RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY, PORT HARCOURT

STRESSED FISH: WARPED BIOMARKERS, AVOIDABLE MORTALITY

AN INAUGURAL LECTURE

By

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DEDICATION

This lecture is dedicated to
C. B. Powell (deceased) – My mentor
My students- my tools for research and discovery

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The Acting Vice Chancellor
Acting Deputy Vice Chancellor
The Registrar and other Principal Officers of the University
Members of the Governing Council
Distinguished Professors and Members of Senate
Deans and Directors
Heads of Departments, Academic colleagues and Staff
My Lords Spiritual and Temporal
Friends and family members
Gentlemen of the Press
Ladies and Gentlemen

1.0 PREAMBLE

Sober reflections on the title "Professor"

From when I began nursing a strong desire to become a lecture, till I was employed as one and eventually was promoted to the rank of Professor, I have pondered what it really means to be a professor. What does the title, Professor evoke in the bearer? Pride or humility? I think it should evoke in the bearer a deep sense of humility, since very few persons attain it, although it is the goal of everyone in the academia, with few exceptions. I have pondered on some questions that bothered my mind on the title, Professor. There are three of such questions, (1) Who is a professor? (2) What does a professor know? (3) How much does a professor really know in his discipline?

Who is a professor?

He is an accomplished and recognized academic, an expert in his discipline. Yet, as accomplished and recognized as he is, Elmer Wheeler observed that **"In whatever a man does without God, he must fail miserably or succeed more miserably"**. If he attributes his success to his academic prowess, then he sees himself as the ultimate source of wisdom that makes him who he is. His accomplishment (success) should be

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founded on God- His righteousness, mercies and providence. If not, his success will mean great pains and sorrows to his colleagues and students in particular. Incidentally, I am a product of God's grace and hard work. I feel greatly humbled by this great privilege of being addressed as a Professor.

“What does a professor know?

A professor gains knowledge by performing and leading advanced research in his field of study. He discovers (searches out) what God has concealed. **“It is the glory of God to conceal a thing, but the honour of man to search it out”.** His discoveries give him honour (promotion), but the glory goes to God. Hence, it is a most abnormal thing to see a pompous, cocky man, called a professor. A professor is supposed to be the most humane, humblest and down-to-earth fellow in the academia. He rose from among the people, belongs to the people and he is in no way better than any of the people. Some ignorant fellows who have a wrong perception of who a professor is may attempt to press him into their own mould of what they think a professor should be. But this must be resisted.

How much does a professor know in his discipline?

A professor knows much about very little and very little about much. Even in his specialty, there may be much he is yet to know. So, he is largely ignorant and therefore, ever a learner. He should be ready to learn from whoever he meets. Infact, he may learn his greatest lessons from his students. That indeed is a humbling revelation! Hence, his students are his most important workshop and tools for research and discovery. Constant reflections on these questions form the foundation of all my actions and interactions with all in the academic environment and outside.

My preparation for the world of academics, a tortuous one began with a prolonged studentship at the Master of Philosophy degree in this University. This lasted for 6yrs. Then this University was fondly and indeed sadly referred to as the “**University of Stress and Tension-UST**”. The tension from undue delay in concluding that programme caused real stress to many, including myself. I got employed in 1996 and enthusiastically settling down to work in my area of interest, Fish nutrition, I met with another round of frustration and stress. It was really stressful to carry out any study in that area as there was lack of necessary equipment. I was compelled to explore other areas of interest that would require minimal use of laboratory equipment. Thus, I got into **Aquatic toxicology**, with special interest in the biomarkers of contaminant effects in cultured fish. Initially, the biomarkers of interest were mostly behavioural changes of fish under contaminants stress and the first trial was by my undergraduate student, Orugbo, Segun Kparobo in 2000. From there, we advanced into pathophysiological changes in several cultured fish resulting from exposure to xenobiotics or environmental toxicants. Later, I got interested in assessing the effects of common on farm procedures on fish and the use of environmentally friendly, plant-based anaesthetics "green anaesthetics" in managing stress in farmed fish, and their impact on the physiology of farmed fish. Results from these studies form the nucleus of this inaugural lecture.

2.0 INTRODUCTION

Aquaculture has expanded and intensified in response to an increasing demand for fish as a source of protein globally because of dwindling catch from capture fisheries (FAO, 2006). The estimated contribution of aquaculture to global supplies of fish, crustaceans and mollusks increased from 3.9% in 1970 to 27.3% in 2000 (FAO, 2002), making aquaculture one of the fastest growing food producing industry in the world (FAO, 2014). Besides, fish play important role in the nutrition of people in the Niger Delta region where aquaculture is intensively practiced (Akinrotimi, *et* al., 2007a; Gabriel *et al*., 2007a). However, intensive aquaculture involves manipulation of fish (Erondu *et al.*, 2007; Gabriel and Akinrotimi, 2011a) and other farm management procedures (handling, liming, confinement, fertilization, transportation and other operations) from the hatchery to the final commercial stage (Wearing, *et al.*, 1996*)*. According to Pickering (1981) these management procedures as crucial as they are, produce some level of disturbances, which can elicit a stress response leading to decreased fish performance (Maule and Shreck, 1990) and in extreme cases lead to mortality (Akinrotimi and Gabriel, 2012a). Infact, stress has been identified as the primary contributing factor of diseases and mortality in aquaculture (Petric, *et al*., 2006).

Fish under intensive culture conditions are exposed to a regime of acute and chronic stressors- chemical, biological, physical and procedural, with

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the last being the most prevalent; consequent of daily routine management practices in fish farming (Gabriel and Akinrotimi, 2011b; Huntingford *et al*., 2012). Fish response to stressors in aquaculture varies according to the source, effect, environment and nature of the stressor. However, the features of stress reactions are generally common to most forms of stressors in fish farm operations. Fish response to stress is an integrated reaction with behavioural, neural, hormonal and physiological elements all combined to provide the best possible chance of survival. Increased interest in understanding and predicting fish response to environmental stress has resulted in an expansive body of research related to stress biomarkers in aquaculture.

This lecture explores stress in fish, the biomarkers employed in assessing stress, especially in cultured fish species and how stress can best be managed for optimum yield in aquaculture.

3.0 CONCEPT AND MECHANISM OF STRESS

Stress is any condition that causes physical or psychological discomfort which results in the release of stress-related hormones or specific physiological responses (Foster and Smith, 2007). Stoskopf (1993) observed that stress was present virtually in the lives of all living organisms and can be described as the latent force that brings about physical, psychological and physiological changes and adjustments. Unfortunately the term “stress” is used inconsistently. It is sometimes taken as the environmental alteration (stressor) itself and at other times the response of the fish, population and ecosystem (Pickering, 1981). Selye (1950) defined stress as “the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of physical and chemical force”. But this definition according to Wedemeyer *et al*. (1990) does not consider the fact that the outcome of stress may be negative for an individual but positive for the population. For example, mortality of individual fish due to exhaustion from overcrowding may actually enhance survival of the population when space or food supplies are limiting.

According to Esch and Herzer (1998), stress then can better be defined as "the effect of any environmental alterations or force that extends homeostatic or stabilizing processes beyond their normal limits, at any level of biological organization". Furthermore, Barton (1997) defined stress as "the response of the cell or organism to any demand placed on it such that it causes an extension of a physiological state beyond the normal resting state". Conversely, stress in fish can be considered as a state of threatened homeostasis that is re-established by a complex of adaptive responses. If the intensity of the stressor is overly severe or long lasting, physiological response mechanisms may be compromised and it can become detrimental to the fish's health and well being, or maladaptive, a state termed "distress" (Barton, 2002). When an organism's capability to maintain homeostasis within a specific range is exceeded, responses are evoked that enable the organism to cope by either removing the stressor or facilitating co-existence with it (Antelman and Caggiula, 1990). Stress can be physical, psychological or environmental; short and sudden or long and chronic. Mild, short-term stress has few serious health effects, compared to long-term stress. However, short-term stress contributes to many of the diseases and deaths in aquarium fish (Gabriel and Akinrotimi, 2011b).

The basic idea of psychological stress is as old as the Greek classical philosophers. Epicurus suggested that coping with emotional factors was a way to improve the 'quality' of life (Johnson *et al*., 1992). But it was much later that these factors became fully integrated in the concept of stress. In 1859, Claude Bernard first introduced the idea of '*milieu interieur"* (internal environment) which had to be kept harmonious in response to the external changes (Johnson *et al*., 1992). Further exploring this, Walter Cannon developed the concept of **homeostasis**, a relatively stable condition by which the body maintains its internal balance. Threatened by any environmental stimulus, those of emotional nature, the body prepares for action through the activation of the sympathetic nervous system (Barton, 2002).

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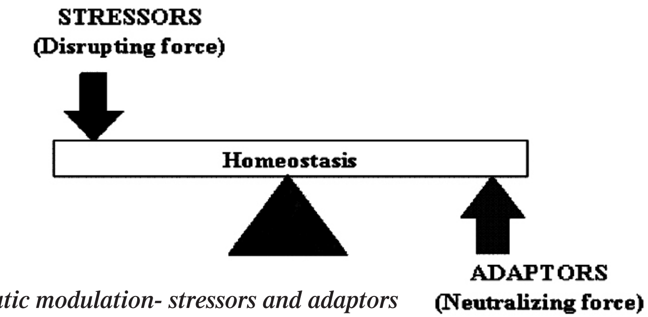

Fig. 1. Homeostatic modulation- stressors and adaptors

The preparation to the 'fight or flight' response mobilizes energy required to restore the threatened or effectively lost homeostasis (Fig. 1). Cannon's ideas, put forward in the first half of the century and never using the word 'stress', gave a major contribution to the understanding of homeostatic and stress mechanisms. Selye (1950) described his concept of stress and the '**General Adaptation Syndrome, GAS** that gave great relevance to the adrenal cortex response (Levine, 2005). This consisted of three stages, '**alarm**', in which the body mobilizes its defense mechanisms; '**resistance**' including various forms of tissue damage if the stressor persists; and '**exhaustion**', when the adaptive energy is depleted, potentially causing **death** (Fig. 2).

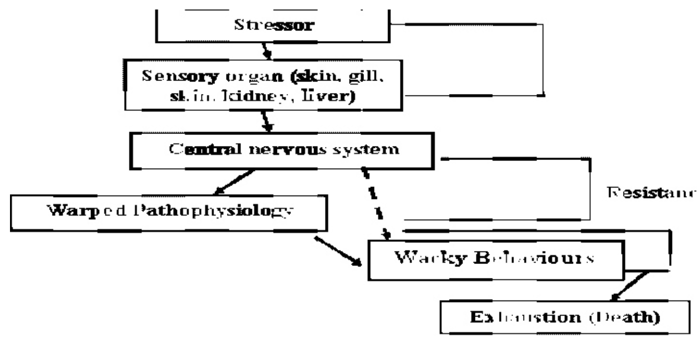

Fig. 2. Schematic presentation of stages of fish response to stress

Mason (1969) was the first to question the generality of the stress response and the lack of relevance to psychological factors in explaining it. He stressed the likelihood of a higher involvement of the central nervous system in what he called the "**emotional arousal to a threat**", which could possibly modulate the stress response (Veissier and Boissy, 2007). The contribution of Lazarus, in the second half of the twentieth century was a milestone to the modern concept of stress. Based on his work for the military in the Second World War, he claimed that individual differences in stress responses were based on the respective '**personal meaning**' attached to particular events. The concept of '**appraisal**' became a central piece of his work, a somewhat **'enriched'** perception also involving a personal evaluation, which is dependent on individual goals and beliefs (Lazarus, 1999). The appraisal process which detects the stimuli and prepares the body to react consists of two stages, the primary appraisal or the process of evaluating the personal relevance of a given stimuli, and the secondary appraisal or the process of assessing the available coping options. His contribution was highly founded in interconnected cognitive and emotional mechanisms, but he also acknowledged that some appraisals can be extremely rapid and unconscious, leading to fast coping responses (Lazarus, 1999). Other appraisal-related theories were developed, mainly differing on the types of criteria that contribute to the evaluation process (Levine, 1991; Désiré *et al*., 2002). More recently, Ursin and Eriksen (2004) presented the '**Cognitive Activation Theory**' involving neurophysiological activation and arousal in which stress is regarded as a healthy process, if not sustained over a long period of time. A major aspect of this theory is the assumption that the stress response depends on learned expectancies related to the stimuli and to the result of the available coping resources (Fig. 3). These expectancies are the major source of individual differences in the stress response (Eriksen *et al*., 2005; Gabriel and Akinrotimi, 2011b).

4.0 WELFARE AND RIGHTS OF FISH

Welfare issues in fish whether in the wild or captivity, are gaining more attention not only among scientists but more especially in aquaculture where fish are held in captivity against their will. This action could be described as infringing on their right to live in their natural environment. According to Mustapha (2013), fish prefer to live in their natural, unperturbed habitats where their needs are naturally provided for. For a successful fish production in culture media, the needs of the fish in culture must be provided. However, continued placement in rearing facilities despite provision of perceived needs might not necessarily provide and maintain their welfare needs and requirements, especially the five freedoms, in spite of the provision of good management. This is because stress factors which could impair their welfare are often found and practiced in aquaculture systems.

STRESSED FISH: Warped Biomarkers, Avoidable Mortality

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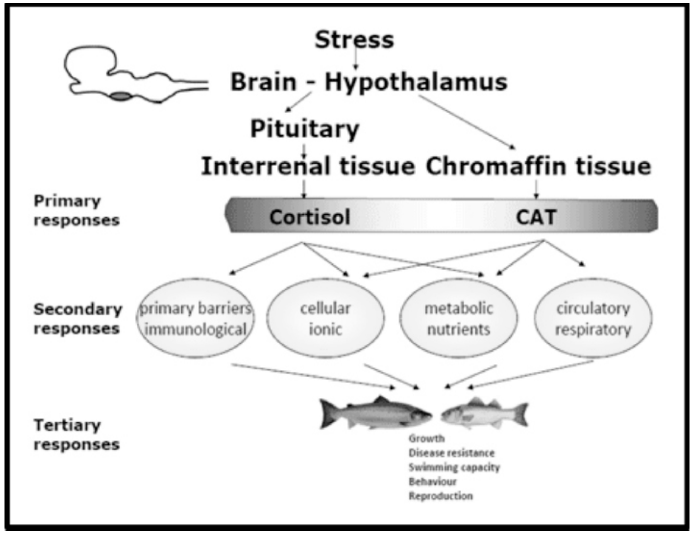

Fig. 3, Stress response in fish (Source,Algers et al., 2015)

Fish welfare depending on context (Huntingford, 2006, 2012) can be categorized as functions-, feelings (emotion)- and nature-based. In the function-based view, the fish is considered to have appropriate welfare if they have the freedom to adapt physiologically to the captive environment and maintain functional biological systems. It is the commonest concept in food production aquaculture where welfare is measured directly by productivity-based assessment, which is by monitoring mortality and growth rates or a wide range of physiological variables (Iwama, 2007; Segner *et al.*, 2012).

In the feeling-based concept (**affective, emotion state view**), fish's emotional concern in the culture environment is the main thrust. The fish besides being free from negative experiences should have positive environment/experiences like social companionship among others. Studies suggest that fish can experience pain-, fear- frustration- anxiety feelings and suffering (Volpato *et al*., 2009 Vindas *et al.,* 2012; Braithwaite, 2014; Maximino, *et al.,* 2010). However, the subject of suffering in fish is being debated among scientists (Braithwaite 2010; Rose *et al*., 2014). Several studies show that fish have functional pre-requisites to feel pains (Portavella *et al*., 2002; Sneddon, 2002; Sneddon *et al*., 2003a, 2003b). The use of physiological variables is less useful within this framework in comparison to feelings which can easily be assessed by indirect inference from behaviour (Huntingford *et al*., 2012; Martins *et al*., 2012) or by preference-based valuation (Volpato *et al.*, 2007). Besides, it is not clear how physiological alterations are reflected in fish behaviours. This framework appears to be the best approach for those concerned about fish welfare and rights. Fish deserve welfare attention especially those in aquaculture since captivity shortens the life span of animals (Algers *et al*., 2015; Chandroo *et al*., 2004).

Fish in the nature-based approach is offered an environment where it can exhibit natural behaviours as freely as possible. Attempts are made to make the culture environment more like the natural by introducing environmental enrichments, EE. Environmental enrichment is the improvement in the environment of animals in captivity. It is defined as the "animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being" (Shepherdson,1998). According to Young (2003) EE, depending on the goal it is commonly grouped as "(i) physical enrichment, including modifications or additions to the tanks, that is, structural complexity; (ii) sensory enrichment, which concerns stimulation of the sensory organs and the brain; (iii) dietary enrichment, encompassing type and delivery of food (note the distinction from nutrient enrichment, which concerns addition of

Table 1. The Five freedoms of animal welfare and the indicators for assessing welfare impairment.

S/N	Five freedoms of Animal welfare	Indicators
1.	Freedom from hunger and thirst	Feed intake, growth rates, condition factor
2.	Freedom from discomfort	Physical damage, fin condition lesions and immune responses
3.	Freedom from pain, injury or disease	Water quality monitoring
4.	Freedom to express normal behaviour	Abnormal behaviour, swimming and feeding behaviour, distribution of the fish within a system (e.g. dumping around inflows), responses of fish to an approaching farmer.
5.	Freedom from fear and distress	Measuring primary and secondary stress responses, Plasma, cortisol glucose, lactate, muscular activity

Source: FAWC, 1996.

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nutrients to the feed); (iv) social enrichment, adding contact and interactions with conspecifics, and (v) occupational enrichment, relating to reduction of physical and psychological monotony by introducing variation to the environment and possibilities for exercise and performance of preferred behaviours". Providing animals the possibility of choice of their environment may be beneficial by increasing their control (e.g. they can choose to avoid certain aggressive conspecifics), but it conclusive evidence of any benefit (Hutchinson, 2005). This can done through the provision of structures for shelters, reduction of aggression, sensory and cognitive stimulation, inducing environmental variability and unpredictability, tank covers (for shade and visual protection), tank floor substrates and organic aquaculture where the culture environment is designed with special regards to the species-specific needs of the fish, for example sufficient space for the fish wellbeing, species-specific lighting condition and other conditions considered relevant to the wellbeing of the fish (Gerber *et al*., 2015; Näslund and Johnson, 2016). According to Young (2003), the aims of enrichment to increase behavioral diversity, the range or number of normal (i.e. natural behavior patterns),; increasing positive utilization of the environment and increasing the ability to cope with challenges in a more normal way (Shepherdson 1989, as modified by Young 2003), and reduction of the frequency of abnormal behavior. Despite the advantages of EE, there are problems associated with their use.

The appropriate way to achieve the best welfare and health of fish in aquaculture is to respect, maintain and improve the rights of fish, otherwise known as the "five freedoms" of fish (FAWC, 1996; Table 1**)**. Noble *et al*. (2012) reported that a fundamental step in improving the welfare of farmed fish is to assess their welfare across a range of husbandry systems and farm practices which can be achieved through the use of practical and easily defined operational welfare indicators to provide an accurate, repeatable, straightforward and relatively inexpensive on-farm assessment of fish welfare.

According to Poli *et al*. (2005), the best strategy for a reliable assessment of fish welfare/suffering and their impact on product quality is a multidisciplinary approach that takes into account the main relative changes of significant indicators of behaviour, the biochemical and physiological *ante mortem* and/or *post mortem* processes involved and of the quality changes. It should be noted that different culture conditions exist for different species based on their differing biological and environmental requirements. Farmed fish that lack welfare could easily be noticed through deviations from their normal biological state based on the life history, species, genetic makeup, behavioural response and coping style (Fig. 4).

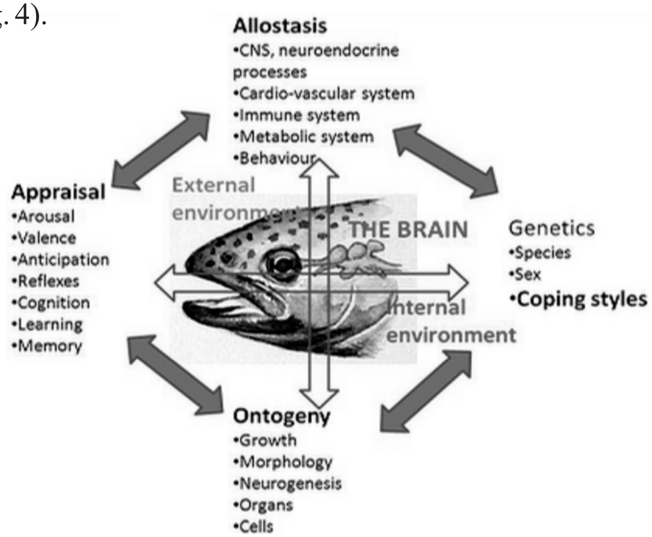

Fig. 4. Stress cycle in fish (Source, Korte et al., 2007).

5.0 SOURCES OF STRESS

Stressors are real or perceived challenges to an organism's ability to meet its real or perceived needs. Fishes are exposed to stressors in nature as well as in artificial conditions such as in aquaculture or in the laboratory (Iwama *et al.,* 2006; Gabriel and Akinrotimi, 2011b). Fish under intensive culture conditions are exposed to a regime of acute and chronic stressors, which have adverse effects on growth, reproduction, immunocompentence and flesh quality (Shreck *et al*., 2001). The most prevalent factors inducing stress in fish are the procedural stressors from daily management practices in fish farming (Table 2).

Table 2. Stressors common in intensive aquaculture

	Stressor	% of Occurrence
A.	**Chemical stressors**	
1.	Poor water quality (Low DO, improper pH)	20%
2.	Pollution	10%
	- Intentional pollution, efficient, wastes, sewage	
	- accidental pollution, spills, insecticide, pesticide	

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3.	Diet composition Imbalance diet	10%
4.	Nitrogenous and other metabolic wastes i.e accumulation of ammonia or nitrite	35%
B	**Biological stressors**	
1.	Population density over crowding	5%
2.	Social dominance	1%
3.	Micro organisms –pathogenic and non pathogenic	2%
4.	Macro organisms -internal and external parasites	2%
C.	**Physical Stressors**	
1.	Temperature	1%
2.	Light	1%
3.	Sounds	0%
4.	Dissolved Gases	1%
D.	**Procedural Stressors**	
1.	Handling/ Acclimation	3%
2.	Transportation	5%
3.	Sorting/Grading	3%
4.	Disease Treatments	1%

Source: Gabriel and Akinrotimi (2011b)

Acclimation

One of the production techniques commonly used in aquacultures is acclimation, a pre-conditioning for stocking of culture systems and experimental purposes. Acclimation therefore, is the modification of biological structures to minimize deviation from homeostasis despite change in environment and physico-chemical parameters (Akinrotimi *et al*., 2007b). It is a general practice to subject fish to be used in laboratory experiments to a minimum acclimation period of seven days. It is believed that during this period the fish may show symptoms of hidden disease that may assist in the separation of apparently healthy fish for any trial or culture. During the acclimation period, the fish may undergo some degree of stress similar to that characteristic of high density stocking in intensive culture systems. Such overcrowding conditions have the likelihood of increasing the incidence of diseases that may produce a number of measurable changes in the physiological processes of the fish (Anyanwu *et al*., 2007).

Artificial Propagation

The use of natural and synthetic hormones for induced breeding in fish cause stress to both the male and female fish. The sacrifice of the male fish to extract the pituitary gland compromises the welfare of the fish, while the anaesthetization and injection of the hormones to induce breeding (hypophyzation) causes much stress, pain and tissue damage. The foreign material (pituitary of another fish or synthetic hormone) introduced into the fish could cause physiological disturbance in the fish, thereby inducing stress. The forceful removal of the eggs and milt (stripping) is also stressful and the eggs and milt are extruded in excess of need and what would have been produced under natural conditions (Keremah *et al*., 2010a).

High stocking density

Stocking density in aquaculture refers to the maximum carrying capacity of the culture system. High stocking densities accompanied with intensive feeding results most often in poor water quality (Ezeri *et al*., 2003). This could lead to increased chronic stress, growth impairment and health problems such as high incidence of parasites and diseases, physical injuries such as fin erosion, poor body condition (Turnbull *et al*., 2005), increased aggression which leads to fin injuries, scale loss, chronic stress and subordinate fish being prevented from feeding by dominant ones; abnormal behaviours like jumping, etc. Carrying capacity is greatly governed by water quality and when it is approached or exceeded, may contribute to lowered metabolism, immunosuppression, increased disease transmission, and lowered survival rates (Gabriel *et al*., 2008a).

Inadequate food and improper feeding regimes

Lack of balanced ration in feeds and inappropriate feeding regimes could impair fish welfare. Diet, feeding technique and management procedures have serious effects on stress responses, subsequent stress tolerance, health and the occurrence of aggressive behaviours in fish (Ashley, 2007). Thus, formulation and composition of feed are important factors that impair fish welfare in aquaculture (Gabriel *et al*.,

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2008b). *C. gariepinus* is a nocturnal feeder, but are usually fed diurnally in aquaculture. This action often leads to reduced feed conversion efficiency thereby affecting fish growth (Mustapha *et al*., 2012). The use of unscientific feeding rates could compromise fish growth, while different feeding regimes have been shown to have a strong impact on the development of diseases in some fish species (Damsgård *et al*., 2004). Improper nutrition has been shown to compromise immune function and has been linked with skeletal deformities in cultured fish (Koumoundouros, *et al.* 1997; Fernández *et al.,* 2008). Feed distribution in a small area can cause competition and increase aggression among fish (Castro and Santiago, 1998) and that in turn could lead to growth variations, reinforcing dominance hierarchies.

Improper Handling

Fish handling is very common procedure in aquaculture. Constant handling of fish by catching, transferring, even with the use of nets, and transportation could cause panic in fish; alter the reproductive performance through stress, thereby impairing their welfare, health and productivity. Handling could even be stressful (Barcellos, *et al.* 2011) and even much more so in the tropics because of the high temperatures. Handling can also result in injury to skin, eyes, fins and muscles which could make the fish vulnerable to diseases (Gabriel and Akinrotimi, 2011b**).**

Withdrawal of Food

This occurs when the food supply is stopped for some period for the purpose of harvesting, transportation and disease treatment. Fasting could be detrimental to fish welfare and increases aggression. Food withdrawal has been reported to cause changes in territorial behaviour strategies and activity patterns in different species of cultured fish (Alanara *et al*., 2001). Fasting period should not exceed two to three days so that the gut is not completely evacuated to avoid a collapse of the gastro-intestinal tract.

Simulated Dark and Light Photoperiods

The use of the above to increase growth, enhance maturation or suppress breeding often impair fish welfare. This is because fish under this phenomenon are not naturally adapted to continuous darkness or light and thus, may undergo many physiological, morphological and behavioural changes that are stressful. *C. gariepinus* develops a very dark colouration on exposure to continuous darkness (Mustapha *et al.,*2012). Continuous light intensity could lead to continuous aggregation of fish at a particular place leading to higher fish density. Artificial photoperiods have been reported to affect the immune system of rainbow trout and hence their susceptibility to pathogenic microorganisms (Burgous *et al*., 2004). Also, high mortalities in juveniles of fish species such as Nile tilapia, *Oreochromis niloticus* and African catfish *C. gariepinus* have been recorded under photoperiod manipulations Mustapha *et al*., 2014. A rapid shift in light intensity should be avoided, as it can alter behaviour by invoking panic or predator type responses, and increase injury and mortality through unintentional collisions (Håstein 2004).

Poor Water quality

Fish generally depend on water for the exchange of gases and ions across the gills, and as a diluting agent for metabolic wastes. Yet, physical and chemical properties of water (water quality) are strongly influenced by the atmosphere, sediments, organisms and their wastes and the flow or exchange rate of a water body (Uedeme-Naa *et al*., 2010). Fish may incur additional energetic costs associated with stress responses from physical and chemical fluctuations in aquatic systems (Barton and Iwama, 1991). Poor water quality, as determined by each species, can prompt the reallocation of energy from secondary (non-essential physiological processes (e.g. growth, reproduction) towards primary (essential) processes (metabolism, immune function). Thus, adequate or preferably "optimal" water quality is essential for raising fish in an environment that will neither cause stress nor alter their normal energy budget. Short-term exposure to poor water quality can result in permanent damage or mortality if physical or chemical variables are allowed to reach lethal levels and/or synergize in a deleterious manner.

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Treatment of parasites and diseases

Diseases and parasites as well as their treatment severely impact fish health and welfare. The handling and various methods of treatment of these diseases and parasites such as immersion, vaccination, injection, etc. are usually stressful to fish. The vaccines and drugs used in the treatment could have their side effects on the fish.

Transport, Sorting and Harvesting

These procedures impact negatively on fish welfare by causing altered physiology (Sutphin and Hueth, 2015). Fish are harmed during capture and handling which result in increased cortisol which further elevates the stressful condition of the fish (Poli, 2009). Furthermore, there may be physical damage to body parts and depending on the severity of the injury, these could result in mortalities, especially during transportation. Besides, there is deterioration in water during transportation which may further stress the fish (Sampio and Freire, 2016). Sorting often facilitates transmission of disease among cultured fish. Net cleaning, channel flushing and pond draining during harvesting could be a source of stress to the fish occurring from events such as influx of suspended solids into the rearing facilities and high amounts of suspended solids can clog the gills leading to poor oxygen intake.

Influx of Toxicants

Pollutants such as pesticides, herbicides, domestic and industrial wastes from run-offs, discharges and drifts often find their ways into the ponds, thereby causing stress to the fish. These pose the problem of an ultimate dis-equilibrium in the natural ecological balance and in the culture medium. Under such conditions, the toxicity of a moderately toxic pollutant could be enhanced by synergism with other toxicants. Of particular concern in the Niger Delta region is pollution from petroleum exploration and exploitation (Davies, *et al.,* 2007; Howard *et al*., 2008, 2009a, 2011, 2012a, 2012b) with the resultant accumulation of hydrocarbon and trace metals in seafoods such as shell and fin fish to detrimental levels (Howard and Gabriel, 2012; Howard *et al.,* 2009b, 2012c, 2013). For example, 712.10μg/g and 415g/g of total hydrocarbon was recorded in the tissues of periwinkle, *Tympanotonus* fuscatus and mudskipper, *Periophthalmus barbarus* from one the oilfields. Aquaculture facilities are not spared from these sources of pollutants. Urgent steps must be taken by relevant authorities to protect the aquaculture industry. This can be made effective if backed up by relevant legislations that must be enforced.

Slaughter methods

Many slaughter methods practiced in aquaculture are stressful to fish, impair their welfare and have an effect on the flesh quality. Southgate and Wall (2001) observed that all slaughter methods are stressful to fish. The slaughter methods used include strangulation, hitting the fish with hard object on the head or cutting of the head region with sharp knife.

6.0 EFFECTS OF STRESS ON CULTURED FISH

Stress upsets the internal environment of fish resulting in disrupted fish physiology and energy balance (Fig. 5, Gabriel and Akinrotimi, 2011b; Orlu and Gabriel, 2011a). Scales and skin are the most commonly damaged by handling stress. This pre-disposes fish to pathogenic organisms. Stress weakens the defensive mechanism of fish (Fig. 6) and in extreme cases leads to mortality, which is more pronounced in the early life stages of fish (Table 3). Effects of stress may lessen with continued exposure but often manifests in retarded growth of fish.

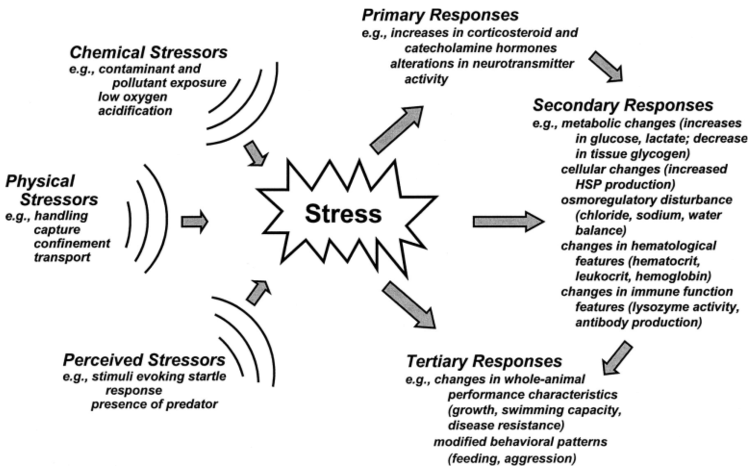

Fig. 5. Effects of physical, chemical and other perceived stressors on fish (Barton, 2002).

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(Physical, chemical and other perceived stressors on fish act on fish to evoke physiological and related effects, which are grouped as primary, secondary and tertiary or whole-animal responses. In some instances, the primary and secondary responses in turn may directly affect secondary and tertiary responses, respectively, as indicated by the arrows).

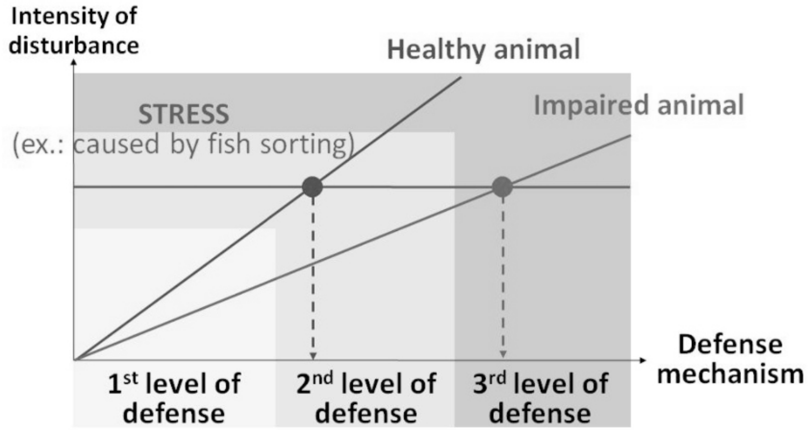

Fig. 6. Effects of stress on defensive mechanism in fish (Source, Techna Group, 2015).

Table 3. Effects of stress on different life stages of fish

Life stage	Stress Effect
Fry	Devastating, with high mortality
Fingerlings	Very fatal, with high mortality
Juveniles	Moderate mortality, reduced growth rate
Adult	Lesser effect compared to juveniles reduced growth rate
Brood Fish	Less gravid / reduced spermatogenesis

Source: Gabriel and Akinrotimi (2011b).

6.1 Warped Biomarkers

Biomarkers are biochemical, cellular, physiological or biochemical variation that can be measured in tissues or body fluid samples, at the level of whole organisms, to provide evidence of exposure or effects from one or more contaminants (Depledge, 1994). Contaminants effects at the lower levels of biological organization (biochemical, cellular and physiological) may occur more rapidly than at the ecological level and can be used as warnings for early detection of negative effects in the population. Various biomarkers ranging from physiological status to physical observations (behaviours) are used to assess the effect of contaminants on fish, which may differ with stressors and fish species.

6.1.1 Biochemistry

Physiological research in fish populations provides essential baseline data for monitoring fish populations over time, enables the rapid assessment of various natural and anthropogenic pressures, explains causal mechanisms, and facilitate evaluation of the efficacy of targeted actions. These tools are applied in cultured environment to understand the factors that influence stress responses in fish, thereby placing stress physiology in an ecologically relevant framework. In the wild and captivity, fish undergo a variety of stress, a source of discomfort or life threatening event. They may be real or not, immediate or anticipated, and even adjusted according to the individual perception of the animal. In any case, these challenging stimuli (stressors) demand a suit of responses (stress responses) which is the essence of coping and adapting to a given environment, essential for the organisms' survival. Animals unable to turn their response system on or off may suffer damages of various degrees (Sapolsky, 1992).

6.1.1.1 Glucose

Glucose is the simplest form of carbohydrate with a major role in the bio-energetic of animals which can be transformed to chemical energy (ATP) and in turn expressed as mechanical energy (Lucas and Watson, 1996). In stressful conditions catecholamine is released into the blood stream (Reid *et al*., 1998). These hormones elevate glucose production in fish through the gluconeogenesis pathways (Fig. 7). To cope with the energy demand produced by the stressor, the production of glucose is modulated by the activities of enzymes and hormones which stimulate gluconeogenesis, and also halts

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peripheral sugar uptake. Glucose is then released into blood stream and enters into cells through the insulin action (Iwama *et al.*, 1999).

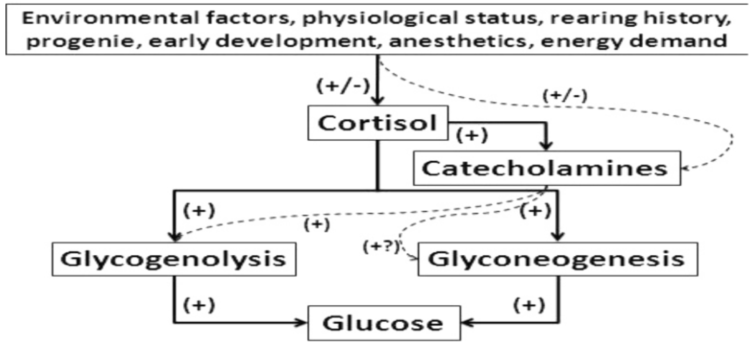

Fig. 7. Dynamics of cortisol and catecholamine in the production of glucose -(+) means positive modulation and (–) means negative modulation (Source, Martinez Porchars et al., 2009).

Plasma glucose concentration is the most commonly measured biochemical response to stressor in fish because it is easy and relatively inexpressive to measure (Ackerman and Iwama, 2001; Gabriel and Akinrotimi 2011c). The concentration of glucose in circulation depends on its rate of production and clearance from the blood stream. The production of glucose under stressful conditions assists the gills and muscles of the fish to cope with increased energy demand (Iwama *et al*., 2004). Fish liver is the main source of glucose production and is achieved by glycogenolysis. However, stress modulated actions such as handling, confinement, overcrowding and pollutions have been associated with increased in plasma glucose concentrations (Table 4, Gabriel and Akinrotimi 2011c).

6.1.1.2 Cortisol

Cortisol is the major glucocorticoid secreted by the interrenal tissues (steroidogenic cells) that are located in the head kidney of fish ((Iwama et al., 1999). The hormone is released by the activation of the hypothalamus pituitary-interregnal axis (HPI/axis). When fish undergoes stress conditions, the hypothalamus in the brain releases corticotropin-releasing factor (CRF) towards blood circulation (Fig. 8). This, further stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland which finally activates the release of cortisol by the interrenal tissue (Martinez-Porchas, et al. 2009). The amount released varies with species, type, and intensity of the stress and modulated by a number of unrelated factors (Table 5). Intrinsic factors such as heritability and extrinsic factors such as environmental and nutritional status have been reported to influence cortisol levels of fish during stress (Martinez-Porchas, et al. 2009). Jentoft et al. (2005) observed that differences in intensity of response might occur in cultured and wild fish. In most fish species during exposure to acute stress cortisol reaches the highest concentration 1 hour after being stressed, and returns to basal levels after 6 hours (Iwama et al. 2006).

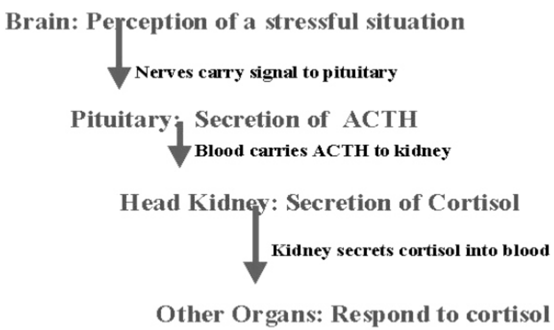

Fig. 8. Cortisol pathway in fish under stress (Anon.)

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EPILOGUE

In retrospect, I remember my class/school mates in the primary and secondary schools I attended in my community; in particular, the boy who taught me how to write on slate in primary school. I wonder where these bright students! Where are they? From among them were children of civil servants, teachers and others whose parents knew the value of education. My parents were fishers who were compelled to send me to school. Yet, I wonder, God in his great mercies picked me up and make me shine. Interestingly enough, it is this child of these lowly fishers who became the first Professor from Asarama Community in Andoni. This must be God at work. Hence, it is not of him that willeth nor of him that runneth of God that showeth mercy. My life is a clear testimony of what firm decision and determination to achieve, hardwork and faith in God can achieve, despite the individuals background your background must not keep your back on the ground. Standing before you is a product of God's grace. Therefore, to the only Wise God, Immortal, Invincible, Mighty and Glorious, we declare:

All the glory must be the Lord

For He is worthy of our praise

No man on earth

Should give glory to himself

All the glory must be to the Lord. Amen!

Table 4. Plasma glucose levels in selected species of fish before and after exposure to stress.

Species	Stressor	Cortisol (mg/l)		Exposure	References
		Pre-stress	Post-stress		
	Exposure outside water	2.10	10.21	Acute	
	Sorting	2.10	4.26	Acute	
C. gariepinus	Serial transfer	2.10	6.81	Acute	Akinrotimi *et al.* (2011a)
	Starvation	2.10	4.42	Acute	
	Over crowding	2.10	5.72	Acute	
T. guineensis	Handling	1.6	4.21	Acute	
Sarotheroon melanotheron	Confinement	1.9	7.01	Acute	Akinrotimi *et al* (2013a)
O. niloticus	Social stressor	1.8	6.7	Chronic	Barretto and Volpato (2006)
C. gariepinus	Salinity/Serial Transfer	65.75	69.07	Chronic	Gagnon *et al.*
Rainbow trout	Chemical/	5.1	7.2	Chronic	Gagnon *et al.* (2006)
	Sorting	5.75	62.40		
Sunshine bass	Temperature	6.1	10.5	Chronic	Davis and Peterson (2006)

6.1.1.3 Electrolytes

Electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity. The basic functions in the body are control of fluid distribution, inter- and extra- cellular acid-base equilibrium, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability (Bronzi and Alati, 2009). The major cations of the intracellular fluid in teleost fish are sodium Na^+, potassium, K^+ and Ca^{2+}; the extracellar anions include hydrogen carbonate (HCO_3^-) and chloride ($C1^-$). The regulation of the internal body composition is essential for normal cellular functions in all organisms. In sea and fresh water there is large osmotic gradient between the extracellular fluid of an aquatic organism and its environment (Davidson *et al.,* 2006).

Maintaining a relatively constant internal environment independent of the external environment is achieved through the combined actions of the gills, gut, and kidneys. The ionic and osmotic concentrations of the body fluids of fresh water are more concentrated than the surrounding environment and consequently, there will be an influx of water and an outward salt diffusion.

Table 5. Plasma cortisol values in selected cultured fish fish before & after stress.

Species	Stressor	Cortisol (nmol/l) Pre-stress	Cortisol (nmol/l) Post-stress	References
	Handling	18	121	
T. guineensis	Confinement	18	110	Gabriel and Akinrotimi (2016a)
	Acclimation	18	102	
	Crowding	18	142	
Sparus auratus	Crowding	13	358	Ortuño *et al.* (2001)
Oncorhynchus mykiss	Chemical exposure	49	110	Benguira *et al.* (2002)
Cyprinus carpio	Stocking density	19	206	Ruane *et al.* (2002)
Salmo salar	Confinement	27	151	Sadler *et al.* (2000)
Sarotherodon melanotheron	Handling	21	133	Gabriel and Akinrotimi (2016b)

Freshwater fish excrete excess water by the production of copious volume of hypotonic urine and salt losses are compensated for by active uptake of electrolytes via the gills. Electrolytes provide the driving force for many transport systems in the body and help the fish maintain a variety of osmoregulatory activities (Evans *et al.,* 2003). Many stressors affect the function of Na^+ transporter mainly because of its crucial role in the regulation of cellular Na^+ and K^+ gradients. In fishes, the major

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I wish to thank my parents, Gabriel Jonah and Laura Gabriel, my brother, Jonah Gabriel and sisters, Mabel, Haniel and Mary who stood by me throughout the challenges I met in life. Chief F. D. Abadi, Manoah Offiongwung and others encouraged my parents to send me to school. Felicia Uwem Gabriel, my beloved wife paid the utmost sacrifice, bearing patiently the long hours I spent in pursuit of my academdic goals. Awajiokinor (my only daughter), Awajiorionor, Awajioniror, Ugwem and Awajimaemi, my sons who "worked fish" with daddy are proudly acknowledged. My teachers and lecturers both at the under- and postgraduate levels are highly appreciated. Worthy of mention is C.B. Powell (deceased), the supervisor of my undergraduate work, who laid the foundation for all that I am in academics today. My family members, among who are Mr. Alexander Amah supported me in several ways in pursuit of my adademic career. I appreciate my academic colleagues without whom the dream of achieving this great height would have been impossible. I wish to gratefully acknowledge my great students I made; who in turn made made me. These are very special, among whom are Dr. Akinrotimi, Andrew Ojo who did the initial draft of this lecture and worked with me until it was completed; Drs. Okey Irom Bassey and Uedemena, Barikpoa, Dr. (Mrs) Nedie P. Akani among others with I whom I shared most exciting moments as we worked together, exploring various aspects of biomarkers of contaminant effects in fish and the use of "green"anaesthetics in the same. I am highly indebted to my Christian family, the Deeper Life Bible Church, where I serve as a Pastor, particularly the State Overseer, Pastor Edison Daminabo and other senior pastors who played very important roles in my spiritual sustenance, the basis for the significant progresss I made in all spheres of life. I thank every other person who has contributed in one way or the other to the success of this lecture. Finally, I thank every person who found time despite the busy schedule to grace this occasion.

packaged for both local use and export. This is one area where the Departments of Forestry and Environmental Management, Plant Science and Biotechnology, Animal and Environmental Biology and Fisheries and Aquatic Environment can collaborate.

2. The use of chemical anaesthetics on food fish should be discouraged or completely banned.

3. Commercially produced plant based anaesthetics could be important source of foreign earnings for the country. Besides, the use of plant based anaesthetics, which is easily biodegradable, will reduce drastically the environmental impacts of chemical anaesthetics as well as safeguard the health of consumers of fish as food.

osmoregulatory organs and tissues such as gills, kidneys, blood plasma and intestine show integration of osmotic function to maintain an optimal hydromineral balance.

The impact of pyrethroid insecticide, cypermethrin on the electrolyte balance of two important clariids, *H. bidorsalis* and *C. gariepinus* were studied under laboratory conditions (Gabriel *et al*., 2009a; Gabriel *et al*., 2012). In *H. bidorsalis* the Na^+ in the gill, kidney muscle; K^+ in the kidney, liver and muscle and Cl^+ in the gill ($p<0.05$) and muscle were negatively affected in comparison with control. However, $Cl^-$was higher in the kidney and liver ($p<0.05$) and K^+ in the gill than the control. The level Na^+ in the kidney, liver and muscle; K^+ in the gill, muscle, Cl in the liver and HCO_3^- in the liver and muscle were lower than the control values. K^+, Cl^- and HCO_3^- in the kidney, Cl^- in the muscle and gill were raised beyond the control values. Level of Na^+ in the kdney, liver, muscle; K^+ in the gill and muscle, Cl^- in the liver and HCO_3^- in the kidney of C. *gariepinus* exposed to cypermethrin declined with increase in toxicant concentration. However, there was elevation of the value of K^+, Cl^- and HCO_3^- in the kidney; Cl^- in the gill and muscle.

6.1.1.4 Enzymes

The changes in enzymatic response may alter the metabolic processes. More recently changes in enzymes concentrations are being employed in the evaluation of toxicological responses (Adams *et al.,* 1996) for rapid detection or to predict early warning of xenobiotic toxicity. They control the formation of biochemical intermediates essential to all physiological functions. The aminotransferases (alanine transaminase, ALT; aspartate transaminase, AST), phosphatases (acid phosphatase, ACP; alkaline phosphatase, ALP) and lactases are the most important group of enzymes in lower animals especially in the teleosts (Warshell, *et al.,* 2006). The transferases catalyze the interconversion of a keto acid into amino acid. The phosphatases are hydrolase enzymes responsible for removing phosphate groups and many types of molecules including nucleotides, proteins and alkaloids. Lactate dehydrogenase is responsible for converting muscle lactic acid into pyruvic acid, an essential step in producing cellular energy.

Pollutant contamination from run-offs and drifts, direct use of ichthyotoxins is commonplace and can affect the metabolism of cultured fish species, manifested by changes in the enzyme profile of impacted subjects. The summary of the findings of the studies (chronic) are presented in Table 6 and summarized below.

1. Roundup caused change ($p<0.05$) in the activities of AST, ALT and ALP activities. That of AST was six and two and half folds, respectively that of ALT and ALP at the highest concentration.

2. General inhibition of AST ALT activities in all the organs studied. ALP activity was reduced in the liver but excited in the kidney. Both excitation and inhibition occurred in the muscles.

3. Plasma ALT, ALP, ALT and LDH activities were generally inhibited ($p<0.05$) below their respective control without a direct relationship with concentration of extracts.

4. ALP, AST and ALT activities were inhibited ($p>0.05$) in the muscle, gill, kidney; AST, ALT in the liver, LDH in the muscle; ALP and LDH were either excited or inhibited in the liver; LDH was excited in the kidney under the effect of the plant extracts.

5. AST, ALP and LDH activities declined with increase in the level of dietary inclusion of cassava in the diet of *C. gariepinus.* The reverse was the case with ALT.

3. Measuring stress physiology in fish has the potential to improve assessment of environmental and human disturbance on cultured fish populations, distinguish the relative influence of stressors and monitor the response of targeted populations to management, mitigation, or recovery strategies. Understanding the mechanisms and context of stress is a fundamental ingredient to designing and implementing effective management strategies.

4. The broad range of physiological tools that have emerged in recent decades have powerful applications toward reducing the complexity of multiple interactions to identify most important stressors for fish. While physiology studies most often provide insight on the responses and condition of individual animals, understanding the physiological causes of stress and individual responses gives understanding of species level tolerances for natural stressors and further conservation and management efforts to mitigate anthropogenic disturbances and manage risk.

5. The indices of fish responses to stimuli varied in their responsiveness to various types of stressors in aquaculture. This is because the effect of different stimuli can occur in an organism through different physiological systems. Different stressor when affecting fish separately cause responses, which can be reversible when appropriate steps are taken to ameliorate the trend. However, it must be well understood that the response of fish to a stressor is a dynamic process that need to be seriously looked into by fish biologists and aquaculturists. If this is done properly, it will undoubtedly increase production and lead to sustainability of aquaculture industries in the world.

9.0 RECOMMENDATIONS

Since several studies have revealed the effectiveness of local plant materials as anaesthetics for fish, the following recommendations are considered very important.

1. For anaesthetics from plant sources to be effectively used in place of chemical anaesthetics, there is the urgent need to explore the variety of available plant materials as anaesthetics for cultured fish. Most of the plants with anaesthetics properties are termed “weeds”. However, these need to be identified, characterized, assessed, processed, produced in commercial quantities and

reported by Akinrotimi *et al.* (2014b) in *C. gariepinus* , where they discovered that the plant has anaesthetisc properties that could be utilized in aquaculture.

8.0 CONCLUSIONS

Plate 17. Indian Almond tree leaves

Having gone the culture lecture, the following conclusions can be made,

1. With the recent upsurge in the world population, especially in the developing countries and the corresponding increase in the need for cheap source of food to satisfy the protein need of the populace, coupled with the declining yield in capture fisheries, aquacultural practice has become massively intensive, as a veritable option in realizing this goal. This level of intensity which involves various fish handling procedures, translates to more stress for the fish.
2. To sustain and increase yields from aquaculture, there is the need to create the awareness and understanding of what constitutes stress in fish, notably in the area of physiological mechanism and responses that lead to changes in metabolism, growth, immune functions, reproductive capacity and normal behaviours.

6. Generally, nuracron depressed the activities of all the enzymes in the organs despite excitation and inhibition of activities recorded in ALP (muscle and gill) and ALT in the gill.
7. ALT activity in the gill and muscle, AST in the muscle and ALP in the liver tissues were either elevated or depressed. The activities of AST (kidney) and AST (gill) were excited; ALT (liver), AST (kidney and liver) and ALP (gill, kidney and muscle) were depressed.
8. Male gravid *C. gariepinus* exposed to leaf extracts had enhanced ($p<0.05$) ALP, ACP, AST ALT) activities in the treated group in comparison to the control. The elevation in the plasma was less compared to that in the liver.
9. Cypermethrin depressed the activities of all the enzymes in the organs below that of their respect control, However, among the treated groups there were differences ($p<0.05$) in the activities of LHD, ALT (gill), LDH, ALP (kidney, liver and plasma) and ALT in the muscle and plasma.
10. Gravid brood female anaethesized with metomidate had concentration- dependent increase in the activities of ALT, AST and LDH

Table 6. Enzyme activities in selected teleosts fish under stress

S/No	Species	Target tissue	Stressor	Enzymes assayed	Duration (days)	Reference
1.	*C. gariepinus*	Plasma	Roundup (glyphosate)	AST, ALT, ALP	70	Gabriel and George (2005)
2.	*Hybrid catfish*	Liver, kidney, gill, muscle	Leaf extracts*	AST, ALT, ALP	21	Gabriel *et al.* (2009b)
3.	*C. gariepinus*	Plasma	Leaf extracts*	AST, ALT, ALP,	14	Gabriel *et al.* (2009b)

S/No	Species	Target tissue	Stressor	Enzymes assayed	Duration (days)	Reference
				LDH		
4.	*C. gariepinus*	Kidney, Liver, gill, muscle	Leaf extracts*	AST, AL T, ALP, LDH	14	Obomanu, *et al.* (2009)
5.	*C. gariepinus*	Serum	Dietary cassava	AST, ALT, ALP, LDH	224	Abu *et al.* (2009a)
6.	*Hybrid catfish*	Kidney, Liver, gill, muscle	Nuracron	AST, ALT, ALP	23	Gabriel *et al.* (2010)
7.	*H. bidorsalis*	Gill, kidney, liver, muscle	Cypermethr in	AST, ALT, ALP	23	Gabriel *et al.* (2011a)
8.	*C. gariepinus*	Liver, plasma	Leaf extracts*	AST, ALT, ALP, ACP	30	Orlu and Gabriel (20011b)
9.	*C. gariepinus*	Gill, kidney, Kidney, Muscle, Plasma	Cypermethr in	AST, ALT, ALP, LDH	10	Gabriel *et al.* (2012b)
10.	*C. gariepinus*		Metomidate	AST, ALT, ALP, LDH	-	Akinrotimi *et al.* (2013b)

**L. alopecuroides*

males in fish and reduce stress in fish (Adebayo *et al.,* 2010 ; Akinrotimi, 2014; Gabriel and Akinrotimi, 2016c).

Plate 16. Avocado Pear tree leaves

Terminalia catappas, Fam. Combretaceae

Indian almond tree is widely distributed in both temperate and tropical regions of the world (Nantarika and Nongnut, 2008). In many parts of the world the leaves are widely used to treat hepatitis A and B, oral infection and intestinal ailments in children. The crushed leaves mixed with coconut oil are used to relieve muscle pain from fractures and sprains (Chitmat *et al.,* 2005). Characterization of the leaf extracts indicates it contains tannins, flavanoids, isovitexin and vitexin which are responsible for the anti-oxidants, anti-inflammatory, antifungal and anaesthetic properties (Ahmed *et al.,* 2005; Akinrotimi, 2014).

The leaves **(Plate 17)** contain substance that promote anaesthetics and wound healing in fish.,For example, in injured Siamese fighting fish (*Betta splendens*), after fighting matches (Chansue *et al.,* 2004) observed that it enhanced thicknening of the keratin layer of the fish scale. Chansue and Tangtronpirus (2006) reported water extracts of the dry leaves rapidly promoted regeneration of fin rays on the tail of common carp (*C. carpio*). Although, the plant have sedative properties, its use as fish anaesthetics was first

Twenty two compounds have been identified in the extracts of clove buds with eugenol and eugenyl acetate as the major aroma constituents. Essential oils are also obtained from the buds, stems and leaves (Bensky et al., 2004) The essential oil from clove seed is used as a topical application to relieve pain and promote healing in herbal medicine (Prashar *et al.,* 2006). The sedative and anaesthetic effects of the buds were investigated by Akinrotimi *et al.* (2015) and Akinrotimi *et al.* (2016) in African catfish (*C gariepinus*) and Tilapia (*Tilapia guineensis*), respectively with a wide range of effective dose, 215–275mgL^{-1}. Also, Akinrotimi *et al.* (2013c) reported that 100-150mgL^{-1} of clove seed extracts caused complete sedation in two species of mullets (*Liza falcipinnis* and *L. grandisquamis).*

Plate 15. Clove Seeds

Pyrus communis **(Avocado pear) Fam., Rosaceae**

Avocado pear is a common exotic plant native to Mexico and Central America (Plate 16). The fruit is an excellent source of monounsaturated fat. In addition, research has shown that avocado leaf extracts improved calcium absorption in rats and addition of avocado to salsa significantly improved lycopene, lutein and carotenes in humans (Ayub-Ali *et al.,* 2010). The leaves contain a fatty acid derivative known as persin, which induced a state of sedation and in higher doses can cause death. Avocado pear leaf has been shown to have anaesthetic properties and could be used to reduce stress and increase survival of gonadectomised

6.1.2 Haematology

Haematological techniques are employed as rapid tools in monitoring fish health in aquaculture and environmental perturbances (Bittencourt *et al.,* 2003; Akinrotimi and Gabriel, 2012a). Since fish are in intimate relationship with their aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in the fish (Ezeri *et al.,* 2004). Results from studies of fish blood suggest the possibility that blood will reveal conditions within the body of the fish long before there is any outward manifestation of stress or disease (Gabriel *et al.* 2004; Ezeri *et al.,* 2004). Haematological parameters have been employed in several cases, when clinical diagnosis of fish physiology was applied to determine the effects of external stressors. (Acker, 2000; Gabriel *et al.,* 2011a; Akinrotimi *et al.,* 2010a). It has been demonstrated that the use of haematological variables as indicators of stress from environmental contaminants can provide information on the physiological response of fish to a changing external environment (Gabriel and Kparobo, 2002; Inyang *et al*., 2010; Gabriel, *et al.*, 2011a; Hrubec and Smith, 2006; Akinrotimi *et al.,* 2010b).

Blood variables can provide important information about the internal environment of the fish. Thus, the evaluation of haematological characteristics in fish has become an important tool for understanding normal, physiological process and toxicological impacts of chemicals (Akinrotimi *et al*., 2013a) on fish. Typically, responses of haematological parameters to stressors are non-specific, however, toxic substances can significantly alter the haematological profile of fish (Akinrotimi *et al.,* 2012a; Akani and Gabriel, 2015) with serious implications for tissue metabolism (Asadi *et al.,* 2006; Akinrotimi *et al.,* 2010c; Gabriel *et al*., 2011b).

The effects of environmental stress and various on-farm procedures on the blood variables of cultured fish formed an extensive part of my studies (Table 7) and are summarized are as follows,

1. Acclimation of *C. gariepinus* and *S. melanotheron* caused either a reduction or rise in the value of the blood variables.

2. The health status of *C. gariepinus* (apparently healthy or sick), source (wild or cultured) influenced the haematological profiles of the fish.

3. In *S. melanotheron* and *O. niloticus* blood profiles, the females consistently had higher values of Hb, PCV, RBC and WBC with higher quantity of blood. Sex affected the responses of *S. melanotheron* to acclimation to fresh and brackishwater

4. Acclimation of *S. melanotheron* to higher should be done by gradual increment rather than abruptly because of the drastic change in the profiles when the latter approach was used.

5. Under exposure to chemicals (cypermethrin, glyphosphate and paraquat) the haematological characteristics of *C. gariepinus* had either concentration-dependent, variable or change in some or all of the variables.

6. Petroleum products (kerosene, water oil dispersion) depending on the concentration caused a decline in the value of the blood variables.

7. Industrial effluents cause a concentration- dependent decline in the blood variable of *T. guineensis* but not in *C. gariepinus* as a result of differences in the characteristics as well as the concentrations applied.

6.1.3 Histopathology

Several authors (Hughes and Perry 1976; Bennett *et al*., 1999; Fernandes and Mazon, 2003) have stressed on the indispensability of including histopathological studies in fish in all physiological pollution investigations. Study of endocrine disrupting substances according to Wester *et al*. (2004) shows that the pathology of fish and mammals are largely similar although class and species-specific anatomical, physiological and pathological need to be considered. The use of fish comes handy as an alternative test animal, for instance when aquatic studies are required, and as a

The roots has purgative, sedative and emetic properties and the major phytochemical constituents of the plant include alkaloids, glycosides, polyphenols and reducing sugar (Eteng *et al.,* 2009). The alkaloids have been reported to include reserpine, a well-known antihypertensive substance found in this plant (Amole and Onabanjo, 2004). Reports on the use of *R. vomitoria* as fish anaesthetic is scarce, except that by Omitoyin *et al.* (2010) and Akinrotimi (2014) in *C. gariepinus*, with safe and effective dose of 150ml/L without mortality.

Plate 14. Roots of *R. vomitoria*

Syzygium aromaticum (zobo spice)**, Fam, Rosaceae**

The plant commonly known as clove is one of the most widely used plants in Nigeria for medicinal purposes (Agbaje *et al.,* 2009). It is an evergreen tree that grows in warm climates and cultivated commercially in Tanzania, Kenya, Northern Nigeria and South America. The tree grows up to 20m and has leathery leaves (Adedapo *et al.,* 2004). Furthermore, the clove seed (**Plate 15)** is the dried flower measuring between 12 to 22 mm in length with four projecting calyx lobes folded to form a hood which hides numerous stammers (Fichi *et al.,* 2007).

7.7.2 Green anaesthetics, untapped goldmine

The recent awareness on safe aquaculture practices has resulted in a renewed interest in finding "green", environmentally friendly anaesthetics with low environmental and health risks. Also, conventional anaesthetics are expensive and not readily available in developing countries including Nigeria. Experimentation with various doses of crude extracts of sedative plant materials (Eze, 1991; Mgbenka and Ejiofor, 1998; Adebayo *et al.,* 2010; Agokei and Adebisi, 2010; Akinrotimi *et al*., 2013c; Gabriel and Akinrotimi, 2016c) indicates that these are viable alternatives to chemical anaesthetics for fish.

Plant extracts was first used by indigenous tribes of South America and now world over as fishing tools (Power *et al.,* 2010) and represent a potential source of new alternative anaesthetics in fish culture. Active ingredients in common plants used in fishery include sesquiterpenoids, furanocoumarin, terpenoids, quinine and triterpenes. However, plants most commonly used have rotenoids, alkaloids and sapponins (Helliwell *et al*.,1998; Akinrotimi, 2014). Rotenone is known to inhibit NADH–Q reductase in the mitochondrial electron transport chain, preventing the mitochondria from using NADH as a substrate. Electron transfer is virtually halted and the organism cannot produce an adequate supply of adenosine triphosphate, which results in asphyxia and paralysis, and in extreme cases death (Neuwinger, 1994).

Rauwolfia vomitoria (devil sizzle stick)**, Family- Apocynaceae**

The plant (Figure 14) is a rain forest shrub that grows in Nigeria having oval leaves with straight variation and cluster of tiny flowers (Akpanabiatu *et al.,* 2009). It is widely distributed in West and East Africa and members of the family usually have medicinal properties. The roots extracts are reportedly used in various medicinal purposes in many African countries. For example, in Nigeria, it is used by traditional healers in treating psychiatric patient, the Democratic Republic of Congo, it is used to treat leprosy. In Ghana it is used to treat athrithis (Opajobi *et al.,* 2011).

suitable second laboratory animal species for hazard identification. Hence, results from such studies give an insight into what pathological changes animals may undergo.

The target organs defined as the "biological organ most adversely affected by exposure to a chemical substance" (USEPA, 2006) mostly studied are the gill and liver because of their respective roles in gaseous exchange and in the metabolism and excretion of xenobiotics; while the other organs are sparingly studied (Heath, 1991; Hampton *et al*., 2006). Histopathological changes in fish in most cases tend to reflect the effects of long term chronic exposure to environmental contaminants (McCairns *et al*., 1988). Nonetheless, in some cases similar changes are recorded in a matter of hours or days depending on the type of pollutant and organ studied (Babatunde *et al*., 2001).

Table 7. Blood profile and effect of stress on some cultured fish species.

S/No	Species	Assessment/ Stressor	Response Variable	Duration (days)	Reference
1.	*C. gariepinus*	Crude *oil water dispers*ion	No change in Hb, PCV	3	Gabriel *et al.* (2001)
2.	(wild/ cultured)	Acclimation	Higher values	7	Ezeri *et al.*(2004)
		Acclimation	Sick, higher WBC	7	Gabriel *et al.*(2004)
3.	*C. gariepinus*	Paraquat (herbicide	Gradual increase	7	Gabriel *et al.*(2006)
5.	*S. melanotheron*	Profile	Higher in Female than male		Gabriel *et al* (2007b)
6.	*S. melanotheron*	Freshwater challenge	Reduction, except WBC	7	Gabriel *et al.* (2007c)
7.	*C. gariepinus*	Kerosene	Reduced Hb, PCV, WBC	14	Gabriel *et al.* (2007d)
8.	*S. melanotheron*	Acclimation (media)	Affected blood variables	7	Gabriel *et al.(*2007e)

S/No	Species	Assessment/ Stressor	Response Variable	Duration (days)	Reference
9.	*S. melanotheron*	Salinity challenge	Reduction except WBC	7	Anyanwu *et al* (2007b)
10.	*S. melanotheron*	Confinement	WBC raised	7	Gabriel *et al.*(2007f)
11.	*S. melanotheron*	Acclimation (sex/method)	Increase WBC	7	Akinrotimi *et al.*(2007b)
12.	*C. gariepinus*	Dietary cassava	No effect	224	Abu *et al.* (2009b)
13.	*C. gariepinus*	Leaf extracts*	Raised WBC, platelets	14	Gabriel *et al.*(2009c)
14.	*C. gariepinus*	Glyphosate	Variable response	70	Gabriel and Erondu (2010a)
15.	*T.guineensis*	Salinity challenge	higher WBC Variable response		Akinrotimi *et al.*(2012b)
16.	*C. gariepinus*	Cypermethrin	Concentration-dependent	10	Akinrotimi *et al.*, (2012a)
17	*C. gariepinus*	Rearing system (WRS)	Higher in females than males		Akinrotimi, and Gabriel. (2012a)
18	*H. bidorsalis*	Cymbush	Variable response	23	Gabriel *et al.* (2011c)
19	*T. guineensis*	Industrial Effluents	Reduction	15	Akinrotimi *et al.* (2013c)
20	*C. gariepinus*	Oilfield waste water	Increase in WBC, reduction in others	28	Akani and Gabriel (2015)
21	*C. gariepinus*	Dietary green leaf	Variable	112	Ariweriokuma *et al.* (2016)

Note: Full blood count done in all except No.1, WRS-Water recirclatory system

sockeye, *Oncorhychus nerka* (Waterstrat 1999; Chansdau *et al.,* 2002) and African catfish, *C. gariepinus* (Akinrotimi, 2014). The recommended concentration for anaethesia of juvenile tilpaia using eugenol is $175mgL^{-1}$ (Ribeiro, *et al.,* 2015), and *C. gariepinus,* 35ml/L (Akinrotimi, 2014).

Metomidate, a water soluble powder is used as sedative in humans as a sedative drug, but it is very expensive and difficult to obtain. Its use in aquaculture started about two decades ago and very effective in fresh and salt water species (Olsen *et al.,* 1995; Akinrotimi *et al*., 2013c). Efficient dosage ranges from 10 to 100ml/L and very large safety margin has been reported in *C. gariepinus* (Gabriel *et al*., 2011e; Gabriel *et al*., 2015), Atlantic halibut and salmon (Masses *et al.,* 1995) with no mortalities.

Sodium bicarbonate (baking soda) readily dissolves in water releasing carbon dioxide (Altun *et al.,* 2009) and it is used as an anaesthetic in temperate and tropical fish. It was first described as a fish anaesthetic by Fish (1942) and has been primarily used for sedation of fish during transport and handling of large number of fish (Bowser, 2001). There is no ban or restriction in its use and it is safe for human (Centinkaya and Sahin, 2005). It is introduced into the water either directly through an air store or indirectly by addition of sodium bicarbonate ($NaHCO_3$) as a source of carbon dioxide which releases carbon dioxide gas slowly (Prince *et al.,* 1995; Akinrotimi, 2014). Keene *et al.* (1998) observed that adult sockeye salmon (*Oncorynchus nerka*) was anaesthetized within 6mins, common carp, *C. carpio (*Juriaan *et al.,* 2000) and 12 mins in juveniles of *C. gariepinus* (Akinrotimi *et al.*, 2014a) in 4mins. Its effectiveness in *C. gariepinus* size-dependent (Aknorotmi, 2014).

and maximum safe concentrations were 40ml/L and 63ml/L, respectively.

Table 14. Selected anaesthetics, their optimum doses, induction and recovery times for cultured fish.

Anaesthetic	Species/Life stage	Dose	Induction time	Recovery time	References
MS-222	*C. gariepinus* Juveniles	200mg/l	<2min	<6 min	
	C. gariepinus Adult	200mg /l	<3min	<7min	Akinrotimi *et al*. (2012c)
Metomidate	*C. gariepinus* Adult	250mg/l	<5min	<10min	Gabriel *et al.* (2015)
Sodium bicarbonate	*C. gariepinus* Juveniles	200mg/l	<5min	<10 min	Akinrotimi *et al*. (2014a)
	C. gariepinus Adult	200mg /l	<6min	<12min	
Eugenol	*C. gariepinus* Juveniles	200mg/l	<2min	<8 min	Akinrotimi, (2014)
	C. gariepinus Adult	200mg /l	<3min	<10min	
Clove seed extracts	*C. gariepinus* juvenile	200mg/l	19mins	233 mins	Akinrotimi *et al.* (2015)
	C. gariepinus adult	150mg/l	122mins	137mins	
Clove oil	*O. mykiss* (30-33cm)	150mg/l	44mins	363mins	Perdikaris *et al.* (2010)
	Gold fish (11-25cm)		117-119mins	257-381mins	

Eugenol is an active ingredient of clove oil with concentration of 90-93% by volume. It is commonly used as an analgesic and antiseptic agent in human dentistry and as a food additive for flavouring and it is extremely safe for humans (Zahl *et al.,* 2009). Eugenol is rapidly absorbed and metabolized after oral administration and it is almost completely excreted in the urine within 24 hours with no apparent side effects (Fichi et al., 2007). Thus, eugenol has long been considered safe for laboratory use (Fisher *et al.,* 1990) and effectively used as anaesthetic in a number of fish species including medaka (*Oryzias latipes*); common carp (*C. carpio*), rabbit fish (*Signus lineatus*);

Gabriel (2006) reported the effects of acute and sublethal levels of roundup (glyphosate) on the histopathology of the liver, gill, kidney, heart, small and large intestine (spleen in adult) of fingerling and adult *C. gariepinus*, respectively, under laboratory conditions. The intensity of the histopathological changes in the organs were generally concentration-dependent. The major changes (**Plates 1- 11**) recorded in the organs as indicated,

1. In the liver there was mild to severe pigmentation of the melanomacrophage centres, MMC; mild to moderate hepatocyte necrosis, hyperaemia and haemorrhages.

2. Severe degeneration and necrosis of tubular epithelial cells, moderate to severe hyperaemia/haemaorrhage, pigmentation of MMC, mononuclear leucocyte infiltration of peri-glomerular areas in the kidney.

3. Mild/moderate hyperplasia of lympho-myeloid tissues, hyperplasia/proliferation of reticular network, mild/moderate pigmentation of MMC, reduced size of lympho-myeloid tissues due to necrosis in the spleen of adult fish.

4. Mild/severe atrophy of secondary lamellae, proliferation of mucus cells which declined with increase in concentrations, moderate/ severe hyperaemia, fibroplasia of primary and secondary lamellae in the gills.

5. The heart had mild haemorrhages within epi- and myocardium, severe necrosis of myocardial fibres, moderate/ severe vascular degenerative necrotic changes.

6. Extensive oedema of submucosa/mucosa with mild/ moderate fibroplasias, moderate necrosis of mucosa glandular cells, moderate hyperplasia with mild haemaorrhages in the stomach.

7. Increased goblet cells/necrosis of lymphoid/goblet cells, degeneration of mucosa lining, mild hyperplasia, moderate hyperaemia, exudation of oedematous fluid into muscular layer of small intestine.

8. Tubular cells of kidney and liver of *C. gariepinus* (**Plate. 12**) exposed to kerosene had moderate to extensive necrosis, hepatocellular steatosis and hyperaemia of central vein; liver had necrotic hepatocytes (Gabriel *et al.* 2007d).

9. There was hyperaemia of central vein and sinusoid, concentration–dependent degenerative changes (hypertrophic, necrotic, completely fused, atrophied or dystophied secondary lamellae) in the same fish exposed to kerosne (Gabriel *et al.* 2007g, **Plate 13**).

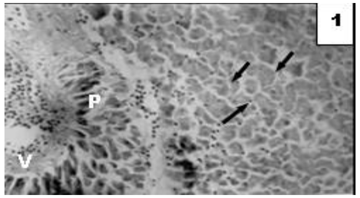


Plate 1. Section of liver of adult *C. gariepinus* in the control- normal hepatocytes in tubular pattern (**arrowed**); pancreatic tissue, **P,** around the portal vein, **V.**

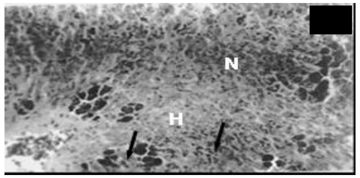


Plate 2. Section of liver of adult *C. gariepinus* exposed to 6.0mg/l Roundup for 70 days- widespread necrosis of the hepatocytes, haemorrhage, **N** and pigment deposition at the melano-macrophage centre.

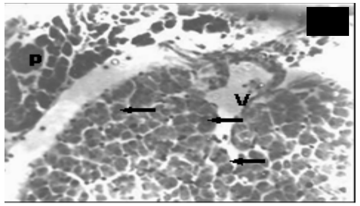


Plate 3. Liver from *C. gariepinus* fingerlings in the control- normal hepatocytes in tubular pattern (**arrowed**), pancreatic tissue, **P** around the portal vein (**V**).

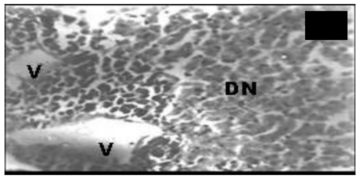


Plate 4. Liver from fingerlings exposed to 16 mg/l Roudup for 96hrs- hepatocytes degeneration and necrosis, **DN.**

7.7 Use of anaesthetics

Increased concern about animal welfare and the pain that fish may experience during routine manipulations in culture medium has necessitated the search for ideal and appropriate anaesthetics (Table 14, Akinrotimi *et al*., 2014a). Uses of anaesthetics enhance safety for both the fish and the handler during aquaculture operations for easy manipulation, with minimal stress for the fish. During surgical procedures, it minimizes movement and physiological changes in response to nociception (Ross, 2001; Mylonass *et al*., 2005; Akinrotimi *et al*., 2013a). It also reduces excitement and hyperactivity-related trauma that can occur during routine handling and thus, mortality and morbidity. Reduction in physical activity minimizes integument damage, associated osmoregulatory disturbance and reduces metabolism, resulting in decreased oxygen demand and production of less wastes.

Anaesthesia is a non-specific word used to describe a broad range of states. Its simplest definition, according to Brown (1993) is absence of sensation, or artificially induced inability to feel pain. Drugs that that cause local and general anaesthesia may have the same or similar properties as general anaesthetics (Smith *et al.,* 2009). Local anaesthetics act locally on the nerves and muscle at the site of application, whereas general anaesthetics act on the nervous system. Generally, anaesthesia in fish offers three separate effects, analgesics (the inability to feel pain), narcosis (unconsciousness) and skeletal muscle relaxation (Iversen *et al*., 2009; Perdikaris *et al*., 2010).

7.7.1 Synthetic anaesthetics

Many first generation chemical anaesthetics are currently not in use due to health hazards, poor efficacy and adverse physiological effects on fish and the handler. Second generation anaesthethic are the chemicals such as metomidate, benzocaine, sodium bicarbonate, eugenol quilnaldine and tricaine methane sulfonate (MS-222) have been extensively used in aquaculture practice (Tsantilas *et al.,* 2006). Currently, only MS-222 (an isomer of benzocaine) which is more soluble in water is licensed for use in food fish culture in the USA and the European Union (Congleton, 2006). It has rapid efficacy, with an induction time as short as 30 seconds depending on species and fish size. In African catfish, *C. gariepinus,* Akinrotimi *et al.* (2012c) reported that the effective

Table 12. Cleaning routine for fish farming tools/systems.

S/No.	Equipment/material	Frequency of cleaning
1.	Earthen pond bottom	Desilt pond bottom once a year after harvesting.
2.	Tank bottom	Regular removal of debris depending on the age of the fish.
3.	Nets	Regular washing and drying of nets.
4.	Farm tools	Regular washing and cleaning of tools used in the farm.
5.	Tanks	Tanks should be washed at least once in every th ree months.
6.	Farm surrounding	Farm environment should be cleared and cleaned regularly.

Source: Gabriel and Akinrotimi (2011b)

7.6 Slaughter method

Slaughter method should inflict minimal pains and suffering on fish which reduce flesh quality. Sharp knives should be used to slaughter fish to ensure less pain. Fish should not be crowded before slaughter for more than two hours (Line and Spence, 2005) since it affects flesh qaulity. Sharp knives, chilling and automatic percussive stunning devices and other innovative methods such as electronarcosis or electrocution that will not require removing the fish from water are better options. A pre-slaughter sedative known as AQUIS, commonly in use in many countries is recommended (EFSA, 2004).

Table 13. Effective methods of transporting fish with minimal stress.

S/N	Mode of transportation	Species	Remark
1.	Oxygenated bags	*T. guineensis* *S. melanotheron*	Very effective
2	Calcium carbonate	*C. gariepinus, O. nilotius*	Effective
3.	Common salt	*Clarias spp. Tilapia spp.*	Less effective
4.	Air conditioned vehicle	Brackish water species T.*guineensis* *S. melanotheron*	Very effective
5.	Early morning transport	Common carp, Tilapia spp. *Clarias* spp. *Heterobranchus* spp.	Effective
6.	Late evening transport	Common carp, Catfish, Tilapia spp.	Effective

Source: Field survey (2015).

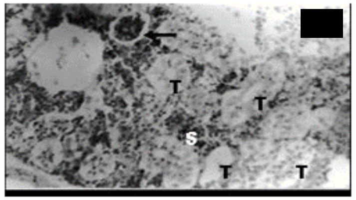


Plate 5. Kidney of adult fish exposed to 3mg/l Roundup for 70days- normal glomerulus (**arrowed**), severe degeneration of tubules, **T**; mononuclear leucocytes/ erythrocytes in the interstitices, **S.**

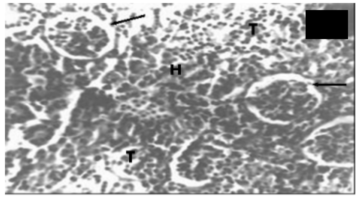


Plate 6. Kidney of fingerling exposed to 16mg/l roundup for 96hrs- normal glomeruli (**arrowed),** hyperaemia and haemorrhages, **H** in interstice, necrosis of the renal tubules with leucocytes infiltration.

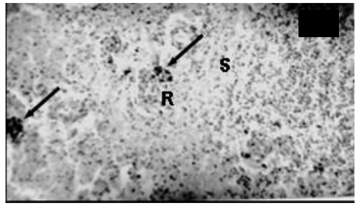


Plate 7. Section of spleen of adult exposed to 4.5mg/l Roundup- proliferation of reticular fibres, **R**-depopulated splenic pulp, **S** and decreased pigment deposition on the melano-macrophage aggregations.

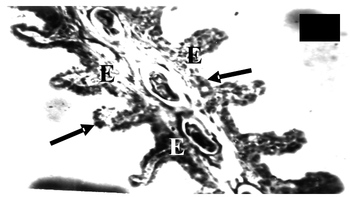


Plate 8. Gill section from adult exposed to 6.0mg/l roundup- oedema of the primary, **EP** and secondary, **ES** lamellae; severe atrophy of secondary lamellae (**arrowed**), hyperaemia of lamella capillaries and necrosis of epithelial cells.

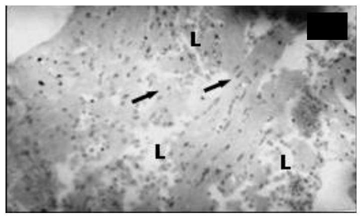
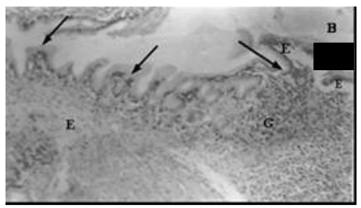


Plate 9. Section of heart of adult fish exposed to 4.5mg/l Rounudup- inflammatory leucocytes, **L** as component of oedema fluid between myocardial muscle fibre bundles.



late 10. Stomach of fingerling exposed to 18mg/l roundup for 96hrs- degeneration and necrosis of mucosa epithelial cells (**arrowed),** oedema of mucosa.

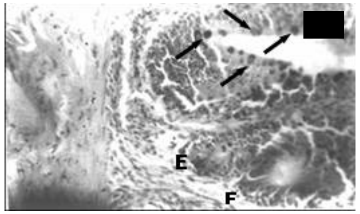


Plate 11. Intestinal section of adult fish exposed to 6.0 mg/l for 70hrs- Necrotic goblet cells (**arrowed**) and oedema, fluid in the submucosa containing leucocytes

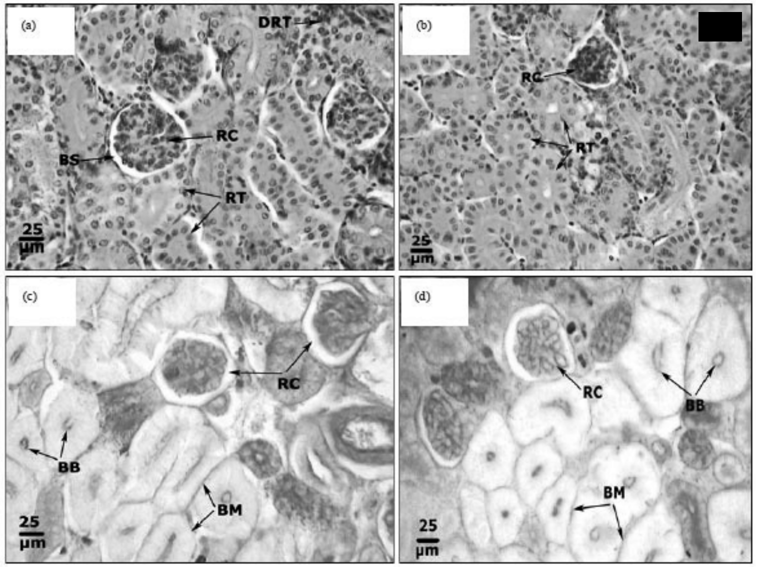


Plate 12. Cross sections of liver from *C. gariepinus* exposed to various levels of kerosene for two weeks. (a) Control, (b) 75 (c) 150 and (d) 300µl/l A (control)-hepatocytes in cords (c), blood sinus (**bs**) and central vein (**cv**). Note the uniform morphology of the nuclei and areas with mild to moderate steatosis. B- Degeneration of cords of hepatocytes and severe necrosis of hepatocytes (**sn**), pycknosis and karyolysis of nuclei and as well as hyalination of hepatocytes (**sh**). Note narrowing of sinusoid channels. C-Hepatocelluar vacuolation (**hv**) and hypertrophy (hh). D-Hepatochellular steatosis, hyperaaemia of the central vein (**H**) and sinusoid (**h**). Note the rounded vacuoles and peripherally displaced nuclei. H and E stain. 200x

Table 9. Ranges of water quality values for optimum growth of selected cultured fish species.

Species	Temp (°C)	Dissolved Oxygen (mg/L)	pH	Salinity (ppt)	Ammonia (mg/l)	Nitrite (mg/l)
Catfish	15-35	3-10	6-8	0-0.5	0-0.03	0-0.6
Oreochromis niloticus	15-35	4-10	6-8	1-5	0-0.03	0-0.6
Tilapia guineensis	15-38	4-10	6-8	1-29	0-0.03	0-0.6
Mullets	8-35	5-10	6-8	0-75	0-0.03	0-0.6
Carp	15-35	3-10		0-17	0-0.03	0-0.6
Tropical Ornamentals	10-35	4-10	6-8	0-0.5	0-0.03	0.00

Source: Boyd (2000)

Table 10. Stocking density of some cultured fish in earthen & tank culture systems

Species	Earthen pond (No. of fish/m^3)	Concrete tank (No. of fish/m^3)
C. gariepinus	30-80	20-40
Tilapia guineensis	30-100	10-30
Heterotis niloticus	30-40	5-10
O. niloticus	30-80	10-30
C. carpio	30-40	20-30
Sarotherodon melanotheron	30 100	10 30

Source: Gabriel and Akinrotimi (2011b)

Table 11. Feeding regime for catfish

Production Stage of	Weight Range (g)	Daily Allowance Body weight(%)	Feeding Frequency (Times/day)	Pellet Size of feed (mm)
Post fry	0.4 - 1	6 -10	3-6	0.5 - 0.8
Fingerlings	1.5 - 3.5	5	3	1 - 1.2
Post fingerling	5 - 10	5	3	2
Early juvenile	10 - 25	5	3	2
	25 - 100	4	3	3
Middle juveniles	100 - 300	4	3	4
Late juvenile	300 - 500	3	3	6
Adult	500 - 1000	2	2	6 - 8
	>1000	2	2	8 - 9

Source: Modified after Adesulu (2001)

Table 8. Behavioural alteration and mortality in early life stages of aquatic organisms under toxicant stress.

S/No.	Species	Stress	Behvavioural change/ mortality	96hrLC$_{50}$$^{\#\#}$	Reference
1.	*C. gariepinus, O. niloticus,* Hybrid catfish	Fertilizer effluents	Yes/No for *C. gariepinus*		Ekweozor *et al.* (2001); Bobmanuel *et al.* (2006)
2.	*C. gariepinus*	Cypermethrin	Yes/Yes	0.034mg/l	Ezeri *et al.*(2004)
3.	*A. gambiae, C.quinquefacia-tus*	Leaf extracts**	-/Yes		Obomanu *et al.*(2006)
4.	*C. gariepinus/* Hybrid	Leaf extracts*	Yes/Yes	1.04mg/l	Gabriel, *et al.* (2008c)
5.	*C. gariepinus*	Leaf extracts*	Yes/Yes	0.65mg/l	Gabriel *et al.* (2008c)
6.	Hybrid	Leaf extracts	Yes/Yes		Gabriel and Okey (2009)
7.	*H. bidorsalis*	Agrolyser#	Yes/Yes	2340.91mg/l	Gabriel *et al.* (2009c)
8.	Hybrid catfish	Leaf extracts*	Yes/Yes	0.77mg/l	Gabriel *et al.* (2009d)
9.	Hybrid catfish	2, 4-D	Yes/Yes	130.71mg/l	Gabriel *et al.(*2010b)
10.	*C. gariepinus*	2, 4-D	Yes/Yes	165.36mg/l	Gabriel *et al.* (2010c)
11.	*C. gariepinus/ H. bidoralis*	Leaf extracts*	Yes/Yes		Keremah *et al.* (2010a)
12.	*C. gariepinus/* Hybrid	Dichlobenil	Yes/Yes	151.67/149. 33mg/l	Gabriel and Edori (2010a)
13.	Hybrid	Agrolyser#	Yes/Yes	2357.29m g/l	Gabriel and Edori (2010b)
14.	*C. gariepinus*	Glyphosate	Yes/Yes	19.58mg/l	Gabriel and Erondu (2010b)
15.	*C. gariepinus*	Salinity	Yes/Yes	Increases with age from 4.39ppt	Gbulubo *et al.* (2011, 2012)
16.	*H. bidorsalis*	Leaf extracts*	Yes/Yes		Okey *et al.* (2013)

Note, Fingerlings were used for the studies except, juveniles in No.2, eggs to fingerling in no. 14. Renewal bioassay used for studies except no.3, ** - *L. alopecuroides and Azarichtha indica,* * - *L. alopecuroides,* # - mocronutrient fertilizer ## -Concentration at which 50% of exposed organism died.

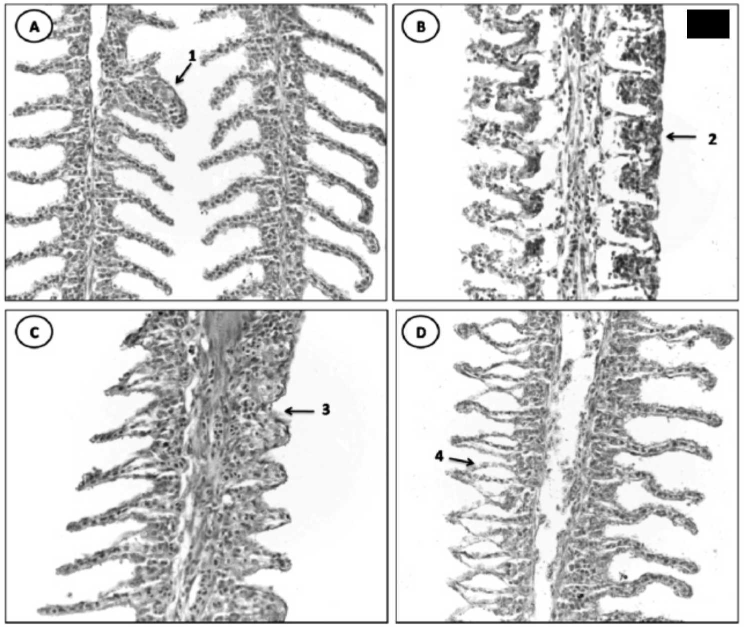


Plate 13. Sections of gills of *C. gariepims* exposed to various levels of kerosene for two weeks (a) Control (b) 70ppm, (c) 150ppm and (d) 300ppm nsl-normal secondary lamella, **asl**-atrophied secondary lamellae **fsl**-fused secondary, **de**-desquamated epithelia from primary lamella, oploedomatous primary lamella. H and E. 200x

6.1.4 Condition and Organ indices

In the aquatic environment, ichthyotoxic botanicals can affect the physiology of fish. This may be reflected by changes in the ratios of the weight of particular organs or tissues relative to total body mass (Goede and Barton, 1990). Besides, organ indices and condition can be used as indicators of change in nutritional and energy status of fish (Adams *et al.,* 1996). Commonly used organ indices include, hepatosomatic index (HSI), gonadosomatic index (GSI) and spleenosomatic index (SSI). The assumption that is generally made with these indices is that lower than normal values indicate a diversion of energyaway from organ or tissue growth in order to combat a stressor. The organ indices are calculated as percentages of body weight (Jenkins, 2004). Studies on the conditions of fish under environmental contaminants (Kleinhauf *et al.,* 1996; Jenkins, 2004) and disease state (Rehulka, 2003) were influenced negatively. However, organ indices and condition of fish under toxicant exposure in laboratory experiments are sparingly reported despite the roles such could play in assessing the general health of the fish under experimental conditions. Exposure of hybrid catfish (Gabriel, *et al*. 2010a) to leaf extracts (*L. alopecuroides)* produced a slight concentration-dependent increase ($p<0.05$) in organ weight mainly in the liver, whereas the pattern in the heart and kidney was not defined, $p>0.05$. The weight of the other organs were not affected ($p>0.05$) by the plant extracts. The organ indices of *C.gariepinus* in control did not differ ($p > 0.05$) from the treated group (Gabriel, *et al*. 2009c) in the SSI. Condition factor and HSI had variable responses relative to extracts concentration, while the heart had a very slight decline in size. RSI and SSI were raised with increased concentration. In another study Ariweriokuma *et al.* (2011) reported that cypermethrin caused a reduction ($p<0.05$) only in the HIS and RSI after 10 days of exposure suggesting that they could be good biomarkers of cypermethrin pollution in *C. gariepinus.*

6.1.5 Wacky Behaviours

Fish altered behaviours resulting from pollution exposure has been extensively reviewed (Gabriel, *et al.*, 2011d). Warner *et al*. (1966) were among the first to use fish behaviours as early warning of sublethal concentrations of pollution and hypothesized that behavioural variables will give an early warning to pollution and that behaviour is a comprehensive variable in the detection of effects of pollution since alterations in behaviour are the consequence of several biochemical and physiological alterations. The aim of studying behavioural effects of pollution is to identify stereotyped behaviours that easily and in a standardized manner can be used for detecting effects of pollution. Marcucella and Abrahamson (1978) defined behavioural toxicity as a "behavioural change, which is induced by stress that exceeds the

7.4 Good sanitation

It involves the routine removal of debris from fish tanks and regular disinfection of containers, nets and other equipment between groups of fish, especially after harvesting and before new stocks arrive (Table 12). Organic debris which accumulates at the bottom of tanks is an excellent medium for reproduction of fungal, bacteria, and protozoan agents, hence prompt removal will reduce the incidence of pathogen-induced stress. In the event of disease outbreak and death such should be removed immediately. Rearing facilities should be left fallow to break the cycle of potential hosts/vectors of harmful microbes or diseases that attack fish. Fish showing signs of disease should be quarantined or discarded to protect the entire population. The use of effective vaccination and improved management procedures will reduce the incidence of diseases and parasites in farmed fish.

7.5 Proper handling and transportation

Fish should be handled with wet hands quickly. Handling should be minimal before slaughtering. Where possible sedatives (anaesthetics) should be used and the fish returned to water with optimum dissolved oxygen. Salt (NaCl) could be added to the water during handling. Also, live fish must never be held by the opercula or tail during handling. During handling, grading and transportation, the fish should not be kept out of water for more than 15 seconds unless anesthetized (Line and Spence, 2005). To reduce stress due to transportation to the barest minimum, appropriate transportation methods should be employed (Aupérin and Baroiller, 2015). The container should be well aerated and shaded to ensure minimal variation. Fish should be transported very early in the morning or late in the evening. Air conditioned vehicles should be used over long distances to reduce mortality as a result of stress (Table 13).

7.2 Appropriate stocking density

To avoid unnecessary stress, fish should be stocked at appropriate stocking density **(Table 10).** The stocking rate for a fish species/life stage is the number of fish which a culture system can hold and maintain. Appropriate stocking density prevents the fish from overcrowding which often leads to struggle for food, oxygen and survival and ultimately culminates in stress. A good stocking density therefore considers space, behaviour, feeding, reproduction, health and normal biological expressions of the fish in culture. It should be monitored frequently to avoid exceeding the carrying capacity of the culture system. However, recent developments in aquaculture like water recirculatory system may help handle high stocking density (Gabriel, *et al.,* 2010d).

7.3 Balanced diet and good feeding practices

Fish should be fed with a high quality diet that meets their nutritional requirements depending on the species, age, size and production function **(Table 11).** Balanced and complete feed have been reported by Gabriel *et al*. (2000), as an appropriate way of minimizing nutritional induced stress in fish. Frequency of feeding is size-dependent. Small fish should be fed more frequently than the bigger ones with fish in tanks fed up to eight times a day. Those in ponds/juvenile cages up to 99g fish up to four times a day, and three times a day in production cages (fish, 100g and above). Feed quality test should be done periodically to check for formulation correctness. Physical checks for moulds, pellet size, feed dust, moisture content and foreign objects should also be carried out on stored feeds. A batch feeding system should be used to balance intake and rationing of feed where the particle size of the food is matched with the size of fish being fed (Inko-Tariah *et al*., 2001). Locally made feeds should be tested for quality control (Gabriel, *et al*., 2007i). Cultured fish should receive adequate quantities of feed appropriate for the species and growing conditions (Gabriel *et al.*, 2008a). Excessive feeding should be avoided to prevent water quality deterioration. The use of species appropriate feeding techniques (Table 11) can limit heterogeneous growth within a group of fish and thus the need for frequent grading (Gabriel and Keremah, 2003). Feeding regime should be species-appropriate to avoid excessive competition and aggression.

normal values of variability". Behavioural impairment according to Depledge (1985) is more sensitive than disability to pollutant effects, because deviations from normal behaviour in response to a specific pollutant are likely the result of physiological disorder that should provide a sensitive, early warning measure of sublethal toxicity (Rand, 1985). According to Robinson (2009) a major advantage of behavioural toxicity is that they are more sensitive indicators of potential impacts on survival in the field than are measures of mortality. Hence, they are important in ecological risk assessment (ERAs). It is believed that behavioural changes in fish are the most sensitive measures of neurotoxicity and this may have motivated the large number of psychophysiological studies on animals (Dutta *et al.,* 1994). Results from several investigations involving the use of several fish species support the concept that toxicant-induced stress on organisms can be quantified by methods other than mortality (Gabriel and Edori, 2010a, 2010b; Keremah *et al*., 2010b; Okey *et al.*2013). Hence, changes in fish behaviour can be used as sensitive biomarkers of acute, lethal and sublethal toxicant exposure.

Woltering (1984) observed that with early life stage toxicity test, growth response studies under the influence of zenobiotics could be eliminated from toxicity assessment of toxicants, the net result being a reduction in the time and cost of screening tests with no appreciable impact on estimating maximum acceptable toxicant concentrations (MATCs) for chemical hazard assessment. Hence, majority of the studies carried out were to assess the effects of toxicants on the behaviours (opercular and tail beat rates) of the early life stages of cultured species (Table 11). The findings from the various studies can be summarized as follows,

1. Fish exposed to toxicants before death followed four main phases in the responses as observed by Besch (1975), the contact phase (brief period of excitability), exertion (visible avoidance characterized by fast swimming, leaping, and an attempt to jump out of the toxicant), loss of equilibrium and lethal (death) phase, when opercular movement and response to tactile stimuli cease completely.

2. Results from several studies involving the clariids seem to suggest that changes in OBF and TBF of fish exposed to various toxicants were directly related to toxicant concentration but inversely related to duration of exposure.

3. Increased swimming and opercular ventilatory rates, lethargic response and loss of equilibrium which in most cases were concentration-dependent.

4. The behavioral changes exhibited by fish exposed to toxicant elicit the potency and sensitivity of the fish to the toxicants which can be used effectively as a biosensor of chemical stress.

5. Variable responses and cessation of TBF before OBF, and subsequent death may indicate the trend in available metabolic energy. This appears to be the usual trend in the responses of *C. gariepinus* to acute concentration of toxicants.

6. OBF appeared to be a more responsive variable in measuring behavioural toxicity in comparison to TBF in C. *gariepinus* exposed to toxicants. The OBF in these studies was usually raised, peaked and then fell with exposure duration for the various concentrations of the toxicants. Fish may have increased the TBF and OBF concurrently, but the latter particularly to increase the rate of water flow over the gills to enhance oxygen uptake from the water.

7. Exposed fish produced copious amount of mucus from goblet cells on the skin and gills as a barrier, but this has grave implications as it may impairs oxygen uptake.

8. The mortalities recorded for the species appeared to be influenced by the type of extracts (toxicants), exposure conditions, fish species and size.

9. The micronutrient fertilizer, agrolyser can be effectively used to enhance yields from aquaculture, since the 96hrLC_{50} for *H. bidorsalis* and hybrid catfish was 390 and 396 times respectively below the recommended application rate of 6mg/l in ponds.

10. When assessing the lethal concentration and safe concentration for it is important to calculate the MLT_{50} (the duration at which half of the exposed organisms may die at a given concentration). This is because toxicants have similar LC_{50} and safe concentration but different MLT_{50}.

11. In assessing toxicity of effluents, direct toxic method is preferred to the traditional as treated samples deemed to have meet set standards before discharged are toxic (Ekweozor *et al.,* 2001; Bobmanuel *et al.,* 2006) and may be toxic to aquatic faunas

12. Acute toxicity studies are useful to understand the level below which a toxicant may be considered 'safe' (the median lethal concentration, $LC_{50.}$ Although this varies under natural conditions, it has gained acceptance among toxicologists as it is most highly reliable cost effective means for assessing the potential adverse effects of xenobiotics to aquatic life.

7.0 MANAGEMENT STRESS IN FISH- AVOIDING MORTALITY

Considering the devastating effect stress can have in cultured fish, the challenge before the aquaculturists is to develop strategies that will maximally reduce stress to enhance sustainable aquaculture production. Aquaculture practices, the strategies for handling acute and chronic stress may differ. Mortality in the fish stock suggests presence of stress that needs to be identified and remedied. This can be done in one or more of the following ways or combination of the options,

7.1 Maintenance of good water quality in culture medium

Good water quality involves preventing accumulation of organic debris and nitrogenous wastes, preventing ammonia build up, maintaining appropriate pH and temperature for the cultured fish **(Table 9).** This can be achieved by the use of environmentally friendly diets (Akinrotimi, *et al*., 2007c; Gabriel, *et al.,* 2007h). It also involves most importantly maintaining dissolved oxygen levels of at least 5mg/L.