CHAPTER ONE

INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world. Worldwide, people obtain 25% of their animal protein from fish and shellfish. Stress has been linked as the primary contributing factor of fish disease and mortality in aquaculture (patric *et al* 2006) . Of all water quality parameters, which affect fish, ammonia is the most important factor after oxygen (Francais-Floyd and Waston (1996). Ammonia is the principal nitrogenous waste product of fishes that represents 60% to 80% of nitrogenous excretion of fish (Handy, and Poxton, (1993 Salin and Williot,1991) . It is also, the main nitrogenous waste material excreted by gills beside urea and amines as an end product of the protein catabolism (De Croux, *et al,* (2004) Under intensive rearing conditions, and particularly when effluent is reused, ammonia concentrations may reach levels that limit fish survival and growth (Haywood 1983).

Ammonia can cause reductions in growth or even death (EPA 1998) United States Environmental Protection Agency) In water, total ammonia consists of non toxic (ionized ammonia) referred to as ammonium (NH^{+}_{4})and toxic un-ionized ammonia (NH_3). The equilibrium between these two forms is pH and temperatures dependant (Agricultural Water Use (1996). Ammonia is measured as total ammonia nitrogen (TAN) which represents the sum of $NH^{+}_{4}$and NH_3, The NH_3 molecule is soluble in lipids, It is 300 to 400 times more toxic than NH_4^+ (Haywood (1983), Thurston, *et al* (1981) , Un-ionized ammonia (UIA-N) can readily diffuse across the gill membranes due to its lipid solubility and lack of charge (Aysel . and Koksal , 2005).

Ammonia accumulates to toxic levels, fish cannot extract energy from feed and will fall into a coma and die (Hargreaves, and Tucker, 2004).

Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage (Joel, and Amajuoyi , 2010), Also it can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart, 1978). Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia, muscle depolarization, hyper excitability, convulsions and finally death (Ip, Chew, and Randall, (2001).

Toxicity of ammonia to fish has been intensively investigated in numerous fish species (Aysel and Koksal (2005) *et al*).

The acute and chronic toxicities of ammonia have been reviewed for fresh water species (Handy, and Poxton, (1993, *et al*)

Ammonia is toxic to a variety of aquatic organisms including fish (Harris *et al.*, 1998) , Un- ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in liquids. It can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart, 1978).

Ammonia is the principal nitrogenous waste product of fishes and is normally oxidized first to nitrite then to nitrate, It is also the main nitrogenous waste material excreted by gills beside urea and amines. Moreover, creatine, creatinine, and uric acid are being excreted through the kidneys (De Croux *et al*., 2004), Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill

hyperplasia, muscle depolarization, hyper excitability ,convulsions and finally death (Ip *et al*.,2001).

Dissolved oxygen (*DO*) concentration is a common indicator of the health of the aquatic ecosystem. *DO* was originally modelled in the Ohio River (US) by Streeter and Phelps (1925), Since then a number of modifications and extensions of the model have been made relating to the number of sinks and sources of *DO* being considered, and how processes involving the nitrogen cycle and phytoplankton are being modelled.

Dissolved oxygen (DO) can be analyzed by two main methods: the Winkler L.W. (1888) or iodometric method ,. and the electrometric method using membrane electrodes.

The choice of procedure depends on the interferences present, the accuracy required, and convenience or expedience (APHA , 1992).
The dissolved oxygen content in water decreases as temperature and salinity increase (APHA ,1992). Dissolved oxygen further decreases with an increase in altitude or decrease in atmospheric pressure (Barton and Taylor , 1994).

In most toxicity studies, acute toxicity data are expressed in concentrations that are lethal to 50% of the organisms in 96 hours. However, few toxicity studies concerning dissolved oxygen reported these LC50 values as was previously indicated by Chapman (1986), The data summarized by Doudoroff and Shumway (1970) were derived from highly variable test procedures, differing in duration, exposure regime and reported endpoints (Doudoroff and Shumway, 1970; Chapman, 1986).

Effective management of DO is a key factor in the operation of commercial Recirculating aquaculture systems RAS, Generally intensive

RAS attempt to maintain system DO at 100% saturation to optimize growth and system performance (i.e. biofilter operation), at higher levels of saturation, loss of DO to the atmosphere can be significant (Parker *et al* 2002).

There is a direct relationship between oxygen consumption of fish, feeding and growth rate. If oxygen is not at near saturation levels, growth rates will be reduced, extending grow-out time and thus reducing potential profit. Another advantage of using pure oxygen is the reduction in pumping costs (i.e. operating, pipe and plumbing size) by delivering water at levels of saturation greater than 100%. The overall size (i.e. buildings, tanks) of the RAS may also be reduced using oxygen, providing further savings during construction.RAS tend to be divided into two levels of intensification based upon methods of oxygen supply:

• Low-density systems (<30 - 40kg/KL) provide oxygen requirements through aeration i.e. oxygen from air), supplied by air blowers and re-aeration components (i.e. diffusers, air lifts, re/degassers etc).

• High-density systems (>60 - 100+kg/KL) receive oxygen as pure oxygen from either liquid oxygen stored on-site or an oxygen generation system.

- As previously described, biological filtration and decomposing of wastes and uneaten feed within RAS impact upon oxygen demand, and needs to be incorporated into the oxygen budget for any system. A rule of thumb is that for each kilogram of feed added, approximately 0.50 - 0.56kg of oxygen will be consumed by fish and bacteria (Losordo *et al* 1992; Parker *et al* 2002), Use of oxygen in RAS should be as efficient as possible. Oxygen transfer devices will be either of an open un-pressurised type such as low head oxygenators, fine diffusers in culture tanks

and packed columns; or closed pressurised types such as U-tubes and oxygenation cones. The design of a RAS should include consideration of the type of

- hatchery and growout
- breeding
- long-term holding
- short-term holding
- display

- Each system type will have its own biosecurity challenges, and not all of the recommendations described in this publication will be practical or feasible in every situation. Understanding general biosecurity principles.

Objectives

- To determine the amount of ammonia in the water in case of feed and without feed.
- Estimation of Dissolved Oxygen in the water for fed and un feed tilapia fingerlings *(Oreochromis niloticus).*
- To determine the amount of ash content on the flesh.

CHAPTER TWO

LITERATURE REVIE

2.1 Taxonomy

Nile Tilapia : *Oreochromis niloticus*

Species: *Oreochromis niloticus* (Linnaeus 1758)

Family: Cichlidae

Order: Perciformes

Class: Actinopterygii

The Nile tilapia *Oreochromis niloticus* is a deep-bodied fish with cycloid scales. Silver in colour with olive/grey/black body bars, the Nile tilapia often flushes red during the breeding season (Picker & Griffiths 2011) It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) (FAO 2012). The average size (total length) of *O. niloticus* is 20 cm (Bwanika *et al.* 2004).

2.2 Natural distribution and habitat

O. niloticus is native to central and North Africa and the Middle East (Boyd 2004) It is a tropical freshwater and estuarine species. It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation (Picker & Griffiths 2011).

2.3 Biology

2.3.1 Diet and mode of feeding

Nile tilapia are known to feed on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films (FAO 2012) and even other fish and fish eggs. Depending on the food source, they will feed either via suspension filtering or surface grazing (GISD 2012), trapping plankton in a plankton rich bolus using mucus excreted

from their gills (Fryer & Iles 1972). *O. niloticus* have been observed to exhibit trophic plasticity according to the environment and the other species they coexist with (Bwanika *et al.* 2007).

2.3.2 Growth

Nile tilapia can live longer than 10 years (GISD 2012). Food availability and water temperature appear to be the limiting factors to growth for O. niloticus (Kapetsky & Nath 1997). Optimal growth is achieved at 28-36°C and declines with decreasing temperature (Teichert-Coddington et al. 1997, FAO 2012). The ability to vary their diet may also result in variation in growth (Bwanika et al. 2007). In aquaculture ponds, O. niloticus can reach sexual maturity at the age of 5-6 months (FAO 2012).

2.4.Water quality in aquaculture

The critical parameters are temperature, suspended solids and concentrations of dissolved oxygen, ammonia, nitrite, carbon dioxide and alkalinity. However, dissolved oxygen is the most important and critical parameter, requiring continuous monitoring in aquaculture production systems.

Water quality is the first most important limiting factor in pond fish production. It is also the most difficult production factor to understand, predict and manage. Water is not just where the fish live. Its quality directly affects feed efficiency, growth rates, the fish's health and survival. Most fish kills, disease outbreaks, poor growth, poor feed conversion efficiency and similar management problems are directly related to poor water quality.

a successful commercial aquaculture enterprise depends on providing the optimum environment for rapid growth at the minimum cost of resources and capital, Water quality affects the general condition of cultured organism as it determines the health and growth metabolic

activities conditions of cultured organism . Quality of water is, therefore, an essential factor to be considered when planning for high aquaculture production, Although the environment of aquaculture fish is a complex system, consisting of several water quality variables, only few of them play decisive role. This is due to fact that fish aerobic metabolism requires dissolved oxygen (Timmons *et al*. 2001).

2.5 Managing the Water Quality Parameters

2-5.1 ammonia

Ammonia is produced by deamination of ingested proteins and is the main nitrogenous waste product of fish. Ammonia can also be introduced into aquatic systems by industrial, municipal, and agricultural sources. Ammonia is typically oxidized to nitrite and then to nitrate by microbial metabolism. Both ammonia and nitrite can be toxic to fish, but toxic concentrations vary among species (Tomasso , 1994).

Ammonia is important to aquaculture because fish are generally cultured at high densities and fed high protein diets. Ammonia is known to reach lethal levels under normal aquaculture conditions (Tomasso 1994). Ammonia exists in an ionized (NH^{+}_{4}) and un-ionized (NH_3) form. Un-ionized ammonia is generally associated with ammonia toxicity because it can freely diffuse across gill membranes into the plasma (Russo and Thurston ,1991).

The proportion of total ammonia-N (TAN) in the un-ionized (UIAN) form increases with temperature and pH (Colt and Tchobanoglous 1976; Russo and Thurston , 1991; Randall, 1991; Tomasso , 1994) therefore, TAN levels should be maintained at lower concentrations for higher temperature and pH levels (Tomasso 1994). The

96 hour median-lethal (96 hr LC50) ammonia concentrations for shortnose sturgeon *Acipenser brevirostrum* was determined to be 149.8 mg/L for TAN and 0.58 for UIAN (Fontenot *et al*. 1998).

Tilapia have a 96 hr LC50 concentration of 2.40 mg/L for UIAN (Redner and Stickney 1979). According to Wise *et al*. (1989), the 96 hr LC50 for UIAN concentration for red drum *Sciaenops ocellatus* was 0.9 mg/L after 24 hr and 0.8 after 48 hr. The 96 hr LC50 for ammonia in white bass *Morone chrysops* fingerling was determined to be 0.63 mg/L (Ashe *et al*. 1996).

Ammonium salts and sulfates are abundant in the environment, Ammonium sulfate is a neutralization product of ammonia and sulfuric acid (WHO, 1986), Sulfate results from the oxidation of elemental sulfur, sulfide minerals, and organic sulfur, e.g. through the combustion of sulfur-containing fuels. Sulfates are found almost universally in natural waters at concentrations ranging from a few tenths to several thousand mg/l (EPA, 2002).

In the frame of the German water quality monitoring program ammonium nitrogen concentrations were measured, In 2000, the rivers Danube, Oder, Weser, Rhine, and Elbe showed concentrations (50th percentile) ranging from 0.04 to 0.07 mg/l Environmental Data Germany UBA, 2003). UBA (2003) states that in Germany 624 000 t ammonia were emitted to air in 1999. Livestock farming (ca. 83 %) and fertilizer use (ca. 12 %) are the main sources for the emissions, Industrial releases of ammonia were less than 3 %.

In the atmosphere, ammonia can react with sulfur dioxide to produce ammonium sulfate contained in atmospheric aerosols, These can return to the earth`s surface as wet or dry deposition (Scott and Cattell, 1979; Gmur, Evans and Cunningham, 1983).

This is considered to be a common protective phenomenon in ammonia exposed gills that prevents ammonia fractions from their further entrance in the gills.

However, the excessive coagulation of mucus over the gills impairs the respiratory function causing death by asphyxiation [Prasad, M. S. (1988), Solangi, and Overstreet, (1982) . This comes in agreement with other studies for fish inhabiting water impacted by domestic sewage (Gordon, *et al* (2000).

El-shafai *et al*. (2004) showed that the lowest-observable effect concentration on the growth performance is 0.144 mg/l UIA-N. Joel and Amojuoy (2010) recorded that level of un-ionized ammonia which is capable of killing fish over a few days start at about 0.6 mg/L. They recorded that un-ionized ammonia levels as low as 0.1 mg/L can cause gill and kidney damage and reduction in growth. They recorded that ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage.

El-shafai *et al*. (2004) reported that the toxic levels of un-ionized ammonia for short-term exposure lie between 0.6 and2 mg/L. They concluded that the toxic level of un-ionized ammonia and its negative effect on the growth performance lies between 0.07 and 0.14 mg/L. While, (Pillay, (1992), considered the maximum tolerance condition to be 0.1 mg/l. Joel and Amojuoy (2010) recorded maximum limit of 0.2 mg UIA-N/l for aquatic life. The results of (Joel, and Amajuoyi, (2010) , firm the present results where they recorded that ammonia concentration of above 0.2 mg/l in fish ponds has a tendency to harm the fish. Szumski *et al*. , (1982) suggested a non-effect concentration of 0.08 mg UIA-N could be applied to warm water fish without effect. Salin, and Williot, (1991) and EPA (United States Environmental Protection Agency) (1998) reported that ammonia can cause reduction in fish growth or even death.

They interpreted that the effect of ammonia on energy utilization may explain growth suppression in fish exposed to ammonia. Thurston *et al*. (1984) revealed no effects on survival, growth or reproductive capacity at concentrations 0.07 mg NH3/l for rainbow trout. Handy, and Poxton, (1993) reported toxic effects of un-ionized ammonia on aquatic organisms in the concentration 0.09 mg/l. Barnabe, (1994), proposed un-ionized ammonia concentration range within which no apparent damage for fresh water species is set at 0.01 mg NH3- N/l. Lamarie *et al*. (2004)] recorded reduced growth of sea bass when ammonia concentration increased.

EL-sherif, *et al* (2008) The results showed that growth performance was significantly ($P \leq 0.05$) decreased with increasing concentration of UIA-N. The feed conversion ratio (FCR) increased with increasing concentrations of UIA-N, the differences were significant ($P \leq 0.05$) among the high concentrations.

Saber *et al*.(2004) who showed that the lowest-observable effect concentration on the growth performance of Nile tilapia is 0.144 mg/l UIA-N and there was no significant differences between the mean individual weight of fish exposed to 0.068 mg/l UIA-N and control0.004 mg/l UIA-N. Atle *et al*.(2003 and 2004) , Sten *et al*. (2004) and Lemarie et al.(2004) reported that fish weight decreased when concentrations of UIA-N/l increased.

EL-sherif , *et al* (2008) reported that mean body weight gain per fish in the various treatment groups were significantly influenced by UIA-N concentrations and decreased with increasing levels of UIA-N. Similar results were obtained by Foss *et al*.(2002), Atle *et al*.(2003 and 2004), Lemarie *et al*.(2004) and Saber *et al*.(2004). Generally, mean body weight gain was significantly reduced in concentrations of 0.1 and 0.15

mg/l UIA-N compared to the control ones. This was attributed to a decrease in daily food consumption.

El-Shafai *et al*. (1995) studied fish production in sewage oxidation ponds and reported 100% mortality for silver carp, which was attributed to an un-ionised ammonia concentration of 0.41 mg N/l. Chronic toxicity of constant exogenous ammonia concentrations was studied in two different batches of turbot juveniles (Person-Le Ruyet et al., 1997a). they reported maximal survival up to 0.33 mg UIA-N l 1 while at 0.73 mg UIA-N l 1 50% mortality was observed. They estimated a 28-day exposure concentration that results in 50% of the SGR of the control between 0.6 and 0.75 mg UIA-N l 1. In three batches of trout exposed to chronic ammonia toxicity, Person-Le Ruyet *et al*. (1997b) reported no mass mortality up to 0.4 mg UIA-N l 1.

In treated sewage-fed ponds, tilapia suffered clear skin ulcers, necrosis and haemorrhage associated with mass mortality at 0.45 mg UIA-N l 1 (Nasr *et al*., 1998). On the other hand, no effects were observed on the growth or survival of fathead minnows exposed to 0.44 mg UIA-N l 1 but clear negative effects on growth and survival were detected at 0.91 mg UIA-N l 1 (Thurston *et al.*, 1986) and the same was observed for striped bass (Hargreaves and Kucuk, 2001)

Smith and Piper (1975) reported that the growth performance of trout exposed to 0.033 mg UIA-Nl 1, showed no change in growth rate within 4 months, however, there was a significant reduction after 6 and 12 months, Hargreaves and Kucuk (2001) proposed that the digestibility of dietary protein and the energy source might have been affected by un-ionised ammonia.

2.5.2 Production and excretion of ammonia in fish

Ammonia production from dietary protein is a major source of amino acids in animals. The intestines of carnivorous fishes are adapted to process diets that are high in protein and low in carbohydrate (Buddington *et al*., 1997),

Animals cannot store excess amino acids, unlike carbohydrates and lipids which can be stored as glycogen and triglycerides, respectively. Thus, dietary amino acids in excess of the amounts needed for growth and maintenance of protein turnover are preferentially degraded over carbohydrates and lipids in the liver (Campbell, 1991). For fishes with high-protein diets, their dietary carbon is extracted from the carbon chain of amino acids after the removal of the α-amino group. Several amino acids, including alanine, are converted to glucose by fish hepatocytes (French *et al*., 1981) and this process is regulated hormonally in much the same way as it is in mammals. Approximately 40–60% of the nitrogen intake from food is excreted within 24 h (Ip *et al*., 2004c; Lim *et al*., 2004b). In addition to diet, muscle proteins can act as a source of amino acids, which are catabolized for the production of ammonia can exit through an aquaporin channel (e.g., AQP8) as NH3, accompanied with proton (H+) transport through an H+ ATP or carbohydrates, in fasting fishes (Houlihan *et al.*, 1995). Under adverse environmental conditions where ammonia excretion is reduced, some fishes can reduce the rate of ammonia production from amino acid catabolism to slow down the build up of ammonia internally (Ip *et al*., 2001c, 2004a,b; Lim *et al*., 2001). During exercise or hypoxia, ammonia can also be produced through the deamination of ammonia produced within the mitochondrial matrix can exit the mitochondrion as NH4+ through a putative NH4+ transporter AMP in the skeletal muscle.

2-5-3 Ammonia Bio filtration

2-5-3.1 Aquaculture Bio filters

Recirculating systems must incorporate both solids removal and biological filtration into the water reconditioning process to achieve proper water quality for fish and plants (Harmon, 2001). Solids removal is accomplished when recirculating water passes through a material that intercepts suspended particles. A biofilter is simply a surface on which bacteria grow. Biological filtration can take place anywhere in the system where recirculating water comes in contact with a surface to which nitrifying bacteria are attached–this may include tank walls, interior surfaces of pipes and even plant roots. However, to provide sufficient bio filtration activity to maintain optimum water quality in intensive recirculating aquaculture, where (TAN) loading can be high, separate bio filter equipment is currently required. bio filters used in recirculating aquaculture are of two main types: fixed film (attached growth) and suspended growth where microorganisms are maintained in suspension (Gutierrez- *et al*, 2006). Suspended growth systems are not common because of their high level of management and reputation for instability. Thus, most of the bio filtration in recirculating systems are aerobic, fixed film bio filters.

These bio filters basically consist of a porous solid phase on which nitrifying bacteria grow and extract nutrients from water passing over this solid phase (Wheaton, 1993). Water may enter the bio filter from the top, side or bottom and exit from the bottom, side or top, depending on design location relative to the water level of the fish tank. There are four basic types of bio filter designs: submerged bed, rotating disc, fluidized bed, and trickling (Tetzlaff and Heidinger, 1990). Submerged bed bio filters are characterized by tank water being pumped through a medium that is

constantly underwater. The rotating disc bio filter consists of a series of parallel circular plates mounted on a shaft and rotating as a round drum with the lower part submerged and the upper part above water. The plates are the substrate the bacteria grow on.

In a fluidized bed bio filter, water enters the bottom of a medium containing cylinder under high pressure and exits out the top after being acted on by the filter. In trickling filters water enters the top and flows down through the medium and keeps the bacteria wet but never completely submerged. Trickling filters are maintained above the water level of the fish tank. Of all the water quality parameters which affect fish, ammonia is the most important after oxygen (Francis-Floyd and Watson, 1996). Ammonia is the main excretion product from fish and uneaten feed. It can quickly become a concern because of the buildup of un-ionized ammonia and nitrite, both of which can be toxic to fish at very low levels (Harmon, 2001;

McGee and Cichra, 2000) as discussed previously. Ammonia is usually not a problem if the biological filters are properly sized for the loading rate and carrying capacity and if adequate water flow is maintained (Fowler, *et al*., 1994). Hockheimer and Wheaton (1998) recommend that the system water move through the biological filter at least 2–3 times per hour. However, Rakocy. (1997) were unable to detect any difference in tilapia growth rate, total weight, or survival between water exchange rates of 0.55 and 1.25 times per hour. Perhaps both of these flow rates were too low to detect a difference. McGee and Cichra (2000) recommend a 3:1 fish tank to biofilter volume ratio as being a more than sufficient design for biofilters. The ammonia generation load is based on the fish feeding rate and could be assumed to be 10% of the protein in the feed becomes the ammonia-N generation rate (Timmons *et al*., 2002). The size of the biofilter depends on the amount of ammonia

added to the system which is closely related to the feeding rate and efficiency of food utilization .

Tetzlaff and Heidinger, 1990, Van Gorder (2000) indicated that feed levels change with fish size as fingerlings consume a much higher percentage of their body weight (5%–8%) than harvestable size fish (0.75%–3%). Another way to determine ammonia – N load is to consider that generally 2.2 to 6.6 kg of ammonia are produced for each 220 kg of feed. Thus, 220 kg of fish being fed 6.6 kg of fish feed per day (3% of body wt/d) produce 0.1 kg of ammonia per day. Chapman (2000) puts these feed levels at 6 to 15% of body weight for young fish (<25 g) and 1% to 3% of body weight forolder fish (>25 g).

Trickling biofilters provide nitrification, aeration, and some carbon dioxide removal in one unit (Losordo, *et al*., 1999). The main disadvantage of trickling filters is that they are relatively large and biofilter media are expensive. The quantity of bacteria available to oxidize ammonia is limited by the surface area of the biofilter medium thus an important factor in biofilter design is to get the maximum amount of surface area into a given volume (Harmon, 2001). However, when particle size is reduced, filter clogging may increase and the ability of oxygenated water to mix well within the filter decreases. Clogging of the medium may occur if the solids are not prefiltered. Volumetric nitrification rates of about 90 g total ammonia nitrogen (TAN)/m3 per day can be expected with trickling filters (Losordo *et al.*, 1999). When designing these filters into a recirculating system for nitrification (assuming 2.5 percent of the feed becomes TAN), a design criteria of 3.6 kg feed/day/m3 of trickling medium should be used. Based on these numbers, and a feeding rate of 3% of fish body weight per day, 1 m3 of trickling medium biofilter should support 120 kg of fish. With a carrying capacity at harvest of 60kg/m3, 1 m3 of trickling medium biofilter would

be required for every 2000 L of fish tank water, a 1 to 2 ratio. Increased removal of ammonia by a trickling biofilter was found with increasing concentrations of ammonia in pond water (Rijn and Rivera 1990) and removal rate was considered substrate-limited with respect to ammonia. Research on freshwater recirculating aquaculture biofilters should focus on cost competitiveness, low head and low energy use operation in support of large scale facilities (Gutierrez-*et al* 2006). The efficiency of biofilters need not be associated with use of expensive commercial biofiltration devices (Prinsloo, *et al*., 1999). When two types of trickling filters were compared, one containing PVC shavings (surface contact area of 1,220 m2), the other a more sophisticated commercially available biofilter made up of Siporax porous sintered.

2.5.3.2 Ammonia levels

The fish create and expel various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and faeces excretion; in addition some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons *et al*. 2002). Ammonia exists in two forms: unionised ammonia (NH3-N), and ionised ammonia (NH4+-N), the sum of these two is called total ammonia nitrogen (TAN). The relative concentration of ammonia is primarily a function of water pH, salinity and temperature (Pillay and Kutty 2005). The excretion of TAN by the fish varies depending on the species in culture. As a general rule, when 1.0 mg of oxygen per litre per minute is consumed by the fish, the fish can produce 0.14 mg of TAN (Timmons . 2002) and specifically for salmonids species, per 1.0 mg of DO consumed per liter they can produce 0.04-0.06 mg of TAN per liter (Aquafarmer, 2004).

2.5.3.3 Ammonia toxicity in fish :-

Ammonia is toxic to fish because of many reasons. At the organismal level, ammonia causes hyperventilation (Hillaby and Randall, 1979; McKenzie *et al*., 1993), hyper-excitability, coma, convulsions and finally death. It also affects the ionic balance in fish, because NH_4^+ can substitute for K^+ (Binstock and Lecar, 1969) in Na^+/K^+-ATPase and in $Na^+/K^+/2Cl^-$ cotransporter (see Wilkie, 1997, 2002 Person Le Ruyet *et al*., 1997). NH_4^+ can also substitute for H^+ in Na^+/H^+ exchanger (NHE), probably NHE2 and/or NHE3 (Randall *et al*., 1999). At the cellular level, ammonia can interfere with energy metabolism through impairment of the tri-11 , carboxylic acid cycle in fish by stimulating glycolysis though the activation of phosphofructokinase I, and thus interferes with energy metabolism (Arillo *et al*., 1981). In addition, NH_4^+ can substitute for K^+ and permeate plasma membrane through the background K^+ channel and affect the membrane potential. Smart (1978) suggested that the mechanism of ammonia toxicity in fish might be similar to that in mammals during hepatic encephalopathy. Several theories, i.e. glutamatergic dysfunction, glutamine accumulation leading to astrocyte swelling, and/or activation of NMDA receptors, have been proposed as mechanisms involved in chronic and/or acute ammonia toxicity in mammalian brains , Rose, 2002). However, it has been proposed recently that mechanisms of and defence against ammonia toxicity in brains of fishes with high ammonia tolerance could be different from those in mammals .

2.6 DISSOLVED OXYGEN (DO)

2.6.1 INFORMATION ON DISSOLVED OXYGEN

Of all life-supporting environmental constituents, oxygen is one of the most essential, In cells, oxygen stores and liberates the energy that drives vital processes of fish, crabs and shellfish such as feeding, growth, swimming and reproduction ,Low dissolved oxygen concentrations can increase mortality, reduce growth rates and alter the distribution and behavior of aquatic organisms, all of which can produce significant changes in the overall estuarine food web (Breitburg , 2002).

The EPA freshwater criteria document, published in 1986, stipulated five limits for dissolved oxygen effects on warm-water species (U.S. EPA 1986). To protect early life stages, the criteria include a 7-day mean of 6 mg liter-1 and an instantaneous minimum of 5 mg liter-1. To protect other life stages, additional criteria were derived.

These are a 30-day mean of 5.5 mg liter-1, a 7-day mean of 4 mg liter-1 and an instantaneous minimum of 3 mg liter-1. Some of the most sensitive survival and growth responses reported for warm-water species in the freshwater criteria document were for early life stages of channel catfish and largemouth bass, both of which are present in tidal-fresh habitats throughout the Chesapeake Bay and its tidal tributaries (Murdy *et al*. 1997)

Truelson (1997) provided a very extensive review of the physical and chemical characteristics of oxygen, and of the analytical methods to determine oxygen concentrations in water. Rather than repeating this information, the most relevant information on chemical and physical

characteristics is summarized below. For more information, the British Columbia criteria document should be consulted (Truelson 1997).

Fish compensate for hypoxia by several behavioural responses: increased use of air breathing, increased use of aquatic surface respiration (ASR), habitat changes, or changes in activity level (Kramer, 1987).

DO levels, although dependent on temperature, are often by themselves an important factor affecting the growth rate of fishes. Moyle and Joseph (1988) measured a significant reduction in growth rate and food conversion efficiency in juvenile largemouth bass (Micropterus almonides) when dissolved oxygen fell below approximately 5mg/l at 26ºC.Presumably, the reduced oxygen below this threshold precludes “extra” aerobic, energy requiring activities such as growth and reproduction above maintenance cost. Some fishes attempt to swim to more favourable environments. However, a report by Mallya (2007) shows that, the period of time during which the oxygen level drops below the required minimum level, will cause the fish to become stressed. It is this stress which causes fish death. More than that, fish reduce food intake, leading to a reduction in growth. Mallya (2007), again stated that, reproduction is inhibited, and both fertilization success and larval survival are compromised. When the oxygen level is maintained near saturation or even at slightly super saturation at all times it will increase growth rates, reduce the food conversion ratio and increase overall fish production. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased. However, fish is not able to assimilate the food consumed when DO is low (Mallya, 2007)

2.6.2 Analytical Methods :

Dissolved oxygen (DO) can be analyzed by two main methods: the Winkler or iodometric method and the electrometric method using membrane electrodes. The choice of procedure depends on the interferences present, the accuracy required, and convenience or expedience (APHA , 1992) the iodometric method is the most precise and reliable titrimetric procedure for DO analysis.

The method is based on the addition of several chemicals resulting in the liberation of iodine equivalent to the original DO content in the sample. The iodine is then titrated to determine the original DO content. Experienced analysts can maintain a precision of " 50 µg/L with visual endpoint detection and a precision of " 5 µg/L with electrometric detection (APHA , 1992). Various modifications of the basic iodometric method exist (APHA , 1992) to minimize the effect of interfering materials (such as oxidizing agents, reducing agents, or organic matter):

2.6.3 Tilapia (Oreochromis niloticus)

It is well known that tilapia can tolerate hypoxic and even anoxic conditions for short periods and are thus better suited than other species to hypereutectic conditions that may exist in static water aquaculture systems (Chorn *et al.* 2006). It is also known that fish found both in the tropics and temperate waters have incipient limiting oxygen levels which may occur as in hypoxia. Incipient limiting levels generally average at 73 mmHg (2.29 g/l at 28C for warm water fish and 90 mmg/l at 6.17 mg/l for cold water fish such as the salmonids) In reference to *Oreochromis niloticus,* studies have shown that the incipient oxygen requirements are between 1.39 mg/l -2,92mg/l.

Studies done by Tsadik and Kutty (1987) on the influence of ambient oxygen on feeding and growth in *O. niloticus* showed that experiments under the various oxygen regimes varied.

2.6.4 Function of fish gills

For most fish species gills work by a unidirectional flow of water over the epithelial surface of the gill, where the transfer of gases occurs (O_2 in, CO_2 out). The reason for this unidirectional flow of water is the energetic nature of the system. The energy that would be required to move water into and out of a respiratory organ would be much more than that used to move air because water holds low oxygen due to its low solubility (Groot *et al.* 1995).

2.6.5 Oxygen uptake in and carbon dioxide release from the fish

During respiration fish, like other animals, take in oxygen and give out carbon dioxide. The process is done by using gills in almost all fish although some can also use the skin and some have lung like structures used in addition to gills. When a fish respires, a pressurised gulp of water flows from the mouth into a gill chamber on each side of the head. Gills themselves, located in gill clefts within the gill chambers,

Of all life-supporting environmental constituents, oxygen is one of the most essential. In cells, oxygen stores and liberates the energy that drives vital processes of fish, crabs and shellfish such as feeding, growth, swimming and reproduction. Low dissolved oxygen concentrations can increase mortality, reduce growth rates and alter the distribution and behavior of aquatic organisms, all of which can produce significant changes in the overall estuarine food web (Breitburg , 2002)

2.6.6 low dissolved oxygen: historical and recently past

Dissolved oxygen levels vary naturally in lakes, estuaries and oceans over varying temporal and spatial scales due to many biological,

chemical and physical processes. In estuaries such as the Chesapeake Bay, freshwater inflow that influences water column stratification; nutrient input and cycling; physical processes such as density-driven circulation; and tides, winds, water temperature and bacterial activity are among the most important factors. These processes can lead to large natural seasonal and interannual variability in oxygen levels in many parts of the Chesapeake Bay and its tidal tributaries. Superimposed on this natural dissolved oxygen variability is a progressive increase in the intensity and frequency of hypoxia and anoxia over the past 100 to 150 years, most notably since the 1960s. This human-induced eutrophication is evident both from instrumental data and geochemical and faunal/floral 'proxies' of dissolved oxygen conditions obtained from the sedimentary record.

The instrumental record, while incomplete prior to the inception of the multi-agency Chesapeake Bay Monitoring Program in 1984, suggests that as early as the 1930s (Newcombe and Horne 1938) and especially since the 1960s (Taft *et al*. 1980), summer oxygen depletion has been recorded in the Chesapeake Bay. Officer *et al*. (1984), Malone (1992), Harding and Perry (1997) and Hagy (2002) provide useful discussions of the instrumental record of dissolved oxygen and related parameter such as chlorophyll a across this multi-decade data record.

2.6.7 Oxygen consumption (MO_2)

The oxygen consumption (MO_2) of fish is variable and depends on many factors such as temperature, MO_2 increases when temperature

increases. Body mass ,MO_2 has an inversely exponential proportion when the body mass increases. Feeding rate,MO_2 increases when the feeding rate increases due to the digestion of food. Growth rate has a directly proportional relationship with MO_2. Swimming velocity and stress levels increased stress levels may enhance the MO_2 of fish, The above factors are the most important that should be taken into account in any aquaculture system (Forsberg 1997, Timmons *et al*. 2002, Pillay and Kutty , 2005). The MO_2 of fish culture in tanks is calculated by the Fick equation, based on the DO concentration of the inflow and outflow water, the flow rate and the total biomass inside the tank, It is also possible to estimate oxygen requirements of fish based on feed intake.

2.6.8 Physical and Chemical Characteristics

Oxygen is the most abundant element in soils and plants (weight content is 49 % and 70 %,respectively: Bohn *et al*. 1979). In addition, oxygen is the most abundant element in water by weight (Truelson 1997). Divalent oxygen combines with two single valent hydrogen atoms to form the extremely stable water molecule, Under normal pH for surface water (4 to 10), water is stable in the redox intensity range of -10 to +17: beyond this range, water is reduced to H2 or oxidized to O_2, respectively (Stumm and Morgan ,1981).

The double-bonded, two-atom molecule in water is the form of interest here, described as dissolved oxygen in the remainder of this document. Dissolved oxygen is the most fundamental parameter in water: it is essential to the metabolism of all aerobic, aquatic organisms (Wetzel, 1975). Low levels of dissolved oxygen result in unbalanced ecosystems,

fish mortality, odours and other aesthetic nuisances (Thomann and Mueller ,1987)**.**

2.6.9 Physiology and Oxygen Demand

Raising the conductance of oxygen into the blood of fish is another possible mechanism to compensate for low DO levels. Oxygen conductance can be increased through increasing the flow of water over the gills (increased ventilation frequency, Jensen *et al*. 1993).

The frequency and amplitude of opercular movements increased with a decrease in dissolved oxygen levels (Doudoroff and Shumway, 1970). Arctic char larvae were exposed to normoxic and hypoxic (25% air saturation) conditions. The hypoxic larvae had coordinated buccal and opercular movements in contrast to the normoxic larvae (McDonald and McMahon 1977). Oxygen conductance can also be increased through an increase in gill oxygen diffusion conductance by: increasing the diffusion area (lamellar recruitment: Booth 1979 as referenced in Jensen et al. 1993), reducing diffusion distance across the gill (Soivio and Tuursala 1981 as referenced in Jensen *et al*. 1993), or increasing the diffusion gradient across the gill by reducing arterial blood oxygen pressure (Jensen *et al*. 1993). Hypoxic arctic char larvae had fewer filaments and lamella than the normoxic larvae. However, stimulated growth after 38 days resulted in the same lamellar surface area (McDonald and McMahon 1977).

2.6.10 Water Temperature

The requirements of invertebrates for oxygen generally increased as temperature increased. LC50s for the caddisfly *Hydropsyche betteni* were determined at different water temperatures (ranging from 10 to 21 C°). The LC50 increased from 1 mg/L DO at 10 C° to 2.9 mg/L DO at 21 C° (Nebeker 1972). Jacob and Walther (1981) tested four invertebrate species at different temperatures. In some cases, LC50 values were dependent on temperature: LC50 expressed as percent saturation increased with increasing temperature. In other cases, LC50s did not change with temperature: for instance, mortality of the mayfly *Ephemera vulgata* only occurred when anoxic conditions were reached regardless of exposure time and test temperature (Jacob and Walther ,1981). Jacob *et al*. (1984) tested 14 invertebrate species at different temperatures. At higher temperatures, lethal oxygen levels generally increased when expressed as percent saturation; expressed as concentration (mg/L), lethal dissolved oxygen levels either increased or were virtually the same at higher temperature (Jacob *et al*. 1984).

Survival time of t he midge *Chironomus anthracinus* in bottles starting with a dissolved oxygen concentrations of 0.15 mg/L was inversely correlated with temperature (Hamburger *et al*. 1995). The 72-hr LC50s of female and juvenile cladoceran *Daphnia magna* increased as temperature increased. The change in LC50s for males with increased temperature was variable (Hoback and Barnhart 1996).

CHAPTER THREE

MATERIALS AND METHODS:

3.1 Experimental site

The study was conducted at fish hatchery of College of Animal Production Science and Technology, fisheries and wildlife department in the Sudan University of Science and Technology, during the period 23/8 to 06 September 2015. At temperature 28c°-30c°.

3.2 Experimental fish

Finger lengths was obtained from fisheries department hatchery in Sudan University of Science and Technology. The fish were conditioned for a week, in plastic tank while oxygen obtained from a compressor the fish was fed feeds manufactured .

At the beginning of the second week the fish were divided to two groups big and small size, and the to groups were divided into to groups i.e. starved and fed for three days before they were individually measured to obtain the initial weight. During the measurement.

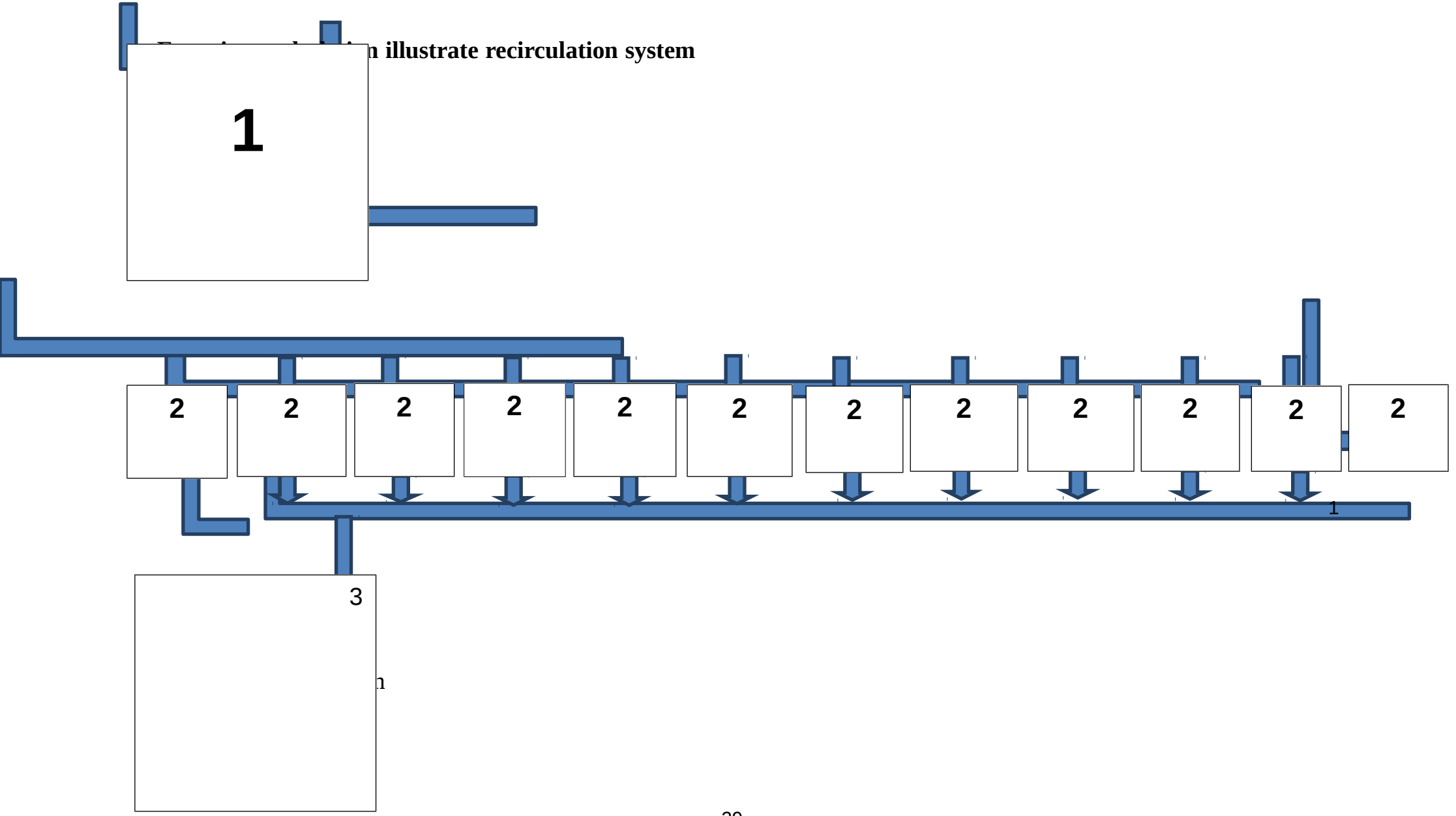
3.3 Experimental design:

The study concerns Nile tilapia *(Oreochromis niloticus)* weighing about 22 ± 10.431 grams average weight, During the acclimatization period, water in the tanks was aerated continuously. Fingerlings were fed with a commercial pellet twice a day.

The system consists of 12 culture tanks, The fish were then distributed into 12 plastic tanks (48x34x30 cm) and the fish were put in a chamber (23x14x17 cm), the small size were put 5 fish per each while the big sizes were 3 fish per each chamber . and bio-filter to remove toxic

waste products (ammonia and nitrites) and water pumped from the biofilter to the reservoir tank with a AZA Submersible Pumping 220 240v, AC 50 AC 50Hz 18 W , for desert cooler Fount, and the reservoir tank were aerated with oxygen pumped from compressor.

n illustrate recirculation system



3.3.1 ammonia concentration measurement

The water for ammonia measurement were obtained from the chamber by siphoning water from the chamber into a glass container capacity (150 ml) with a flow rate of 25ml per second, then ammonia measured using The Palintest 1000 Ammonia Meter with a precision dual range Photometer for measuring Ammonia concentration in water (rang in gavailable are 0 – 15 mg/l N and 0 – 50 mg/l N).

Picture . No (2) AMMONI METER

3.3.2 Dissolve oxygen (DO)

Dissolve oxygen measured using dissolved oxygen meter (DO 5509) with input from 0 – 20 mg/l. by putting oxygen probe handle into the water in the glass container.

Picture .No (3): Oxygen Meter

3.3.3 Ash determination:-

A crucible was weighed empty ,the sample and crucible were placed in a muffle furnace at 550c° to 24 hours. Until white grey or reddish ash was obtained ,the crucible was removed from furnace ,the ash

weight from the weight of crucible with sample after ashing minas the empty crucible

W2-w1= ash weight

W1=weight of empty crucible

W2=weight of crucible + sample after ashing

3-4 Statistical analysis

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS, 1988All statistics was carried out using SPSS version 21.

CHAPTER FOUR

RESULTS & DISCUSSION

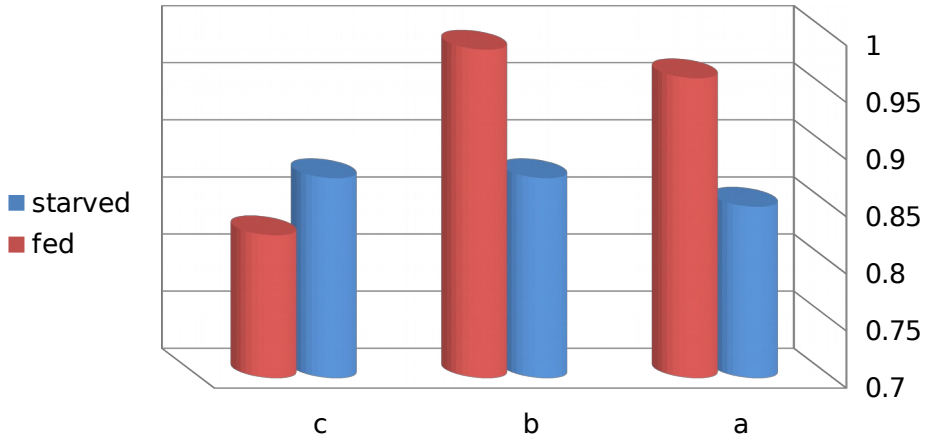
Results

In this study the chemical water parameters measurements of ammonia and Dissolved oxygen concentrations were recorded in all experimental fingerlings groups .

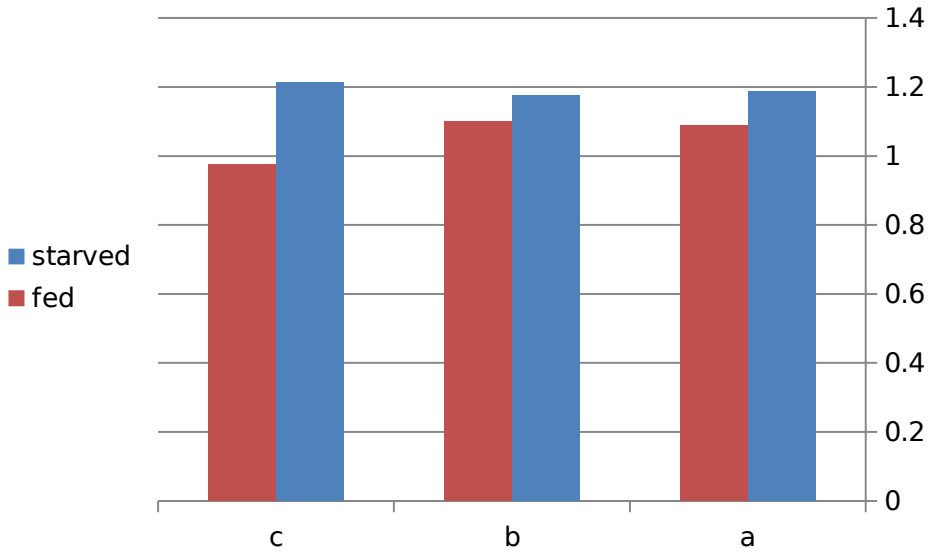
4.1.Estimation of ammonia concentration

In this study the measurements of ammonia in the water were calculated by determining the mean and standard deviation (table4. 3) Analysis of variance revealed that ammonia concentration obtained in big starved fingerlings after 9 to 24 hours measurement and there is No significant different($p>0.05$) the concentrations of ammonia obtained in big fed fish after 3, 6 and 24 hours of measuring and there is no significant different ($p>0.05$) and the mortality of fish above maximum Fig(1).

In the present study the statistical analysis revealed that there was no significant difference in all ammonia concentrations measured in the small fish starved group. in the small fed fish the statistics analysis revealed that there is significant different at 6 and 24 hours. ($p<0.05$) (table4.3) . Fig ,(2)



Fig(1):Ammonia level for big tilapia fingerlings



Fig(2):Ammonia level for small tilapia fingerlings

Table (4-3) mean and their standard deviation of NH3 during the experiment

Hours e	3 M±St.d	6 M±St.d	9 M±St.d	12 M±St.d	15 M±St.d	18 M±St.d	21 M±St.d	24 M±St.
Starve	1.07±0.06[a]	1.07±0.12[a]	0.83±0.06[b]	0.87±0.06[b]	0.867±0.15[b]	0.77±0.12[c]	0.73±0.06[c]	0.73±
Fed	1.13±0.06[a]	1.13±0.15[a]	1.00±0.50[a]	1.03±0.23[a]	0.87±0.06[b]	0.80±0.10[b]	0.77±.1155[c]	0.67[d]
Starve	1.47±0.55[a]	1.20±0.17[a]	1.43±0 . 51[a]	1.07± 0.15[a]	1.10 ±0.10[a]	1.20 ± 0.10[a]	1.00 ±0.10[a]	1.07± 0.15[a]
Fed	1.17±0.06[a]	1.27±0.25[a]	1.00±0.10[a]	1.03±0.12[a]	1.07±0.15[a]	1.07±0.23[a]	1.00±0.10[a]	0.83

A ,b ,c .d means superscript is significantly different $(p<0.05)$.

Table (4-4) mean and their standard deviation of O_2 during the experiment

Hours e	3 M±St.d	6 M±St.d	9 M±St.d	12 M±St.d	15 M±St.d	18 M±St.d	21 M±St.d	24 M±St.
Starve	0.53±0.153[c]	0.50± 0.17[c]	0.50± 0.17[c]	0.467± 0.058[d]	0.37±0.115[e]	0.33± 0.153[e]	0.20± 0.0000[e]	0.20± 0.000
Fed	0.53± 0.153[c]	0.63± 0.115[b]	0.43± 0.058[d]	0.50± 0.0000[c]	0.37± 0.058[e]	0.37± 0.058[e]	0.13± 0.058[e]	0.23±
Starve	0.83± 0.115[a]	0.53± 0.115[c]	0.57± 0.058[c]	0.63± 0.058[b]	0.57± 0.208[c]	0.57± 0.0577[c]	0.37± 0.057[e]	0.33±
Fed	0.50± 0.0000[c]	0.47± 0.058[c]	0.53± 0.058[c]	0.60± 0.100[b]	0.57± 0.058[c]	0.47± 0.115[d]	0.27± 0.058[e]	0.30± 0.000

a, b ,c ,d, e, means superscripts is significantly different $(p<0.05)$.

4.2 Estimation of Dissolved Oxygen(DO)

Dissolved oxygen (DO) is most crucial factor in natural waters for the growth and survival of fishes.

The dissolved oxygen content in the water of different experimental tanks in the present investigation varied from 0.53±0.153 to 0.20± 0.0000 in big staved first measure and final one, from 0.63± 0.115 to 0.13± 0.058 in big fed fingerlings and the statistic analysis revealed that there are significance different($p<0.05$) Table.4.3 and Fig.(3).In small starved the reading of DO varied from 0.83± 0.115 to 0.33± 0.057 and in small fed varied from 0.60± 0.100 to 0.30± 0.0000 and showed that there are significanc different($p<0.05$), Table.(4.4).Fig.(4).

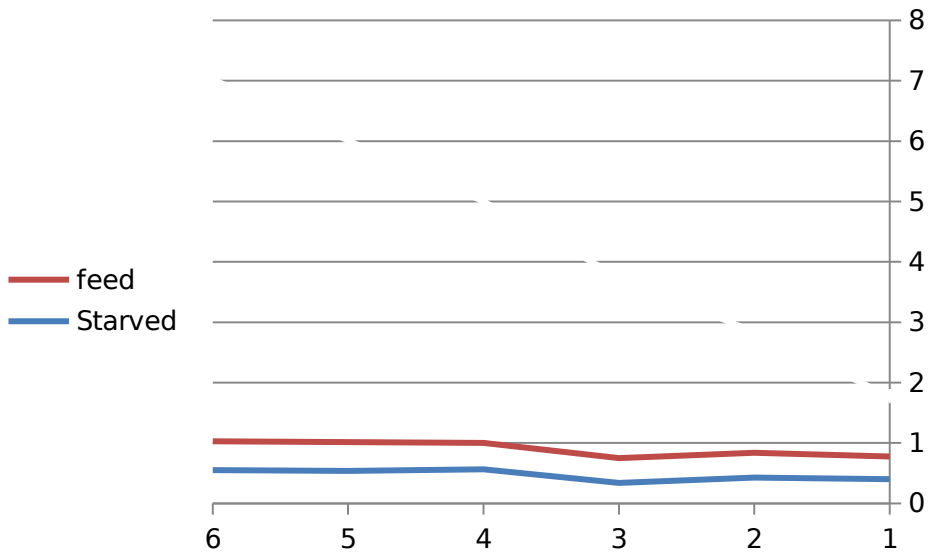


Fig (3) : Comparison of Do level for big and small tilapia fingerlings (starved –fed)

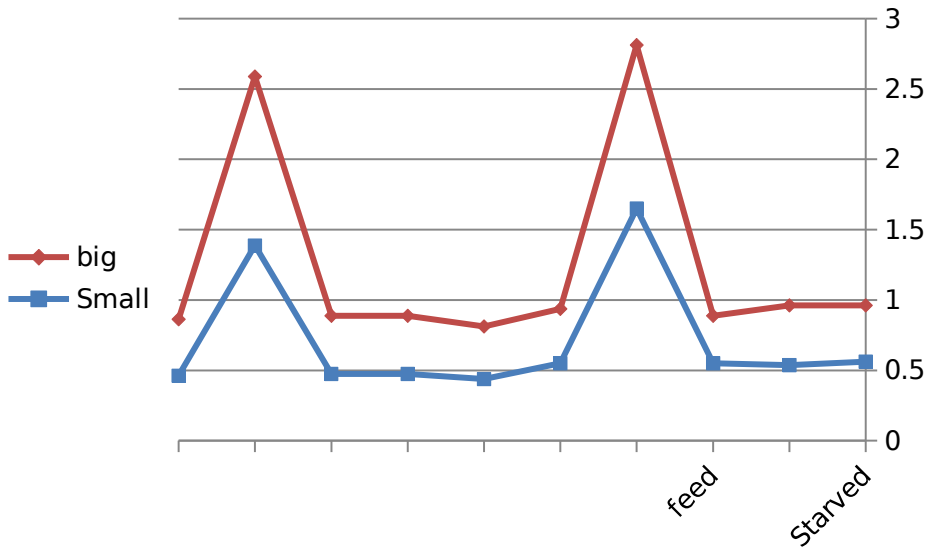
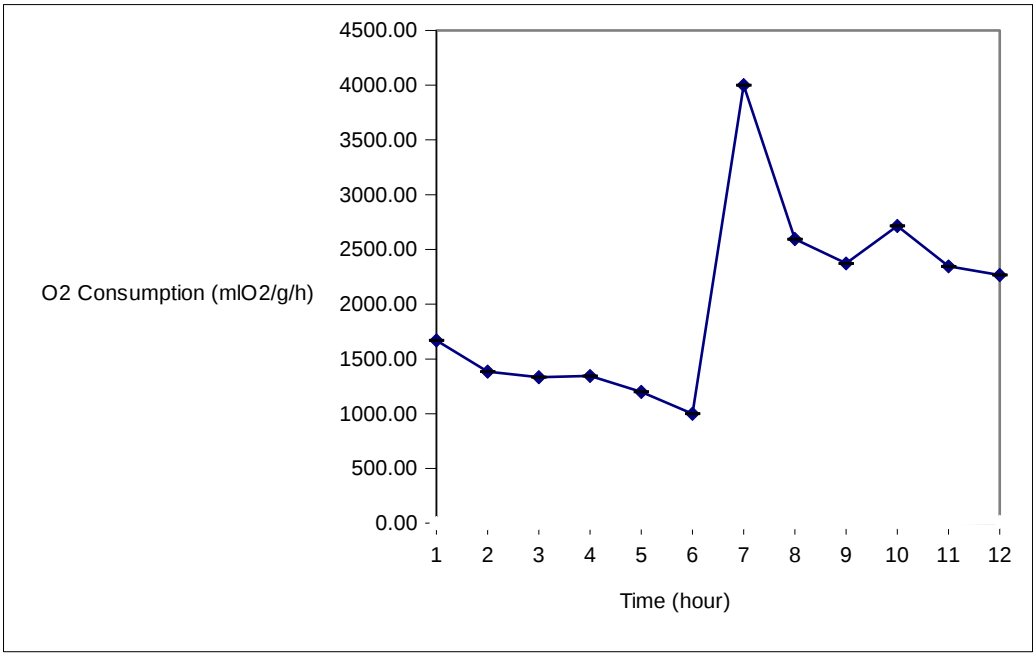
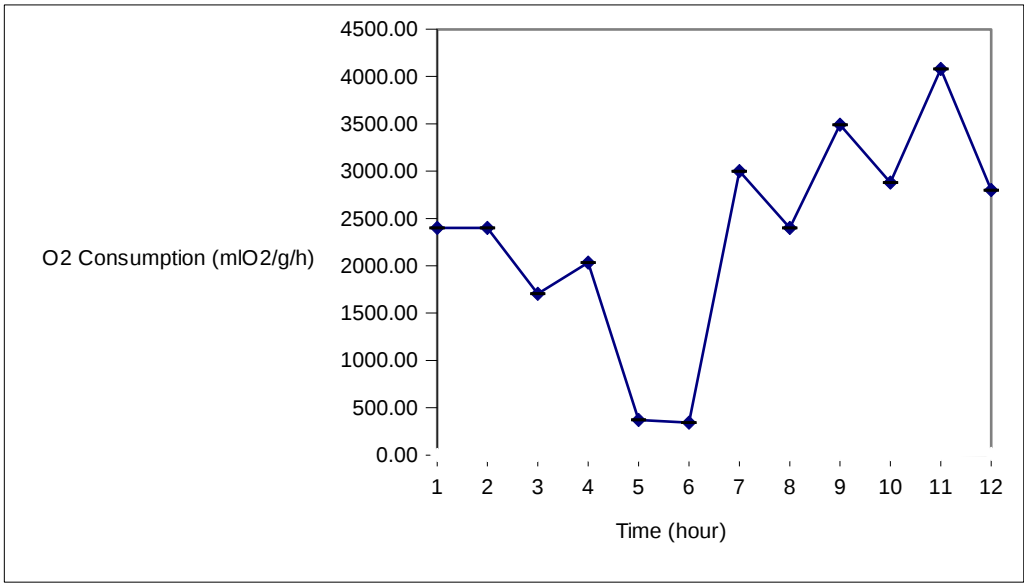


Fig (4) : Comparison of means for Do level for big and small tilapia fingerlings (starved –fed).

In the trial the water flow rate was 25ml per second and the Oxygen consumption ranged from 2594.59 to 1000.00 mlO2/m for fingerlings starved for 72 h the fingerlings was 3490.91ml O2/m to 342.86 ml O2/m when fed commercial to fingerlings I found in this experiment that fish fed consume high percentage of oxygen.



Fig(5) O_2 consumption for big and small tilapia fingerlings starved



Fig(6) O_2 consumption for big and small tilapia fingerlings fed

4.3 Estimation of Ash :

In this study the approximate composition of ash was found out in all treatment individually, the statistical analysis showed that there was significanct difference in all groups experiment. .

Table (4.5): shows the determination of ash for big and small Oreochromis niloticus.

Fish Parameters	Big starve M±St.d	Big fed M±St.d	Small starve M±St.d	Small fed M±St.d
Ash	0.73±0.08[b]	0.51±0.035[c]	0.82±0.07[a]	0.38±0.28[d]

The mean different is significant at 0.05 level

DISCUSSION

In the present trial a bio-filter was used to remove toxic waste products (ammonia and nitrite) from the experimental tanks in all groups. Ammonia is usually not a problem if the biological filters are properly sized for the loading rate and carrying capacity and if adequate water flow is maintained (Fowler, et al., 1994).

Hockheimer and Wheaton (1998) recommended that the system water move through the biological filter at least 2–3 times per hour. However, Rakocy et al. (1997) were unable to detect any difference in tilapia growth rate, total weight, or survival between water exchange rates of 0.55 and 1.25 times per hour. In this trial the water flow rate was 25ml per second, In this experiment the removal of ammonia and organic waste were done by siphoning the same result was obtained by (Rijn and Rivera 1990) increased removal of ammonia by a trickling bio-filter was found with increasing concentrations of ammonia in pond water. Ammonia parameter is very crucial factor in aquaculture that affects the general conditions of cultured organism, as it determines the health and growth conditions of cultured organism. El-Shafai et al. (2004) showed that the lowest-observable effect concentration on the growth performance is 0.144 mg/l UIA-N. (Joel, O. and Amajuoyi, C.S. (2010) recorded that level of un-ionized ammonia which is capable of killing fish over a few days start at about 0.6 mg/L. They recorded that un-ionized ammonia levels as low as 0.1 mg/L can cause gill and kidney damage and reduction in growth.

And the result of ammonia concentration was agreement with the Saber et al.(2004) who showed that the lowest-observable effect concentration on the growth performance of Nile tilapia is 0.144 mg/l UIA-N and there was no significant differences between the mean individual weight of fish exposed to 0.068 mg/l UIA-N and control0.004 mg/l UIA-N. Atle et al.(2003 and 2004) , Sten et al. (2004) and Lemarie et al.(2004) reported that fish weight decreased when concentrations of UIA-N/l increased. And the result was disagreed with

following authors EL-SHERIF, et al (2008) who showed that growth performance was significantly ($P \leq 0.05$) decreased with increasing concentration of UIA-N. The feed conversion ratio (FCR) increased with

increasing concentrations of UIA-N, the differences were significant ($P \leq 0.05$) among the high concentrations.

Gorder (2000) indicated that feed levels change with fish size as fingerlings consume a much higher percentage of their body weight (5%–8%) than harvestable size fish (0.75%–3%). Chapman (2000) puts these feed levels at 6 to 15% of body weight for young fish (<25 g) and 1% to 3% of body weight for older fish (>25 g) this result was investigated in this study. The ammonia generation load is based on the fish feeding rate and could be assumed to be 10% of the protein in the feed becomes the ammonia-N generation rate (Timmons et al., 2002). In the this investigation there was no mass mortality were recorded in all tanks the same result was observed by Person-Le Ruyet et al. (1997b) reported no mass mortality up to 0.4 mg UIA-N l 1. In treated sewage-fed ponds, tilapia suffered clear skin ulcers, necrosis and haemorrhage associated with mass mortality at 0.45 mg UIA-N l 1.

Oxygen is the most abundant element in soils and plants (weight content is 49 % and 70 %,respectively (Bohn et al. 1979). In addition, oxygen is the most abundant element in water by weight (Truelson 1997).

Studies done by Tsadik and Kutty (1987) on the influence of ambient oxygen on feeding and growth in O. niloticus showed that experiments under the various oxygen regimes varied. Mallya, (2007) shows that, the period of time during which the oxygen level drops below the required minimum level, will cause the fish to become stressed. It is this stress which causes fish death this result was disagreement with the present trail. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased. However, fish is not able to assimilate the food consumed when DO is low (Mallya, 2007), this result was obtained by the present study in the fed fish

CHAPTER FIVE
Conclusion and Recommendation

5.1 Conclusion

The concentration of ammonia in fish pond should be closely monitored to avoid both chronic acute toxic effects. Care should be taken to closely monitor the level to always be below 0.2mg/l. It is imperative to ensure that the pH and temperature values are adequately controlled to avoid negative synergistic effect with the presence of ammonia in the pond

Results obtained in this study demonstrated that the Nile tilapia (O. niloticus) metabolic rate, expressed as the rate of oxygen

consumption, was influenced by the feeding status, whether starved or fed and by the size of fish.

In conclusion, the observed variation in individual oxygen consumption rates for Nile tilapia(O. niloticus) fry and fingerlings in fed and starved conditions suggests that significant individual variation exists at starvation fish, where resistance potential was present at very early stages of development.

Ash percentage of the weight of the fish was used to determine the proportion of oxygen consumer by Fish .

It could be concluded that Nile tilapia(O. niloticus) fingerlings with average weight 14-10.431 g, were suitable to culture at water optimum for growth performance and survival rate than other water conditions.

5.2 Recommendations

1. When managing aquatic system and transportation of fish species , the water quality should be considered in terms of control , dissolved oxygen, temperature and ammonia.

2. The study recommended that for transportation of fry should be starved before 48 hrs to decrease ammonia effect.

3. The expansion of the search so as to increase the time of the experiment and measuring some outputs, such as nitrates, pH and temperature with the use of different types of diets with the use of separate types of tilapia in terms of sex (male and female), and size.

CHAPTER SIX
REFERENCES

Arillo, A., Margiocco, C., Melodia, F., Mensi, P. and Schenone, G.(1981). Ammonia toxicity mechanisms in fish: studies on the rainbow trout (Salmo Gairdneri Rich.). Ecotoxicol. Environ. Saf. 5, 316-328.C

APHA, American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Ed. APHA, AWWA, WEF. Washington, D.C.

Agricultural Water Use (1996): Ammonia. Aquaculture 2(1): 6p

Ashe, D., J. R. Tomasso, A. G. Eversole, and T. I. J. Smith, 1996. Toxicity of ammonia to fingerling white bass: effect of selected

environments on uptake dynamics. The Progressive Fish-Culturist. 58:277-280.

Atle, F. , T.H. Evensen, T. Vollen and V. Oiestad. 2003. Effects of chronic ammonia exposure on growth and food conversion efficiency in juvenile spotted wolfish. Aquaculture 228, 215-224 ,

Atle , F., S. I. Siikavuopio, B. S. Saether and T.H. Evensen. 2004. Effect of chronic ammonia exposure on growth in juvenile Atlantic cod. Aquaculture 237, 179-189.

Aquafarmer. 2004. The farming of Arctic charr. Technical Institute of Iceland, the Holar University College and The Aquaculture Development Centre of Ireland. November 2007 – January 2008; http://www.holar.is/~aquafarmer/

Aysel C. and Koksal G. (2005): The acute toxicity of ammonia on tilapia (Oreochromisniloticus) larvae and fingerlings. Turk. J. Vet. Anim. Sci, 29: 339- 344

Binstock, L. and H. Lecar. (1969). Ammonium ion currents in the squid giant axon. J. Gen. Physiol. ~: 342.

Bohn, H. L., B. L. McNeal, and G. A. O'Connor. (1979). Soil Chemistry. John Wiley & Sons. New York. 329p.

Booth, J.H. 1979. *The effect of oxygen supply-epinephrine and acetylcholine on the distribution of blood flow in trout gills. J. Exp. Biol. 83, 31–39.*

Barnabe, G. (1994): On growing fish in intensive system. Aquaculture**:** biology and ecology of cultured species. In Barnabe, G. (Ed.). Ellis Harwood Series in Aquaculture and Fisheries Support. Pp. 353- 356.

Barton, B. A. and B. R. Taylor. (1994). Dissolved Oxygen Requirements for Fish of the Peace,Athabasca and Slave River Basins. Northern

River Basins Study Project Report No. 29.Northern River Basins Study. Edmonton, Alberta. 104p+App.

Buddington, R. K., Krogdahl, A., and BakkeMcKellep, A. M. (1997). The intestines of carnivorous fish: structure and functions and the relations with diet. Acta Physiol. Scand. 161, 67–80.

Breitburg, D. L. (2002). Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries* 25:767-781

Brusilow, S. W. (2002). Reviews in molecular medicine – hyperammonemic encephalopathy. Medicine 81, 240-249

Butterworth, **D.S., Kmrubel, J.L. and Punt, A.E. (2002)**. What is needed to make a simple density-dependent response population model consistent with data for the eastern North Pacific gray whales? J. Cetacean Res. Manage. 4(1): 63-76.

Boyd, E.C. 2004. *Farm-Level Issues in Aquaculture Certification: Tilapia. Report commissioned by WWF-US in 2004. Auburn University, Alabama 36831.*

Bwanika, G.N., Makanga, B., Kizito, Y., Chapman, L.J. & Balirwa, J. (2004). Observations on the biology of Nile tilapia, *Oreochromis niloticus*, L., in two Ugandan Crater lakes. African Journal of Ecology 42: 93–101.

Bwanika, G., Murie, D. & Chapman, L.(2007). Comparative Age and Growth of Nile tilapia (*Oreochromis niloticus L.*) in Lakes Nabugabo and Wamala, Uganda. Hydrobiologia 589: 287-301.

Colt, J. and G. Tchobanoglous, (1976). Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture. 8:209-224

Chapman, **G.(1986**). Ambient Water Quality Criteria for Dissolved Oxygen. EPA 440/5-86-003. Washington, D.C.

Campbell, J. W. (1991). "Excretory nitrogen metabolism" in Environmental and Metabolic Animal Physiology. Comparative Animal Physiology, 4th Edn., ed. C. L. Prosser (New York: Wiley-Interscience), 277–324.

Chapman, **F.A.(2000**). Culture of hybrid tilapia: a reference profile. Circular 1051. Gainesville: Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/FA012

Chorn E. Lim *et al* .(2006). Feeding Practices. The Hawthorn Press. 547-559.

Doudoroff, **O. and D. L. Shumway. (1970)**. Dissolved Oxygen Requirements of Freshwater Fishes. FAO Technical Paper No. 86. Food Agriculture Organization, United Nations. Rome, Italy.291p.

Desjardins, R.L.and Macpherson, J.I. (eds) (2001). Evaluating and improving the parameterization of biogeochemical processes associate with the flux of N2 O from Canadian agroecosystesms. Final report to the Climate Change Action Fund, Greenhouse Gas Sources and Sinks. March, 2001. 62 pp.

Desjardins R.L., H. Janzen, R. Lemke and R. Riznek. (2001a). Regional and National Estimates of the Annual Nitrous Oxide Emissions from Agroecosystems in Canada using the Revised IPCC Methodology. pp. 3-22.

In De Croux, P.; Julieta, M. and Loteste, A. (2004): Lethal effects of elevated pH and ammonia on juveniles of neotropical fish Colosoma macropomum (Pisces, Caracidae). J Environ. Biol. Jan., 25(1): 7-10.

Division of Specialized Information Services (SIS) of the US National Library of Medicine (NLM), (2005) Toxicology Data Network (TOXNET®): Hazardous Substances Data Bank (HSDB®) [online]. Bethesda, MD: SIS. Available from http://toxnet.nlm.nih.gov/ (Accessed 1 February 2007)

El-Gohary, F., El-Hawarry, S., Badr, S., Rashed, Y.,(1995). Wastewater treatment and reuse for fish aquaculture.Water Sci. Technol. 32 (11), 127– 136.

EPA (United States Environmental Protection Agency) (1998): Updates of ambient water quality criteria for ammonia. USA United States Environmental Protection Agency, 822-R-98-008. 52- 107. Meade, J. (1985): Allowable ammonia for fish culture. Prog. Fish Cult. 47: 135-145 . Salin, D. and Williot, P. (1991): Acute toxicity of ammonia to Siberian sturgeon (Acipenser baen) P. Williot, Ed. A cipenser, Comagref Publ. 1991: 153-167.

Environmental Protection Agency, (1999): Achieving Clean Air and Clean Water. The Report of the Blue Ribbon Panel on Oxygenates in Gasoline. US EPA 420-R- 99-021.

Environmental Protection Agency U.S. (EPA). April (2002). Role of Background in the CERCLA Cleanup Program. Office of Emergencyand Remedial Response, Washington, DC. OSWER 9285.6-07P (see Appendix B of this guidance).

EPA Environmental Protection Agency (2002). U.S. Environmental Protection Agency. Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Sulfate (Draft). April 2002.

El-Shafai, S., El-Gohary, F., Nasr, F., Peter, N. and Gijzen, H. (2004): Chronic ammonia toxicity to duckweed-fed tilapia (O. niloticus). Aquaculture, 232: 117- 127.

EL-SHERIF, M.S. AND EL-FEKY, AMAL M. Anim. Prod. (**2008) &** Fish Res. Dept., Fac. Agric., Suez Canal University .,Ismailia, Egypt**,** 8th International Symposium on Tilapia in Aquaculture.

Excretion. In: Wright, P.A., Anderson, P.M. (Eds.), Fish Physiology, vol. 20. Academic Press, New York., 109-148.

Fryer, G & Iles, T.D. 1972. The Cichlid Fishes of the Great Lakes of Africa: Their biology and Evolution. T.F.H. Publications, Hong Kong

French, C. J., Mommsen, T. P., and Hochachka, P. W. (1981). Amino acid utilization in isolated hepatocytes from rainbow trout. Eur. J. Biochem. 113, 311–317.

Felipo V, Miñana MD, Cabedo H, et al. (1994). L-Carnitine increases the affinity of glutamate for quisqualate receptors and prevents glutamate neurotoxicity. Neurochem Res 19(3):373-377

Fowler, **P., D. Baird, R. Bucklin, S. Yerlan, C. Watson, and F. Chapman. (1994).** Microcontrollers in recirculating aquaculture systems. University of Florida Energy Extension Service EES–326:7 pgs.

Francais-Floyd R and Waston C. (1996): Ammonia. UF/ UFAS. Pp 1-

Forsberg, O.I. (1997). The impact of varying feeding regimes on oxygen consumption and excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon Salmo salar L. Aquaculture Research 28: 29 – 41.

Fontenot, Q. C., J. J. Isely, and J. R. Tomasso, (1998). Acute toxicity of ammonia and nitrite to shortnose sturgeon fingerlings. The Progressive Fish-Culturist. 60:315-318.

Felipo V, Monfort P, Fernandez-Marticorena I, et al. (2000). Activation of NMDA receptors in rat brain in vivo following

acute ammonia intoxication. Characterization by in vivo brain microdialys is. Eur J Neurosci 12(Suppl11):40.

Foss , A., T. H. Evensen and V. Qiestad.(2002). Effects of hypoxia and hyperoxia on growth and food conversion efficiency in the spotted wolfish Anarhichas minor (Olafsen) Aquac. Res. 33, 437 – 444.

FAO (2005-2012). Cultured Aquatic Species Information Programme. *Oreochromis niloticus*. Cultured Aquatic Species Information Programme. Text by Rakocy, J. E. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 18 February 2005. [Cited 11 September 2012]

Gmur N.F, Evans LS, and Cunningham E.A (1983). Effects of ammonium sulfate aerosols on vegetation - II. Mode of entry and responses of vegetation. Atmospheric Environm. 17, 715-721.

Groot .C. Margolis L., and W.C. Clarke(1995). *Physiology, Ecology of pacific salmon*. Department of Fisheries and oceans, Biological sciences Branch Pacific biological station Nanaimo, British Columbia, Canada.

Gordon, C. B., Yiannis, C. K.; Arthur, J. V. and Chris, D. M. (2000): Gonadal anomalies in Pillay, T.V. (1992): Aquaculture and the environment. 1st Ed. University Press. Cambridge, P 189. Seawright, D.E. Stickney, R., 1998. Nutrient dynamics in integrated aquaculturehydroponics systems. Aquaculture, 160: 215- 237.

Gorder, S.D. (2000). Small scale aquaculture. The Alternative Aquaculture Association. Breinigsville, PA, USA.

Gutierrez-Wing, M. and R. F. Malone.(2006). Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacultural Engineering 34:163–171.

GISD (2012). Global Invasive Species Database–*Oreochromis niloticus* - Available from: http://www.issg.org/database/species/ecology.asp?si=1322&fr=1&sts=sss&lang=EN

Hillaby, **B. A. and Randall, D. J. (1979).** Acute ammonia toxicity and ammonia excretion in Rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Ca. 36, 621–629.

Haywood G. (1983): Ammonia toxicity in teleost fish: A review. Con. Tech. Rep. Fish.Aquat. Sci. 1177: 1-35. Thurston, R.V., Philips, G.R. and Russo, R.C. (1981): Increased toxicity of ammonia to rainbow trout (S. gairdneri) resulting from reduced concentrations of dissolved oxygen. Can. J.Fish. Aquat. Sci., 38: 983-988.

Handy, R.D. and Poxton, M.G. (1993): Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Fish. Biol. Fish. 3: 204-241. Salin, D. and Williot, P. (1991): Acute toxicity of ammonia to Siberian sturgeon (Acipenser baen) P. Williot, Ed. A cipenser, Comagref Publ. 1991: 153-167.

Handy, R.D. and Poxton, M.G. (1993): Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Fish. Biol. Fish. 3: 204-241. Haywood G. (1983): Ammonia toxicity in teleost fish: A review. Con. Tech. Rep. Fish. Aquat. Sci. 1177: 1-35 .

Hamburger, K., P. C. Dall, and C. Lindegaard. 1995. Effects of oxygen deficiency on survival an glycogen content of *Chironomus anthracinus* (Diptera, Chironomidae) under laboratory and field conditions. Hydrobiologia 297:187-200.

Houlihan, D. F., Carter, C. G., and McCarthy, I. D. (1995). "Protein turnover in animals," in Nitrogen Metabolism and Excretion, eds P. J. Walsh and P. Wright (Boca Raton, FL: CRC Press), 307–395.

Hoback, W. W. and M. C. Barnhart. 1996. Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*. J. N. A. Benthol. Soc. 15:117-26.

Harding, L. W. and E. S. Perry. 1997. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. Marine Ecology Progress Series 157:39-52

Harris, J.O., G. B. Maguire, S. Edwards and S. M. Hindrum.(1998). Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, Haliotis laevigata Donovan. Aquaculture 160, 259-272.

Hochheimer, **J.N. and F. Wheaton. 1998.** Biological filters: trickling and RBC design. Proc. Second International Conference of Recirculating Aquaculture:291–318.

Hermenegildo C., Monfort P. and Felipo V. (2000) Activation of N methyl-D -aspartate receptors in rat brain in vivo following acute ammonia intoxication: characterization by in vivo brain microdi-alysis. Hepatology 31 , 709–715.

Hargreaves, J.A., Kucuk, S.,(2001). Effects of diel un-ionised ammonia fluctuation on juvenile hybrid stripped bass, channel catfish and blue tilapia. Aquaculture 195, 163– 181.

Harmon, **T., (2001)**. A look at filtration in aquaponic systems: bead filters. AquaponicJ., 3:16–19.

Hagy, J. D.(2002). Eutrophication, hypoxia and trophic transfer efficiency in Chesapeake Bay.Ph.D. dissertation, University of Maryland, College Park, Maryland.

Hargreaves, J.A. and Tucker, C.S. (2004): Managing ammonia in fish ponds. SRAC Publication No. 4603, pp 1-7.

Ip, Y. K., Lim, C. B., Chew, S. F., Wilson, J. M., and Randall, D. J. (2001c). Partial amino acid catabolism leading to the formation of alanine in *Periophthalmodon schlosseri* (mudskipper): a strategy that facilitates the use of amino acids as an energy source during locomotory activity on land. *J. Exp. Biol.* 204, 1615–1624.

Ip, Y. K., Chew, S. F., and Randall, D. J. (2004a). Five tropical fishes, six different strategies to defend against ammonia toxicity on land. Physiol. Biochem. Zool. 77, 768–782.

Ip, Y. K., Chew, S. F., Wilson, J. M., and Randall, D. J. (2004b). Defences against ammonia toxicity in tropical fishes exposed to high concentrations of environmental ammonia: a review. J. Comp. Physiol. B 174, 565–575.

Ip YK, Randall DJ, Kok TKT, Bazarghi C, Wright PA, Ballantyne JS, Wilson JM, Chew SF (2004c) The mudskipper *Periophthalmodon schlosseri* facilitates active NH_4^+ excretion by increasing acid excretion and decreasing NH_3 permeability in the skin. J Exp Biol 207:787–801

Ip, Y. K., Lim, C. K., Lee, S. L. M., Wong, W. P., and Chew, S. F. (2004c). Postprandial increases in nitrogenous excretion and urea synthesis in the giant mudskipper Periophthalmodon schlosseri. J. Exp. Biol. 207, 3015–3023.

Jacob, U. and H. Walther. 1981. Aquatic insect larvae as indicators of limiting minimal content of dissolved oxygen. Aquatic Insects 4:219-24.

Jacob, U., H. Walther, and R. Klenke. 1984. Aquatic insect larvae as indicators of limiting minimal contents of dissolved oxygen - Part II. Aquatic Insects 6:185-90.

Jensen, F. B., M. Nikinmaa, and R. E. Weber. 1993. Environmental Perturbations of Oxygen Transport in Teleost Fishes: Causes, Consequences and Compensations. Fish Ecophysiology, eds. J. C. Rankin and F. B. Jensen. Chapman&Hall. London.

John , A. H. and K. Semra. 2001. Effects of dial un-ionized ammonia fluctuation on juvenile hybrid striped bass, channel catfish, and blue tilapia. Aquaculture 195, 163-181.

Joel, O. and Amajuoyi, C.S. (2010): Determination of the concentration of ammonia that could have lethal effect on fish pond. ARPN J. Engineer. Appl. Sci. pp 1-4.

Kramer, **D. L. 1987.** Dissolved oxygen and fish behavior. Environmental Biology of Fishes 18:81- 92.

Kapetsky, J.M. & Nath, S.S. 1997. A strategic assessment of the potential for freshwater fish farming in Latin America. COPESCAL Technical Paper. No. 10. Rome, FAO. 128p

Losordo, **T. M., Masser, M., Rakocy, J., (1992)**. Recirculating aquaculture tank production systems: An overview of critical considerations, Southern Regional Aquaculture Centre Publication 451.

Lorsordo, **T.M., M.P. Masser and J.E. Rakocy. (1999).** Recirculating aquaculture tank production systems: a review of component options. S. Region Aquaculture Center Publication No. 453:12 pgs.

Lim, C. B., Anderson, P. M., Chew, S. F., and Ip, Y. K. (2001). Reduction in the rates of protein and amino acid catabolism to slow down the accumulation of endogenous ammonia: a strategy potentially adopted by mudskippers (Periophthalmodon schlosseri and Boleophthalmus boddaerti) during aerial exposure in constant darkness. J. Exp. Biol. 204, 1605–1614.

Lamarie, G., Dosdat, A., Coves, D., Dutto, G., Gasset, E. and Person-LeRuyet, J. (2004) Effect of chronic ammonia exposure on growth of European seabass (Dicentrarchus labrax) juveniles. Aquaculture 229(1-4): 479- 491. Stickney, R.R. (1994): Principles of aquaculture. John Wiley & Sons. New York, 155-162.

Lim, C K, Wong, W. P., Lee, S. M. L., Chew, S. F., and Ip, Y. K. (2004b). The ammonotelic African lungfish Protopterus dolloi increases the rate of urea synthesis and becomes ureotelic after feeding. J. Comp. Physiol. B 174, 555–564.

McDonald, D. G. and B. R. McMahon. 1977. Respiratory development in Arctic char S*alvelinus alpinus* under conditions of normoxia and hypoxia. Can. J. Zool. 55:1461-67.

Moyle, **Peter B. and Joseph J. Cech, Jr. (1988**). Fishes: An Introduction to Ichthyology.2nd edition.

Malone, T. C. (1992). Effects of water column processes on dissolved oxygen: Nutrients, phytoplankton and zooplankton. In: Smith, D., M. Leffler, G. Mackiernan (eds.) Oxygen Dynamics in Chesapeake Bay: A Synthesis of Research. University of Maryland Sea Grant College Publications., College Park, Maryland. Pp. 61-112.

Margulies, D. (1993). Assessment of the nutritional condition of larval And early juvenile tuna and Spanish mackerel (Pisces: Scombridae) In the Panama Bight. Mar. Biol. 115:317−330.

Margulis, Lynn, and Sagan, Dorion, (1993). The Garden of Microbial Delights: A Practical Guide to the Subvisible World, Kendall/Hunt, ISBN 0-8403-8529-3

McKenzie EA, et al. (1993) The centromere and promoter factor, 1, CPF1, of Saccharomyces cerevisiae modulates gene activity

through a family of factors including SPT21, RPD1 (SIN3), RPD3 and CCR4. *Mol Gen Genet* 240(3):374-86

McGee, **M., and C. Cichra. (2000).** Principles of water recirculation and filtration in aquaculture. FA-12. Gainesville. Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, University of Florida. Retrieved April 5, 2007 from http://edis.ifas.ufl.edu/FA050

Mallya Y. J. (2007). The effects of dissolved oxygen on fish growth in aquaculture. Kingolwira National Fish Farming Centre, Fisheries Division Ministry of Natural Resources and Tourism, Tanzania.

Newcombe, **C. L. and W. A. Horne. (1938)**. Oxygen-poor waters of the Chesapeake Bay. *Science* 88:80- 81.

Nebeker, A. V.(1972). Effect of low oxygen concentration on survival and emergence of aquatic insects. Trans. Am. Fish. Soc. 101:675-79.

N**asr, F.A., El-Shafai, S.A., Abo-Hegab, S., (1998)**. Suitability of treated domestic wastewater for raising Oreochromis niloticus. Egypt. J. Zoology 31, 81– 94.

Officer, C. B., R. B. Biggs, J. L. Taft, L. E. Cronin, M. A. Tyler and W. R. Boynton. 1984. Chesapeake Bay anoxia: Origin, development, and significance. Science 223:22-27.

Prasad, M. S. (1988): Sensitivity of branchial mucous to crude oil toxicity in a freshwater fish, Colisa fasciatus. Bull. Environ. Contam. Toxicol., 41: 754-758 .

Pillay, T.V.R., 1992. Aquaculture and the Environment. Fishing Book News London, 189 pp.

Pcrson-Le Ruyet J., R. Galland, A. Le Koux, H. Chartois (1997). Chronic ammonia toxicity in juvenile turbot (Scoph- tlzulrnuv vnaximus). Ay~~uculture 154, 155- 17 1.

Person-Le Ruyet, J., Galland, R., Roux, A., Chartois, H., 1997b. Chronic ammonia toxicity to juvenile turbot (Scophthalmus maximus). Aquaculture 154, 155–171.7– 314.

Prinsloo, **J.F., W. Roets, J. Theron, L.C. Hoffman, and H.J. Schoonbee. (1999).** Changes in some water quality conditions in recycling water using three types of biofiltration systems during the production of the sharptooth catfish Clarias gariepinus (Burchell). Part1. Relative efficiency in the breakdown of nitrogenous wastes by the different biofiltration units. Water SA 25(2):239–252.

Parker, **E., Couturier, M., Benfey, T., (2002)**. Oxygen management at a commercial aquaculture farm producing Atlantic Salmon (Salmo salar) smolts. In: Proceedings of the third International conference on recirculating aquaculture (Editors) Libey, G. S., Timmons,M. B

Pillay, T.V.R. and Kutty, M.N. 2005. Aquaculture, Principles and Practices, 2nd Edition. Blackwell Publishing Ltd, Oxford, UK. 630 p.

Patric D, Chris NG, Songa Y, Kjersti K, Gro F, Rune W, Merc H. and Bemtssen G. (2006): Sensitivity of Atlantic Salmon (Salmon solar) to dietary endosulfan as assessed by hematology, blood biochemistry and growth parameters. Aquat. Toxicol. 80 (3): 207-216.

Picker, M.D. & Griffiths, C.L. (2011). Alien and Invasive Animals – A South African Perspective. Randomhouse/Struik, Cape Town, South Africa. 240 pp.

Redner, B. D. and R. R. Stickney, 1979. Acclimation to ammonia by Tilapia aurea. Transactions of the American Fisheries Society. 108:383-388.

Ruffier, **P.J., Boyle, W.C. and Kleinschmidt, J.K. (1981)**: Short-term acute bioassays to evaluate ammonia toxicity and effluents standards. J. Water Poll. Contr. Fed. 53: 367-377.

Rijn, **J.V. and G. Rivera. (1990)**. Aerobic and anaerobic biofiltration in an aquaculture unit – nitrite accumulation as a result of nitrification and denitrification. Aquacultural Engineering 9: 217–234.

Randall D.,(1991). The impact of variations in water pH on fish. Aquaculture and Water Quality. 90-104.

Russo, R.C. and Thurston, R.V. (1991): Toxicity of ammonia, nitrite and nitrate to fishes. In: E. Burne and J.R. Tomasso(Eds), Aquaculture and Water Quality. WAS Publication, 58- 89**.**

Rakocy, J.E. (1997). Integrating tilapia culture with vegetable hydroponics in recirculating systems. World Aquaculture Society, Tilapia Aquaculture in the Americas 1:163–184.

Rson-Le Ruyet, J., Delbard, C., Chartois, H., Le Dellion, H., 1997a. Toxicity of ammonia to turbot juveniles: Effects on survival, growth and food utilisation. Aquat. Living Resour. 10,307– 314

Randall, **D. J., Wilson, J. M., Peng, K. W., Kok, W. K., Kuah, S. S. L., Chew, S. F., Lam, T. J. and Ip, Y. K.(1999).** The mudskipper, Periophthalmodon schloesseri , actively transportsNH4+ against a concentration gradient. Am. J. Physiol. 277, R1562–R1578.

Rose, **L., and W. E. Dugger, Jr.(2002)**. ITEA/Gallup Poll Reveals What Americans Think About Technology. Reston, VA: International Technology Education Association.

Streeter, **h.w. and PHELPS, E.B. (1925)**. A study of the pollution and natural purification of the Ohio River, III. Factors concerned in the phenomena of oxidation and reaeration. Washington, D.C., US Public Health Service.

Smith, C. E., Piper, R. G. (1975). Lesions associated with chronic exposure to ammonia. In: W. E. Ribelin, Migah, G. (eds.) The pathology of fishes. Univ. of Wisconsin Press, Madison, WI, p. 497-514

Smart, A.(1978). The dawn of Italian painting, 1250–1400. Oxford: Oxford University Press; Ithaca, N.Y.: Cornell University Press.

Smart, G. R. (1978): Investigations on the toxic mechanisms of ammonia to fish gas exchange in rainbow trout (Salmo gairdneri) exposed to acutely lethal concentrations. J. Fish Biol., 12: 93-104. Ip, Y. K.; Chew, S. F. and Randall, D. J. (2001): Ammonia toxicity , tolerance, and Gairdneri Richardson. Pp. 103-19 In Stress and Fish., Ed. A. D. Pickering. Academic Press Inc. London.

Scott WD and Cattell FCR (1979). Vapor Pressure of Ammonium Sulfates. Atmospheric Environm.13, 307-317.

Soivio, A. and M. Nikinmaa.(1981). The Swelling of Erythrocytes in Relation to the Oxygen Affinity of the Blood of the Rainbow Trout, Salmo

Stumm, **W. and J. J. Morgan. (1981).** Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters. 2nd Ed.John Wiley & Sons. New York 780p

Szumnski, D.S., Barton, D.A., Puntam, H.D. and Polta, R.C. (1982): Evaluation of EPA unionized ammonia toxicity criteria. J. Water Pollut. Control Fed., 54: 282- 291.

Solangi, M. A. and Overstreet, R. M. (1982): Histopathological changes in two estuarine fishes, Menidia beryllina (Cope) and Trinectes maculatus (Bloch and Schneider) exposed to crude oil and its water-soluble fractions. Journal of Fish Diseases., 5: 13-35.

Salin, D. and Williot, P. (1991): Acute toxicity of ammonia to Siberian sturgeon (Acipenser baen) P. Williot, Ed. A cipenser, Comagref Publ. 1991: 153-167. EPA (United States Environmental Protection Agency) (1998): Updates of ambient water quality criteria for ammonia. USA United States Environmental Protection Agency, 822-R-98-008. 52- 107.

Saber , A. El-Shafai , A. El-Gohary Fatma, A. N. Fayza, N. Peter van der Steen and J. G. Huub. (2004) . Chronic ammonia toxicity to duckweed-fed tilapia (O. niloticus). Aquaculture 232, 117-127.

Sten , I. S. , D. Trine, F. Atle and M. Atle.(2004). Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin Strongylocentrotus droebachiensis. Aquaculture 242 ,313-320.

Taft, J. L., W. R. Taylor, E. O. Hartwig and R. Loftus. (1980). Seasonal oxygen depletion in Chesapeake Bay. Estuaries 3:242-247.

Thurston, R.V., Russo, R.C., Luedike, R., Smith, C., Megn, E., Wang, K.C. and Rown, C. (1984): Chronic toxicity of ammonia to rainbow trout. Trans. Am. Fish. Soc., 113: 56- 73.

Thurston, R.V., Russo, R.C., Meyn, E.L., Zajdel, R.K., Smith, C.E., 1986. Chronic toxicity of ammonia to fathead minnows. Trans. Am. Fish. Soc. 115, 196– 207.

Thomann, **R. V. and J. A. Mueller. (1987)**. Principles of Surface Water Quality Modeling and Control.Harper & Row, Publishers. New York

Tsadik Gagma M.N. Kutty (1987). Influence of ambient oxygen on feeding and growth of tilapia, Oreochromis niloticus ARAC/87/WP/10.United Nation Development Programme. Food and Agriculture Organization of the United Nations, Nigerian

institute for oceanography and marine research project RAF/87/009.

Tetzlaff, **B.L., and R.C. Heidinger. (1990)**. Basic principles of biofiltration and system design. SIUC Fisheries and Illinois Aquaculture Center, Bulletin 9:17 pgs.

The testicular tissue of white Perch, Morone americana collected in Coote's Paradise, lake Ontario Canada. 21st Annual Meeting, Nashville, Tennessee., 12-16 November.

Tomasso, J. R., (1994). Toxicity of nitrogenous wastes to aquaculture animals. Reviews in Fisheries Science. 2(4):291-314.

Truelson, **R. L. (1997)**. Ambient Water Quality Criteria for Dissolved Oxygen. Water Quality Branch, Water Management Division, Ministry of Environment, Lands and Parks. 107p.

Teichert-Coddington, D.R., Popma, T.J. & Lovshin, L.L. 1997. Atributes of tropical pond-cultured fish, pgs 183-198. In: Enga H.S. & Boyd, C.E.(eds.), Dynamics of Pond Aquaculture. CRC Press, Boca Raton, Florida, USA.

Timmons, L., Court, D.L., and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans.* Gene 263, 103-112

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. and Vinci, B.J. (2002). Recirculating Aquaculture Systems, 2nd Edition. Cayuga Aqua Ventures, Ithaca, NY 14850, USA. 800 p. NRAC Publication No. 01-002.

Timmons, M.B., J.M. Ebeling, F.W. Wheaton, S.T. Summerfelt, and B.J. Vinci. (2002).Recirculating aquaculture systems, 2nd Edition. Northeast Regional Aquaculture Center Publication No. 01–002:769 pgs.

UBA (2003). Environmental Data Germany 2002.

Van Gorder, S.D. 2000. Small scale aquaculture. Alternative Agriculture Association, Breinigsville, PA: 190 pgs

Winkler, L.W. (1888). Die Bestimmungdes in Wasser gelösten Sauerstoffen. Berichte der Deutschen Chemischen Gesellschaft, 21: 2843–2855.

Wetzel, R. G. (1975). Limnology.W.B. Saunders Company. Philadelphia.

WHO - world health organization (1986). Environmental Health Criteria 54. Ammonia. Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization. Geneva, 1986.

Wise, D. J., C. R. Weirich and J. R. Tomasso., (1989). Toxicity of ammonia to red drum Sciaenops ocellatus fingerlings with information on uptake and depuration. Journal of the World Aquaculture Society. 20(4):188-192.

Wheaton, **F.W. (1993)**. Aquacultural engineering. Krieger Publishing Company, Malabar, FL: 708 pgs

Wilkie M. P. (1997). Mechanisms of ammonia excretion across fish gills. Comp. Biochem. Physiol. 118A, 39–5010.1016/S0300-9629(96)00407-0 [Cross Ref]

Washington, DC. EPA (United States Environmental Protection Agency) (1998): Updates of ambient water quality criteria for ammonia. USA United States Environmental Protection Agency, 822-R-98-008. 52- 107.

Wilkie M. P. (2002). Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. J. Exp. Zool. 293, 284–30110.1002/jez.10123 [PubMed] [Cross Ref]

Summary of O2 Data (Starvation Treatment)

Sampling (at 3 hour interval)treatments

Class size	Replicate	3	6	9	12	15	18	21	24
Big	a	0.700	0.400	0.400	0.500	0.300	0.500	0.200	0.200
	b	0.500	0.700	0.700	0.500	0.300	0.300	0.200	0.200
	c	0.400	0.400	0.400	0.400	0.500	0.200	0.200	0.200

Class size	Replicate	3	6	9	12	15	18	21	24
small	a	0.700	0.600	0.600	0.700	0.800	0.500	0.300	0.300
	b	0.900	0.400	0.600	0.600	0.400	0.600	0.400	0.400
	c	0.900	0.600	0.500	0.600	0.500	0.600	0.400	0.300

Summary of O2 Data (fed Treatment)

Sampling (at 3 hour interval)treatments

Class size	Replicate	3	6	9	12	15	18	21	24
Big	a	0.500	0.500	0.400	0.500	0.300	0.400	0.100	0.300
	b	0.700	0.700	0.400	0.500	0.400	0.300	0.100	0.200
	c	0.400	0.700	0.500	0.500	0.400	0.400	0.200	0.200

Class size	Replicate	3	6	9	12	15	18	21	24
small	a	0.500	0.400	0.500	0.500	0.600	0.400	0.300	0.300
	b	0.500	0.500	0.600	0.700	0.500	0.400	0.300	0.300
	c	0.500	0.500	0.500	0.600	0.600	0.600	0.200	0.300

Summary of NH_3 Data (Starvation Treatment)

Sampling (at 3 hour interval)

24	21	18	15	12	9	6	3	Replicate	Class Size
0.6	0.7	0.7	1	0.8	0.8	1.2	1	**A**	**big**
0.8	0.7	0.9	0.7	0.9	0.9	1	1.1	**B**	
0.8	0.8	0.7	0.9	0.9	0.8	1	1.1	**C**	

24	21	18	15	12	9	6	3	Replicate	Class Size
1.1	0.9	1.1	1	1.2	2	1.1	1.1	**A**	**small**
1.2	1.1	1.2	1.2	1.1	1.3	1.1	1.2	**B**	
0.9	1	1.3	1.1	0.9	1	1.4	2.1	**C**	

Summary of NH_3 Data (feeding Treatment)

Sampling (at 3 hour interval)

24	21	18	15	12	9	6	3	Replicate	Class Size
0.7	0.7	0.7	0.8	1.3	1	1.3	1.2	**A**	big
0.7	0.9	0.8	0.9	0.9	1.5	1.1	1.1	**B**	
0.6	0.7	0.9	0.9	0.9	0.5	1	1.1	**c**	

24	21	18	15	12	9	6	3	Replicate	Class Size
1	1.1	1.2	1.1	0.9	0.9	1.3	1.2	**A**	**small**
0.7	1	1.2	1.2	1.1	1	1.5	1.1	**B**	
0.8	0.9	0.8	0.9	1.1	1.1	1	1.2	**C**	

Picture No (1): recirculating water system for Tilapia fingerlings experiment