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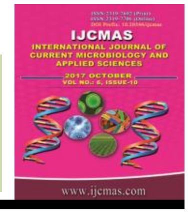

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Stress in Aquaculture Hatcheries: Source, Impact and Mitigation

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A B S T R A C T

Stress is a major concern in aquaculture as many stressors are present in hatchery which can predispose the fish to compromised growth and health and ultimately promote disease. Larval stages may be more sensitive to stress as compared with other stages, however little is known about behavior and stress at this time. Fish which undergo metamorphosis are most vulnerable to stress. Although with the increasing interest in fish culture, the study of stress in the hatchery is an important field for future. Proper management practices if followed in the hatchery will reduce the stress and will ultimately lead to the better survival of fish.

Introduction

Hatcheries are an important component of aquaculture system as they are designed and operated for cost-effective rearing of larvae. The expansion and sustainability of aquaculture depends on providing suitable conditions for the development of larvae. In hatcheries, the larval fish are reared in conditions which are at significant deviation from the natural environment. However, the environmental conditions of the culture system must comply with the physiological and behavioral needs of the fish larvae in order to achieve high survival and growth. Providing the larvae with the environment that best suits its growth and development without compromising the economics of the production system is a big challenge which the industry is facing all over the world.

The larval phase, which is spent in the hatchery, is the most critical phase of the entire production cycle after spawning This vulnerability of fish larvae is because of lack of a fully functional immune system (Bricknell and Dalmo, 2005); digestive system (Holt, 2011); osmotic system and compromised host microbe interaction (Vadstein *et al.*, 2013). These factors, along with handling, induce stress in fish larvae resulting in high and sudden mortalities. Stress is an important factor, which should be considered in the successful rearing of larvae in hatcheries. Stress is defined as the nonspecific response of the organism to any demand made upon it (Selye, 1973). Although, the initial phase of stress, called as eustress, is beneficial to the organism, distress happens when certain factors cause physiological changes in an organism that ultimately compromise the organism's integrity (Selye 1984). Most of the research on stress is mainly focused on distress phase (Martinez Porchaset *et al.,* 2009). The main aim in the hatchery is to produce a reliable and sufficient good quality of fish seed but certain stressors affect the whole process of production resulting in high mortality rates, and economic loss in aquaculture sector. The intensive culture of larvae exposes them to considerable stressors of chemical, physical and biological nature and in addition, some of these stressors can occur in an intermittent manner or as pulses, which may further amplify their negative effects. Therefore, the recognition of theses stress sources, and their mitigation are critical in the successful operation of hatchery.

Sources of stress in hatchery

Most common stressors, which are present in hatchery, include poor water quality (Tomasso *et al.,* 1981a, 1981b), xenobiotic toxic molecules (Schlenk *et al.,* 1999; Griffin *et al.,* 1999), and handling (Davis *et al.,* 1993). These stressors can be of a short-term acute nature, like handling or may be long-term chronic type, like an extended period of poor water quality. Since the transport, feeding and treatment are routine activities of a hatchery operation, the larvae are more exposed to both chronic and acute stressors. A similar risk for poor water quality also exists in the hatcheries because of the high stocking densities, which are common in intensive culture (Kenneth, 2006).

Water quality is an important factor in the production results of any hatchery and is the foundation of healthy fish-pathogen-environment relationship. Water quality requirements of the larvae are species specific. Generally, larvae or fry of any species have stringent water quality requirements than their larger con-specifics. Any change in the water quality constituents can greatly alter the normal physiology of larvae causing acute and chronic stress.

Water temperature

Water temperature regulates major physiological processes of fish larvae and a two to three-order magnitude change in temperature will have a profound affect on physiological mechanisms (Blaxter, 1992**).** Temperature affects the time of hatching, development of larvae, cellular function, muscle ontogeny and development and ontogeny of internal organs (Herzig and Winkler 1986). Besides this, external morphology, meristic characters (Torres-Núñez *et al.,* 2014), appearance of skeletal deformities, sex determination, delayed feeding (Dou *et al.,* 2005), changes in the rates of enzymatic reactions, swimming performance, growth (overall survival and lifespan of fish are affected by temperature (Meeuwig *et al.,* 2013).

pH of water

The pH is an important variable in water quality as many reactions are pH dependent and normal waters contain both acids and bases. The biological processes in water tend to increase either acidity or basicity and the interactions among these opposing acidic and basic substances determine pH. Carbon dioxide concentration, which is acidic, plays an important role in regulating pH. The alkalinity of water results primarily from bicarbonate and carbonate ions derived from the reaction of carbon dioxide in water tends to buffer water against excessive pH change (Boyd, 2011). Fish larvae are highly sensitive to pH changes than juveniles and adults and at this stage significant effects are most likely to be detected. This can partly be understood due to insufficient acid-base regulation prior to the formation of gills (Falk-Petersen, 2015). Studies have shown many impacts of predicted CO2 concentrations on larval fish on sensory abilities like olfaction (Munday *et al.,* 2009), behaviour (Dixson *et al.,* 2012), otoliths (Checkley *et al.*, 2009; Bignami *et al.*, 2013) development, tissue and organ structure (Frommel *et al.,* 2012; 2014), survival of eggs specifically the hatching success (Chambers *et al.*, 2013), and survival of very early larval stages (Bromhead *et al.,* 2015). However, other studies were not able to find an effect on survival (Munday *et al.,* 2009). Low-pH seawater slow down the embryonic and early larval development causing appreciable larval mortality (Miller *et al.*, 2016)

Photoperiod

Light plays an important role as a biological regulator controlling feeding, digestion, and reproduction in fish. Light influences the basic signalling systems by affecting the light sensitive brain tissues including the retina of the eye containing serotonin, an important neurotransmitter. Light comprises different components such as quantity (light intensity), quality (spectral composition), light distribution (point source or evenly), and cycling (photoperiod and season).

The outdoor light conditions are quite defined depending on latitude, season, depth, and algal blooms. However, hatchery conditions often provide very different conditions from natural systems like light intensity is lower, spectral composition is different with e.g. lack of UV radiation, and 24-hour light is commonly used. This may remove any diurnal signals from the fish larvae, with unknown impacts on larval development. Unfavourable light conditions which do not facilitate good feeding behaviour induces stress conditions since most marine fish larvae are visual feeders (Tamazouzt, 2000)

Besides the physicochemical aspects of water, several routine practices in the hatchery like overcrowding, handling and transport can cause stress in fish (Wedemeyer, 1996a). High stocking density produces a wide variety of effects on fish such as alterations in behavior (Alanara and Brannas 1996), poor feed utilization(Jorgensen *et al.,* 1993), behavioural (Schreck *et al.,* 1997) and hormonal changes (Leather and Cho, 1985) resulting in poor growth (Bjornsson, 1994) and survival(Sodeberg and Meade 1987). High stocking density also causes immunosuppression (Tort *et al.,* 1996) and induces mobilization of energy sources (Vijayan *et al.,* 1988, 1990). Capture and handling elicite rapid, marked elevations of plasma cortisol and glucose level in the fish (Barton *et al.,* 1993). Transportation causes a physiological stress response by accumulation of carbon dioxide and ammonia in fish hauling tanks, increasing concentrations of circulating cortisol level and reducing survival rate (Schreck *et al.*, 1989).

Behavioral interactions occur between fish in a hatchery. Agonistic encounters, interspecific competition, social hierarchies, and territoriality dominance is a significant source of social stress in fish indicated by manifold changes in physiological and neuroendocrine processes (Summers, 2002; Blanchard, 1991). Fishes show aggressive behaviors and predation causing unnecessary stress and physical injury. Fin nipping, scale loss from ramming, reduced growth, pathological changes in gastrointestinal tissue, and increased susceptibility to diseases can occur in defeated individuals (Earley *et al.,* 2004).

Stress response

Stress response involves all three regulatory systems, neural, endocrine and immune and is common in all the vertebrates including fish. Perception of stress by the sensors of the nervous system causes the immediate secretion of corticosteroid releasing hormone (CRH) by the preoptic nucleus of the hypothalamus. The stimulated CRH receptors of the pituitary gland induce the release of adrenocorticotropic hormone (ACTH) into the circulation that subsequently stimulates release of cortisol by the interrenal cells (Pickering, 1981). The other axis that is related to stress is the sympathetico-chromaffin axis. The sympathetic nerve fibers innervate the chromaffin cells and stimulate the release of cateholamines via cholinergic receptors (Reid *et al.,* 1996; Catecholamines, release is rapid and the circulating levels of these hormones increase immediately with stress (Randall and Perry, 1992).

Stress response promotes the survival and recovery of individuals during and after challenging events (Greenberg *et al.,* 2002).However, this response is characterized by release of glucocorticoid hormones (Axelrod and Reisine 1984), resulting in secondary system-level and tertiary whole-animal changes. These changes include increase in carbohydrate catabolism (Barton 2002), mobilization of energy reserves (Wendelaar Bonga, 1997), increases in aerobic and anaerobic metabolism(De Boeck *et al.,* 2001), increase in standard metabolic rate (Lankford *et al.,* 2005) and reduction in feeding (Lankford *et al.,* 2005). Chronic elevation of circulating cortisol concentrations also reduces growth despite normal feeding behaviour (Bernier *et al.,* 2004) showing that there is a metabolic cost of stress. Variable changes in plasma levels of Growth hormone (GH) occur as a result of stress. GH is released from pituitary and stimulates the release of insulin-like growth factor (IGF-1) which in turn affects cell growth and differention. Thyroid hormones throxine (T_3) and its peripheral metabolite triiodothyronine (T_4) is also required for normal growth (Leatherland, 1994). Starvation causes an increase in plasma GH levels (Fabridge and Leatherland, 1992). Growth inhibiting effects can occur due to stress through depression of T_3 & T4, either directly or indirectly from a stress- reduced reduction in food intake. (Pickering, 1991).Stress effects mediated by cortisol treatment suppress T_3 and T_4 levels (Vijayan and Leatherland, 1989), depress somatic growth and often associated with hyperglycemia (Lidman *et al.,* 1979).

Stress and immune system

The specific and non-specific immune system in fish can be compromised in fish due to stress factors present in the hatchery leading to establishment of infection**.** Different kind of stressors induces a variety of immune changes and the outcome of a stress response will depend on the intensity of the stressor and its duration. (Wendelaar Bonga, 1997) Stress response, therefore, can either activate or suppress immune function (Dhabhar *et al.,* 2008). Majority of stressors, however, produces deleterious effects. During the activation phase, enhanced innate humoral immunity such as increased levels of lysozyme and C3 proteins can occur after acute stress (Demers and Bayne, 1997). Stressful hatchery conditions like crowding (Vazzana *et al.,* 2002), variable water temperature (Varsamos *et al.,* 2006), environmental contaminants, oxidative stress (Franco *et al.,* 2009) and deficient diet (Montero *et al.,* 1999)which cause chronic stress normally show suppressive effect.

Most of these effects are reduced immunocompetence, increased level of cortisol, decreased level of lysozyme. Stress reduces the number of circulating B-lymphocytes, decreases the antibody response, complement hemolytic activity, IgM and induce apoptosis (Franco *et al.,* 2009).

Stress related Diseases

Fish and microorganisms present in the rearing environment are most often harmless or beneficial. However, this fish-microorganism interaction when influenced by stress factors result in infections and epizootic disease (plumb 1994). Fish pathogens are generally classified as obligate or facultative pathogens and the stress-mediated fish diseases are mostly caused by facultative bacterial pathogens (plumb, 1994), external fungi, (Pickering and Willoughby., 1982), protozoan parasites (Alavarez *et al.*, 1993). Some of the common bacterial diseases occurring in hatcheries can be stress-mediated such as vibiosis (*vibrio* species) and motile *Aeromonas* septicaemia (*Aeromonashydrophila),* Enteric red mouth*(Yersinia ruckeri),* Enteric septicemia (Wedemeyer *et al.*, 1984., Pickering *et al.*, 1991) Furunculosis (*A. salmonicida*) is often correlated with stressful water quality conditions and fish culture procedures (Nomura *et al.*, 1992.,). Bacterial gill disease (*Flavobacterium branchiophilum)* occurs in hatchery mostly with management errors such as overfeeding, overcrowding, inadequate water flow rates, low DO levels, increased unionized ammonia and accumulation of suspended particulate matter that causes gill irritation. (Klonz, 1993). Behavioural conflicts and in presence of pathogen can result in MAS in defeated individuals (Peters *et al.*, 1988). *Ichthyophthirius, Gyrdoactylus and Lernae* are particularly some of the parasites having a relationship between temperature and infection (Plumb, 1994). Stress and cortisol treatment has been shown to increase susceptibility to parasitic infections (Saeij *et al.*, 2003a). A number of noninfectious diseases such as skeletal abnormalities, swim bladder stress syndrome and coagulated yolk disease occur due to improper management of rearing conditions (Soares *et al.*, 1994).

Stress and Reproductive inhibition

In the hatchery, brooders may be in-house or may come from the wild conditions. Wild brooders are the main stay of most of the marine hatcheries. When the wild fish are brought into the hatchery, it generates stress that will inhibit their sexual maturation for a certain time (latency period) (Pickering *et al.*, 1987). Effect of number of stressors like low pH (Freeman, 1983), contaminants and industrial effluents on reproduction has been documented. Stress associated with capture or confinement of wild brooders and handling for the collection of gametes (Mellotti *et al.*, 1992) influences reproduction by affecting circulatory levels or changing seasonal pattern of reproductive hormones. Effect of stress is mediated mainly through HPI axis resulting in increase in plasma cortisol level and catecholamines. Increased plasma cortisol has shown suppressive effects on plasma sex hormones like testosterone, oestradiol and gonadotropin, pitutitary level of gonadotropin, circulating vitellogenin and ovary weight *in vitro* (Carragher and Sumpter, 1990). Repeated acute stress prior to spawning results in delayed ovulation, reduced egg size in females and lowered sperm counts in males (Pankhurst and Carragher, 1992). Progeny from the stressed fish is also known to have a lower survival rate as compared to unstressed fish (Short *et al.*, 1995).

Reduced feeding behavior and growth

Stress caused by handling such as transferring brooders from one tank to another tank and size grading to avoid competition results in loss of feeding behavior for various period of time depending on the severity of stress and physiological state of fish. Stress disrupts feeding behavior like appetite, visual and chemosensory ability, restricted area searching, responding to and capturing prey and handling and ingestion of prey (Beitinger, 1990). Low pH of water has shown to depress attraction to food scent (Jones *et al.,* 1987). Natural mortality in fish occurs mostly during larval stages, due to predation and starvation (Hunter, 1981). Starvation of larvae in hatcheries can occur due to poor acceptance and inadequate quality of food leading to huge mortality(Ola and Davis,1992).Starvation in larvae can also cause behavioural abnormalities such as depression and cessation of positive phototaxis, abnormalities in gas bladder development leading to lack of buoyancy control and early death (Fletcher, 1997).

Stress and behvioural response

Fish respond to stressors by change in their behavior like swimming performance, thermoregulation, orientation, avoidance, chemoreception, feeding, predator evasion, and learning (Schreck *et al.,* 1997) In many cases, these changes are adaptive and therefore, increase the probability of survival(Olla., 1996) and are also sensitive indicator of physiological and biochemical changes occurring in response to stress (Marcucella & Abramson,1978). Pathogenic microorganisms and parasitic infestation can cause behavioral changes in fish (Post, 1987) which may include increased activity, flashing at the surface and ultimately decreased activity and lethargy (Thoesen, 1994). Leaping and rolling at the surface can occur as a result of parasitic infestations (Furevik *et al.,* 1993).

Changes in temperature preferences (Reynolds *et al.,* 1978) and infection with pathogens (Tsai and Hoh, 1995) has also been documented. Stress can interfere with learning responses (Schreck *et al.,* 1997), however there is little information that directly relates the effect of stress on learning ability or memory of fish.

Indicators of stress in fish

Measuring stress in fish is to determine how the health, performance, and welfare of fishes are being influenced by different stressors like handling, rearing, and transport (Portz *et al.,* 2006), as well as anesthesia (Trushenski *et al.,* 2012) affecting captive broodstock health, survival of larvae and production efficiency. The main aim of assessing is to reduce it and maximize growth and survival. Quantification of stress basically refers to quantification of primary, secondary, and tertiary responses following HPI axis activation like gene expression, immune function, metabolism, growth, reproduction, performance, behavior. Measurement of pre- and poststressor levels of the indicator are required for quantification, however, mostly the latter response is measured once following stressor exposure (Iwama *et al.,* 2007; Schreck, 2010). Physiological indicators of stress include all the responses between the cellular and molecular level and the whole-animal level.

Cellular and molecular indicators

Oxidative stress, by-product of metabolism, occurs when ROS production overwhelms the counter balancing capacities of antioxidants damaging biological molecules like lipids, proteins, RNA, and DNA (Costantini, 2008,). Oxidative stress is quantified by multiple markers through a variety of colorimetric assays measuring ROS, antioxidant levels; or measuring damage to biomolecules (Lesser, 2006) in plasma, serum, urine, tissue homogenates, or cell cultures (Valavandis *et al.,* 2006). Heat shock proteins (HSP) sensitive to a range of stressors like rapid temperature and salinity challenges, and handling can be a reliable indicator of stress (Donaldson *et al.,* 2008). HSP's are basically molecular chaperones having role in folding, repairing, and catabolizing protein (Moseley, 1997). HSPs controlled by Heat Shock Factor

1 (HSF1) induces cellular stress response and expression increases to maintain cellular homeostasis (Iwama *et al.,* 2004). In fish, a number of genes like cytochrome c. transcription factor JUNB, NUPR1 have been investigated as potential biomarkers for various stressors (Jefferies *et al.,* 2012). Changes in the gene expression related to inflammation, protein degradation, immune response onset of recovery poststress related to gluconeogenesis, glycogenolysis, and energy metabolism in the liver have been observed (Wiseman *et al.,* 2007).

Primary and secondary physiological indicators

Primary and secondary stress indicators are very useful in assessing responses related to specific aquaculture or handling practices, or acute disturbances in the field. Cortisol and catecholamines are commonly measured as stress indicator (Barton, 2002). Cortisol responds more slowly than catecholamines to specific stressors, it can be quantified in laboratory or field settings although the later provides very accurate information about the response to acute stressors. (Romero and Reed, 2005) Poststress glucocorticoid levels can provide information about specific stimuli (e.g., capture and handling stress, different holding conditions, acute exposures. Secondary stress indicators commonly measured include glucose elevation (Barton, 2002); lactate elevation, osmolality or specific ions, leukocytes (Davis *et al.,* 2008).

Whole-organism indicators

A number of aspects of fish performance such as changes in growth, disease resistance, metabolism, cardiac activity, swimming performance, behavior, fitness, and even survival are measured in whole-organism (or tertiary) responses to stressors (Sadoul and Vijayan, 2016).

Mitigation of stress factors

Proper capturing and handling of brooders

Handling and grading are highly stressful and more complicated because of the aquatic medium. Handling requires a foreign element into the water to catch the fish and if not done correctly, excessive stress can jeopardize fish, disrupts the protective mucous coating and fish scales and increases susceptibility to parasitic or pathogenic infection (Post, 1987). To avoid disrupting the mucous coat of fish and losses of scales, use of wet hands or soft cotton gloves while handling fish is always recommended. Stress should always be minimized while capturing and handling brooders and this can be partly achieved by selecting suitable gear during the operation. The appropriate makeup of netting varies with species, and depending on presence or absence of scales and scale type. Knotted mesh can dislodge fish scales resulting in parasitic and pathogenic invasion The best fishing gear for catching the wild fish is the fish barrier which is a fixed trap placed at the outlet of coastal lagoons. When fish enters the trap following their migratory instinct, they can be captured without much stress. The seine net that is used in ponds and tanks can also give good results but should be handled very carefully, while harvesting only a small number of fish per haul. To avoid, the use of knotless stretched mesh nylon nets, as well. Healthy adult fish can also be captured by hook and line, but care should be taken to reject all fish with major injuries in their mouth, gut or, even worse, gills, as well as those which have lost too many scales when hauled on board (Tucker, 1985; Tucker and Robinson, 1990). Temperature effect the stress response to handling and capturing, as water hold less less oxygen at higher temperatures (Wheaton, 1977; Lawson, 1995). Most of these activities should be performed during the cooler portions of the day or initiated at night so as to finish in the early morning. Temperature-related stress can occur any time when fish are handled, but fish are most vulnerable during high temperature resulting in mortality in both the short and long-term (Piper *et al.,* 1982).

Proper transportation

Much of the research has been focused on overcoming the challenges of harvesting and transporting fish due to wide inter-specific variations in physiological responses to these stressors (Barton, 2002). Level of plasma cortisol in different species can vary by as much as two orders of magnitude due to identical stressors. Some fishes can be transported easily other than maintaining proper water quality and temperature, however, others species require specific additional precautionary steps so as to prevent ionic and osmotic imbalances resulting in mortality (Carmichael and Tomasso, 1988).

Repeated handling and transport should be avoided and there should be a recovery period. Physiological responses of fish after acute netting, handling and transport can recover after a period of 6 h to1 day, however, it may also vary from 10 days to 2 weeks if they are persisting, but not lethal (Schreck *et al.,* 1997).

Transporting a fish is a multiple-phase operation and should be designed in such a way to minimize stress (Piper *et al.*, 1982).The temporary holding and transport containers should be selected carefully to reduce additional and potentially fatal stress to recently caught fish. They should be heat insulated and filled with water from the same location where the fish have been caught. The containers should be round in shape or square with rounded corners so as to avoid skin abrasions and mechanical shocks and large enough to allow the fish a fair degree of movement. A correct choice of fish loading density (fish weight per unit volume of water) is of paramount importance in minimizing the stress. The fish density should be inversely proportional to transport time and water temperature. Fish should not be fed for about 24 h prior to harvest and transport to avoid feces and fouling of the transport water. In the container oxygen saturation should be around 100% and the animals should be brought to the hatchery as soon as possible: speed and care are always recommended. A variety of water additives (anesthetics, hypnotic drugs, mineral salts, polymers), fasting, hypothermia, and reduced light intensity have been developed to reduce the physiological effects of transportation on fish health (Wedmeyer, 1997)

Maintaining water quality requirements

To achieve optimal growth and survival of newly hatched larvae, it is important to have good water quality in the hatchery. The quality of water should be more or less stable without any fluctuation. The hatchery should have both heating and cooling plant to control the temperature fluctuations because natural water may have a sub-optimal temperature for growth and survival for the different life stages (Lekang, 2007).

Larvae are sensitive to high gas saturation in the water and as a result can become diseased quickly and thus aerators should be used properly to avoid such problems in hatchery. Gas bubble disease can occur if the nitrogen gas concentration in the water is above 101–102 % (Gunnarsli *et al.*, 2008; Noga, 2010). CO_2 super-saturation is not common because, its solubility is higher than that of oxygen and nitrogen but the tolerance of aquatic organisms for CO_2 in the water is, however, limited (Good *et al.,* 2010). The Ph of water also plays a significant role in the hatchery and thus ph should be measured regularly and adjustment should be done to avoid large fluctuations.

Control of microorganism

In the hatchery, diseases can be controlled by the disinfection of the inlet water. The most common system is ultraviolet (UV), ozone. UV lamp can be used to send out UV radiation having a sufficient dosage to inactivate microorganism. Ozone gas (O3), a very strong oxidizing agent produced by ozone generator can also be used in the hatchery for disinfection of water. Ozone can also reduce water turbidity, watercolor, organic carbon, metal ions and algae (Summer felt, 2003; Masters *et al.*, 2008)

Stabilization of water

Disinfection of water reduces large amount of microorganisms but still the water will have dead, inactivated bacteria and decomposed organic matter which can be a good food source for the remaining bacteria. Microbial stabilization or maturation is a method where the disinfected water is allowed to pass through a filter where biological organisms growing naturally can stabilize the water quality by inhibiting growth of bacteria. There should also be a retention time for general stabilization of water quality before it is passed into hatchery (Olafsen, 2001; Brunvold, 2010)

Use of immunotimulants

The immunomodulation of larval fish is a potential method for improving larval survival by increasing the innate defence of the developing animals until its adaptive immune response is sufficiently developed to mount an effective response to the pathogen.

The immunostimulants, as dietary supplements can be used during periods of high stress, such as grading, reproduction, sea transfer and vaccination (Bricknell and Dalmo, 2005).

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