and Mc Cormick ,1998). The combined effect of acclimatisation temperature (10^{o}C,20^{o}C and 32^{o}C) and application of handling cortisol and aldosterone disturbed the serum ionic balance in gold fish, *Carassius auratus* as seen by loss of serum chloride at all temperature and serum potassium at 10 ^{o}C (Umminger and Gist,1972).

Stress and Immune response

Components of immune system are primarily responsible for immune response. Leucocyte includes lymphocytes which are of two types B-Lymphocytes that produce antibodies. These are specific for particular antigen. The other components of immune system are T-lymphocytes monocytes,granulocytes(Eosinophils,basophils and neutrophils). The T-lymphocytes are of three types the T helper cell (TH1) that helps the phagocytes ,TH2 cell that helps in differentiation and division of B cells and T-cytotoxic cell that kills the virus and other infected cells . Phagocytes are a group of leucocytes that include monocytes (long lived phagocytes, when in tissue are termed as macrophages), neutrophils (short lived phagocytes, dies after engulfment and destroying material),the T-cells and monocytes produce soluble mediators cytokines that activate phagocytosis.

Bidide phagocytosis and antibodies are normally present in cells. Some protein termed as acute phase protein such as C-reactive protein and complement system *etc*.are activated during infection. The complement system activates the specific immune response (Classical

pathway) and non-specific immune system alternative pathway (Male, 2001). The function of macrophages is the destruction of pathogen that they mediate by number of process such as production of active oxygen and nitrogen radicals, production of enzymes such as lysozymes, urokinase *etc.*and production of cytokines *etc.*(Gordon,2001).The other components of the immune system besides leucocytes are the tissue cells that produce interferon and cytokine like substances (Mole, 2001).

Haematological changes

Stress activates the neuroendocrine system. There is a communication between neuroendocrine and immune systems (Weytes *et al.*,1999). Receptor for cortisol are found on the blood leucocytes (Schereck, 1996; Weytes,1999) and these are reported to increase in the spleen and leucocytes after stress (Maule and Schreck,1991) and decrease in peripheral blood cells following stress (Weytes,1988b). The difference is probably due to the trafficking of receptor rich leucocytes from blood to lymphoid organs (Weytes,1999).

The general effect of cortisol on the immune system is the reduction of lymphocytes (lymphocytopenia) and increase in neutrophils, *i.e.*, neutrophilia (Ellasesser and Clem,1987). Acute handling stress reduces lymphocytes in brown trout (Pickering *et al.* ,1981) and similarly lymphocytes are reduced in chronic stress example crowding stress in red porgy *Pagrus pagrus* (Rotllant *et al.*,1997). Stress reduces the production of immunoglobulin by lymphocytes and production of various intercellular mediators such as prostaglandin and lymhokines (Kaattari and Tripp,1987).

Primary antibody response in Atlantic salmon *Samon salar* is down regulated in acute stress while secondary antibody response decreases in both acute and chronic stress (Einarsdotter *et al* ,2000).

In vivo cortisol administration alters the number, distribution and differentiation of lymphocytes where as in vitro administration increases the apoptosis of mitogen activated lymphocytes (Weytes *et al.*,1999). On the contrary, no effect on lymphocytes apoptosis was observed following cortisol administration by Alford *et al.*, (1994).

Non specific immunity

The non-specific immunity responses are also affected by stress but results are conflicting . Crowding stress decreases the activity of alternative complement system and haemoagglutination activity as studied on red porgy *Pagrus pagrus* (Rotlland *et al*,1997) confinement stress along with sea louse infection causing inhibition of lysozyme activity

coupled with augmentation of respiratory burst activity of macrophages (Ruane *et al.*,2000). C-reactive protein content got increased in *Channa puntatus* exposed to sub-lethal level of environmental toxicants, *viz.* mercuric chloride, cadmium chloride *etc.* (Ghosh *et al.*,1993) Histone like protein is identified from the mucous of catfish *Ictalurus punctatus*. This protein has broad spectrum antibacterial activity and its activity got reduced during stress. This protein can be used as a novel stress marker (Robinette and Noga,2001)

Cortisol has an immune-regulatory function rather than an immune-suppressive role and neutrophilic when cultured in presence of cortisol which reduced apoptosis (Weytes *et al.*,1998b). On the contarary, Schreck (1996) observed no significant affect on phagocytosis of rainbow trout macrophages *in vitro* experiments with cortisol.

Stress and Metabolism

Hyperglycemia or the rise in blood glucose level is the most evident example of secondary responses . Glucose can be estimated at the field level using glucometers to study the stress response (Iwama *et al.*,1995). The rise in glucose is due to glycogenolysis in the earlier stage and glycogenolysis in the later stage (Barton and Iwama,1991; Reubush and Heath,1996). Numerous workers have reported rise in glucose level on application of various kind of stressors. Handling stress increase glucose level (Wedemeyer,1972; Wedyemer,1976; Mazeaud *et al.*,1977; Carey and Mc Cormick,1998). Similarly glucose level got enhanced after transportation (Specker and Schreck,1989 and Iverson *et al.*,1998). Thermal stress also induced blood glucose level (Barton and Schreck,1987; Strange,1980). Stress influences metabolism of three major biomolecules, *viz.*, carbohydrates, proteins and lipids.

Carbohydrate metabolism

Glycogen is resumed carbohydrate in fish tissues and maximum amount is stored in liver (Moon and Foster, 1995). Many workers have observed the breakdown of glycogen during stress. The primary source of elevated glucose is glycogenolysis (Mazeaud *et al.*,1977; Mazeaud and Mazeaud,1981). Glycogen depletion was caused in the initial two hours of confinement in Tilapia but started increasing after 24 hrs confinement indicating that glycogenolysis is initially caused due to the effect of catecolamines (Vijayan *et al.*,1997). Similarly, glycogen reserves got depleted due to crowding in red porgy as per Rotlant and Trot (1997) and due to handling in stripped bass and Chinook salmon (Reubush and Heath,1996; Barton *et al.*,1986) and further transportation of tilapia (Orji,1998). 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 limitedability to utilize carbohydrate source .It lacks proper transporter 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 lactatate levels generally rise during stress. More ever, the importance of carbohydrate reserves in fish is felt during acute stress and hypoxia as there is a need for anaerobic respiration and only glycolysis cycle can then function (Moon and Foster,1995).

Stress also increases the tissue lactate level (Reubush and Heath,196;Davis and Shreck,1997; Iverson *et al.*,1998; Wells and Baldwin,1995). The increase in lactate dehydrogenase is primarily due to muscle glycolysis (Milligan and Girard,1993) . The rate of increased glucolysis is also indicated by increased activity of the enzyme lactate dehydrogenase an important enzyme of the glycolysis pathway. LDH has two isoform H type and M-Type function in tissue where there is a graet need for anerobic metabolism. Stress hormone like Catecholamine s and cortisol have an important role in regulating both type LDH and oxygen tension is the only environmental factor shown to promote the synthesis of M-Type (Giri and Singh,1979). Lactate dehydrogenase activity increases due to starvation and confinement (Vijayan *et al.*,1997;Vijayaraghavan and Rao.,1998). Lactate is the proffered substrate for glycogenesis in liver (Moon and Foster,1995; Vijayan,1997) The lactate is used for glucose production or glycogen repletion in liver (Vijayan and Moon,1992; Vijayan *et al.*,1993)

Glycogen synthesis from lactate is mainly through Cori Cycle (Muscle lactate →Liver glucose→muscle glycogen). However , most fish have low cori cycle activity and the fate of lactate can only be for the immediate energy production in peripheral tissue . The repletion of muscle glycogen and weak activity of cori cycle in most fishes leave the mechanism of repletion of glycogen in fish muscle still unanswered (Moon and Foster,1995).

Stress and Protein metabolism

Protein is the most important energy source in fish (Belinski,1974) and teleost have developed capacity for converting amino acid to glucose (Bever and Dunn .,1981). Amino acids are important source of energy for fish tissues however, quantitative impact of amino acid on total oxidative energy is still not understood (Thillort and Raaiji,1995). Amino acid is converted to glucose by glucogenesis or is used for energy production through conversion to TCA cycle intermediates by tranaminases. Transaminases act between the protein and

carbohydrate metabolism (Kumar,1999), for example alinine amino transferase transfers amino group of alanine to α Keto glutarate and converts in alanine to pyruvate which is used for gluconeogenesis or enters the TCA cycle (Das,2002).

The utilization of protein during stress depends on the type of stressor. During acute stress as hypoxia or exhausting exercise carbohydrate sources are utilized and protein is also used as flux through TCA cycle. (Thillort and Raaij,1995; Moyes and West,1995). During chronic stress, the amino acid is utilized as energy source. Confinement stress (24hrs) in tilapia caused increase in free amino acid (Vijayan *et al.*,1997). Environmental stressors such as pesticides ,herbicides, heavy metals and poor quality alter protein metabolism resulting in the decrease of total and soluble proteins and in turn increase in the activity of aminotranferase (De-Smet and Blust,2001; Rani *et al.*,2001; Reddy and Bashamohideen,1995;Chandravathy and Reddy,1994; Reddy *et al.* ,1991; Dange and Masurekar,1985; Kumar,1999). Artificial implantation of cortisol increased the free amino acids pool but couldnot increases the amino transferase activity (Anderson, 1992). High stocking density of brook charr reduced the feed intake and growth rate but the energy required was not met from protein sources as evident from the result of amino transferase activity (Vijayan *et al.* ,1990).

Stress and Lipid metabolism

Fat represents a critical source of ATP in fish. The major circulatory lipid of fish are free fatty acid and triacylglycerols (TAG)and most of them get shuttled between tissues as free fatty acids (rapid delivery) or as TAG and phospholipids (Slow delivery) (Weber and Zwingelstein,1995). About 30% of circulating free fatty acids is unsaturated with chain lengths of 20-22 carbon (Singer *et al.*,1990 ; Singer and Ballantyne,1991). Most of the long chain unsaturated fatty acids are utilized for membrane phospholipids and eicasonoid compound synthesis where as the shorter chain fatty acids are used primarily for energy metabolism (Weber and Zwingelstein,1995). The effect of stress or exercise on plasma free fatty acid fluxes should be very different in different species as they have different swimming abilities and lipid storage strategies (Tashima and Cahil,1965). Lipid catabolism, as in case of protein metabolism was not important during acute stress such as hypoxia or burst activity, as the oxygen required to metabolize lipid was lacking (Thillart and Raije,1995).Triglycerides are used as substrates for gluconeogenesis during the high stocking density of brook charr, *Salvelinus frontinalis* (Vijayan *et al.*,1990).

The effect of hormone like catecholamine and cortisol on free fatty acids changes is far from uniform and clear (Mazeaud and Mazeaud,1981). Catecholamine have been shown to

increase free fatty acids in common carp and lamprey (Farkas,1967; Plisetskaya and Mazina,1969). Adrenaline does not have any effect on free fatty acids in rainbow trout (Perrier *et al.*,1972). Similar results were obtained for cortisol , which were shown to increase free fatty acid level in fish (Butler,1973) and have no effect on FFA in rainbow trout (Sheridan,1986). Stress related hormones such as catecholamines and cortisol stimulated lipolytic activity resulting free fatty acid (Kate and Messino,1981) but in fishes significant decrease in free fatty acid may be observed probably due to lipolytic activity (Farkas,1967).

The cholesterol level gets increased in stress. Handling stress results in hypercholesterolemia in Coho Salmon but not in steel head trout (Wedemeyer,1972). Cholesterol is the precursor of steroids hormones including sex and adrenal hormones (Montgomery *et al.*,1980). Perrier *et al.*, (1972) observed the increase in cholesterol after adrenal injection in rainbow trout but observed no change in free fatty acid content. Phospholipid functions mainly for membrane integrity. Muscle lipid content got decreased Coho salmon due to exercise. Lower chain fatty acids were exhausted first but loss of longer chain fatty acids decreased following vigorous exercise which was probably due to tissue damage as these fatty acids were utilized for phospholipid hydrolysis (Krueger *et al.*,1968).

Tertiary Responses to stress

Stress and Disease

Cortisol induces immunosuppression which results in lymphocytopenia reduced antibodies production, immunoglobulins and decreased ability to resist pathogen, (Pickering and Duston,1983; Maule *et al.*, 1987; Reddy and Leatherland,1998). Pathogens are abundantly present in the immediate environment but can multiply in the organism when the resistance of the organism is decreased by environmental stress (Jeney and Jeney,1995). Aquacultural practices such as handling, Chasing, transportation *etc.* caused the transient elevation in cortisol level which further caused immunosuppression and made fish more susceptible to disease (Reddy and Leatherland ,1988; Schreck *et al.*,1996). Cortisol level elevation has been observed in much disease infection such as saprolegniosis in brown trout (Pickering and Christie,1980) and bacterial infection (William *et al.*,1967). Similarly cortisol administration and subsequent exposure to pathogens have shown mortality in case of bacterial diseases like *Vibrio anguillarum* (Maula *et al.*,1987) *Edwardsiella ictaluri* (Antonio and Hedrick ,1994) fungal infection saprolegniosis (Pickering and Duston,1987).

Stress and Growth

Stress results in the growth suppression of animals (Peters and Schwaezer,1985) and a coefficient of condition of growth (Pickering *et al.*,1989). The reduction in growth may be due to the decrease feed intake following stress. The other reason of growth inhibition may be the catabolic effects of cortisol and catecholamine . The hormones such as thyroxin and growth hormone that are growth promoter get inhibited during stress (Reddy and Leatherland,1998).

Stress and transportation

Transportation of live fish has two main objectives, first to keep the fish alive and second is to do as economically as possible. (Carmicaheal,1984). Handling and transportation are usually stressful events that comprises the ability of fish to perform essential life functions after release (Specker and Schreck,1980; Barton *et al.*,1980; Schreck,1989). Transportation stress elicits the same response as other forms of stress (Maule *et al.*,1988). Primary response such as elevation of cortisol after transportation was observed by (Barton and Peter,1982; Schreck *et al.*,1989; Schreck,1999). Other physiological responses decreased survival and disease resistance are also resulted following transportation. Survival of Coho salmon was reduced when released just after transportation in migratory stream as compared to fish acclimatized after transportation. (Schreck *et al.*,1989).

Stress during transporation

Transportation is a stress comprising both physical (handling) and social (confinement) stress. (Jeney *et al.*,1997). According to Ramchandran (1969), Rao (1980) and Lingaraju (1995) the following are the major causes of mortality during transportation .

- Exhaution of dissolved O_2 in water medium due to respiration and oxidation of metabolic waste products by microorganisms.
- Accumulation of harmful gases like CO_2 and NH_3.
- Physical injury caused by bad handling of fish.
- Hyperactivity and strain due to overcrowding in a confined space.
- Sudden fluctuation of water temperature in the carriers.

Reference

Barton B.A. and Peter R.E. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri Richardson*, to various transport conditions, anaesthesia, and cold shock. *Journal of Fish Biology* 20,39-51.

Barton, B. A., and Zitzow, R. E. 1995. Physiological responses of juvenile walleyes to handling stress with recovery in saline water. *The Progressive Fish-Culturist*, *57*(4), 267-276.

Brett, J. R., 1958. Implications and assessments of environmental stress. In : The investigation of fish power problems. H. R. MacMillan Lectures in Fisheries, University of British Columbia. Pp. 69-97.

Chavin, W., 1973. Teleostean endocrine and para-endocrine alterations of utility in Environmental Studies. W. Chavin (Ed.), Responses of Fish to Environmental Change, Thomas, Springfield, IL, pp. 199–238.

Chester Jones, I., W. Mosley, I. W. Henderson, and H. O. Garland. 1980. The interrenal gland in pisces. In General, Comparative and Clinical Endocrinology of the Adrenal Cortex, vol. III (ed. I. C. J. a. I. W. Henderson), pp. 396-523. London: Academic

Davis L.E. and Schreck C.B. 1997. The energetic response to handling stress in juvenile coho salmon. *Transactions of the American Fisheries Society* 126, 248-258.

Henderson, I. W., and Garland, H. 0.1980. The interrenal gland in Pisces. Part 2. Physiology. In: General, Comparative and Clinical Endocrinology of the Adrenal Cortex (I. Chester Jones, and IW Henderson, eds.), Vol. 3.

Iversen M., Finstad B., Nilssen K.J. 1998. Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* 168: 387–394.

Janssen., Waterman., 1988. Hormonal regulation of gluconeogenesis and glycogenolisis in carp *Cyprinus carpio* liver pieces cultured *in vitroComp. Biochem. Physiol.*, 91A: 451–457.

Lamers, A.E., Flik,G., Atsma, W., Wendelaar Bonga, S.E., 1992. A role for di-acetyl α-melanocyte-stimulating hormone in the control of cortisol release in the teleost *Oreochromis mossambicus*. *J. Endocr*. 135, 285–292.

Leatherland, J. F. (1988). Endocrine factors affecting thyroid economy of teleost fish. American Zoologist, 28(2), 319-328.

Leatherland, J. F.,,Farbridge K.J. ,Vijayan M.M. and Flett A.A. 1993. Adaption growth and thyroid hormones. In: Muir, J. F. and Roberts, R.J. (eds.). *Recent Advances in Aquaculture* IV Institute of Aquaculture,Blackwell Scientific Publications, Cambridge, pp. 176-184.

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. *Transactions of the American Fisheries Society*, *106*(3), 201-212.

Mommsen, T. P., Walsh, P. J., Perry, S. F., Moon, T. W., 1988. Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *General and comparative endocrinology 70*(1), 63-73.

Olivereau ,M. and Olivereau, J.M., 1991 Responses of brain pituitary immmunoreactive corticotropins releasing factors in surgically inter renal ectomised eel: Immuno cytochemical study. *General and Comparative Endocrinology* 81:295-303

Osborn, R. H. and Simpson, T. H., 1974. The effect of stress and food deprivation on the thyroidal status of plaice and of rainbow trout. *Cons. Perm. Int. Explor. Mer CM 1974 Gear and Behaviour Comm.*, B9.

Pickering, A. D. 1993.Growth and stress in fish production. *Aquaculture,111*(1), 51-63.
Pickering,A.D.,1992.Rainbow trout husbandry: management of the stress response. *Aquaculture*, *100*(1), 125-139.

Pottinger, T.G. and Pickering, A.D. 1990. The effect of cortisol administration on hepatic and plasma estradiolbinding capacityin immature female rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* 80, 264-273.

Reddy P.K., Leatherland J.F. 1998. Stress Physiology. *In*: Leatherland JF, Woo PTK (eds) *Fish diseases and disorders.* Vol.2. CABI Publishing, Oxford, UK, Pp 279-301.

Rotllant, J., Tort, L., 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. *Journal of Fish Biology*, *51*(1), 21-28.

Ruane, N. M., Nolan, D. T., Rotllant, J., Costelloe, J., Wendelaar Bonga, S. E. 2000. Experimental exposure of rainbow trout *Oncorhynchus mykiss*(Walbaum) to the infective stages of the sea lous *Lepeophtheirus salmonis* (Kroyer) influences the physiological response to an acute stressor. *Fish and shellfish immunology*, *10*(5), 451-463.

Schreck, C. B. 1976. The stress concept in fisheries: Implications in environmental monitoring. *Proc. West. Ass.State Game fish Comm*. 56, 186-188.

Selye, H., 1950. Stress and the general adaptation syndrome. *Brit. Med. J.* 1: 1383-1392.

Selye, H., 1973. The Evolution of Stress Concept. *American scientist* 61(6), 692-699.

Tomasso, J. R., Davis, K. B., and Simco, B. A. 1981. Plasma corticosteroid dynamics in channel catfish (Ictalurus punctatus) exposed to ammonia and nitrite. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(9), 1106-1112.

Umminger, B. L.and Gist, D. H. 1973. Effects of thermal acclimation on physiological responses to handling stress, cortisol and aldosterone injections in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology Part A: Physiology*, *44*(3), 967-977.

Vijayan, M. M., Ballantyne, J. S. and Leatherland, J. F. 1991. Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. *Gen. comp. Endocr*. 82, 476–486

Wedemeyer, G. A., 1976. Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. *Journal of the Fisheries Board of Canada*, *33*(12), 2699-2702.

Wedemeyer, G., 1972. Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Board of Canada*, *29*(12), 1780-1783.