

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

**EFFECT OF FERTILIZATION FREQUENCY ON THE PRODUCTIVITY OF
EARTHEN PONDS CULTURED WITH ALL-MALE *OREOCHROMIS NILOTICUS*
(NILE TILAPIA).**

A thesis submitted to the Department of Theoretical and Applied Biology,
Kwame Nkrumah University of Science and Technology, in
partial fulfilment of the requirements for the award

of

Masters of Philosophy Degree in Limnology and Fisheries

By

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DECLARATION

I hereby declare that this thesis is my own work towards the award of MPhil in Limnology and Fisheries and has been composed under supervision. It has not been submitted previously either wholly or partially for a degree in the Kwame Nkrumah University of Science and Technology or elsewhere, except where due acknowledgement has been made in the text.

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Head of Department

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Date

DEDICATION

I dedicate this work to my dear wife, Alberta Osei Kusi, my three sons and my parents.

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ACKNOWLEDGEMENT

I wish to express my profound appreciation to God Almighty for His protection throughout the project. His praises shall continually remain in my mouth. My thanks goes to the staff members at Tano-Odumasi Pilot Aquaculture Centre for their assistance and inputs they provided in this research. I do appreciate the role of Mr. Agyei Francis (Manager), Gabriel and Emma in this quest. I am grateful to my supervisor, Dr. Samuel Aikens who besides reading and marking the scripts, explore all possible avenues to help make my efforts fruitful. It was due to his constructive criticisms that brought improvement to this work. To my family, especially my wife; Alberta Osei Kusi, my parents, my in-laws and my siblings. I say thank you for your support in prayer, financially and emotionally.

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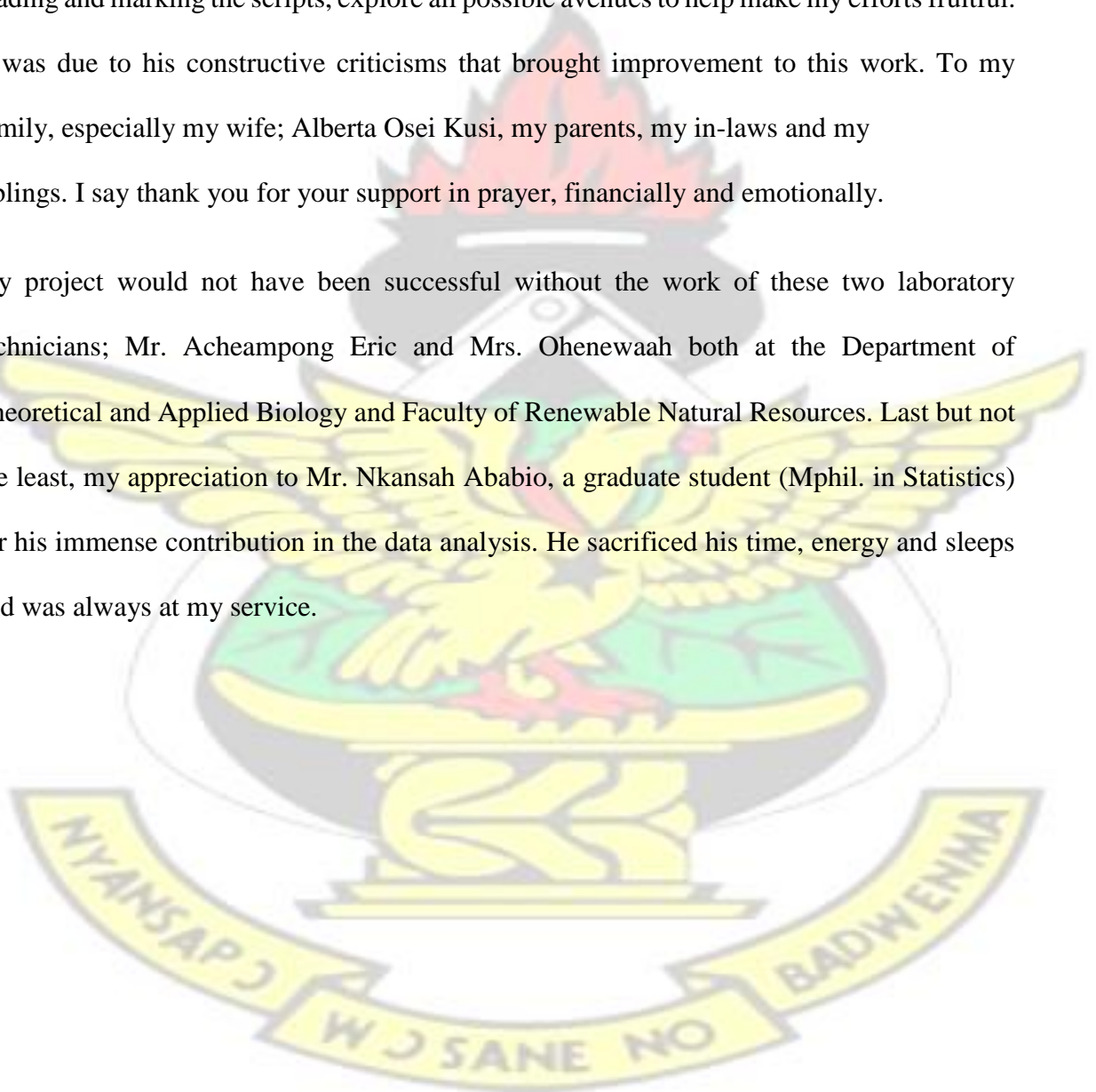


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LIST OF ABBREVIATIONS

DO	Dissolved Oxygen
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
OMF	Once a Month Fertilization Frequency
OMF1	Once a Month Fertilization Frequency One
OMF2	Once a Month Fertilization Frequency Two
OMF3	Once a Month Fertilization Frequency Three
SGR	Specific Growth Rate
TDS	Total Dissolved Solids
TMF	Twice a Month Fertilization Frequency
TMF1	Twice a Month Fertilization Frequency One
TMF2	Twice a Month Fertilization Frequency Two
TMF3	Twice a Month Fertilization Frequency Three
WHO	World Health Organization

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ABSTRACT

This study was conducted to evaluate the frequency of fertilization effect on the productivity of earthen ponds cultured with all-male *Oreochromis niloticus* (Nile tilapia) at Tano-Odumasi between June to December, 2014 in Ashanti region of Ghana. The effect of manuring regime was assessed on the water qualities of pond (which included nutrients in the form of nitrates and phosphates), plankton production, fish monthly growth rate and yield as well as the microbial contamination of the ponds water, fish skin and muscles. There were three replications per treatment and each pond was stocked with 600 fingerlings of the average 10.3g. All the experimental ponds received the same total manure inputs (600 kg). Fertilization frequencies used were 50 kg and 100 kg on biweekly and monthly basis respectively. Bagging method of manuring and supplementary feeding (2% of fish wet body weight) were used throughout the experimental periods of 180 days. Samples were collected on monthly basis apart from the microbial data of the ponds water, fish skin muscles which were done once. The data gathered were analyzed using independent T-test of variance at 95 % confidence level. Results indicated that ponds that were fertilized biweekly intervals gave greater net yield (7898.4 ± 110 kg/ha), specific growth rate (0.833% day⁻¹) and individual fish yield (315.59 g) than once a month fertilized ponds net yield (6504 ± 62.6 kg/ha), specific growth rate (0.797% day⁻¹) and individual fish yield (269.64 g) respectively. All the physico-chemical parameters and nutrients of the ponds water did not

differ significantly and remained in an acceptable range for *Oreochromis niloticus* culture except ammonia concentration which was high. At the end of the study, it was also revealed that ponds water, fish skin and muscles had been contaminated by coliform bacteria including *Total coliform*, *Faecal coliform*, *Enterococci coliform* and *Faecal enterococci* when analyses was done using Most Probable Number (MPN) method. Although no *Salmonella* was present prior treatment of the fish is required before consumption.



CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Fish demand has been increased nowadays all over the world due to the recognition of its nutritional value. It provides protein and other nutrients in human diet (FAO, 2006). Since capture fisheries alone cannot meet the world's protein demand there is the need for increased fish production through aquaculture (FAO, 2012).

Fertilization of ponds using either inorganic fertilizers, organic fertilizers or both is a management practice that enhances biological productivity. It enables fish farmers to increase fish yield by ensuring natural food in the pond ecosystem (Bocek, 2009). However, because the cost of inorganic fertilizers is high particularly in developing countries there has been a shift to utilize manure (Das and Jana, 1996). In fish culture greater part of production cost is contributed by feed. About 65% to 75% of the intensive fish culture inputs are contributed by feed. Reduction of protein cost is therefore a major objective in fish nutrition (Yadava and Garg 1992). One way of doing this is to maximize productivity through the use of inorganic fertilizers and manures (FAO, 1997).

Fish feed and inorganic fertilizers cost in fish culture can be drastically reduced by judicious use of organic wastes. According to Yadava and Garg (1992), the use of organic manure economically relieves the farmer because it reduces 50% costs of inorganic fertilizer and supplemental feeding. Poultry manure, dung from cows, sheep, pigs, among others are used to establish biological productivity in ponds. These are known suitable substitutes for the costly feeds and inorganic fertilizers. Organic fertilizers are sources of essential nutrients

including nitrogen, phosphorus and carbon for the growth of algae. The nutrients they release when used to fertilize fish ponds promote planktonic growth (Ansa and Jiya, 2002). Organic fertilizers that are mostly used in Ghana in ponds fertilization include poultry waste, sewage, cow dung and pig dung.

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1.2 Problem Statement and Justification

A fishpond ecosystem should be well managed in order to ensure better yield of fish. The two major considerations that are involved in the management of fishpond are the total load of nutrient requirements and how frequent it should be applied. However, there have been inconsistencies in frequency of manuring (Wohlfarth and Schroeder, 1979) and the differences are usually based on the cost of labour or measure of waste disposal without considering what the pond ecosystem requires to produce fish.

Although manure is used to fertilize fishponds in Ghana no research has been carried out on the frequency of application and its effect on ponds productivity. There is no documentation on the manure fertilization frequency as most fish culturists do trial-and-error fertilization regimes (Wohlfarth and Schroeder, 1979). Some even rely on what they observe visually and apply the manure according to the pond colour but when the fish pond is over or under fertilized, it results in low yield of fish (Das and Jana, 2003). It is necessary to establish the best regime in which manure must be applied to reduce manure use and wastage as well as optimizing pond productivity.

There have also been scrutinizations by some people as they criticize that the use of manure in pond fertilization could lead to problems in the environment (Wohlfarth and Hulata, 1987). Different views have been expressed on the dangers associated with the use

of manure to fertilize fishponds (Jinyi *et al.*, 1988). Some people strongly caution against the consumption of farm-raised fish in which animal manure or faeces has been used to fertile the fishponds (Ampofo and Clerk, 2010). This is because several indicator microorganisms or pathogens are found in animal wastes (Quines, 1988).

1.3 Objective

The main objective was to assess the effect of fertilization frequency on the productivity of earthen ponds cultured with all-male *Oreochromis niloticus*.

Specifically, the research was aimed at achieving the following objectives:

1. To determine the effect of fertilization frequency and seasonality on the physico-chemical qualities of the pond including dissolved oxygen, temperature, pH, transparency, total dissolved solids, salinity, electrical conductivity and ammonia.
2. To determine the effect of fertilization frequency and seasonality on the level of nutrients in the form of nitrates and phosphates released.
3. To determine effect of fertilization frequency on diversity and abundance of phytoplankton and zooplankton in the ponds.
4. To determine the effect of fertilization frequency on microbial contamination in the pond water, fish muscles and skin.
5. To determine the effect of fertilization frequency on fish monthly growth rate and total yield.

1.4 Research Hypothesis

The study seeks to test and validate the following theoretical hypotheses:

- i. H_0 : Fertilization frequency does not have effect on the water quality of the pond.

H_1 : Fertilization frequency has effect on the water quality of the pond.

- ii. H_0 : Fertilization frequency does not have effect on the plankton abundance in fishpond.

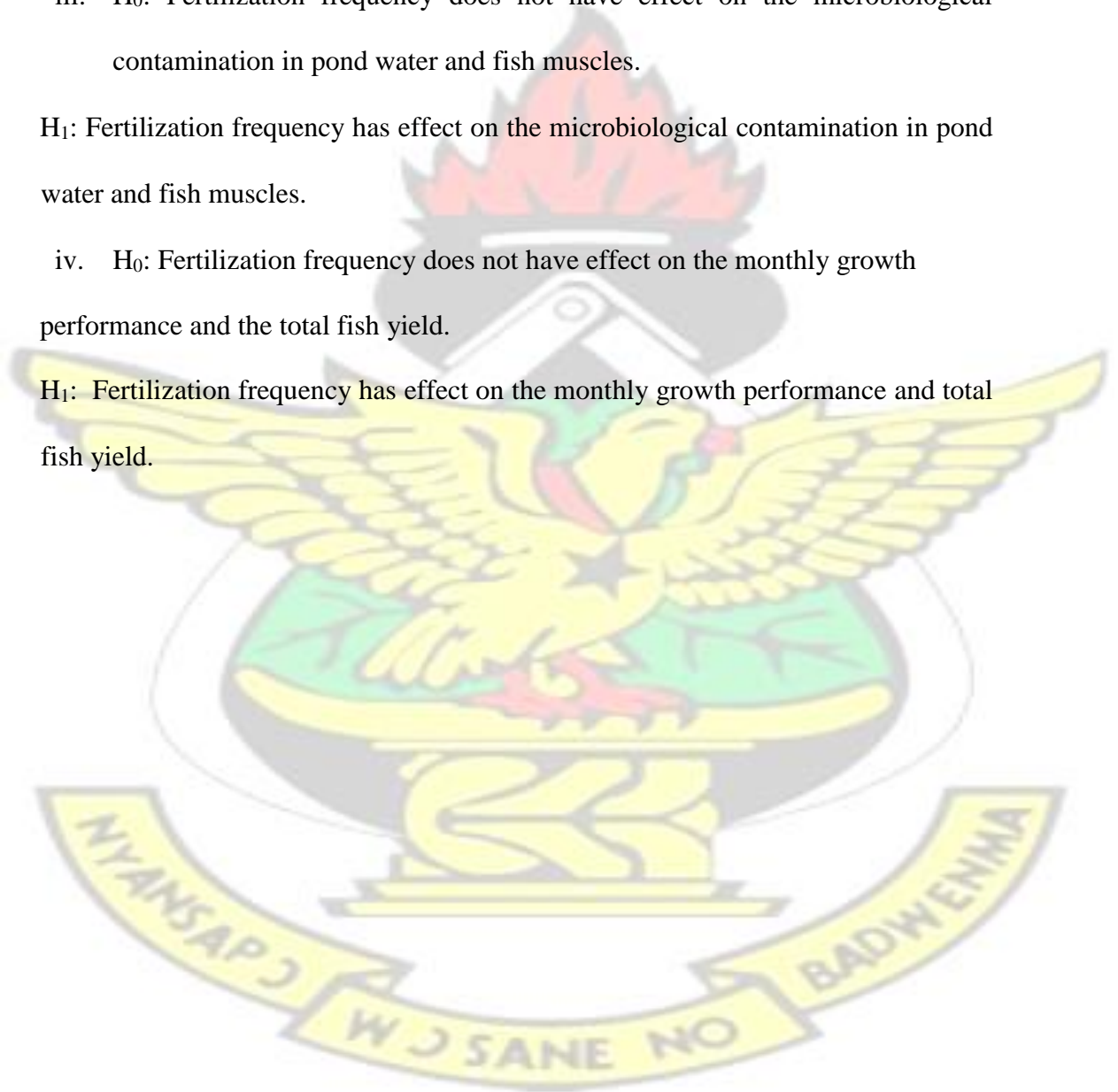
H_1 : Fertilization frequency has effect on the plankton abundance in fishpond.

- iii. H_0 : Fertilization frequency does not have effect on the microbiological contamination in pond water and fish muscles.

H_1 : Fertilization frequency has effect on the microbiological contamination in pond water and fish muscles.

- iv. H_0 : Fertilization frequency does not have effect on the monthly growth performance and the total fish yield.

H_1 : Fertilization frequency has effect on the monthly growth performance and total fish yield.



CHAPTER TWO

LITERATURE REVIEW

2.1 The use of chemical fertilizers and organic fertilizers in fishponds

Chemical (inorganic) fertilizers and organic fertilizers (manures) are applied in fishpond to ensure its productivity. Each of them has its own advantage, disadvantage or particular use (Bocek, 2009). Chemical fertilizers are usually expensive especially in developing countries even though small quantities are required. Fish can consume microorganisms such as bacteria and fungi in the organic manure (Brunson et al., 1999). Unlike chemical fertilizers which induce only phytoplankton bloom, organic fertilizers induce both phytoplankton and zooplankton blooms (Bocek, 2009). The use organic manures have the following advantages (Brunson *et al.*, 1999);

1. They are available at little or no cost.
2. They take shorter period to produce the natural food especially for production of zooplankton in rearing ponds than using inorganic fertilizers.
3. The decomposition of organic materials increases natural fish food mainly phytoplankton and subsequently, it releases carbon dioxide.
4. Organic fertilizers improve the pond soil.
5. Fish species such as tilapia can consume them directly as food.
6. They also promote bacterial and protozoan growth which can be utilized directly by fish or fish food organisms.

In spite of all these benefits, a problem could arise if large quantity is applied in pond fertilization. When such occurs, depletion of oxygen as a result of the manures decomposition may lead to the production of harmful substances in the pond (Brunson *et al.*, 1999). However, organic manures become the best choice of fertilizer if they are managed properly in pond fertilization (Bocek, 2009). Among organic fertilizers, poultry manure is considered as the best fertilizer in pond fertilization because its content is the combination of both urine and faeces which releases high amount of nitrogen (Knud-

Hansen *et al.*, 1993). It is also considered as a complete fertilizer because it has both the qualities of organic and inorganic fertilizers (Njoku and Ejiogu, 1999; Njoku, 2008).

In application of organic manure in fish pond, several methods could be used. Some have advantages as compared to the others. Among the methods used are bagging, broadcasting and the compost crib. All of these methods are used in the establishment of green colour or algal bloom of the ponds and study conducted in Malawi reported that high survival rate of fish was observed when bagging method was used in pond fertilization (Ludoviko and Kang'ombe, 2012).

2.2 Effects of manuring and fertilization

Fishponds are fertilized to stimulate the growth of natural food known as planktons to be consumed by fish (Brunson *et al.*, 1999). In semi-intensive systems, using animal manure ensures the growth of the pond's natural food and fish (Nwachukwu, 1997). There is direct relationship between organic manure load and the yields of fish and excessive use of organic manure increases microbial activity (Ansa and Jiya, 2002). The use of organic manure releases nutrients in the form of nitrate and phosphate and increase phytoplankton and zooplankton biomass of the pond water. Manure use economically relieves the farmer because 50% cost of inorganic fertilizer and supplemental feeding is reduced (Yadava and Garg 1992).

2.3 Fertilization frequency

When adopting a system in pond fertilization involving manure dosage which is fixed, other factor to be considered is the interval by which the manure should be applied (KnudHansen

and Batterson, 1994). This is because when cells of the algae are growing, dividing or reproducing, their nutrients requirements change (Boyd and Musig, 1981). The cells of algae take about 3 to 4 days to divide and reproduce depending on the conditions of the ponds according to Fogg, (1975). Depending on the type of manure and method of manuring, fertilization frequency can or cannot affect fish growth and water quality (Milstein *et al.*, 1995). One study conducted in Thailand reported that fertilization frequency had no effect on the net fish yield or net primary productivity (Knud-Hansen and Batterson, 1994) but Garg and Bhatnagar, (2000), reported of a significant effect of fertilization frequency on fish yield in study conducted in India. In another study conducted in Honduras, fertilizer application frequency had effect on the primary productivity of the ponds and fish yield (Teichert–Coddington *et al.*, 1990).

2.4 Effect of manure application on water quality

Organisms in aquatic ecosystem under culture conditions perform best when they are not subjected to stressful environment (Bhatnagar and Davi, 2013). Maintaining good water quality is the best way to avoid stressful conditions in the water ((Davenport, 1993). Fish and culture organisms could only tolerate certain range of water quality parameters. A drastic change affects fish growth and performance (Kiran, 2010). The method as well as frequency of manuring affects the rate at which changes of water quality occurs (MertinezPalacios *et al.*, 1998). The most critical water quality parameters in aquaculture production systems are dissolved oxygen, temperature, pH, turbidity, total dissolved solids, salinity, electrical conductivity, nitrate, phosphate and ammonia concentration (Mmochi *et al.*, 2002). Dissolved oxygen (DO) amount in ponds is one of the most important factors for the growth and survival of fish. Fish need dissolved oxygen for life processes (Bhatnagar and Singh, 2010). Cloudy weather, plankton die-off and heavy stocking result in low levels of dissolved

oxygen can stress or kill fish (Bhatnagar and Davi, 2013). Some signs exhibited by *Oreochromis niloticus* when there is low dissolved oxygen concentration in the ponds include gasping for air at the surface, sluggishness and poor eating, crowd near water inflow pipe, slow growth and outbreak of diseases (Ingthamjitr, 2003). Dissolved oxygen level needed for good performance of fish is usually greater than 5 mg/l (Bhatnagar *et al.*, 2004). Although some fish species can survive as in a low dissolved oxygen concentration of 1.0 mg/l for some time, the desirable limit to support better growth is 5.0 mg/l (Ekubo and Abowei, 2011). The metabolic rate of poikilothermic aquatic animals and plants is controlled by temperature (Bhatnagar and Davi, 2013). Tilapia performs better when temperature is between 20 to 32 °C (Mjoun *et al.*, 2010). According to Huet, (1994) the tolerable temperature range for *Oreochromis niloticus* is from 12 to 42 °C. Temperature as well as certain toxic level of ammonia could influence the metabolic and feeding rates of fish. The recommended temperature range for fish farming is from 25 to 30 °C according to FAO, (2006).

Fishpond pH also influences fish growth and survival (Mmochi *et al.*, 2002). According to Schofield (1976) acidic water destroys gills tissues, causes inflammation and increases in mucus secretion in the gills. *Oreochromis niloticus* can survive in pond pH ranging from 5 to 10 and their best growth occurs in pH ranging from 6 to 9 (Popma and Masser, 1999).

Salinity also plays an important role in the culture organisms' growth through osmoregulation of body minerals from the surrounding water (Jamabo, 2008). It is important to maintain salinity in the pond water within an acceptable range for better survival and growth of fish (Bhatnagar and Singh 2010). Freshwater tilapia can tolerate salinity up to 5 ppt or 0.05 mg/l, beyond this amount is not desirable Boyd, (1998). Conductivity is a measure of the total ionic content of water, and therefore shows the freshness or otherwise

of the ponds (Ogbeibu and Victor, 1995). A conductivity range of 3.8 to 10 mS/cm is virtually poor in chemicals (Sikoki and Veen, 2004) while a range of 100 to 2000 mSiemens/cm is desirable and that of 30 to 5000 mSiemens/cm is considered acceptable for pond fish culture (Stone and Thomforde, 2004). Turbidity, another physicochemical parameter of fresh water culture (Mmochi *et al.*, 2002) could be caused by planktons and solids that are suspended in the column of the water bodies. It includes clay and silt particles in ponds (Stickney *et al.*, 1979). Planktons tend to be distributed evenly in ponds, so algal bloom or turbidity is monitored indirectly by measuring the clarity of the water using Secchi disk (Boyd, 1998). Transparency ranging from 20cm and 60cm is considered for optimum freshwater pond management (Boyd, 1998). However the preferred range for aquaculture is species dependent. Other turbidity due to suspended silts or other solids is harmful as it could suffocate the fish and eventually result in death. In this case, the suspended particles could cover the gill filaments of the fish thereby preventing effective gaseous exchange in the gills, hence the suffocation to death (Boyd, 1998). There is increased in fish growth performance in low turbid water because it facilitates easy light in order to enhance photosynthesis by algae (Bash *et al.*, (2001). Total Dissolved Solid (TDS) is also one water quality factor that influences fish production. In fish culture, TDS value less than 200 mg/l is optimal range for cold and warm-water fish in intensive aquaculture while a maximum value of 400 mg/l is permissible for diverse fish production (James, 2000).

2.5 Nutrients and nitrogenous compounds in pond water

The two most important nutrients are nitrogen and phosphorus and both of them should be sufficiently available in freshwater to ensure a balanced ecosystem (Boyd, 1998). Although nitrate is not toxic to fish in ponds when it exceeds 90 mg/l it becomes toxic (Stone and Thomforde, 2004). According to Santhosh and Singh (2007) nitrate level from 0.1 to 4.0

mg/l is considered favourable for fish culture. Phosphorus is present in fishponds in phosphate (PO_4) form and nutrients containing phosphorus as well as nitrogen promote planktonic growth (Bhatnagar *et al.*, 2004; Boyd, 1998). Phosphorus is an essential plant nutrient which promotes algal growth to increase the productivity of aquatic life. The phosphate level of 0.06 mg/l is considered favourable for growing fish in ponds (Stone and Thomforde, 2004) and the range of 0.05 to 0.07 mg/l is optimum and productive while 1.0 mg/l is good for plankton production (Bhatnagar *et al.*, 2004). Ammonia is the wasteproduct from the bacterial breakdown of organic materials such as uneaten food, faeces, among others (Bhatnagar and Devi, 2013). Total ammonia comprises of un-ionized (NH_3) form which is toxic and the ionized (NH_4^+) form which is not toxic (Boyd, 1998). At certain temperature and pH concentrations make high levels of ammonia toxic to the life in water bodies (Boyd, 1998). The concentration of ammonia less than 0.2 mg/l is considered undesirable for fish culture in ponds (Bhatnagar and Singh, 2010).

2.6 Microbiological quality of fishponds

Manure serves as a substrate for bacteria and protozoa growth which provides protein rich food for the cultured fish. Excessive application of manure increases the microbial activity (Wohlfarth and Schroeder, 1979) and causes pathogenic bacteria infection in fish tissues of (Ampofo and Clerk, 2010). Numerous diseases are acquired from microorganisms found in water and fish (Olayemi *et al.*, 1991). Coliforms are bacteria that are used as possible indicators of faecal contamination (Morgan, 1990). They themselves are not harmful but they indicate the possible presence of organisms that cause diseases. The presence of these coliforms bacteria also shows the presence of disease pathogens and that consumption of such fish might be risky (Cairncross and Quano, 1991).

2.7 Feeding and diet of *Oreochromis niloticus*

Oreochromis niloticus (Tilapia) is capable of feeding a diet from both natural and artificial. Tilapia is predominantly herbivorous, which means that they eat food from plant sources (Jauncey and Ross, 1982). Both juveniles and adults have diurnal feeding pattern (Cimbaro, 2000). *Oreochromis niloticus* is phytoplanktivore and a facultative detritivore fish (Abdel-Tawwab, 2000). *Oreochromis niloticus* in Agulu Lake basin in Nigeria fed mainly on a wide variety of phytoplankton and zooplankton (Anibeze, 2001). In addition to grazing on phytoplankton (Moriarty, 1973), tilapia feed on benthic, attached and detrital aggregates (Bowen, 1982). It has been argued that tilapias are perhaps the only true herbivorous fishes (Bitterlich and Gnaiger, 1984). This was because the content of the guts of naturally feeding *Oreochromis* and *Sarotherodon* species comprise mainly algae and algal detritus (Khallaf and Alme-na-ei, 1987).

2.8 Major constraint in the production of *Oreochromis niloticus*

The major drawback in the culture of *Oreochromis niloticus* is their highly precocious reproductive efficiency (Hepher and Pruginin, 1982). This results in overcrowding, leading to long grow-out periods and small fish yield that fetch lower prices (Teichert-Coddington *et al.*, 1997). Stocking ponds with monosex mainly all-male *Oreochromis niloticus* is a method for controlling excessive recruitment in ponds (Chervinski, 1982). Male tilapias growth rate is naturally faster as compared to that of the females (Hanson *et al.*, 1983; Toguyeni *et al.*, 1997) making them better choice for commercial fish farming. The females spawn at frequent intervals even if the eggs are not fertilized. Thus energy is diverted from growth to egg production (Hepher and Pruginin, 1981). Using fingerlings that are all males is the best way to solve this problem (Green *et al.*, 1997). In Ghana, the Fishery Department in the Ministry of Food and Agriculture applied the oral administration of 17-alpha methyl

testosterone to sexually undifferentiated *Oreochromis niloticus* fry as the standard means of producing all-male tilapia since 1998.

2.9 Stocking density of fish

The growth performance of tilapia in a fish pond is affected by the stocking density (Rakocy and McGinty, 1989). Beyond the carrying capacity of the water body, growth will be adversely affected even if other factors such as food and others are available (Diana *et al.*, 1996a). Stocking depends on duration of production, natural productivity of water and the size of the fish to be produced (Huet, 1994). Although the rate of fish growth declines as fish get larger as most energy is channeled into physiological activities according to Wootton (1992), high stocking density reduces individual growth rates as reported by Diana *et al.*, (1997).



CHAPTER THREE

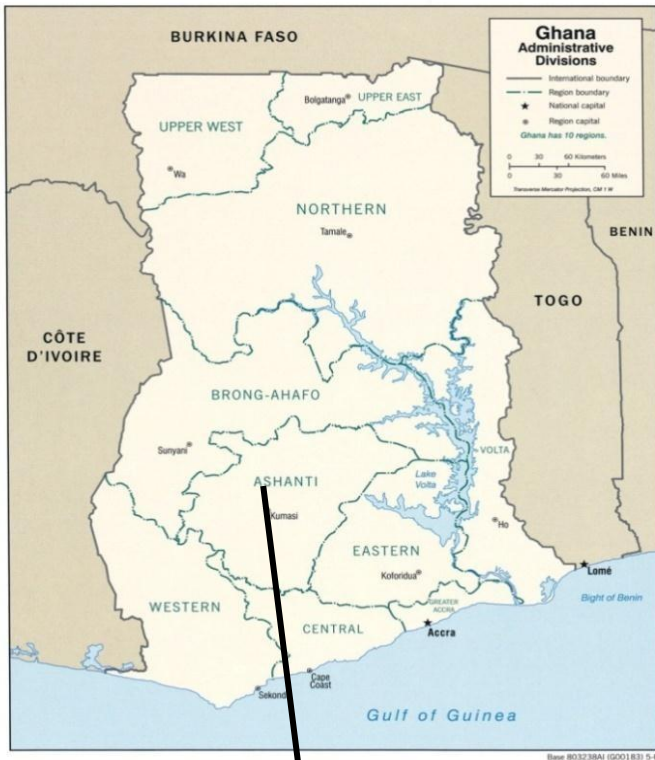
MATERIALS AND METHODS

3.1 Study Area

This research was carried out at Pilot Aquaculture Centre at Tano-Odumasi in the Sekyere South District in Ashanti Region of Ghana from July to December, 2014. Tano-Odumasi is

located 35 km away from Kumasi, and 3 km from Agona, from the Kumasi-Mampong highway (Figure 1). Specifically, the District lies between latitudes $6^{\circ} 50''$ N and $7^{\circ} 10''$ N longitudes $1^{\circ} 40''$ W and $1^{\circ} 25''$ W. The District has a total land area of 780 km^2 . The climate of the study area is equatorial with two major rainfall patterns which occur in the months of May till July and from September till October. Typically, minimal rainfall is experienced from September month. Mean temperature within the area is 27°C . The vegetation of the District lies within the rainforest belt, and it can be described as moist semi-deciduous. The District is drained by the Offin, Oyon and Abankro Rivers. The two geological formations in the District are the Voltain and Dahomeyan formations.

Pilot Aquaculture Centre at Tano-Odumasi was selected for this study because of the availability of support fishery facilities. The site is the largest fingerling hatchery centre in the Ashanti region and has well-constructed earthen and concrete ponds as well as aquaculture equipment and materials. Rooms and places for keeping inputs such as feeds and manure are also available. The security of the experimental ponds was guaranteed with the help of centre security personnel and the fishery officers. The research centre is also endowed with good quality and quantity water. The soil type is mainly sandy-clay for water retention and the supply of water is throughout the year which is pollution free. The area is not prone to flooding because the topography is not too steep. The place is also accessible for easy transportation of inputs to perform experimental activities and data collection.



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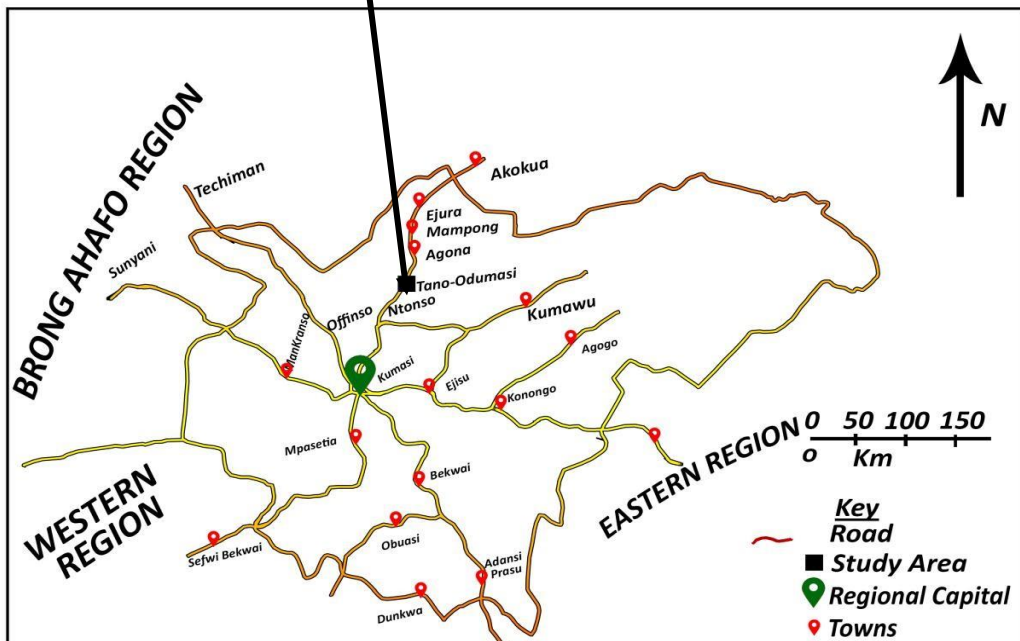


Figure 1: The study area location from Ghana and Ashanti region maps.
3.2 Ponds Preparation

Six ponds at the Pilot Aquaculture Centre were used for the study. The size of each of the ponds was 250 m². The ponds were drained, cleaned and lime applied. Pumping machine

was used to drain the ponds water completely. The purpose of draining the ponds water was to get the existing fish transferred to other ponds. It was also to pave way for the liming to be done and to replace the used or waste water with fresh water. The ponds were cleaned to remove all plants and animal residues. Fifty kilograms of calcium carbonate (powdered lime) each was applied to each of the six ponds. The lime was spread evenly on the ponds bottom and dike (Plate 1). Prior to the lime application, the ponds bottom was saturated with water to ensure that the lime applied react with pond soil. Liming was done to neutralize the acidity of the ponds to more desirable level and also control parasite particularly leeches (Boyd, 1998). The ponds outlets and inlets were screened using 12 mm wire-mesh to prevent foreign organisms from entering or to prevent the escape of used fish from ponds. Two weeks after liming, the ponds were refilled with fresh water from the canals up to 1.2 m depth using pumping machine. Adosu stream and rainwater were the main sources of water to fill the ponds during the experimental period. The ponds water levels were maintained throughout the experimental period.

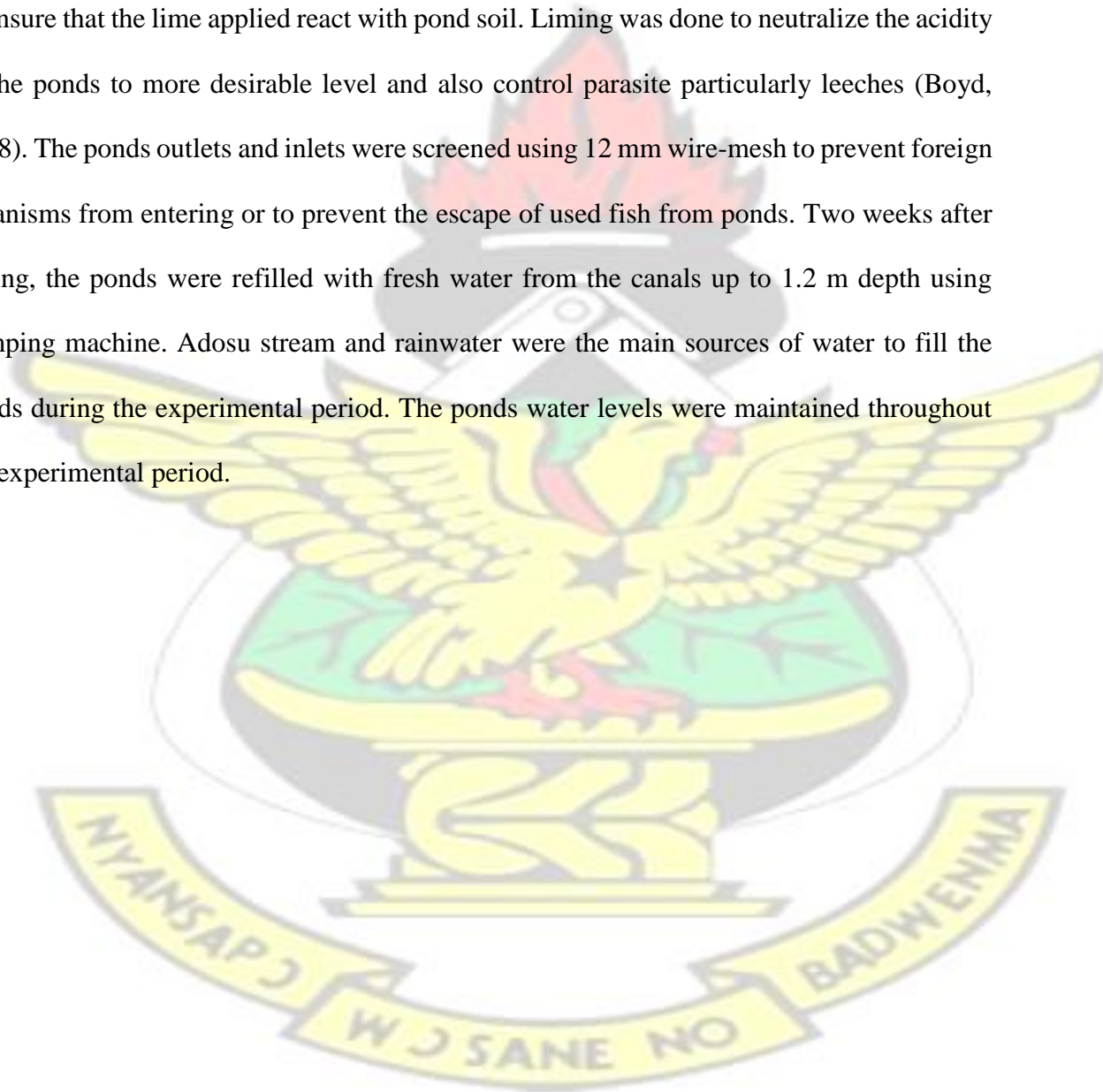




Plate: 1 Pond preparation involving liming

3.3 Experimental Design and layout

Two fertilization frequency treatments with three ponds each per frequency were used in this study. The first treatment ponds received manure on monthly basis whilst the second treatment ponds were also fertilized biweekly in a month. The first set of ponds was labeled as Once Month Fertilization One (OMF1), Once Month Fertilization Two (OMF2) and Once Month Fertilization Three (OMF3) whilst the second set of ponds was labeled as Twice Month Fertilization One (TMF1), Twice Month Fertilization Two (TMF2) and Twice Month Fertilization Three (TMF3) (Figure 2).

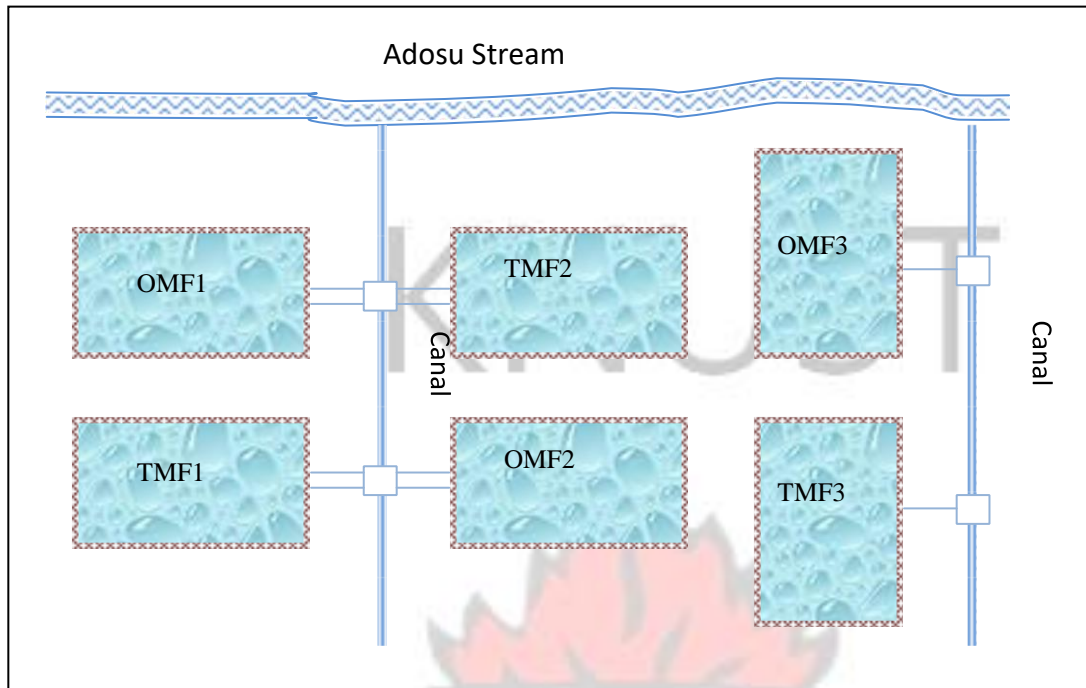


Figure 2: Schematic presentation of the experimental ponds layout in the study area.

Chicken manure was used to fertilize the ponds. The manure application rate used for the once a month fertilized ponds was 100 kg whilst 50 kg was used for the twice a month fertilized ponds. In all, 600 kg total quantity of manure loads was giving to each of the ponds throughout the experimental or grow-out period (Table 1).

Table 1: Fertilization frequency and rate of chicken manure application.

MANURE APPLICATION FREQUENCY	APPLICATION INTERVAL	RATE	TOTAL FOR ENTIRE DURATION
Twice a month	Biweekly	50 kg	600 kg
Once a month	Monthly	100 kg	600 kg

Bagging method was used in the manure application. In this method, the manure was placed in bags and perforated before being put into the ponds with tied thread pegged at the corners of the ponds (Plate 2). Shaking was done to release the nutrients. The nutrients dissolved and seeped out of the bags and mixed by the water currents (Ludoviko and Kang'ombe, 2012).



Plate 2: Manure application using bagging method

Sex-reversed all male *Oreochromis niloticus* (Nile tilapia) fry less than 0.5 g supplied were raised in hapas up to 10.3 g average weight. This was done to control the predation by frogs and also to increase their survival rate. All male tilapia were used because they naturally grow faster than females (Toguyeni *et al.*, 1997). As recommended by the FAO, the stocking

density of *Oreochromis niloticus* in semi-intensive culture ponds usually ranges from 5,000 to 30,000 fish per hectare (Diana *et al.*, 1997). Therefore each of the six experimental ponds was stocked with 600 fingerlings. The fingerling were not thrown into the ponds but were allowed to swim out of the rubber bucket to the ponds. Supplemental feeding (Coppens) was given to the stocked fish daily at 2% of their wet body weight. The amount of feed was adjusted or increased monthly in proportionate to their weight increments. Feeding was performed manually. Feeding was also done twice daily about 8 to 9 am in the morning and 5 to 6 pm in the evening. In every week, there was two days skip in the supplemental feeding to ensure that the fish eat the natural food present.

3.4 Water Quality Analysis

Water samples were obtained at monthly intervals between 7 to 9 am from different places and depth of each pond. The samples from each pond were mixed gently in a plastic bucket. A subsample of 500 ml from each of the ponds water was placed in a well sterilised polyethylene bottle and labeled using permanent marker. The water samples were put in an „ice chest“ and transported to the laboratory for the analysis of ammonia, nitrates and phosphates. Other limnological variables such as temperature DO, pH, EC, and salinity and transparency were measured directly on the field using Hanna 9828 multi-parameter instrument. The probe attached to the meter was immersed directly in the ponds and the respective readings that appeared on the meter after the instrument had been stabilized were taken and recorded. Three readings were taken from different locations and the averages were recorded.

Secchi disk was also used to measure the transparency of the pond (Plate 3). The disk was submerged into the water until the barely disappeared. The depth was recorded again after

being lowered for it to disappear and it's barely reappeared. The two depth measurements were added and divided by two to obtain the Secchi Disk Average which is also the Secchi Disk Visibility or transparency of the pond water.



Plate 3: Measurement of pond transparency using Secchi disk

3.5 Plankton Analysis and Enumeration.

Five hundred millilitres of water samples for plankton analysis were collected on monthly (30-days intervals) from different locations and depths of each pond. The samples collected were gently mixed and 5ml subsamples were poured into amber glass test tubes. Lugol iodine solution and formalin (5% buffered) were used to preserve phytoplankton and zooplankton samples respectively. An advantage of Lugol's solution is that flagellates preserved with it

retain their flagella (Vollenweider, 1969). Two drops each of the preservatives were added to 5 ml water samples each in 5 different glass test tubes to preserved phytoplankton and zooplankton respectively. This was done at the research site (plate 4). The samples were sealed, labeled (using wax pencil) and transported in dark cool conditions less than 10⁰C to the laboratory for analyses. The preserved plankton samples were stored in the refrigerator at 4⁰C and the analysis was done within three weeks.



Plate 4: Plankton sampling and preservation

Plankton Enumeration and Estimation

Equipment for Plankton Analysis

Labomed C×L Monocular microscope with camera attachment at 200 × magnifications, Sedgwick-Rafter Counting Cell (S-R cell) & cover glass, 500 ml plastic bottle for sample collection, 10 ml sterile transfer pipette, Plastic Petri dish.

Procedure for plankton enumeration

Pipette was used to fill the S-R cell with approximately 1 ml of well mixed sample filling the slide but not overfilling. Cover glass was placed diagonally across the cell after which it was mounted on the Labomed C X L Monocular microscope (Plate 5) with Optika VisionLite software to capture the images on the cell at 200X magnifications. The phytoplanktons and zooplankton were identified using Benskin, (2009) and Park, (1999) determination keys, respectively followed by counting. The captured images (planktons) on the computer monitor were counted. Three strips each from each sample were counted and they were used for quantitative estimation using the following method and the following formula given by Benskin, (2009).

$$\text{Units/ml} = \frac{C \times 1000\text{mm}^3}{L \times D \times W \times S}$$

„C“ represents the number of planktons counted;

„D“ represents the depth of a strip (S-R cell length) = **1 mm**;

„L“ represents the length of each strip (S-R cell length) = **50 mm**;

„S“ represents the number of strips counted = **3**;

„W“ stands for the width of a strip (Whipple grid image width) = **0.355 mm** (for 20X ocular)



Plate 5: Plankton identification and enumeration

3.6 Sampling and Growth Parameters of Fish

After every 30 day, samples of sixty stocked fish were taken at random from each of the ponds using drag net. Catching of the fish was done by dragging the net through the ponds (Plate 6) and the fish caught were put in the rubber pan containing water fetched from the ponds in which the fish were caught. The fish wet body weight was measured using electronic balance (Plate 7).



Plate 6: Fish sampling using seine net

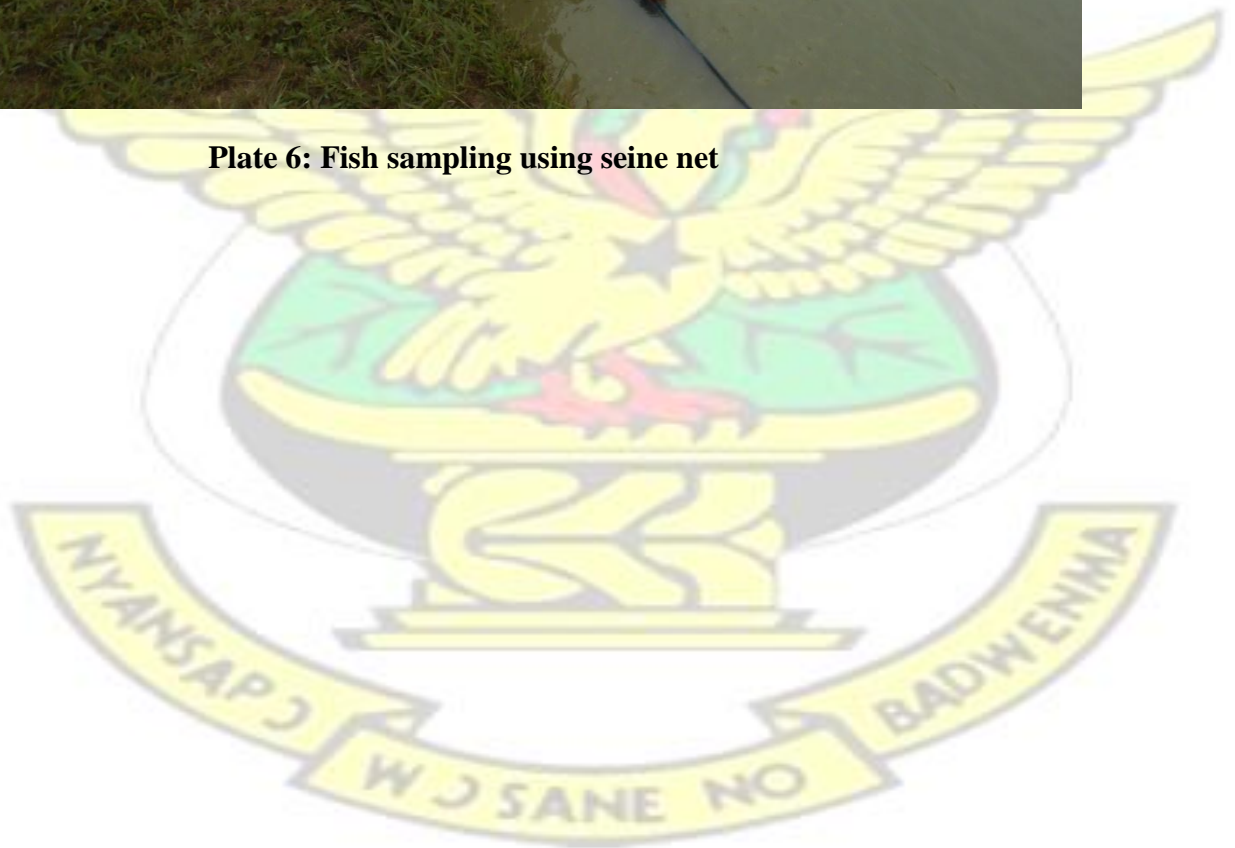




Plate 7: Determination of fish weight

The fish caught were identified by cutting part of their caudal tail during the first three months and the dorsal tail during the last three months so that they would not be sampled in the subsequent months. After taking the sampled fish wet weight, they were released back into their respective ponds from where they were caught. This was done in the morning to avoid causing stress to the fish. The growth parameters of fish measured were as follows:

Specific Growth Rate = $\frac{\ln(\log \text{ Fish final weight}) - \ln(\log \text{ Fish initial weight}) \times (100)}{\text{Time between weighing (days)}}$

Weight gain = Fish final weight – Fish initial weight.

Percentage weight increase = $\frac{(\text{Fish final weight} - \text{Fish initial weight})}{\text{Fish final weight}} \times 100\%$.

All the number of fish that died were removed from the ponds without replacement and recorded. Trial continued for 180 days. After the termination of the study, the ponds were drained using pumping machine and the fish from the two treatments (with the three replicates) were harvested using seine net, counted and weighed using direct reading balance to determine the fish yield. Total fish production and rate of survival were determined by the use of the following formulae:

Fish net yield per hectare = Harvested fish weight (kg) minus initial fish weight (kg) divided by unit area (ha)

Survival rate = (initial number of fish minus number of dead fish) divided by initial number of fish $\times 100$.

3.7 Microbial Sampling and analysis of ponds water, fish skin and muscles

500ml water sample was fetched from each of the experimental ponds from different locations on the 5th month (November) of the production period. Before sampling, the bottles were labeled using permanent marker. The water samples were put in an „ice chest“ and were being transported to the microbiological laboratory for the microbial analysis.

Eight (8) live fish of the same sizes were taken at random from the harvested fish. The swap from the fish skin was taken using clean cotton wool and was put in 10 ml sterile peptone water in sterile rayon (Plate 8). They were also sealed, labeled and placed in an „ice chest“ in ice packs after which they were transported along with the sampled fish to the University laboratory for microbial analysis. The results were recorded as the microbial contamination of the fish skin. The skins of the fish were decontaminated by placing them in ethanol and lightly flames. They were dissected aseptically and meat was petrified. 10 g of each sample was taken and 90 ml of distilled water added after which the analyses were also done. The results were recorded as the microbial contamination of the fish muscles.

The Most Probable Number (MPN) method (Grasso *et al.*, 2000) and (Woomer *et al.*, 1994) was used to determine the coliform bacteria in the samples of ponds water, fish skin and muscles. The method involves addition of specified quantities of fish meat to the test tubes that contain a nutrient broth. They were then incubated at a specified temperature for a period of time. The gas or turbidity that was present or absent in each tube was used to determine an index known as the Most Probable Number (MPN).



Plate 8: Taking of a swap from the fish surface using sterile cotton bud

3.8 Data Analysis

T-test was used to compare the physico-chemical parameters, nutrients and microbial levels of the pond water between the OMF and TMF. The physico-chemical parameters and nutrients were also compared between rainy and dry seasons. The abundance of

phytoplankton and zooplankton in the ponds were also compared between the OMF and TMF with the use of t-test. Likewise, the fish growth parameters were also compared between the OMF and TMF using t-test.

KNUST



4.1 Water quality parameters of ponds water

The physico-chemical parameters assessed include dissolved oxygen, temperature, pH, transparency, total dissolved solids, salinity and electrical conductivity. For all these parameters, there were no significant differences between the values of OMF and TMF treatments ($p > 0.05$) and all of them were within the desirable range for fish culture (Table 4.1). Also, for the nutrients (nitrate and phosphate) and ammonia, there were no significant differences between the values of OMF and TMF treatments ($p > 0.05$). Both nitrate and phosphate values in OMF and TMF treatments were also within the desirable range for fish culture except ammonia values of the two treatments that exceeded the desirable range (Table 2).

Table 2: Mean of water quality values during the experimental period and means compared using independent T-test

Parameter	Once a month fertilization	Twice a month fertilization	p- value	Desirable range for fish culture
DO	5.7 ± 0.7 mg/l	5.8 ± 0.6 mg/l	0.75	5 mg/l
Temperature	27.8 ± 1.3 °C	27.7 ± 1 °C	0.96	25 – 30 °C

pH	7.9 ± 0.7	7.6 ± 0.3	0.38	6.5 – 9.5
Transparency	21.6 ± 1.3cm	20.7 ± 1.1 cm	0.21	20 – 60 cm
TDS	43.3 ± 21.2 mg/l	42.7 ± 17.2 mg/l	0.95	< 200 mg/l
Salinity	0.0317 ± 0.01 mg/l	0.028 ± 0.01 mg/l	0.56	0.05 mg/l
EC	75.7 ± 34.3 mS/cm	75.4 ± 24.3mS/cm	0.99	30 – 5000 mS/cm
Ammonia	0.32 ± 0.05	0.28 ± 0.04	0.19	< 0.2 mg/l
Nitrate	0.325 ± 0.07 mg/l	0.28 ± 0.04 mg/l	0.22	0.1 – 4 mg/l
Phosphate	0.12 ± 0.08 mg/l	0.17 ± 0.14 mg/l	0.49	0.05 – 0.07 mg/l

Some water quality parameters observed seasonal changes whilst in others no seasonal changes occurred (Table 3). For dissolved oxygen, pH, transparency, nitrate and ammonia, there were no significant differences between the values of rainy and dry seasons ($p > 0.05$). However, for the temperature, TDS, salinity, EC and phosphate seasonal changes were observed and the differences between the values of rainy and dry seasons were significant ($p < 0.05$, Table, 3). In all these water quality parameters, dry season recorded greater mean values compared to the rainy season. Both OMF and TMF treatments phosphate values were low in the first three months (0.047 to 0.08 mg/l) compared to the last three months (0.13 to 0.4 mg/l) (Figure 7). OMF rainy season temperature values ranged from 25.93 to 27.67 °C and TMF values ranged from 26.34 to 27.58 °C. For dry season temperature values, OMF recorded a range from 28.04 to 29.94 °C and TMF values ranged from 28.01 to 29.43 °C (Figure 3).

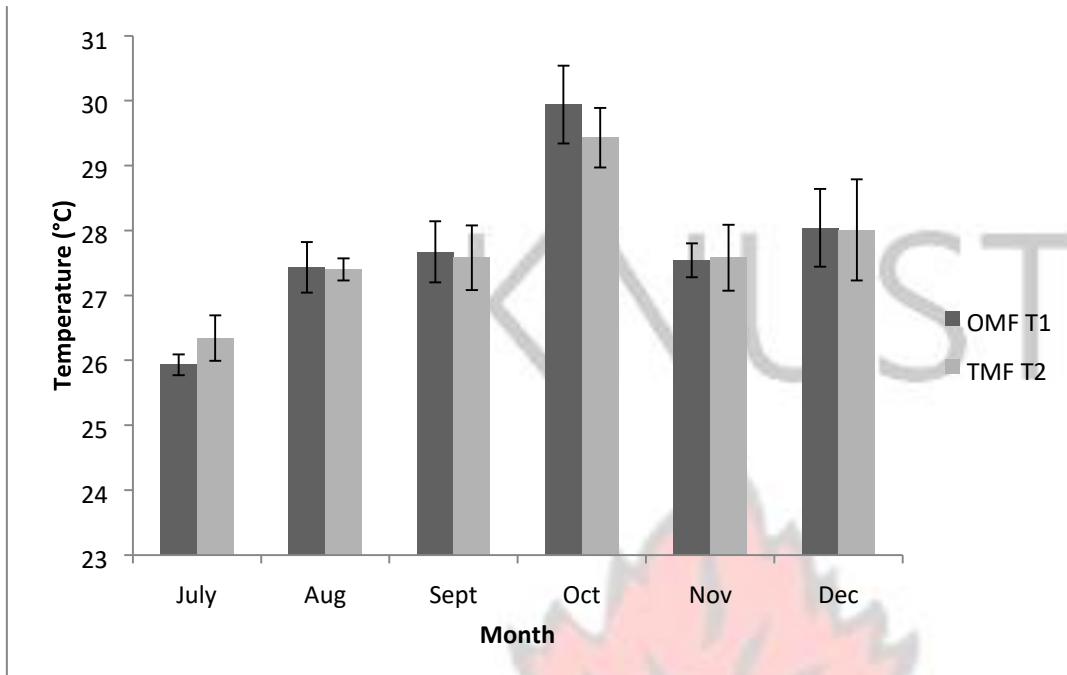
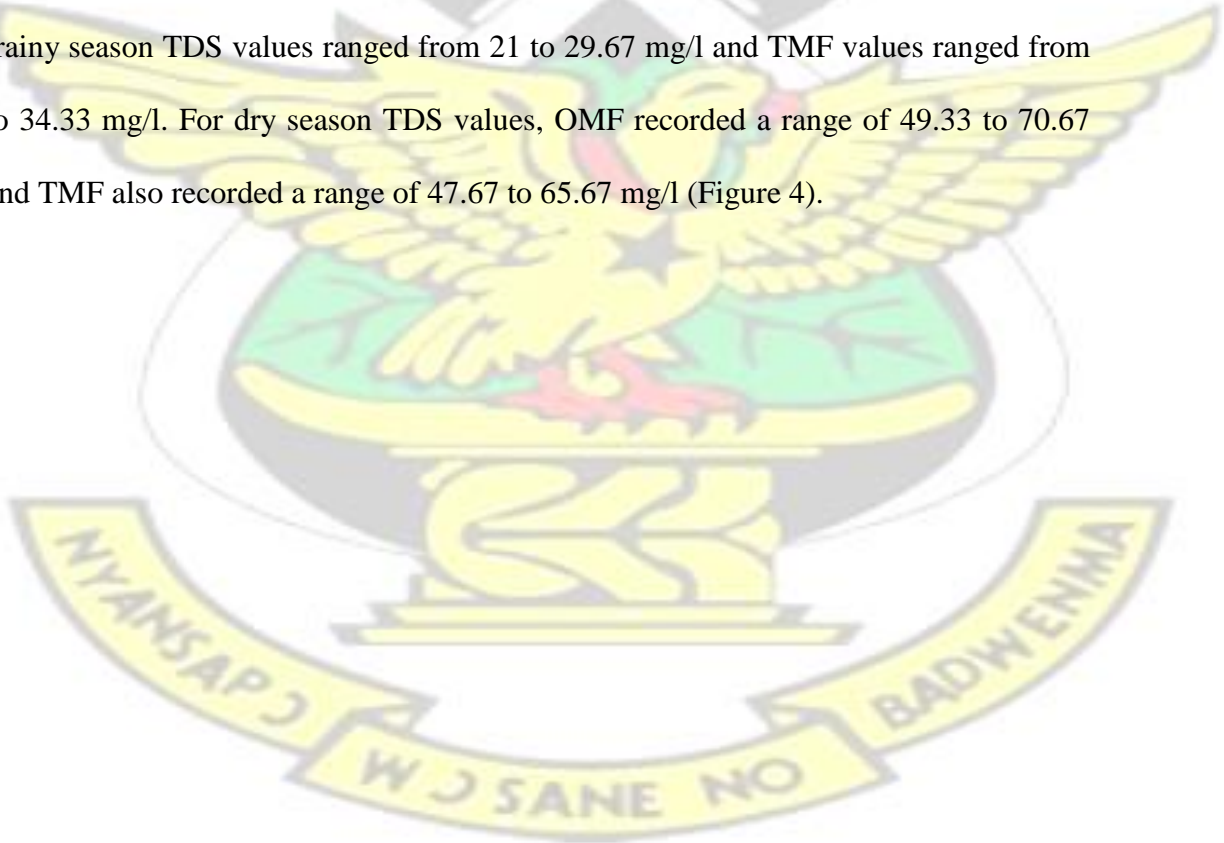


Figure 3 Mean temperature recorded for each treatment.

OMF rainy season TDS values ranged from 21 to 29.67 mg/l and TMF values ranged from 23.3 to 34.33 mg/l. For dry season TDS values, OMF recorded a range of 49.33 to 70.67 mg/l and TMF also recorded a range of 47.67 to 65.67 mg/l (Figure 4).



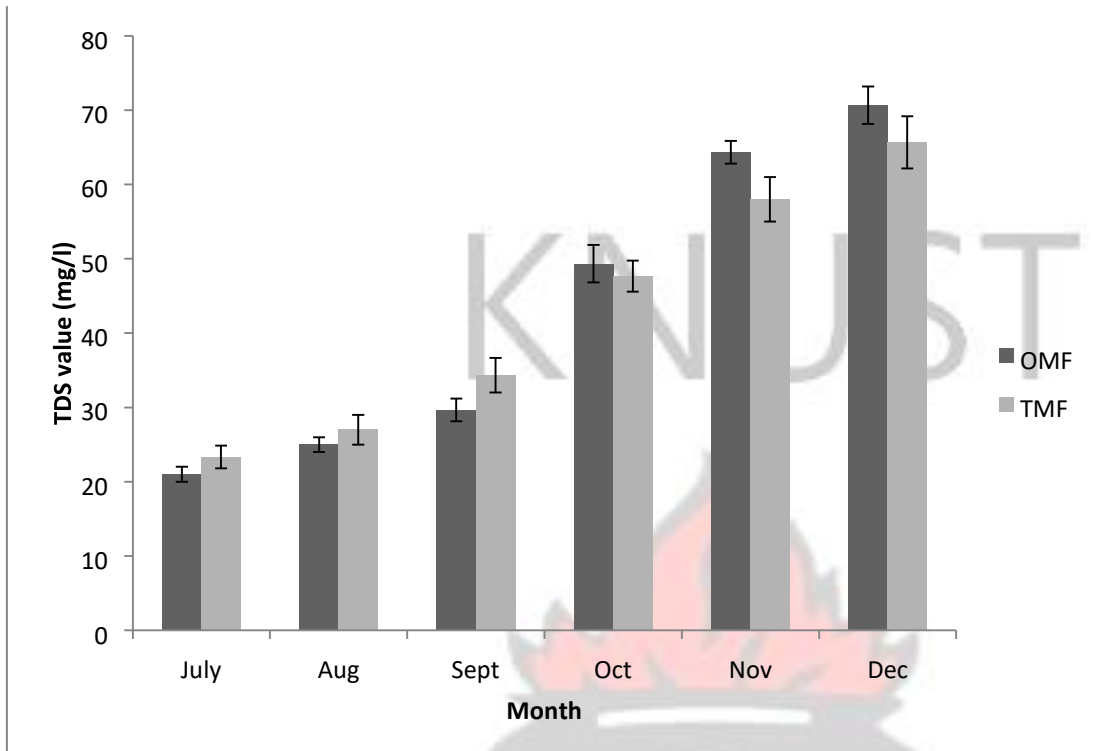


Figure 4 Mean total dissolved solids recorded for each treatment.

OMF rainy season salinity values ranged from 0.013 to 0.037 mg/l and TMF recorded a range of 0.017 to 0.027mg/l. In the dry season, OMF recorded salinity values which ranged from 0.03 to 0.05 mg/l and TMF recorded a range of 0.027 to 0.04 mg/l (Figure 5).

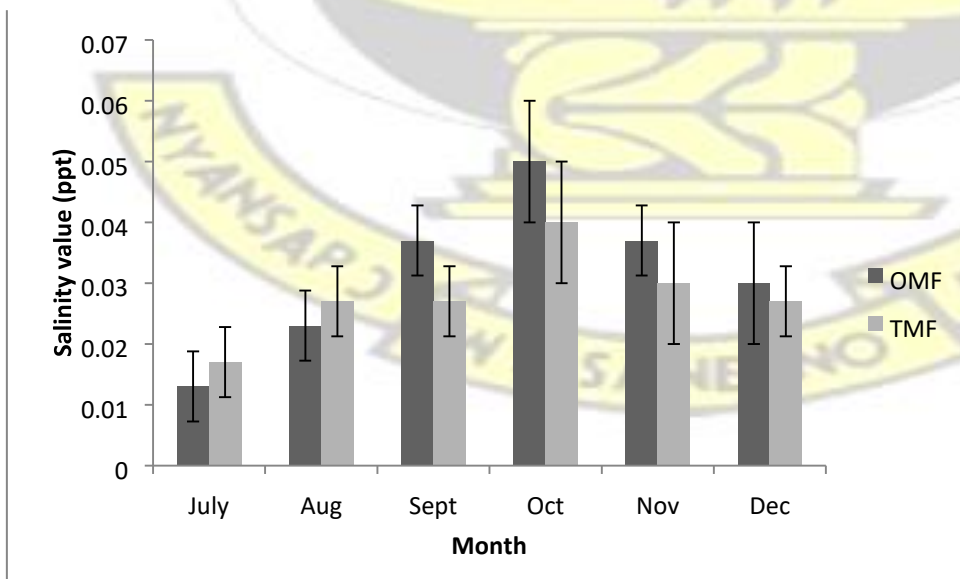


Figure 5 Mean salinity recorded for each treatment.

OMF recorded rainy season EC values which ranged from 32.67 to 60 mS/cm and TMF also recorded a range of 51.67 to 67 mS/cm. In the dry season EC values, OMF recorded a value which ranged from 81 to 128 mS/cm and TMS values ranged from 67 to 115 mS/cm (Figure 6).

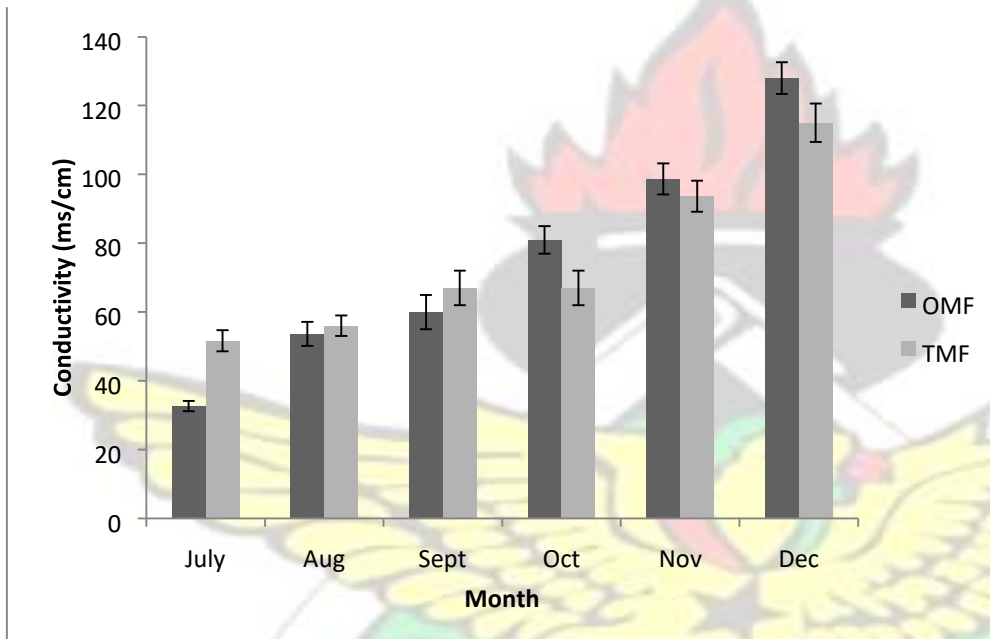


Figure 6 Mean electrical conductivity recorded for each treatment.

For rainy season phosphate values, OMF recorded a range of 0.046 to 0.073 mg/l and TMF values ranged from 0.053 to 0.08 mg/l. In the dry season OMF recorded phosphate values ranging from 0.127 to 0.263 mg/l and TMF values ranged from 0.15 to 0.4 mg/l (Figure 7).

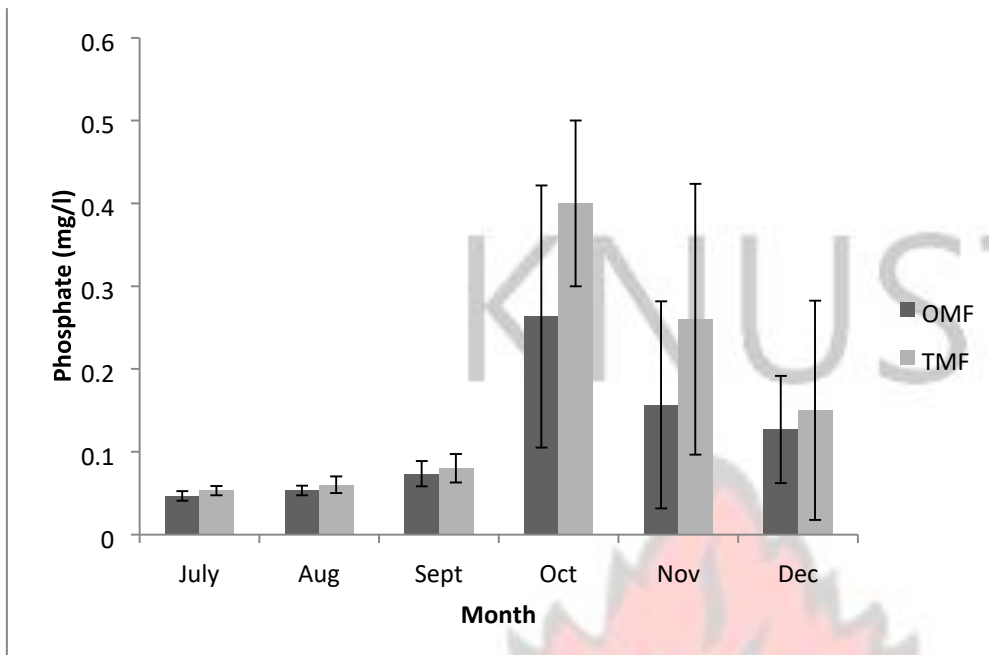


Figure 7 Mean phosphate recorded for each treatment.

Table 3 Seasonal changes in the water quality parameters

Parameter	Rainy season	Dry season	p-value
DO	5.9 mg/l	5.6 mg/l	0.4
Temperature	27.1 ⁰ C	28.4 ⁰ C	0.02
pH	7.6	8.0	0.2

Transparency	21.7 cm	20.6 cm	0.12
TDS	26.7 mg/l	59.3 mg/l	0.0
Salinity	0.024 mg/l	0.36 mg/l	0.04
EC	53.5mS/cm	97.2mS/cm	0.002
Ammonia	0.293 mg/l	0.302 mg/l	0.8
Nitrate	0.305 mg/l	0.303 mg/l	0.96
Phosphate	0.06 mg/l	0.23 mg/l	0.011

4.2 Phytoplankton and zooplankton production

The phytoplankton groups found in the experimental ponds during the production periods were Diatoms (Bacillariophyta), Blue-green algae (Cyanobacteria), Green algae (Chlorophyta) and motile algae (Euglenophyta) (Table 4). Chlorophyta were the dominant group of phytoplankton in the culture ponds in both OMF and TMF followed by Cyanobacteria, Motile algae and then Diatoms. Phytoplankton was represented by 30 genera which included 7 Diatom genera, 5 Cyanobacteria genera, 12 Chlorophyta genera and 6 motile algae genera. In terms of abundance, TMF recorded greater grand mean value compared to OMF throughout the production period. However, there were no significant differences between the values of TMF and OMF treatments for all the phytoplankton ($p > 0.05$, Table 4).

Table 4 Phytoplankton diversities and abundance in the experimental ponds.

Family	Genus	Abundance			
		OMF Quantitative mean	TMF Quantitative mean	p-value	Significance

		estimation			estimation	
		n			n	
		(Unit/cell)			(Unit/cell)	
Diatom (Bacillariophyta)	<i>Cyclotella</i>	18.7	355.3	-	12.7	241.3
	<i>Diatoma</i>	3.7	70.3	-	4.7	89.3
	<i>Navicula</i>	0.7	13.3	-	1.3	24.7
	<i>Stephanodiscus</i>	8.3	157.7	-	9.3	176.7
	<i>Surirella</i>	3.3	62.7	-	2.7	51.3
	<i>Synedra</i>	7.3	138.7	-	2.7	51.3
	<i>Tabellaria</i>	3	57	-	0	0
	Sub-mean	45±20	855	0.4	33.3±6.8	634.6
Blue-green algae (Cyanobacteria)	<i>Anabaena</i>	4.7	89.3	-	0	0
	<i>Aphanacapsa</i>	16.7	317.3	-	6.7	127.3
	<i>Aphanothece</i>	600.7	11413.3	-	745.3	14160.7
	<i>Merismopedi</i>	26	494	-	8.3	157.7
	<i>Microcystis</i>	36	684	-	26.7	507.3
	Sub-mean	684±17	12997.9	0.6	787±208	14953
Green algae (Chlorophyta)	<i>Chlorella</i>	490.7	9323.3	-	397.3	7548.7
	<i>Chlorococcum</i>	511.7	7722.3	-	526.3	9999.7
	<i>Closterium</i>	20	380	-	63	1197
	<i>Coleochaeta</i>	58.3	1107.7	-	62	1178
	<i>Micractinium</i>	505.7	9608.3	-	457.7	8696.3

Table 4 cont'd.

	<i>Netrium</i>	9.3	176.7	-	12.7	241.3
	<i>Planktosphaeria</i>	777	14763	-	797.7	15156.3
	<i>Pleurotaenium</i>	17.3	328.7	-	12.7	241.3
	<i>Staurotaenium</i>	6.7	127.3	-	33.7	640.3
	<i>Tetraedron</i>	149.3	2836.7	-	298.3	5667.7
	<i>Ulothrix</i>	2.3	43.7	-	2.3	43.7
	<i>Xanthidium</i>	131.3	2494.7	-	232.3	4413.7
	Sub-mean	2679.7±	50912.4	0.5	2896	55024
		390.6			±274.1	
Motile algae	<i>Astasia</i>	4.3	81.7	-	0.3	5.7
(Euglenophyta)	<i>Carteria</i>	45.7	868.7	-	24.3	461.7
	<i>Ceratium</i>	3.3	62.7	-	6	114
	<i>Euglena</i>	10.7	203.3	-	7	133
	<i>Mallomonas</i>	0	0	-	1.7	32.3
	<i>Trachelomonas</i>	10	190	-	16.7	317.3
	Sub-mean	74	1406.4	0.6	56 ±36.1	1064
		±39.2				
Grand mean		3482.7±	66171.3	0.131	3772.3±	71673.7
		236			120.3	

Zooplankton groups found in the experimental ponds during the production periods were Rotifers, Cladocera and Copepoda (Table 5). There were 4 genera of zooplankton which included *Keratella* and *Brachionus* that belong to Rotifer, *Daphnia* (Water flea) belonging to Cladocera and *Cyclopoida* which belong to Copepoda. Among the zooplankton groups (families), rotifers were the dominant in terms of diversity and abundance in both OMF and

TMF. OMF recorded greater mean count of zooplankton compared to TMF and the difference was significant ($p < 0.05$, Table 5).

Table 5 Zooplankton diversities and abundance in the experimental ponds.

Family	Genus	Abundance			
		OMF mean	Quantitative estimation (Unit/cell)	p value	TMF mean - Quantitative estimation (Unit/cell)
Rotifers	<i>Keratella</i>	50.3	955.7	-	39.3 746.7
	<i>Brachionus</i>	33.3	632.7	-	27.7 526.3
Sub-mean		83.7 ± 5.1	1590.3	0.01	67 ± 4.4
Cladocera	<i>Daphnia</i>	17.3 ± 1.2	328.7	0.03	13 ± 2247
Copepoda	<i>Cyclopoida</i>	7.3 ± 2.5	124.1	0.2	4.7 ± 89.3
Grand mean		108.3 ± 6.4	2043.1	0.01	84.7 ± 7

4.3 Monthly growth rate and total yield of *Oreochromis niloticus*

The data trend indicated that from the first month till the last month of production, TMF monthly mean weight gain values were greater than OMF values and the differences were significant ($p > 0.05$, Table 6). Fish growth performance in terms of weight gain was mostly

affected in October as it recorded the lowest mean weight gain values in both OMF and TMF. Although there was an increasing trend in fish weight from the first month till the last month of production, it became evident that monthly growth rate in terms of percentage weight gain of the *Oreochromis niloticus* declined when they were increasing in sizes of growth. During the first two months, TMF recorded greater weight gain percentage increase in growth than OMF but in the subsequent months, it was vice versa.

July recorded the highest weight gain percentage increase of 74.42 % in TMF and 70 % in OMF. On the other hand, December recorded the lowest weight gain percentage increase of 16.42 % in TMF and 17.61 % in OMF. More fish died in OMF than as in TMF throughout the experimental period (Table 6).

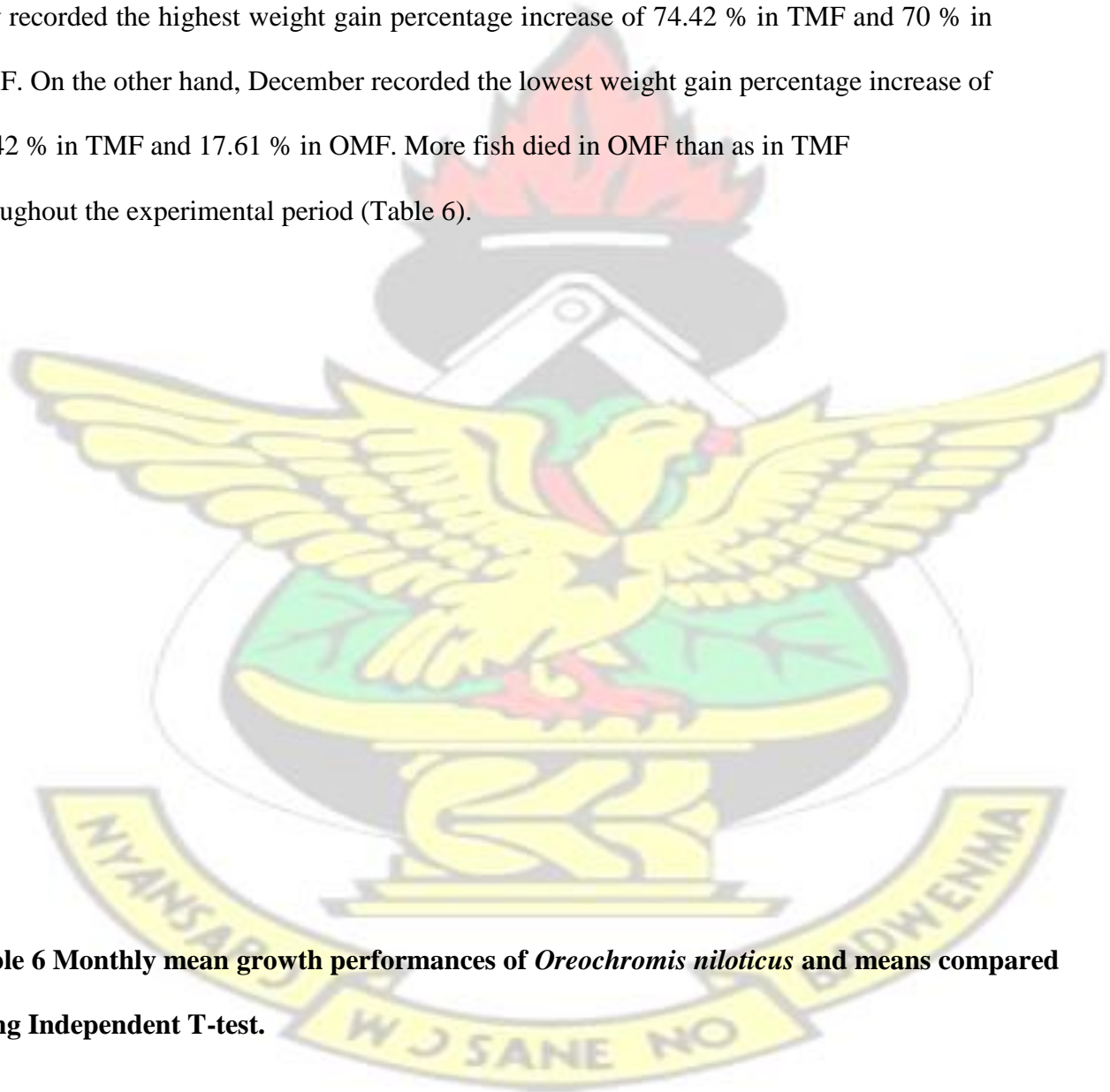


Table 6 Monthly mean growth performances of *Oreochromis niloticus* and means compared using Independent T-test.

Month/Treatment	Average body weight gain	Average weight(g)	p-value	% increase	weight No. of fish died
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July	OMF	34.10±2.2	23.8 ± 2.2	70	2
	TMF	40.26±1.8	29.96 ± 1.8	74.42	0
0.019					
Aug	OMF	84.77±1.6	50.66 ± 0.6	59.8	1
	TMF	110.9±2.5	70.64 ± 1	63.7	2
0.00					
Sept	OMF	148.68±3.4	63.92 ± 1.8	43	2
	TMF	178.67±1.8	67.76 ± 1.1	38	1
0.036					
Oct	OMF	185.38±2	36.7 ± 1.5	19.8	3
	TMF	219.05±2.8	40.38 ± 1.1	18.43	2
0.027					
Nov	OMF	230.65±2.8	45.27 ± 1.1	19.63	3
	TMF	272.37±3.6	53.32 ± 1	19.58	2
0.001					
Dec	OMF	279.94±2.3	49.29 ± 0.8	17.61	0
	TMF	325.89±4.5	53.52 ± 1.4	16.42	1
0.01					

For the yield of *Oreochromis niloticus* per individual, TMF recorded greater value as compared to OMF. The same trend was recorded concerning fish biomass weight gain and net yield in which TMF recorded greater values than OMF. In these yield values, the differences were significant ($p < 0.05$, Table 7). Data trend indicated that OMF yield values were more consistent and precise as it recorded lower standard deviation compared to TMF. With regard to the specific growth rate (SGR), TMF was comparatively greater than OMF.

Both OMF and TMF treatments recorded high survival rates but that of TMF was greater than OMF.

Table 7 Total yields of *Oreochromis niloticus* and means compared using independent T-test.

Treatment	Survival (%) (kg/ha)	Individual Fish Yield (kg)	Biomass rate (%) Weight (kg)	Fish Biomass		Specific Initial Fish growth	Net yield
				Weight (kg)	Weight gain(kg)		
OMF	99.4	0.283	6.16	168.8	162.6	0.80	6504±62.6
TMF	99.6	0.328	5.70	195.7	190.0	0.853	7598.4±110
pvalue	-	0.00	-	0.00	0.00	-	0.00

4.4 Microbiological quality of pond water, fish skin and muscles

Microbes that were present in both OMF and TMF were total coliform, faecal coliform, *Enterococci* coliform and faecal enterococci. For the mean count of those microorganisms in the ponds water, there were no significant differences in microbial load between the OMF and TMF treatments ($p > 0.05$, Table 8).

Table 8: Mean of microbial count in ponds water and means compared using Independent T-Test.

Microorganism	OMF(cfu100 ml ⁻¹)	TMF(cfu100 ml ⁻¹)	p-value
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<i>Total coliform</i>	7.4 ± 1.7	6.5 ± 0.8	0.5
<i>Faecal coliform</i>	6.8 ± 0.3	7.0 ± 1.2	0.8
<i>Enterococci coliform</i>	6.3 ± 0.2	5.8 ± 0.6	0.2
<i>Faecal enterococci</i>	1.7 ± 0.02	1.6 ± 0.13	0.2

Also, for the coliform bacteria found on the fish skin, there were no significant differences in load between the OMF and TMF treatments ($p > 0.05$). However, for total coliform found in fish, OMF recorded greater compared to TMF and the difference was significant ($p < 0.05$). For the faecal coliform, *Enterococci* coliform and faecal *enterococci* in the fish muscles, there were no significant differences between the OMF and TMF ($p > 0.05$, Table 9).

Table 9 Microbial count in fish skin and muscle: means compared using Independent T Test.

Microorganism	Fish skin			Fish muscles		
	OMF(cfu100 ml ⁻¹)	TMF(cfu100 ml ⁻¹)	p-value	OMF(cfu100 ml ⁻¹)	TMF (cfu100 ml ⁻¹)	pvalue
<i>Total coliform</i>	9.3 ± 0.12	9.1 ± 0.69	0.6	9.5 ± 0.14	8.8 ± 0.2	0.01
<i>Faecal coliform</i>	7.8 ± 0.3	7.5 ± 0.3	0.4	8.4 ± 0.5	7.7 ± 0.3	0.08
<i>Enterococci coliform</i>	6.5 ± 0.2	6.4 ± 0.2	0.2	6.5 ± 0.4	6.7 ± 0.6	0.6

<i>Faecal enterococci</i>	3.8 ± 0.04	3.7 ± 0.03	0.23	3.8 ± 0.01	3.7 ± 0.06	0.05
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CHAPTER FIVE

DISCUSSION

5.1 Fertilization frequency effect on physico-chemical parameters of ponds water

Fertilization frequency had no effect on dissolved oxygen concentration. Also, dissolved oxygen concentrations in both treatment ponds (OMF: 5.7 ± 0.7 mg/l, TMF: 5.8 ± 0.6 mg/l) suggested that they were within the range suitable for fish culture (5 mg/l: FAO, 2006). This was in agreement with Bhatnagar *et al* (2004) who reported the same dissolved oxygen level of 5 mg/l as good for fish production. Ekubo and Abowei (2011) also in their earlier work confirmed the same amount of dissolved oxygen as adequate level in fishponds. The seasonal changes did not affect the dissolved oxygen level of the ponds water adversely and this could

be attributed to the bagging method of manuring used in this study. According to Ludoviko and Kang'ombe (2012), this method of manuring makes the nutrients in manure dissolve and seep out of the bags to be mixed by the water currents. That was why there was no changes in dissolved oxygen concentration in the two treatment ponds.

Also, fertilization frequency had no effect on the temperature of the ponds water. However, seasonal changes effect was observed in which dry season ponds water temperature was higher compared to that of rainy season. The fluctuation could be due to the evaporative loss of water during the period of drought in dry season. Both treatment ponds temperature (OMF: 27.8 ± 1.3 °C, TMF: 27.7 ± 1 °C) were within the acceptable range for fish culture (25 – 30 °C: FAO, 2006) throughout the production period suggesting that they were beneficial to tilapia culture. This was in agreement with Mjoun *et al* (2010) and El-Sayed and Kawanna (2008) who reported the similar range (24 to 32°C) as optimum for tilapia growth. Moreover, fertilization frequency had no effect on pond water pH. In this study, both treatment ponds pH levels (OMF: 7.9 ± 0.7 , TMF: 7.6 ± 0.3) became evidence that they were within the suitable range for fish culture (6.5 – 9.5: FAO, 2006). The result was in agreement with Santhosh and Singh (2007) and Bhatnagar *et al* (2004) who reported the same pH range of 6.5 to 9.5 as suitable for fish culture. Popma and Masser (1999) also confirmed that *Oreochromis niloticus* can perform best in the same pond pH range (5 to 10). Also, no seasonal fluctuation of pH was observed in rainy and dry seasons of the production period. This was due the bagging method used to apply the manure. According to Ludoviko and Kang'ombe (2012), this method ensures that manure dissolves and seep out of the bags gradually to be mixed by the water currents.

Fertilization frequency had no effect on the transparency of the ponds water and the season in which manure was applied also had no effect on the pond transparency or visibility. The transparency of both treatment ponds in this study (OMF: 21.6 ± 1.3 cm, TMF: 20.7 ± 1.1 cm) suggested that they were within an acceptable range for aquaculture (20-60 cm: Boyd, 1998). This Secchi disk visibility range supported the fact that they were optimum for the management of fish pond culture. However, this study contradicts Bash *et al* (2001) assertion that there is increased in fish growth performance in low turbid water because it facilitates easy light in order to enhance photosynthesis by algae. In this study, TMF treatment ponds though recorded relatively high turbidity compared to OMF treatment ponds their fish monthly growth performance and total yield were greater than OMF with low turbidity. The turbidity in the experimental ponds which promoted fish growth could therefore consist of planktons and faecal matter of culture organisms (*Oreochromis niloticus*). According to Stickney *et al* (1979) and Kadri and Emmanuel (2003), plankton bloom is established through fertilization.

When employing a fixed rate fertilization strategy, the frequency in which the manure was applied had no effect on the pond water total dissolved solids. However, seasonality had effect on the total dissolved solids concentration. When ponds were fertilized in dry season, they gave greater total dissolved solids than when they received manure in rainy season. This is because the evaporative losses of water during the period of drought make the water level decline leading to high concentration of dissolved solids. Although fertilization frequency had no effect on ponds water TDS, both ponds treatments TDS (OMF: 43.3 ± 21.2 mg/l, TMF: $42.7 \pm$ mg/l) implied that they were within the desirable range for fish culture (< 200 mg/l: James, 2000).

Moreover, fertilization frequency had no effect on the salinity concentration of the ponds water. Both treatment ponds salinity concentration (OMF: 0.0317 ± 0.01 mg/l, TMF: 0.028

± 0.01 mg/l) suggested they were within the acceptable range for fish culture (0.05 mg/l: Boyd, 1998). However, seasonal changes had effect on the pond water salinity concentration because when the manure was applied in dry season, it gave high salinity than when it was applied in rainy season. This is because a decline in water level during the dry season due to water loss through evaporation led to this phenomenon.

Ponds electrical conductivity was not affected by the frequency of manuring. The two treatment ponds EC (OMF: 75.7 ± 34.3 mS/cm, TMF: 75.4 ± 24 mS/cm) indicated that they were within the range acceptable for fish culture (30-5000, Stone and Thomforde, 2004). However, seasonal changes had effect on the pond water EC. This study revealed that when apply manure in dry season it gives greater EC than when it was applied in rainy season. This is because during the dry season the evaporative loss of water decrease water level to increase the water conductivity level.

5.2 Fertilization frequency effect on nutrients and nitrogenous compound.

Fertilization frequency had no effect on nutrient in the form of nitrate concentration of pond water and the two treatment ponds nitrates levels (OMF: 0.325 ± 0.07 mg/l, TMF: 0.28 ± 0.04) suggested that they were within the favourable range for fish culture (0.1 – 4.0 mg/l, Santhosh and Singh, 2007). The season in which the manure was applied also had no effect on the nitrate level of ponds water. According to Ludoviko and Kang'ombe (2012), this method manure application makes the nutrient (nitrate) dissolve and seep out gradually of the bags to be mixed by the water currents. So the same nitrate quantities from the manure were released gradually in both OMF and TMF treatment ponds which did not cause any changes in nitrate concentration between them.

For pond water phosphate, fertilization frequency had no effect on its concentration level and both treatment ponds phosphates levels (OMF: 0.12 ± 0.08 mg/l, TMF: 0.17 ± 0.14 mg/l) supported the fact that they were within an acceptable range for aquaculture (0.05 – 0.07 mg/l, Bhatnagar *et al* 2004). This agrees with Bhatnagar *et al* (2004) who found the same phosphate level as optimum and productive. However, seasonal fluctuation in phosphate concentration was recorded and dry season was higher than the rainy season. This is because during the dry season the evaporative loss of water decrease water level to increase the water the phosphate concentration.

Fertilization frequency had no effect on the ammonia concentration of ponds water. Both treatment ponds (OMF: 0.32 ± 0.05 , TMF: 0.28 ± 0.04) ammonia levels exceeded the limit suitable for pond fishery (< 0.2 mg/l, Bhatnagar and Singh, 2010). Although, *Oreochromis niloticus* has the ability to tolerate high ammonia concentration in pond water according to El-Sayed (2006), it became evident that high ammonia concentration levels were harmful and had an adverse effect on the growth performance of the fish. This particularly occurred in the October in which growth rate was reduced in both OMF and TMF treatment ponds. This month recorded the lowest fish weight gains in OMF and TMF treatments. According to Boyd (1998), high ammonia concentrations become toxic to aquatic life and detrimental to the ecological balance of pond ecosystem at certain level of temperature and pH. This is because at high temperature and pH, greater amount of ammonium ions are converted into ammonia gas thus causes an increase in toxic ammonia levels within the freshwater pond. It could also be argued that due to an increased muscular activity at maturity which halt growth and tend to compact muscles based on the Wootton (1992) assertion. However, fish monthly growth rate in terms of weight gain declined drastically in October compared to other production months. This affirms Boyd (1998) assertion that ammonia can be toxic at certain temperature and pH. High ammonia values recorded during production period was attributed

to the bacterial breakdown of manure used in the fertilization, uneaten feed, dead planktons, fish faeces and excreta. This confirms assertion of Bhatnagar and Devi (2013). The breakdown of *Oreochromis niloticus* metabolic products resulting from high stocking density as they advance in growth could also contributed to high ammonia concentration.

5.3 Effect of fertilization frequency on plankton production

Fertilization frequency had no effect on the phytoplankton abundance in the fishpond. This study agrees with Knud-Hansen and Batterson (1994) who found no fertilization frequency effect on the phytoplankton abundance in their earlier work. However, it disagrees with Garg and Bhatnagar (2000) who reported that fish ponds which were fertilized twice a month recorded higher phytoplankton count compared to those that were fertilized once a month. Although the difference was not significant, twice a month fertilized ponds observed relatively greater phytoplankton count which accounted to its high turbidity compared to once a month fertilized ponds. This enhanced increase in monthly fish growth rate and total yield in those ponds compared to the ponds that were fertilized once a month. It is therefore in agreement with Wootton (1992) who reported that rapid fish growth and performance was an indication that natural food (phytoplankton) was present. The grazing of phytoplankton by the zooplankton could also affect the standing stock of phytoplankton in once a month fertilized ponds that observed relatively low count. This is because the plankton data indicated that a rise in zooplankton number coincided with a decline in phytoplankton number. Among the 30 families of phytoplankton identified in both once a month fertilized and twice a month fertilized ponds, green algae (Chlorophyta) was the dominant in both diversity and abundance. Although Diatoms (Bacillariophyta) and motile algae (Euglenophyta) recorded more diversity (genera) than Blue-green algae (Cyanobacteria) in

both once a month and twice a month fertilized ponds in terms of abundance, Blue-green algae recorded greater value than the two families.

For the zooplankton result ponds that received fertilizer once a month was greater than those that received manure twice a month. This contradicts the finding of Garg and Bhatnagar (2000) who reported that fish ponds that were biweekly fertilized in a month recorded greater zooplankton compared to those that were fertilized once a month. It became evident that greater population of zooplankton affected the phytoplankton number in both once a month and twice a month fertilized ponds. This is because phytoplankton is the main food of zooplankton in the food chain of aquatic ecosystem. Generally, extremely low zooplankton number was recorded as compared to the phytoplankton count. This could be attributed to their mobility which makes them move to the various parts of the ponds to escape sample apparatus. Because of that the time of sampling could affect it. Among the three families of zooplankton that were identified in all the experimental ponds, Rotifer was the dominant group followed by Cladocera and then Copepoda which was the least group.

5.4 Fertilization frequency effect on the monthly growth rate and total yields of *Oreochromis niloticus*.

Comparison of the fertilization frequency effect on the yield and monthly growth performance of *Oreochromis niloticus* revealed that fish ponds that received fertilizer twice a month was greater than those that received fertilizer once a month. These results are in agreement with Garg and Bhatnagar (2000) who in their finding reported that fish ponds that were fertilized twice a month gave greater fish biomass, specific growth rate and total fish production compared to ponds that were fertilized once a month. The study also agrees with

the finding of Teichert-Coddington *et al* (1990) who reported that fertilization frequency has influence on fish production. However, the present study contradicts the finding of Knud-Hansen and Batterson (1994) who found no fertilization frequency effect on the yield of fish in their earlier work. Twice a month fertilized ponds greater fish growth rate and yield could be attributed to the fact that they contained more natural food (phytoplankton) which enhanced the growth of the fish compared to once a month fertilized ponds. This confirms Wootton (1992) assertion that better growth performance is an indication of abundant natural food. From the result of this study, it is evident that monthly growth rate in terms of percentage weight gain of the *Oreochromis niloticus* declined as they became larger. This could be due to increased muscular activity at maturity which halts growth and tend to compact the muscles. It conforms to Wootton (1992) assertion that the rate of fish growth declines as they increase in sizes because most energy is channeled into physiological activities such as respiration, excretion and digestion due to increased muscular activity. Fish growth performance in terms of body weight gain was higher during the first three months of the production period than as in the last three months and growth was mostly affected in the October. Although Wootton (1992) affirms that fish growth rate decline when they are increasing in growth sizes, in this study several factors could also account to this phenomenon. One of the contributing factors identified is ammonia toxicity that has been discussed earlier. Another factor suspected to might have resulted in the drastic decline in fish growth rate more especially in the October could be overcrowding of fish stocked in the ponds as they advance in growth. This also coincided with decline in water level during dry season. Fish therefore were stressed and their growth impaired when their volume to water ratio was reduced. This agrees with finding of Diana *et al* (1997) that high stocking density though increases yield per unit area yet reduces individual growth rates.

No fish disease was encountered during the period of the fish production but few injuries such as abrasions and loss of scales were found on some fish in both OMF and TMF treatments. These were particularly prevalent during November and December. This could be caused by parasitic of leeches in the water. However, high survival rate recorded in both OMF and TMF could be attributed bagging method of manuring used in this study. This conforms to the finding of Ludoviko and Kang'ombe (2012) who reported that fish in bagging method of manuring ponds gave higher survival rate than broadcasting and crib methods of manuring.

5.5 Fertilization frequency effect on microbiological quality of pond water, fish skin and muscles

This study recorded high load of total coliform, Faecal coliform, *Enterococci* coliform and faecal *enterococci* in the pond water, fish skin and muscles in both OMF and TMF. However, fertilization frequency had no effect on the microbial loads them except total coliform found on the fish muscles in which OMF contamination level was higher than TMF. This makes the consumption of fish from OMF more risky than TMF. However, both treatments microbial load were above the WHO (1997) recommendation and this indicates serious contamination. The microbial contamination of the pond water, fish skin and fish muscles could be attributed to the faeces in the poultry manure thus contributing to the high build up of these coliforms. This is in agreement with Morgan (1990) who reported that contamination of water bodies was as a result of livestock faeces that contributed to the high incidence of total and faecal coliform build up. The presence of the *coliform* group of bacteria in the products of fish renders them unsafe to humans and this confirms earlier work of

Olayemi *et al* (1991) who reported several indicator organisms in fish tissues intended for human consumption. This constitutes a potential danger not only in causing diseases, but also the possible transfer of antibiotic resistance from fish to humans.

This research has also shown that fish cultured in poultry manure fertilized ponds are susceptible to infection with pathogenic bacteria. Although some may be beneficial saprobes that are involved in the numerous recycling processes, the contamination of fish by coliform bacteria is what of much concern in limnology and fisheries. Microbial indicators found in pond water suggested that the pathogenic bacteria present bioaccumulate in the fish muscles and skin. This in agreement with Ampofo and Clerk (2010) who reported the presence of pathogenic bacteria in the fish tissues cultured in poultry manure-fertilized ponds. The fact that *Salmonella* was not found in the samples *per se* does not guarantee the safety of fish health and humans consuming the fish and their products. Indicator organisms such as total coliform, faecal coliform, *Enterococci* coliform and faecal *enterococci* in the ponds water, fish skin and fish muscles in both OMF and TMF present a health hazard to humans. This agrees with Cairncross and Quano (1991) assertion that the presence of the indicator organisms show the presence of pathogenic microorganisms and that eating such fish might be a threat to humans' health.

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CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This research has indicated that all the water quality characteristics including dissolved oxygen, temperature, pH, transparency, total dissolved solids, salinity, electrical conductivity, ammonia and nutrients in the form of nitrate and phosphate of the ponds water did not differ significantly in response to the fertilization frequency. The Null Hypothesis is therefore rejected since no differences existed at 95% confidence interval. From the results in this study, seasonal changes had no effect on the pond water dissolved oxygen, pH, transparency, nitrate and ammonia. However, there was seasonal changes effect on the temperature, TDS, salinity, electrical conductivity and phosphate of the pond water. In all these parameters, dry season values were greater than that of rainy season.

This research has also revealed that all the parameters of water quality were found to be within the acceptable range for the production of fish apart from ammonia concentration

which was higher than the desirable level for fish culture in both fertilization once a month and twice a month fertilized ponds. This research has shown that water quality parameters had a direct effect on the primary productivity (production of planktons) of the ponds which also correlated with the monthly body weight, specific growth rate and the total yield of the fish.

It was also revealed in this research that the phytoplankton number in the ponds was not affected by the fertilization. This accepts the Null Hypothesis against the Alternate

Hypothesis. For the zooplankton number in the ponds water, it was affected by the frequency of fertilization and once a month fertilized ponds greater than those that were fertilized twice a month. This study has shown that the fertilization frequency has a significant effect on the monthly growth rate and total yield of *Oreochromis niloticus* cultured in six months production period. Ponds that were fertilized twice a month gave greater monthly growth rate and total fish yield than those that received manure once a month. The Null Hypothesis is therefore rejected since difference existed at 95% confidence interval.

This study has once again shown that the interval of applying manure into a pond has no effect on the bacterial contamination. However, it has shown the presence of indicator organisms' contamination at high magnitudes in both once a month and twice a month fertilization frequencies. Total coliform, faecal coliform, *Enterococci* coliform and faecal *enterococci* were found in the fish skin, muscles and the ponds water.

6.2 Recommendations

Based on the findings in this present study it is therefore recommended to fish farmers to undertake twice a month fertilization frequency because it gave greater monthly growth rate

and total yield of fish than once a month fertilized frequency. In terms of product (muscles) quality, fish from twice a month fertilized ponds also gave relatively low *coliform* bacteria load than once a month fertilized ponds. Manure should also be treated before application. Solar treatment is one treatment method which is less costly. Prior treatment of fish is recommended before consumption. Fish farmers should be very cautious about the manure rate to use in ponds fertilization since it leads to a build-up of ammonia which can be lethal to the culture organisms. Moreover, fish stocking density should be reduced if farmers intend to use animal manure fertilization to stimulate the growth of natural food in the ponds for long culture period. The study suggests the need to plan the season in which fish culture should be done to minimize cost of refilling the ponds to compensate the evaporative loss of water. This is necessary because in dry season water level decline and refilling the ponds would increase the production cost than in rainy season. It is also recommended that fertilization frequencies should be tried on other species such as *Clarias gariepinus* which is also cultured in Ghana to assess if the same effect could be obtained. This is because it is a benthic feeder whilst *Oreochromis niloticus* used in this study is euphotic feeder. A further study is again needed by examining other pathogenic contamination of the fish tissues cultured in manure fertilized ponds.

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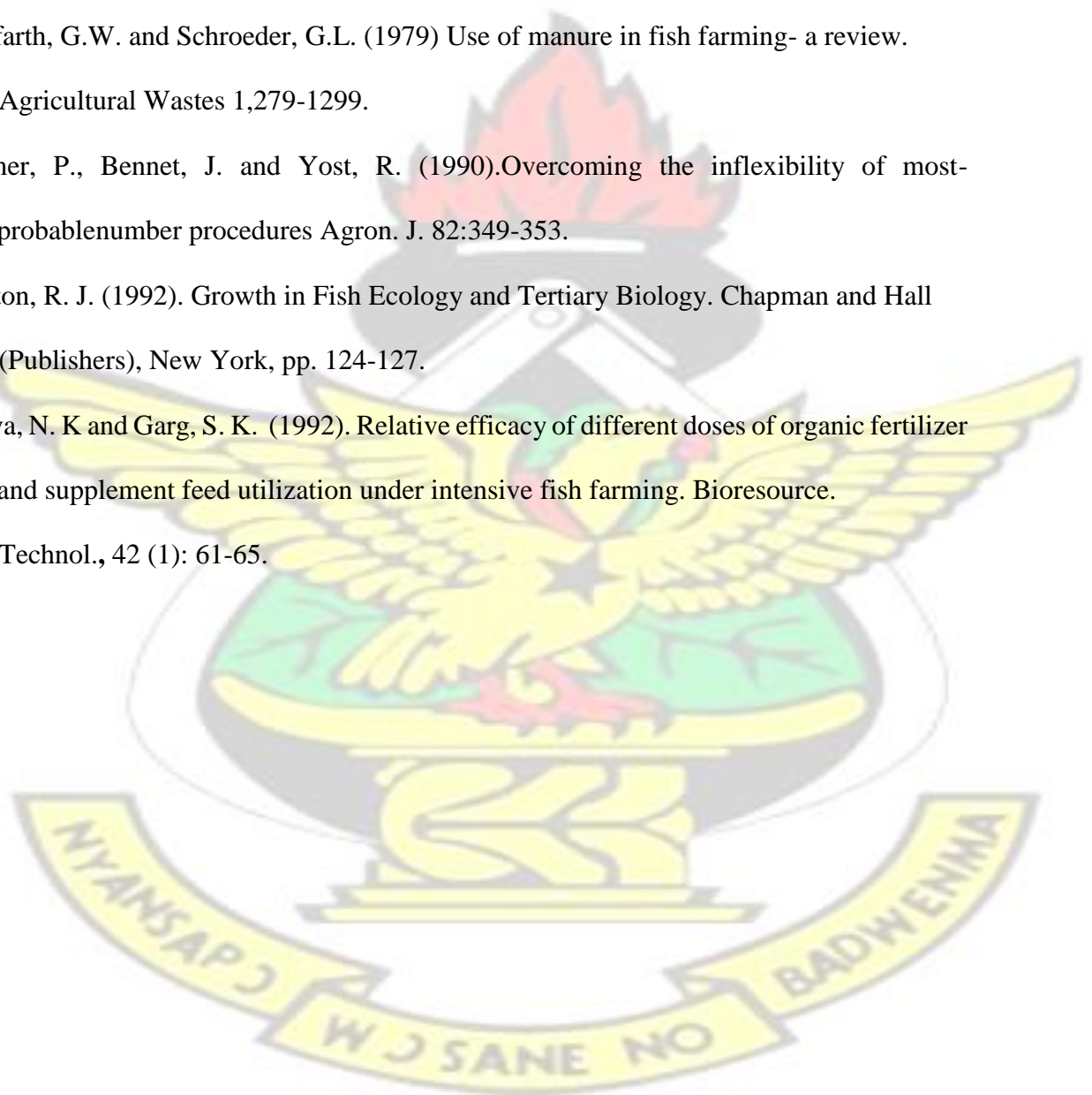
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APPENDICES

APPENDIX A: Independent T-Test for the Water Quality.

APPENDIX A1: Independent T-Test for the Dissolved oxygen.

Group Statistics

Dissolved oxygen	N	Mean	Std. Deviation	Std. Error
				Mean
Grand mean OMF	6	5.6583	.66475	.27138
mean TMF	6	5.7767	.57123	.23320

Independent Samples Test

Dissolved oxygen	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Grand mean										
Equal variances assumed	.197	.666	-.331	10	.748	-.11833	.35782	-.91560	.67894	
Equal variances not assumed			-.331	9.779	.748	-.11833	.35782	-.91806	.68139	

APPENDIX A2: Independent T-Test for the Temperature.

Group Statistics

Temperature	N	Mean	Std. Deviation	Std. Error Mean
Grand mean OMF mean TMF	6	27.7583	1.29190	.52742
	6	27.7233	1.00540	.41045

Independent Samples Test

Temperature Grand mean	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Equal variances assumed	.747 .110	.052	10	.959	.03500	.66831	-1.45409	1.52409	1.53636	
Equal variances not assumed		.052	9.431	.959	.03500	.66831	-1.46636			

APPENDIX A3: Independent T-Test for the pH.

Group Statistics

pH	N	Mean	Std. Deviation	Std. Error	
				Mean	
Grand mean	OMF	6	7.9317	.71188	.29062
	TMF	6	7.6400	.32062	.13089

Independent Samples Test

pH	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Grand mean	Equal variances assumed	1.300	.281	.915	10	.382	.29167	.31874	-.41853	1.00187
	Equal variances not assumed			.915	6.948	.391	.29167	.31874	-.46317	1.04651

Group Statistics

APPENDIX A4: Independent t-test for the Transparency.

Transparency	N	Mean	Std. Deviation	Std. Error Mean
Grand OMF mean	6	21.6000	1.28062	.52281
TMF	6	20.6500	1.14324	.46673

Independent Samples Test

Transparency	Levene's t-test for Equality of Means										
	Grand mean	Test for Equality of Variances	F	Sig.	t	df	Sig. (2tailed)	Mean Differen ce	Std. Error Differen ce	95% Confidence Interval of the Difference	
										Lower	Upper
Equal variances assumed			.185	.676	1.356	10	.205	.95000	.70083	-.61155	2.51155
Equal variances not assumed					1.356	9.874	.205	.95000	.70083	-.61426	2.51426

Independent T-T

Group Statistics

APPENDIX A5: Independent T-Test for the Total Dissolved Solids.

TDS	N	Mean	Std. Deviation	Std. Error Mean
Grand mean OMF	6	43.3333	21.19287	8.65195
TMF	6	42.6667	17.19389	7.01938

Independent Samples Test

TDS Grand mean	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.799	.392	.060	10	.953	.66667	11.14127	-24.15763	25.49097
				9.592					
Equal variances not assumed			.060		.954	.66667	11.14127	-24.30133	25.63466

APPENDIX A6:

est for the Salinity.

Group Statistics

Salinity	N	Mean	Std. Deviation	Std. Error Mean
Grand OMF mean	6	.0317	.01280	.00523
TMF	6	.0280	.00738	.00301

Independent Samples Test

Salinity	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Grand mean									
Equal variances assumed	1.875	.201	.608	10	.557	.00367	.00603	-.00977	.01711
Equal variances not assumed			.608	7.990	.560	.00367	.00603	-.01024	.01758

Independent T-T

Group Statistics

APPENDIX A7: Independent T-Test for the Electrical Conductivity.

Conductivity	N	Mean	Std. Deviation	Std. Error Mean
Grand mean OMF	6	75.6683	34.27648	13.99332
mean TMF	6	75.3900	24.30204	9.92127

Independent Samples Test

Conductivity Grand mean	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.767	.402	.016	10	.987	.27833	17.15355	-37.94217	38.49883
Equal variances not assumed			.016	9.013	.987	.27833	17.15355	-38.51729	39.07395

Group Statistics

APPENDIX A8: t-test for the Nitrate.

Nitrate	N	Mean	Std. Deviation	Std. Error
Grand OMF mean	6	.3250	.06777	.02767
TMF	6	.2823	.04036	.01648

Independent Samples Test

Nitrate Grand mean	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	1.443	.257	1.325	10	.215	.04267	.03220	-.02908	.11442
Equal variances not assumed			1.325	8.150	.221	.04267	.03220	-.03135	.11669

Independent T-T

Group Statistics

KNUST

APPENDIX A9: Independent t-test for the Phosphate

Phosphate	N	Mean	Std. Deviation	Std. Error Mean
Grand OMF mean	6	.1200	.08241	.03364
TMF	6	.1672	.13797	.05632

Independent Samples Test

Phosphate	Levene's Test for Equality of Variances	t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
Grand mean									Lower	Upper
Equal variances assumed	1.823	.207	-.720	10	.488	-.04727	.06561		-.19345	.09891

Group Statistics

Equal variances not assumed									

KNUST



Independent T-T

APPENDIX A10:

est for the Ammonia.

Group Statistics

Ammonia	N	Mean	Std. Deviation	Std. Error
Grand OMF mean	6	.3150	.05468	.02232
TMF	6	.2767	.03724	.01520

Independent Samples Test

Ammonia	Levene's Test for Equality of Variances									
	Grand mean	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Equal variances assumed	1.129	.313	1.419	10	.186	.03833	.02701	-.02184	.09851	
Equal variances not assumed			1.419	8.817	.190	.03833	.02701	-.02296	.09962	

APPENDIX B: Independent T-Test for Seasonal Changes of Water Quality.

APPENDIX B1: Independent T-Test for Temperature Seasonal Changes.

Group Statistics

KNUST

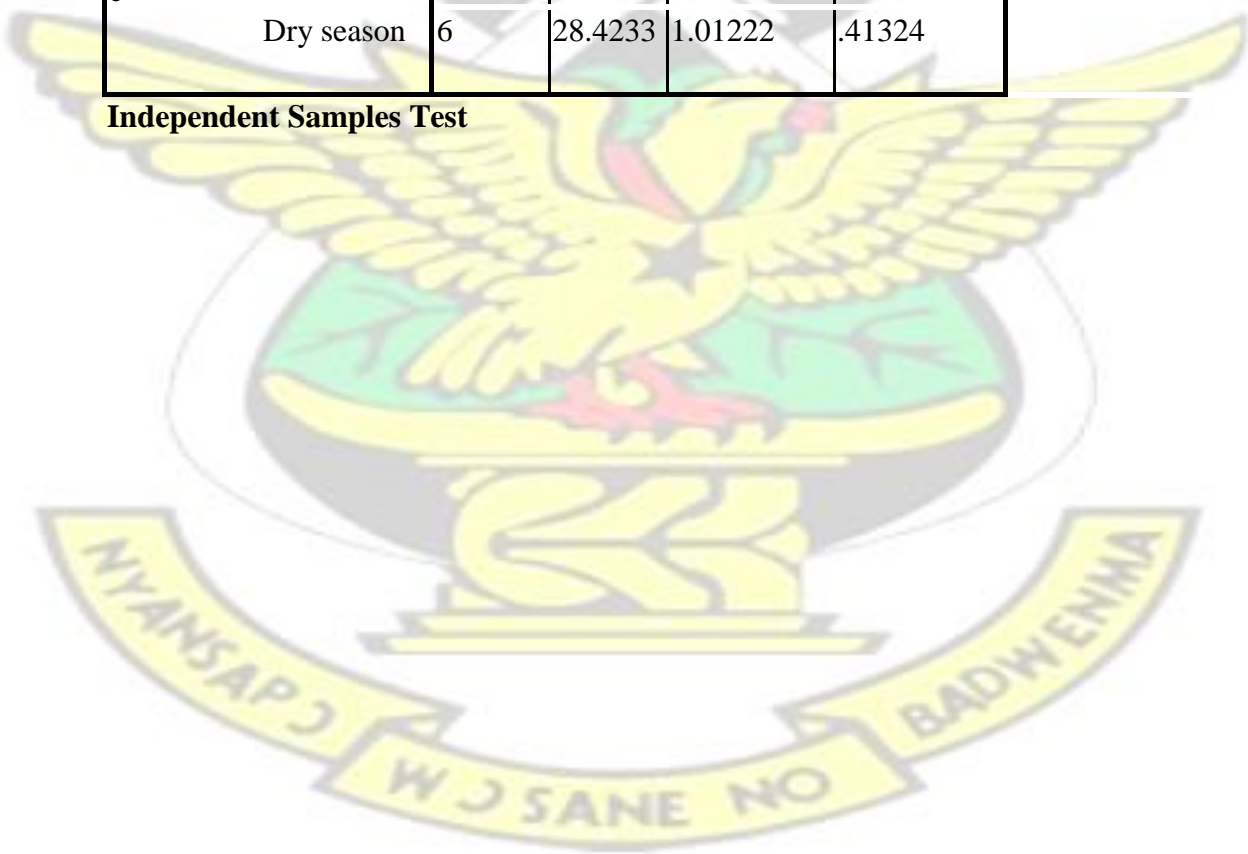
Levene's Test for Equality of Variances		t-test for Equality of Means							
F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
							Lower	Upper	
1.189	.301	-2.679	10	.023	-1.36667	.51009	-2.50323	-.23011	

Independent T-T

Equal			-2.679	9.110	.025	-1.36667	.51009	-2.51846	-.21487
variances Temperat									
assumed									
ure	Equal								
	variances not								
	assumed								

	Physico	N	Mean	Std. Deviation	Std. Error Mean
Temperatur e	Rainy season	6	27.0567	.73252	.29905
	Dry season	6	28.4233	1.01222	.41324

Independent Samples Test



APPENDIX B2:

est for TDS Seasonal Changes

Group Statistics

	Physico	N	Mean	Std. Deviation	Std. Error
					Mean
TDS	Rainy season	6	26.7217	4.77204	1.94818
	Dry season	6	59.2783	9.28978	3.79254

Independent Samples Test

		Levene's Test for Equality of Variances								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower		Upper
TDS	Equal variances assumed	4.018	.073	-7.636	10	.000	32.55667	4.26365	-23.05666	42.05667
	Equal variances not assumed			-7.636	7.467	.000	32.55667	4.26365	-22.60115	42.51218

Independent T-T

APPENDIX B3: est for Salinity Seasonal Changes.

Group Statistics

	Physico	N	Mean	Std. Deviation	Std. Error Mean
Salinity	Rainy season	6	.0240	.00846	.00345
	Dry season	6	.0357	.00855	.00349

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.015	.904	-2.376	10	.039	-.01167	.00491	-.02261	-.00073
Equal variances not assumed			-2.376	9.999	.039	-.01167	.00491	-.02261	-.00073

APPENDIX B4:

est for EC Seasonal Changes.

Group Statistics

	Physico	N	Mean	Std. Deviation	Std. Error Mean
EC	Rainy season	6	53.8350	10.84457	4.42728
	Dry season	6	97.2233	22.15662	9.04540

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
										Lower	Upper
EC	Equal variances assumed	2.53	.142	-4.308	10	.002	-43.38833	10.07075	-65.82737	-20.94930	
	Equal variances not assumed			-4.308	7.266	.003	-43.38833	10.07075	-67.02654	-19.75012	

Independent T-T

APPENDIX B5: Independent T-Test for Phosphate Seasonal Changes

Group Statistics

	Physico	N	Mean	Std. Deviation	Std. Error
Phosphate	Rainy season	6	.0610	.01301	.00531
	Dry season	6	.2278	.10194	.04162

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	10.542	.009	-3.975	10	.003	-.16678	.04195	-.26026	-.07331
Equal variances not assumed			-3.975	5.163	.010	-.16678	.04195	-.27361	-.05995

APPENDIX C: Independent T-Test for Plankton Abundance

APPENDIX C1: Independent T-Test for Phytoplankton Grand Total

Group Statistics

Phytoplankton	N	Mean	Std. Deviation	Std. Error Mean
Grand OMF total	3	3482.6667	236.00071	136.25507
TMF	3	3772.3333	120.25944	69.43182

Independent Samples Test

		Levene's Test for Equality of Means								
		Test for Equality of Variances								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Phyto	Equal variances assumed	2.863	.166	-1.894	4	.131	-289.66667	152.92554	-714.25605	134.92271
	Equal variances not assumed			-1.894	2.973	.155	-289.66667	152.92554	-778.84949	199.51615

APPENDIX C2: Independent T-Test for Diatoms

Group Statistics

	Phytoplankton	N	Mean	Std. Deviation	Std. Error Mean
Diatom	OMF	3	45.0000	20.07486	11.59023
	TMF	3	33.3333	6.80686	3.92994

Independent Samples Test

		Levene's Test for Equality of Variances								
		t-test for Equality of Means								
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Equal variances assumed	Equal variances	2.041	.226	.953	4	.394	11.6666	12.2383	-	45.64584
	not assumed			.953	2.454	.425	11.6666	12.2383	-	56.00849

APPENDIX C3: Independent T-Test for Cyanobacteria

Group Statistics

	Phytoplankton	N	Mean	Std. Deviation	Std. Error Mean

OMF	3	684.0000	179.66914	103.73203
Cyanobacteria TMF	3	787.0000	208.93300	120.62753

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper	
Cyanobacteria	Equal variances assumed	.176	.696	-.647	4	.553	-103.00000	159.09536	544.71953	338.71953
	Equal variances not assumed			-.647	3.912	.553	-103.00000	159.09536	548.65427	342.65427

APPENDIX C4:Independent T-Test for Blue-green Algae

	Phytoplankton	N	Mean	Std. Deviation	Std. Error Mean
Blue green OMF		3	2679.6667	390.56668	225.49378
algae TMF		3	2896.0000	294.09352	169.79497

Independent Samples Test

		Levene's Test for Equality of Variances								t-test for Equality of Means	
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
									Lower	Upper	
Bluegreen	Equal variances assumed	.211	.670	-.766	4	.486	-216.33333	282.27252	-1000.04750	567.38083	
	Equal variances not assumed			-.766	3.716	.489	-216.33333	282.27252	-1024.22152	591.55485	

APPENDIX C5: Independent T-Test for Motile Algae

Group Statistics

	Phytoplankton	N	Mean	Std. Deviation	Std. Error Mean
	OMF	3	74.0000	39.23009	

Motile algae	3	56.0000	36.09709	22.64950
TMF				20.84067

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Motile algae	Equal variances assumed	.057	.822	.585	4	.590	18.00000	30.77878	-67.45559	103.45559
	Equal variances not assumed			.585	3.973	.590	18.00000	30.77878	-67.68849	103.68849

APPENDIX C6: Independent T-Test for Rotifers

Group Statistics

	Zooplankton	N	Mean	Std. Deviation	Std. Error Mean
	OMF	3		5.13160	2.96273

Rotifers	3	83.6667	4.35890	2.51661
TMF		67.0000		

Independent Samples Test

		Levene's Test for Equality of Variances								t-test for Equality of Means	
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
										Lower	Upper
Rotifer	assumed s Equal variances	.082	.789	4.287	4	.013	16.66667	3.88730	5.87379	27.45955	
	variances not assumed			4.287	3.898	.014	16.66667	3.88730	5.76149	27.57185	

APPENDIX C7: Independent T-Test for Cladocera

Group Statistics

Zooplankton	N	Mean	Std. Deviation	Std. Error Mean
OMF	3	17.3333	1.15470	.66667
TMF	3	13.0000	2.00000	1.15470

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.400	.561	3.250	4	.031	4.33333	1.33333	.63141	8.03526
Cladocer assumed a Equal variances not assumed			3.250	3.200	.043	4.33333	1.33333	.23623	8.43044

APPENDIX C8: Independent T-Test for Copepoda

Group Statistics

	Zooplankton	N	Mean	Std. Deviation	Std. Error Mean
Copepoda	OMF	3	7.3333	2.51661	1.45297
	TMF	3	4.6667	1.52753	.88192

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.643	.468	1.569	4	.192	2.66667	1.69967	-2.05238	7.38572
Equal variances not assumed			1.569	3.298	.207	2.66667	1.69967	-2.47654	7.80987

APPENDIX C9: Independent T-Test for Zooplankton Grand Total Group Statistics

	Zooplankton	N	Mean	Std. Deviation	Std. Error Mean
Grand mean	OMF	3	108.3333	6.42910	3.71184
	TMF	3	84.6667	7.02377	4.05518

Independent Samples Test

		Levene's Test for Equality of Variances									
		t-test for Equality of Means									
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
										Lower	Upper
Grand mean	Equal variances assumed	.000	1.000	4.305	4	.013	23.66667	5.49747	8.40323	38.93010	
	Equal variances not assumed			4.305	3.969	.013	23.66667	5.49747	8.35625	38.97708	

APPENDICS D: Independent T-Test for Net Yield of the *Oreochromis niloticus* culture.

Group Statistics

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Net Yield	OMF	3	6503.8000	62.55124	36.11398
	TMF	3	7598.4000	109.99831	63.50756

Independent Samples Test

Net Yield	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	1.011	.372	-14.983	4	.000	-1094.60000	73.05771	-1297.44072	-891.75928
Equal variances not assumed			-14.983	3.171	.000	-1094.60000	73.05771	-1320.16665	-869.03335

APPENDIX E: Independent T-Test for the monthly Weight Gain of the *Oreochromis niloticus* culture.

APPENDIX E1: July Weight Gain Independent T-Test.

Group Statistics

July	Treatment	N	Mean	Std. Deviation	Std. Error Mean
		3	23.80333	2.171689	1.253825

Weight OMF gain TMF	3	29.96000	1.787540	1.032037
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Independent Samples Test

July Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	upper
Equal variances assumed	.107	.760	-3.791	4	.019	-6.156667	1.623939	-10.665444	
Equal variances not assumed			-3.791	3.857	.021	-6.156667	1.623939	-10.731927	1.581407

APPENDIX E2: August Weight Gain Independent T-Test.

Group Statistics

August	Treatment	N	Mean	Std. Deviation	Std. Error
					Mean

Weight gain	OMF	3	50.66333	.624046	.360293
	TMF	3	70.64333	1.008481	.582247

Independent Samples Test

August Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.466	.532	-29.180	4	.000	19.980000	.684706	21.881048	-18.078952
Equal variances not assumed			-29.180	3.336	.000	19.980000	.684706	22.040056	-17.919944

APPENDIX E3: September Weight Gain Independent T-Test.

Group Statistics

September	Treatment	N	Mean	Std. Deviation	Std. Error
Weight gain	OMF	3	63.91667	1.841530	1.063208
	TMF	3	67.76333	1.086385	.627225

Independent Test Samples

September Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.570	.492	-3.116	4	.036	-3.846667	1.234432	-7.273999	-.419334
Equal variances not assumed			-3.116	3.242	.047	-3.846667	1.234432	-7.614562	-.078772

APPENDIX E4: October Weight Gain Independent T-Test

Group Statistics

October	Treatment	N	Mean	Std. Deviation	Std. Error
					Mean

Weight OMF gain	3	36.70000	1.532188	.884609
TMF	3	40.38333	1.083620	.625629

Independent Samples Test

October Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.359	.581	3.400	4	.027	3.683333	1.083487	6.691576	-.675091
Equal variances not assumed			3.400	3.600	.032	3.683333	1.083487	6.827956	-.538710

APPENDIX E5: November Weight Gain Independent T-Test

Group Statistics

November	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Weight OMF gain	3	45.26667	1.089235	.628870	
TMF	3	53.30667	.959705	.554086	

Independent Samples Test

November Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Differenc e	Std. Error Differenc e	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.126	.741	-9.593	4	.001	8.040000	.838146	-10.367067	5.712933
Equal variances not assumed			-9.593	3.938	.001	8.040000	.838146	-10.381694	5.698306

APPENDIX E6: December Weight Gain Independent T-Test

Group Statistics

December	Treatment	N	Mean	Std. Deviation	Std. Error
					Mean

Weight gain	OMF	3	49.29333	.766312	.442430
	TMF	3	53.53000	1.389568	.802268

Independent Samples Test

December Weight gain	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.718	.445	-4.624	4	.010	4.236667	.916176	-6.780378	-1.692955
Equal variances not assumed			-4.624	3.114	.018	4.236667	.916176	-7.093126	-1.380207

APPENDICES F: Independent T-Test for Microbial Contamination of Ponds Water

APPENDIX F1: Independent T-Test for *Total coliform* Group

Statistics

	Total coli	N	Mean	Std. Deviation	Std. Error Mean
Coli	OMF	3	7.3650	1.73465	1.00150
	TMF	3	6.5110	.77337	.44650

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	4.099	.113	.779	4	.480	.85400	1.09653	-2.19045	3.89845
Coli Equal variances not assumed			.779	2.765	.497	.85400	1.09653	-2.80970	4.51770

APPENDIX F2: Independent T-Test for *Faecal coliform*

Group Statistics

	Faecal coli	N	Mean	Std. Deviation	Std. Error Mean
	OMF	3	6.7957	.30341	.17517

Coli	3	6.9627	1.21749	.70292
TMF				

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Coli Equal variances assumed	7.842	.049	-.231	4	.829	-.16700	.72442	-2.17831	1.84431
Coli Equal variances not assumed			-.231	2.247	.837	-.16700	.72442	-2.97707	2.64307

APPENDIX F3: Independent T-Test for *E. coli*

Group Statistics

	E.COLI	N	Mean	Std. Deviation	Std. Error
Coli	OMF	3	6.3493	.24446	.14114
	TMF	3	5.7900	.63290	.36540

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	4.587	.099	1.428	4	.227	.55933	.39171	-.52824	1.64690
Coli Equal variances not assumed			1.428	2.584	.262	.55933	.39171	-.80895	1.92762

APPENDIX F4: Independent T-Test for Faecal enterococci

Group Statistics

	Faecal enterococci	N	Mean	Std. Deviation	Std. Error Mean
Coli	OMF	3	1.7210	.01905	.01100
	TMF	3	1.5633	.12606	.07278

Independent Samples Test

	Levene's Test for Equality of Variances									
	t-test for Equality of Means									
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
Coli	Equal variances assumed	10.131	.033	2.142	4	.099	.15767	.07361	-.04671	.36204
	Equal variances not assumed			2.142	2.091	.160	.15767	.07361	-.14616	.46149

APPENDICES G: Independent t-test for Microbial Contamination of Fish Skin

APPENDIX G1: Independent T-Test for *Total coliform*

Group Statistics

	Total coli	N	Mean	Std. Deviation	Std. Error Mean
Skin	OMF	3	9.3087	.11717	.06765
	TMF	3	9.0967	.68725	.39679

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	9.360	.038	.527	4	.626	.21200	.40251	-.90555	1.32955
Equal variances not assumed			.527	2.116	.648	.21200	.40251	-1.43187	1.85587

APPENDIX G2: Independent T-Test for *Faecal coliform*

Group Statistics

	Faecal coli	N	Mean	Std. Deviation	Std. Error
Skin	OMF	3	7.7630	.33949	.19600
	TMF	3	7.4920	.30331	.17512

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
Skin	Equal variances assumed	.113	.754	1.031	4	.361	.27100	.26284	-.45875	1.00075
	Equal variances not assumed			1.031	3.950	.361	.27100	.26284	-.46239	1.00439

APPENDIX G3: Independent T-Test for *Enterococci coliform*

Group Statistics

	Ecoli	N	Mean	Std. Deviation	Std. Error Mean
Skin	OMF	3	6.1530	.18160	.10484
	TMF	3	6.3973	.22100	.12759

Independent Samples Test

	Levene's Test for Equality of Variances	t-test for Equality of Means

	Faecal enterococci	N	Mean	Std. Deviation	Std. Error Mean					
Skin	OMF	3	3.7700	.04194	.02421					
	TMF	3	3.7253	.03456	.01995					
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
Equal variances assumed	Skin	.047	.840	-1.480	4	.213	-.24433	.16514	-.70285	.21418
									Equal variances not assumed	
				-1.480	3.855	.216	-.24433	.16514	-.70973	.22106

APPENDIX G4: Independent T-Test for *Faecal enterococci*

Group Statistics

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
Skin	Equal variances assumed	.154	.715	1.424	4	.228	.04467	.03138	-.04245	.13178
	Equal variances not assumed			1.424	3.859	.230	.04467	.03138	-.04372	.13305

APPENDICES H: Independent Y-Test for Microbial Contamination of Fish Muscles

APPENDIX H1: Independent T-Test for *Total coliform* Group

Statistics

	Total coli	N	Mean	Std. Deviation	Std. Error Mean
muscles	OMF	3	9.5127	.13861	.08003
	TMF	3	8.8460	.19746	.11400

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
muscles	Equal variances assumed	.978	.379	4.786	4	.009	.66667	.13929	.27995	1.05339
	Equal variances not assumed			4.786	3.586	.011	.66667	.13929	.26169	1.07165

APPENDIX H2: Independent T-Test for *Faecal coliform*

Group Statistics

	Faecal coli	N	Mean	Std. Deviation	Std. Error Mean
muscles	OMF	3	8.4297	.50058	.28901
	TMF	3	7.6577	.28757	.16603

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.773	.429	2.316	4	.081	.77200	.33331	-.15341	1.69741
muscles Equal variances not assumed			2.316	3.190	.098	.77200	.33331	-.25380	1.79780

APPENDIX H3: Independent T-Test for *Enterococci coliform*

Group Statistics

	E coli	N	Mean	Std. Deviation	Std. Error
muscles	OMF	3	6.4657	.44140	.25484
	TMF	3	6.6863	.61628	.35581

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed muscles	.485	.525	-.504	4	.641	-.22067	.43766	-1.43580	.99447
Equal variances not assumed			-.504	3.624	.643	-.22067	.43766	-1.48710	1.04577

APPENDIX H4: Independent T-Test for *Faecal enterococci*

Group Statistics

	Faecal enterococci	N	Mean	Std. Deviation	Std. Error Mean

muscles	OMF	3	3.8177	.00971	.00561
	TMF	3	3.6760	.06159	.03556

Independent Samples Test

		Levene's Test for Equality of Variances									
		F	Sig.	t	df	Sig. (2tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
										Lower	Upper
muscles	Equal variances assumed	10.766	.030	3.936	4	.017	.14167	.03600	.04172	.24161	
	Equal variances not assumed			3.936	2.099	.054	.14167	.03600	-.00639	.28973	