THE HEALTH AND SURVIVAL OF FISH EXPOSED TO WASTEWATER FROM MOTETEMA WASTEWATER TREATMENT PLANT, SEKHUKHUNE DISTRICT

by

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DISSERTATION

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

ZOOLOGY

in the

FACULTY OF SCIENCE AND AGRICULTURE

(**School of Molecular and Life Sciences**)

at the

UNIVERSITY OF LIMPOPO

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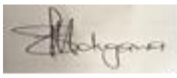
2020

DECLARATION

I declare that the **dissertation** hereby submitted to the University of Limpopo, for the degree of **Master of Science** in **Zoology** has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.



10 November 2020

M.P. Mokgawa (Ms) **Date**

ACKNOWLEDGEMENTS

Greatest thanks to the man above for blessing me with the strength and wisdom to complete my Master’s degree.

I wish to thank and acknowledge the following people and organisations whose involvement made this research project a success:

- My supervisors, Prof W.J. Luus Powell, Prof J.R. Sara and Prof P.J. Oberholster, for all their support, guidance, patience and valuable comments, it is highly appreciated.
- Mr W.J. Smit for always going the extra mile to assist with everything the project needed throughout the study period.
- The DSI-NRF SARChI Chair (Ecosystem Health), Flemish Interuniversity Council (VLIR-OUS), Belgium for providing financial support for my study.
- David Kunutu, Dipono Letlhage, Precious Mokonyane and Matseilane Phala for helping out with experiments in the laboratory, much appreciated.
- To my family, thank you for always being my best cheerleaders, for the love and support through my study years. For always encouraging me and motivating me to be the best I can.
- My daughter, thank you. You made it so easy for mommy to carry on with her studies. As little as you are its like you knew and understood what mommy had to do. Love always.

ABSTRACT

The pressing state of South Africa's freshwater resources due to pollution from the release of raw sewage or poorly treated domestic wastewater, has resulted in the urgent need for the implementation of innovative ways to mitigate this problem. A proposed solution by the Council of Science and Industrial Research (CSIR) is to introduce different cultures of algae in wastewater treatment ponds where facilities have aged and become dilapidated. In turn fish are introduced into sewage maturation pond treated with algae to reduce algal biomass. The assumption being that if fish can survive under these conditions, the nutrients assimilated will be converted into fish biomass when ingested, in an attempt to decrease aquas nutrient loads. The aim of the study was to assess the health and survival of *Oreochromis mossambicus* exposed to wastewater from the Motetema wastewater treatment plant (WWTP) and to establish the extent to which this species consumes micro-algae within the water column. The aim was accomplished by assessing the consumption of algae by *O. mossambicus* based on algal cell density counts in fish aquaria and to establish the feeding ratio of fish based on stomach content fullness as well as to monitor the survival rate of *O. mossambicus* exposed to various concentrations of sewage water over a 96-hour period.

To establish fish survival under wastewater conditions, a 96-hour experiment was conducted in glass tanks (60 L), whereby the health and mortality of *O. mossambicus* exposed to different concentrations of domestic wastewater from Motetema WWTP was investigated. One set of aquariums supplied with compressed air via the use of diffusers while the other set of aquariums were void of aeration so as to simulate conditions at the treatment plant. Treatments comprising of four concentrations of 25, 50, 75 and 100% wastewater and a control of 0% were used. Water quality parameters were monitored every four hours and mortalities were recorded. Water samples were collected twice a day and sent to Capricorn Veterinary laboratory for nutrient analysis. Gill samples were also collected and sent to Onderstepoort Veterinary Institute for histological sections analysis. To assess the consumption of algae, 18 tanks (60 L) were set up in the laboratory, whereby three aquariums served as a control void of algae with the remaining tanks dosed with two algal species (*Chlorella vulgaris* and

Chlorella protothecoides) of concentrations of 33%, 66% and 100%, over a period of ten days. Counts of algal density before and during the course of the experiments were done using a handheld flourometer. Upon mortalities and on the fifth day, fish were randomly selected from fish tanks, euthanised and stomach contents analysed. The stomach of fish was rated based on the percentage fullness and categorised as being empty, ¼ full, ½ full, ¾ full, full or gorged. When mortalities occurred, fish were dissected and their stomach contents analysed for fullness. Upon completion of the trial, two fish per treatment were euthanised and their stomach contents evaluated for algal consumption. Fish remaining at the end of the fish trial were counted and weighed to calculate the weight gain and specific growth rate. Survival rates were also determined. Water quality parameters were monitored three times a day over the duration of the trial. Water samples were collected every second day and send to Capricorn Veterinary laboratory for nutrient analysis. All mortalities were recorded over the duration of the trial period.

In exposure and survival trials, physico-chemical parameters of water from the experimental tanks were within the acceptable limit for the growth and survival of *O. mossambicus*, except for dissolved oxygen and ammonia concentrations. Ammonia levels and mortality rates were significantly higher ($p < 0.05$) in treatments with wastewater, with ammonia levels exceeding those considered toxic for *O. mossambicus*. High ammonia concentrations resulted in definite histopathological changes in the gills as well as fish mortalities. After exposure to wastewater moderate signs of aneurism of the gill lamella, mild epithelial lifting, focal hyperplasia and clubbing of the terminal end of the secondary lamellae were recorded. Lesions are explained as a defence mechanism where gills increase the distance between the external environment and the blood, thus serving as a barrier to the entrance of the contaminants. Furthermore, results indicated that effluent levels >25% were detrimental to the fish. Fish survival decreased when exposed to effluent water, with higher number of mortalities recorded in tanks with no aeration. A 100% survival was observed in tanks with 25% treated wastewater in both aerated and non-aerated aquariums. The presence of fish mortalities in treatments >25 domestic wastewater shows that conditions at Motetema WWTP will be unfavourable for fish. Thus, results from the study indicate that domestic wastewater would need to be diluted to less concentrated levels to ensure the survival of fish and mechanical aerators needs to be deployed to increase oxygen levels in treatment ponds.

Water quality parameters for the second set of experiments fell within the recommended range for the growth and survival of fish. However, low oxygen levels were recorded from the control group with minimum values of 1.8 mg/l and maximum concentrations of 3.6 mg/l. Furthermore, temperature and pH ranges recorded during this study fell within the desired range for growth and reproduction of *Chlorella vulgaris* and *Chlorella protothecoides*. Nutrient concentration (phosphates, sulphates and nitrate) for this study were low, however ammonia and nitrite were above the acceptable level for fish growth and survival. The presence of fish resulted in increased levels of ammonia. High ammonia levels were not mitigated by algae in the tanks. However, the high ammonia levels decreased with the increasing number of fish mortalities. There were slight decreases in chlorophyll-*a* concentrations over the study period, from tanks comprising of algae and, tanks comprising of algae and fish. Decreases in chlorophyll-*a* concentrations in tanks with fish were linked to consumption of algae by the fish. This was verified by the presence of algae in the stomach of fish euthanised during and at the end of the experiment. In tanks without fish, decreases in chlorophyll-*a* concentrations could be attributed to plankton die offs, as aquariums had algae that had settled at the bottom of the tanks.

Although, consumption of algae by fish was observed in this study, no full stomachs were recorded over the experiment period. Tanks with 66% algal concentrations had low survival rates. Better survival was observed from treatments with 33% algal concentrations. Toxic secretions could have attributed to the high mortality rate or low survival rates during the study period in tanks with 66% and 100% algal concentrations. In addition to this, ammonia and nitrite values that were above tolerable limits for fish could have also contributed to the high mortality rates (>80%). The use of fish in the tanks as a means to assimilate the algae, seemed to have an opposite effect than the desired one. As the presence of fish in tanks increased ammonia levels, therefore, the effluent would need to be further treated before it can be re-used or released into the environment. Further experiments would need to be conducted to establish whether negative influences could be neutralised, when other species such as *Scenedesmus* spp. are used together with *Chlorella* spp. for the treatment of wastewater, in order to make the environment suitable for fish survival.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Freshwater ecosystems in South Africa are under pressure. This is because the demand of water continues to increase due to population and economic expansion (Ashton *et al.* 1993). As a consequence, the quality and quantity of freshwater resources in South Africa has deteriorated due to pollution from point sources originating from industrial and domestic wastewater discharge, and non-point pollution sources such as agricultural runoff (Rodda *et al.* 2011). Anthropogenic stressors such as mining, urbanisation, industrial and agricultural activities have resulted in the Olifants River System being one the most polluted rivers in the country (Grobler *et al.* 1994; Ashton & Dabrowski 2011). Furthermore, the uncontrolled release of poorly treated and raw sewage has exacerbated the deterioration of the water quality in the Olifants River System (Ashton & Dabrowski 2011). Thus, without radical improvement in wastewater treatment technologies, untreated or poorly treated wastewater will continue to decrease the benefits and increase costs associated with the use of freshwater resources (Oberholster & Ashton 2008).

Pindihama *et al.* (2011) highlights that aging or a lack of wastewater infrastructure, poor planning and a shortage of skilled personnel has contributed to the poor functioning of most wastewater treatment facilities in South Africa. Another contributing factor is limited financial funding, whereby costs of purifying polluted surface waters to acceptable standards and investing in treatment plant infrastructure has proven to be expensive for most municipalities, especially those in rural areas. Therefore, alternative measures to ease the burden of most wastewater treatment plants in South Africa should therefore remain a priority (DWA 2012).

1.2 Processes involved in the treatment of wastewater

Wastewater treatment is the process that is used to remove contaminants from wastewater or sewage. The aim of wastewater treatment is to produce an effluent that is within the acceptable limit thereby preventing pollution of the receiving water bodies (Khopkar 2004). The major processes in a conventional wastewater treatment plant generally involves: preliminary treatment, primary treatment, secondary treatment, tertiary treatment and disinfection of wastewater (Tempelton & Butler 2011).

1.2.1 Preliminary treatment of wastewater

Preliminary treatment of wastewater involves the removal of solid and large materials delivered by sewers that could obstruct with flow through the plant or damage equipment (WISA 2002). These materials are composed of floating objects such as rags, wood, faecal material and heavier grit particles. Large objects are removed by passing wastewater through bars spaced at 20–60 mm. Grit is removed by reducing the flow velocity to a range at which the grit will settle, this is usually done at a velocity of 0.2–0.4 m/s (WISA 2002; Oakley 2018).

1.2.2 Primary treatment of wastewater

After the removal of coarse materials from the wastewater, sewage passes to sedimentation tanks, which aims to remove settleable solids by gravity (WEF 1996). Pathogen removal during primary treatment varies greatly for different organisms.

1.2.3 Secondary treatment of wastewater

The secondary treatment process aims to achieve a certain degree of effluent quality by removing the remaining settleable solids and suspended organic compounds. Secondary treatment is usually performed by micro-organisms in an aerobic habitat (WISA 2002).

1.2.4 Tertiary treatment of wastewater

According to Tempelton and Butler (2011), tertiary treatment is the further removal of suspended solids or nutrients before discharge to the receiving water bodies. Tertiary process aims to remove all organic ions, which can be accomplished biologically or chemically. Biological tertiary treatment process appears to perform well compared to chemical processes which are often too costly to be implemented in most places.

1.2.5 Disinfection of wastewater

Disinfection is the treatment of the effluent for the destruction of all pathogens. Disinfection procedures applied to wastewaters result in a substantial reduction of all microbes so that bacterial numbers are reduced to a safe level (WISA 2002).

1.3 Effects of nutrient rich wastewater on receiving water bodies

The removal or reduction of nutrients from wastewater is essential, this is because untreated or partially treated domestic wastewater discharged into receiving water bodies, results in nutrient enrichment and eutrophication of the receiving water bodies (Oberholster *et al.* 2009; Oberholster 2013). Furthermore, the presence of high levels of ammonia and nitrogen in wastewater discharges is undesirable (Pai *et al.* 1999), mainly because it has ecological impacts and can affect public health. Ammonia is toxic to fish and many other aquatic organisms. It is also an oxygen-consuming compound, which can deplete dissolved oxygen in the water. The depletion of dissolved oxygen in water bodies can be a problem since dissolved oxygen is vital for the survival of all life forms in the aquatic ecosystem.

Another ecological impact of ammonia and nitrogen in wastewater is eutrophication. All forms of nitrogen are taken up as a nutrient by different algae. The excessive growth of algae due to the increased amount of nitrogen discharged into the water, contributes to the reduction of oxygen in the water. Although nitrate is not toxic, its conversion to nitrite is a concern to public health. Nitrite is a potential public health hazard when consumed by infants (Sedlak 1991). In humans and fish, nitrite can oxidise iron(II) and form methaemoglobin which binds oxygen less effectively than normal haemoglobin. The resulting decrease in oxygen levels in young children which may lead to shortness of breath, diarrhoea, vomiting and in extreme cases death (Kelter *et al.* 1997). Moreover, in fish, gills and blood will turn brown, hindering with respiration, which will account for the gasping behaviour observed in fish (Lawson 1995).

According to Fried *et al.* 2003, all plants need phosphates to grow, however excess phosphates causes algae to grow out of control and create oxygen imbalances in the water system which destroys other life forms and produce harmful toxins. Excessive amounts of algae cloud the water in an effect called algal bloom, which reduces the sunlight available the other plants and sometimes kills them. When the algae die, the bacteria that breaks them down use up the dissolved oxygen in the water, depriving and sometimes suffocating other aquatic life. These occurrences are commonly associated with the disposal of poorly treated municipal sewage water (Pinette

1993). Therefore, the harmful effects of all these incidents pose the importance of the proper treatment of wastewater, prior to the effluent being discharged.

1.4 Conventional and pond-based techniques used in the treatment of wastewater in South Africa's wastewater treatment works (WWTWs)

Conventional methods are generally used in South Africa to treat wastewater. These methods include centrifugation, in-pond chemical precipitation of suspend materials, flotation and filtration (Raposo *et al.* 2010). Centrifugation is a high speed process whereby the force generated from rapid rotating cylindrical bowl separates waste solids from the liquid (U. S. EPA, 1987). Centrifuges have been used in wastewater treatment since 1930s and require large capital investment and skilled labourers to operate. In contrast to centrifugation, the flotation process in the treating of wastewater has been around for a number of years. The flotation process is designed to remove suspended solids, decrease the biochemical oxygen demand (BOD), oils and greases from wastewater (Rubio *et al.* 2007). This method offers an advantage over filtration method as it provides better treatment efficiency. The filtration process involves water flowing through a filter that is designed to remove suspended solids and enhance the effectiveness of disinfection. Filters are usually made up of sand, gravel and in some cases crushed anthracite, which is a hard form of coal. Alternatively, chemical precipitation of suspended materials is when specific chemicals are added to pond effluents to remove particulate matter. However, these processes of adding chemicals in ponds increases the running costs (Middlebrooks *et al.* 1974).

The implementation of conventional methods is often expensive and demand a high level of technology that requires highly skilled personnel that most municipalities in rural areas lack. Oberholster *et al.* (2018) reported that a significant proportion of wastewater treatment plants in South African rural areas make use of pond-based systems alone. These ponds referred to as oxidation ponds comprise of a series of ponds, all of which are relatively shallow bodies of water contained in earthen basins. These ponds are suitable for rural areas since it is low cost, needs a sizable land requirement (± 30-50 ha) and is not labour intensive (Pham *et al.* 2014). As a consequence, researchers have been looking into the possibility of using high rate algal ponds (HRAPs) as an alternative to treating wastewater generated by rural communities.

1.5 Measures to mitigate poor conditions of most wastewater treatment plants in rural areas of South Africa and Sekhukune District

The pressing state of most wastewater treatment works (WWTWs) in South Africa, especially those in rural areas, has resulted in the urgent need for the development and implementation of innovative ways to resolve wastewater problems. As a consequence, the Department of Water and Sanitation (DWS) of South Africa has implemented a programme called Green Drop. This initiative was developed to enable the DWS to continuously improve and track the progress of WWTWs annually. The DWA (2010) Green Drop reported that most municipalities in the Limpopo Province received a low score for the management of their WWTPs. Additionally, the report stated that hundreds of million litres of untreated or poorly treated effluent are being discharged into rivers and streams every day. A subsequent DWA (2012) Green Drop report stated that most WWTWs in Limpopo Province still remain in a critical state with the disposal of wastewater remaining a matter of urgency.

The Greater Sekhukhune District Municipality in the Limpopo Province is experiencing immense challenges in the treatment of domestic effluent. A DWA (2012) Green Drop report states that all 17 WWTWs in the Greater Sekhukhune municipality are at high risk of failure. This was concluded based on the following: (1) none of the 17 WWTPs has the means to measure actual flow rates from which the operational capacity of the plant can be calculated, for this reason it is assumed that all plants are exceeding its design capacity, (2) 14 of the 17 WWTWs do not have adequate monitoring programmes in place, which monitor the impact of the discharged wastewater on the environment down-stream of the plant and (3) the majority of the municipal WWTWs could not provide any proof of monitoring records, operation or maintenance schedules, operating procedures, budgets and expenditure records to support a positive green drop score.

With the DWA (2012) report highlighting the critical state in which most WWTWs are in, the government sought innovative ways to address the problems faced by most treatment plants of treating domestic effluent in accordance to national standards. In 2015 the Water Research Commission (WRC) in collaboration with the Council of Science and Industrial Research (CSIR) selected the Motetema WWTWs as the pilot study site for the implementation of an algae-based wastewater treatment system. The treatment plant was chosen due to the aging infrastructure that was no longer capable of facilitating treatment of the large amounts of wastewater that is being generated by the surrounding community. A detailed description of the treatment plant will be given in the methods section of the dissertation.

1.6 Low cost method for treating wastewater

High rate algal ponds (HRAP) have gained considerable attention in recent years as an alternative wastewater treatment process (Garcia *et al.* 2000; Young *et al.* 2017), as they offer a sustainable and efficient option to treating wastewater (Jones *et al.* 2014), by creating optimal conditions for the production of algae to remove nutrients and wastes from HRAP systems. The HRAP approach was developed at the University of California in the middle of the twentieth century (Oswald *et al.* 1957). Since then these systems have been implemented in many countries including Israel (Shelef & Azov 1987), the United Kingdom (Fallowfield & Garrett 1985a), France (Picot *et al.* 1991), China and New Zealand (Craggs *et al.* 2003a), Spain (Garcia *et al.* 2008), Morocco (El Hamouri 2009) and Australia (Young *et al.* 2016). Furthermore, numerous laboratory and pilot projects at several sewage treatment plants have been constructed in California, Germany and South Africa (Oswald 1988a; Shi *et al.* 2007; Zhu *et al.* 2008; Jones *et al.* 2014; Jones 2016). High rate algal ponds are used to treat a variety of wastes including those from domestic (Chen *et. al* 2003), tannery (Rose *et al.* 1996), dairy (Craggs *et al.* 2003b) and piggery sources (Fallowfield & Garrett 1985b). Studies undertaken by Oswald (1988); Muttamara *et al.* (1995); Hoffmann (1998); Chinnasamy *et al*. (2010) and Wang *et al.* (2010), have shown that these systems have high removal efficiencies of nitrogen, phosphorus and heavy metals to acceptable standards for the treatment of wastewater.

The most common microalga strains used for wastewater treatment are *Chlorella* spp. and *Scenedesmus* spp. (Garcia *et al.* 2000). Lau *et al.* (1997) and Garcia *et al.* (2000) studied the ability of *Chlorella* spp. in nutrient removal efficiency and have reported that microalgae have a nutrient removal efficiency of 86% for inorganic nitrogen and 78% for phosphorus. Colak and Kaya (1988) reported a removal efficiency of 97,8% in domestic wastewater when treated by algae. Interest in the treatment of wastewater using microalgae emerged from the fact that conventional treatment methods have some disadvantages. These are: (1) variable removal efficiency depending on the nutrients, (2) costly to operate, (3) chemical and physical processes often leading to secondary pollution, (4) treatment processes leading to incomplete utilisation of natural resources (Phang 1990). Therefore, these disadvantages related with the treatment of wastewater by conventional treatment methods indicate how effective the introduction of biological treatment systems can be, when biological processes are compared with physical and chemical processes (De la Noue *et al.* 1992).

Algae have shown to be beneficial in the treatment of wastewater, however, high concentrations of organic matter in the form of algae in post-HRAP water pose a major pollution risk to the receiving environment. High concentrations of algae in water can alter the physical and chemical properties of the receiving water bodies, by decreasing oxygen levels and increasing the water pH, turbidity and rate of sedimentation, thus leading to the effluent not meeting the required standard for the treatment of wastewater (Pan *et al.* 2006). For that reason, the removal of algae from the final ponds, before wastewater is discharged into receiving water bodies is essential. The removal of algae following the treatment of wastewater, is one of the major restrictions in the treatment process (Martinez 2016). Several harvesting techniques which are based on mechanical, chemical and electrical methods are used for recovering algae (Gerardo *et al.* 2015). However, they have major constraints such as high energy requirements and high costs. These constraints can hinder the use of algae for low-value applications in the context of wastewater treatment (Uduman *et al.* 2010).

Alternative approaches whereby phytoplanktivorous fish are used to remove algae has been researched by Leventer and Telsch (1990), Starling (1993) and Hambright *et al.* (2002). Findings from these studies showed that the introduction of silver carp (*Hypophthalmichthys molitrix*) in eutrophic lakes can be used to control algal densities. According to Berday *et al.* (2005), silver carp is a versatile omnivorous fish species, which is able to reduce phytoplankton and zooplankton. This is due to their specialised gill apparatus and digestive tracts which are adapted to a phytoplankton diet. Filter feeding by fish is a passive process, as phytoplanktivores swim with their mouths and operculars wide open, passively forcing water into their mouths and over their gills (Hambright *et al.* 2002).

This method of using phytoplanktivorous fish to remove algae in HRAPs has proven to be effective. However, the quality of the effluent following treatment would need to be favourable for the health and survival of fish, as water quality parameters above the tolerable level can negatively impact the health of fish (Dempster *et al.* 1995).

Physiological stress caused by changes in water quality parameters can affect the growth and reproduction of fish, induce changes in the gill structures of fish by causing and forming gill lesions, and reduce the ability of fish to filter feed (Moriaty 1973; Dempster *et al.* 1995; Barlas 1999; Roberts 2001). Furthermore, gastric secretions which are vital for the digestion of algal cell walls, can be compromised in stressed fish reducing the rate of algal removal in post-HRAP effluent (Jones *et al.* 2016). The integration of fish in the treatment of wastewater has been practised for decades in some regions of the globe, especially in Asian countries (Khalil & Hussein 1997). In China there exists one of the largest low cost sewage treatment system, whereby fish is used to harvest algae after algae is used to treat the water. The system in China is sorely based on wastewater. Wastewater is nutrient rich and enhances the growth of algae, which ultimately serve as food for the planktivorous fish introduced in treatment ponds thereby improving the physico-chemical and biological water quality (Fukushima *et al.* 1999). As a result, supplementary feeds are not required (Little *et al.* 2002; Bunting 2004; Bunting *et al.* 2008).

In South Africa, the use of sewage effluent for the production of fish was first reported by Hey (1955). Published records of animal manure and treated sewage effluent began to appear during the 1960s, explaining in detail how pig and poultry can be combined with fish production (Hepher and Schroeder 1975; Mortimer *et al.* 1963; Woynarovich 1976). Other studies done in wastewater maturation ponds were conducted by Prinsloo *et al.* (1989) and Prinsloo and Scoonbee (1991). However, these studies focused on the production of common carp (*Cyprinus carpio*) and sharptooth catfish (*Clarias gariepnus*) in sewage maturation ponds, as opposed to treatment purposes where fish can be used as a means to control algal densities. Although this process of treating wastewater in combination of filter feeding fish is commonly practised in some regions of the globe, it is not commonly practised in Africa, especially in South Africa (Edwards & Pullin 1990). *Hypophthamichthys molitrix* and *C. carpio* (filter feeders) are commonly used in Asian countries to control algal densities. However, these fish species are not indigenous to South Africa. *Oreochromis mossambicus* is the only fish in Southern Africa that is able to ingest phytoplankton to a degree (FAO 1992; Skelton 2001). Although filter feeding fish have shown to have microalgae removal capabilities, it is not certain whether *O. mossambicus* will be able to cope with wastewater conditions and whether it will be able to utilise and reduce algal densities in treatment ponds.

1.7 Problem statement

In South Africa there are approximately 850 municipal wastewater treatment plants, of which less than 50% meet the national standards for treating wastewater (Mthembu et al. 2013). In urban areas, wastewater treatment plants are better developed and equipped with regards to infrastructure than those in rural areas (DWA 2009). This is mainly due to cost constraints, limited resources, a shortage of skilled personnel and the increase in rural population. As a consequence, the treatment of wastewater in rural areas requires innovative ways to resolve increased nutrient load generated from domestic effluent (Kalbar et al. 2012). Due to the discharge of wastewater not meeting the minimum standard of treating wastewater, health and hygiene challenges in those municipalities increase. For example, certain municipalities around Eastern Cape and Kwa Zulu experienced health problems related to sewage spills. In uKhahlamba District Municipality in the Eastern Cape, sewage spills that were reported in 2008 led to the eruption of wastewater related sicknesses and deaths in the local communities. Pathogens such as *Shigella*, *Salmonella*, *Vibrio cholera* and enteric viruses are known to cause severe diarrhea in children and adults, as experienced before in South Africa with outbreaks *Shigella dysenteriae* and *Vibrio cholera* which resulted in 13 and 288 mortalities, respectively (DPLG 2001; Momba et al. 2006). According to Mail and Guardian (2004), typhoid fever also remains endemic to many parts of South Africa, including KwaZulu Natal, Limpopo, Mpumalanga and the Transkei. The health spokesperson in Delmas, Mpumalanga reported that there were 30 suspected cases of typhoid fever and nine confirmed cases. A further 380 cases of diarrhea were reported (Mail & Guardian 2004). This highlights how crucial it is that measures be taken to ensure that domestic wastewater be treated to acceptable quality for consumption and re-use in rural areas, where water treatment plants are seldom able to produce supplies of water of acceptable quality.

To address this problem government has tasked the CSIR to investigate novel approaches that do not require expensive infrastructure, highly skilled personnel and electricity. To this end the CSIR identified WWTWs in Mpumalanga province as a site to initiate a pilot project where wastewater treatment ponds would be inoculated with different cultures of algae. The precept behind this approach is that algae will assist with the removal of nutrients from the wastewater and introduce fish in the final stages of the treatment plant to consume the algae, prior to the wastewater exiting the plant and entering a small tributary of the Olifants River. The assumption being that the nutrients assimilated by the algae will be converted into fish biomass when ingested, enabling the treated wastewater to be safely discharged or re-used for non-human purposes (Oswald 1988).

1.8 Rationale of study

Conventional treatment systems that are commonly used in South Africa to treat wastewater are expensive in terms of installation, operation and management costs. Moreover, the treatment system requires a high degree of expertise to operate (Mara 2004; DWA 2014). Besides the capital investment and expertise required for the design and construction of these wastewater treatment systems, government struggles to train and employ skilled labourers to maintain and operate these WWTPs (Henze *et al.* 2008). Therefore, these systems are increasingly being replaced by biological treatment systems because they are an economically and environmentally sustainable alternative that are in most instances, more appropriate. The use of specific algae treatment to treat municipal domestic wastewater provides an alternative practice to improving water quality effluent of existing rural pond systems not only in South Africa but in Southern Africa (Oberholster *et al.* 2018). This would be suitable for use because they offer a low cost, low maintenance, highly efficient and an entirely natural solution to treating wastewater. The only energy HRAP systems use is direct solar energy, no equipment, electricity and highly skilled personnel are generally required, which helps decrease expenditure. However, HRAP systems results in water with high algal densities. Thus the removal of microalgae from post-HRAP ponds using filter feeding fish, where the fish passively consume the algal biomass and converting algae into fish biomass, has been shown to have potential using Mozambique tilapia (Jones *et al*. 2016). However, HRAP system are characterized by extreme water conditions such as oxygen that can affect fish health. It has not been established if Mozambique tilapia is the most suitable species to perform this function and whether it is possible to mitigate the conditions in the HRAP effluent making it more suitable for fish culture and thus improve rates of algal removal by Mozambique tilapia (Solamolai *et al.* 2003). Thus, a more detailed research is needed to understand the process of utilising fish to aid with the treatment of wastewater and to examine whether water conditions at the Motetema WWTW will allow for the survival of *O. mossambicus*. Lastly to examine whether the latter fish species will be able to utilize and reduce algal densities in treatment ponds.

1.9 Aim of the study

The aim of the study was to assess the health and survival of *O. mossambicus* exposed to wastewater from a Sekhukhune Municipality treatment plant and to establish the extent by which these species consume microalgae used to inoculate the water column as a means to remove nutrients and suspended solids.

1.10 Objectives

The objectives of the study were to:

i. monitor the survival rate of *O. mossambicus* exposed to various concentrations of domestic sewage water over a 96-hour period.
ii. assess the consumption of algae by *O. mossambicus* based on algal cell density counts in fish aquaria and to establish the feeding ratio of fish based on stomach fullness.

1.11 Dissertation outline

To achieve the aim of the study and to address the objectives, the dissertation consists of three chapters and a concluding chapter which contains the overall remarks that highlight the main findings of the study as well as recommendations for future research. References used are listed at the end of each chapter. Chapters are outlined as follows: **Chapter 1:** Introduction and purpose of the study. It outlines the purpose of the study, problem statement and lastly aims and objectives. **Chapter 2:** Contains the methods and materials. It furthermore provides a brief description of the algae and fish species used for this study period. From **Chapter 3 and 4** a brief introduction was given on the aspects to be investigated, a brief summary of the methods and materials used, results obtained discussed and conclusions drawn to answer the aims and objects of the study. **Chapter 3** contains water quality results obtained from the 96-hour experiment, the rate survival of *O. mossambicus* when exposed to various concentrations of wastewater and the impact wastewater has on the gill morphology of *O. mossambicus* after the 96-hour period. **Chapter 4:** provides results and discussion on the consumption of algae by *O. mossambicus* based on algal cell density counts in aquaria and stomach fullness over a 10-day period. Furthermore, results of the survival rates, feeding rates and growth parameters of *O. mossambicus* presented and discussed. **Chapter 5:** provides a short summary of the results and conclusions drawn and provides recommendations for future studies.

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CHAPTER 2

METHODS, MATERIALS AND SITE DESCRIPTION

2.1 Introduction

As a means to establish whether the concept could be feasible, two sets of experiments were conducted at the University of Limpopo, in Limpopo Province, South Africa. Experiments were carried out in glass aquaria (60 L) in the laboratory. Prior to the commencement of the experiments wastewater and microalgae were collected at Motetema WWTWs in 200 L containers and transported to the University of Limpopo. Furthermore, six hundred *Oreochromis mossambicus* of ± 5–10 cm in length were purchased from Eppagelia fish farm (Pty LTD) in Gauteng Province and transported to the University of Limpopo in well aerated 200 L tanks. Fish were kept in well aerated tanks in the laboratory so as to acclimatise them to experimental conditions. This was done two weeks prior to initiating the trials. During this period water temperature was maintained at 24 – 30°C and the fish fed to satiation using commercial pellets once a day.

2.2 Site description

The Motetema WWTW (location 25° 6’ 3.87” South and 29° 28’ 6.78” East), is situated near the small town of Elias Motsoaledi, in the Sekhukhune District (Figure 2.1), Limpopo Province, South Africa. The WWTW treatment system has 12 ponds (Figure 2.1), which are organised into two groups of six. Only one group works at a given time, while the other set of ponds are drained of their content and left to dry out. The entire treatment system relies on gravity to allow for water to flow from one pond to the next, which is suitable for the conditions at the WWTW since there is no electricity available at the plant.

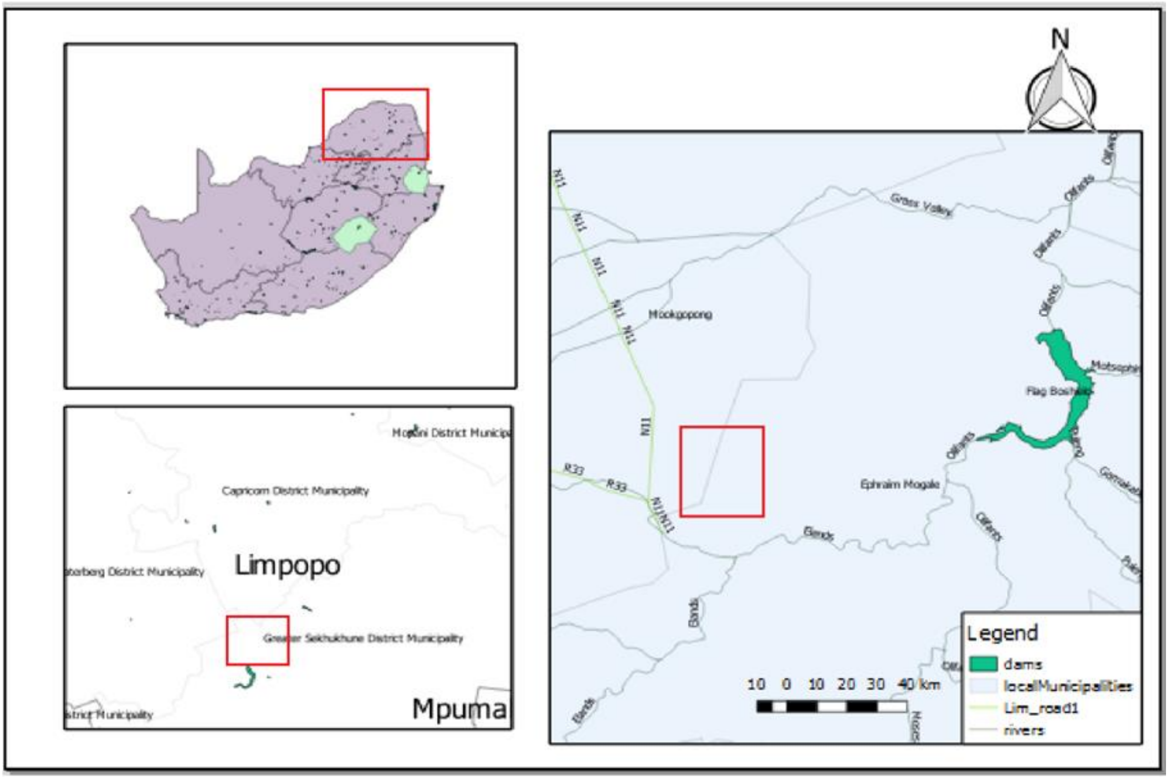

Figure 2.1: Map showing the geographical location of Motetema WWTW in Limpopo Province, South Africa (Google Map, 2018), yellow dot indicating the location Motetema WWTW.

The Motetema WWTW comprises of four types of ponds. These are (1) anaerobic ponds (2) primary facultative ponds, (3) secondary facultative ponds and (4) aerobic maturation ponds, each having different treatment and design characteristics (Figure 2.2). The WWTW was designed to treat domestic waste for a population of about 11400 people (amounting to 1 560 households) and an average effluent of 2.5 megalitres a day (Genthe & Oberholster 2016). However due to the increase in growth of the local population, the plant receives about 4.5 megalitres of effluent a day, which is double the amount it was originally designed to treat. The algae-based treatment process at Motetema WWTW involves two algal species from the Phylum Chlorophyta, namely *Chlorella vulgaris* and *Chlorella protothecoides*. The two algal species were isolated and cultured at the laboratory and in a raceway on the premises of the CSIR Pretoria, thereafter transported to Motetema WWTW. At the site algae are transferred into and kept in 5000 litres standing transparent tanks (algae bioreactors) that have been installed with a network of pipes and valves that release the algae into maturation ponds 4 and 5 (Figure 2.2). The ongoing dosing of the algae into ponds 4 and 5 is done bi-monthly, ensuring an effective treatment process. The last pond (Pond 7), does not contain much algae and should results from this study be viable, this will be where the fish will be introduced. This is done to prevent high concentrations of algae to be discharged into the Olifants River System.

Figure 2.2: Layout of the ponds at Motetema wastewater treatment plant (from Genthe & Oberholster 2016).

2.3 Algae selection

Algae are a diverse group of organisms that lack roots, leaves or stems. There are numerous types of algal species ranging from single celled microalgae to complex multicellular forms. Algae are photosynthetic organisms that are present in most aquatic environments, such as seawater and freshwater (Carlsson *et al.* 2007). Algae also adapt to different climate conditions and occur in very hot to cold regions. The algal cells comprise chlorophyll-*a* as a primary photosynthetic pigment. Sheehan *et al.* (1998) classified algae into two types based on their sizes, i.e. macroalgae and microalgae. Microalgae are abundant in both marine and freshwater environments (Metting 1996), having a cell size of 2–30 micrometres (Grima *et al*. 2003). One of the common strains used in the treatment of wastewater is *Chlorella* spp.

Chlorella vulgaris are spherical cells of 3 –10 micrometres in diameter (Figure 2.3).

Chlorella vulgaris reproduce asexually, in which the mother cell produces four daughter cells, with a doubling mass cell time of about 19 hours (Illaman *et al.* 2000; Daliry *et al.* 2017). According to Hultberg *et al.* (2014) and Daliry *et al.* (2017), rapid growth and independence from seasons makes these algae appropriate for use in the food industry, aquaculture, cosmetics, pharmaceuticals, wastewater treatment and the production of biofuels. This strain of algae comprises of a high percentage of proteins, minerals and vitamins (Lee *et al.* 2001).

Chlorella protothecoides are non-motile, spherical cells 2 – 10 micrometres in diameter (Kay 1991; Becker 2007). *Chlorella protothecoides* grows autotrophically (requiring light and inorganic carbon for growth) and heterotrophically (with no light and organic carbon source) under different culture conditions (Figure 2.3). These algal species are commercially important as they are used as food supplement for humans and aquaculture feeds (Kyle 1992; Chen 1996) and are a very good candidate for the production of biofuel (Wen *et al*. 2002).

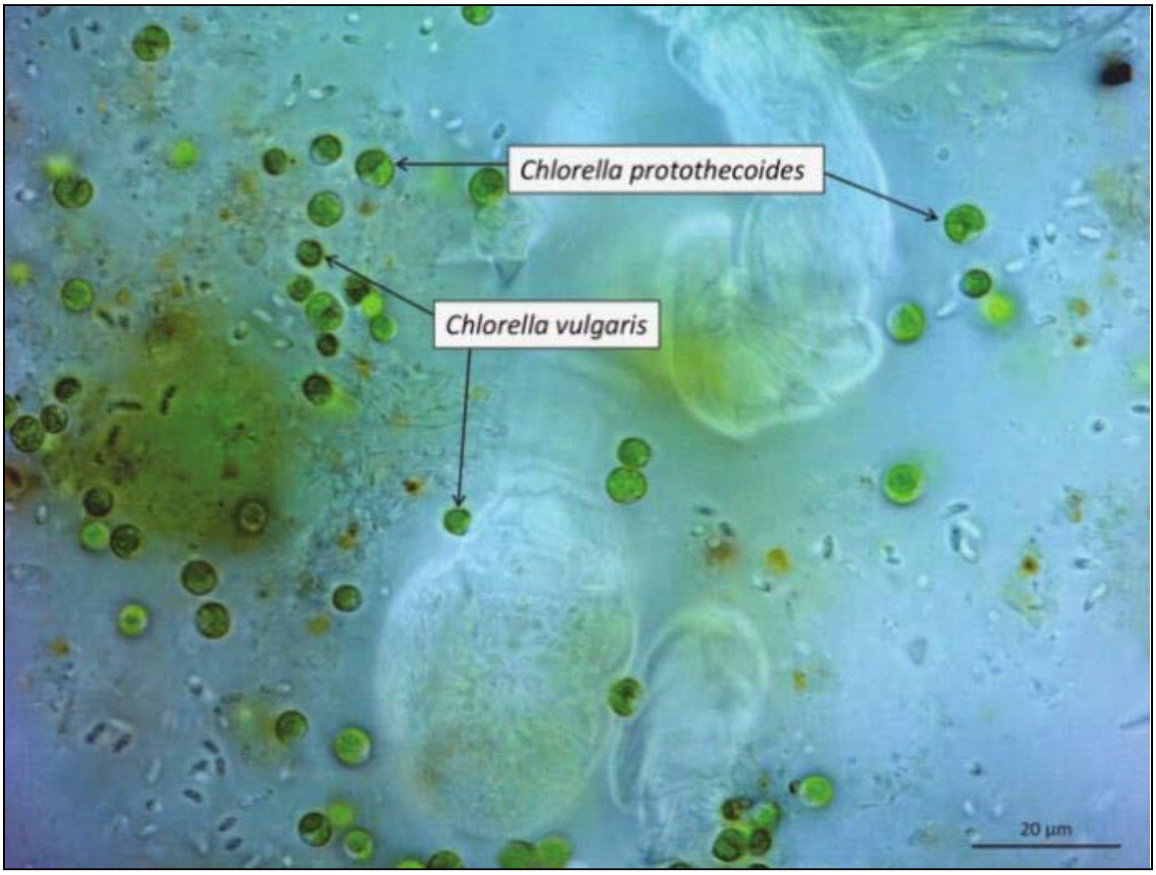

Figure 2.3: Microscopic image of *Chlorella vulgaris* and *Chlorella protothecoides* (Oberholster *et al.* 2016).

2.4 Fish selection

Oreochromis mossambicus, Mozambique tilapia, is distributed along the east coastal rivers from the lower Zambesi system south to the Bushmans system, Eastern Cape (Figure 2.4) (Peters, 1852). Furthermore, this species is found south of the Phongolo system and is naturally confined to closed estuaries and coastal reaches of rivers (Skelton 2001). This species has been introduced to tropical and temperate localities throughout the world and thrives in standing waters. The Mozambique tilapia survives lower temperatures below 15 ^{o}C in brackish and/or marine waters but prefers warmer temperatures above 22 ^{o}C (Skelton 2001). The Mozambique tilapia generally feeds on algae, especially diatoms and detritus (Skelton 2001). These species are hardy and display a remarkable tolerance for organic and inorganic pollution (Noorjahan *et al.* 2003; Somanath 2003). The Mozambique tilapia are widely used in aquaculture and is a valued angling species (Skelton 2001).

2.5 Experimental designs

To determine the survival of fish under wastewater conditions and their ability to utilise and reduce algal densities, two sets of experiments were carried out and divided into exposure and feeding trials. Exposure trials were based on establishing the survival rates of *O. mossambicus* exposed to various concentrations of wastewater. The second part of the experimental trials were designed to assess the daily consumption of algae by *O. mossambicus* based on cell count density and stomach fullness. Control treatments comprised of matured tap water only, while other treatments included concentrations of algae or wastewater and matured tap water. There were no water changes performed throughout the duration of the study. There were no water changes performed throughout the duration of the study. Ethical approval (Project number: AREC/12/2018: PG) was obtained from Animal Research and Ethic Committee (AREC), University of Limpopo, thus at all times humane measures were implemented when handling and sacrificing fish.

2.5.1 Exposure and survival trials

Trials comprised of two sets of experiments each having five treatments, whereby glass aquaria (60 L) was filled with 0, 25, 50, 75 and 100% domestic wastewater collected from maturation pond seven at Motetema treatment plant. The aquariums were each stocked with eight fish and each treatment was replicated three times. Trials comprised of two sets of experiments, whereby one set had aquariums supplied with compressed air via the use of diffusers while the other set of tanks were void of aeration so as to simulate conditions at the treatment plant. To establish changes in water quality, water samples were collected twice a day (morning and afternoon) in acid pre-treated polypropylene bottles and sent to Capricorn Veterinary Laboratory (ISO/IEC 17025: 2005), for analysis of nitrate, nitrite, ammonium, phosphate and sulfate. Temperature, dissolved oxygen, pH, total dissolved solids, conductivity and salinity were measured in each of the aquariums using a handheld multiparameter instrument (YSI 5100). In situ water quality parameters were taken hourly for the first 24 hours and thereafter every four hours until completion of the experiment. Upon mortalities of all the fish in aquaria, the last water quality parameters were taken, thereafter no measurements were recorded from those aquariums for the entire duration of the experiment.

During the course of the experiment, dead fish were removed immediately from their tanks and time recorded. Instances were 100% mortality occurred, final water quality readings were taken and recorded. Measurements of the length in millimetres and weight in grams of fish were recorded prior to initiating and on termination of the trials. The mass of each specimen was determined in grams using a Mettler ^{TM} balance. Upon completion of the trial, fish that were still alive were euthanised by severing the spinal cord. Measurements of their length (mm) and weight (g) were taken and recorded. Gill samples of the dead fish were collected for histopathology analysis and fixed in Karnovsky's fixative. Each treatment required that the survival rate of fish be monitored over a 96-hour period and the survival rate determined using equation [4].

2.5.2 Algae based feeding trials

These experiments involved 21 glass aquaria (60 L), whereby three aquariums served as a control, void of algae. The remaining glass aquaria were dosed with different algal concentrations: 33, 66 and 100%. The feeding trial was conducted over a period of 10 days and each aquarium were stocked with twelve fish. Fish were not fed 24 hours before the start of the experiment. Aquariums were well aerated to prevent oxygen deficiencies as a result of algal respiration.

(i) Establishing the stomach fullness index of *O. mossambicus* over the study period

To establish the stomach fullness index, five fish were dissected and their stomach contents analysed before the experiment commenced. On the fifth day, two fish from each aquarium containing algae and from the control were randomly selected and euthanised. The standard and total lengths (mm) of fish were measured using a measuring board and the fish mass was weighed to the nearest gram using a Mettler ™ balance. Specimens were dissected and their stomach contents removed. Thereafter fish mass, void of gut content and innerds was weighed to the nearest 0.01 g. The digestive tract (oesophagus, stomach and intestine) was separated from other visceral organs. A paper towel was then used to clean and dry the digestive tract. The mass of the intestinal tract was weighed. Thereafter the digestive tract was dissected and washed over a petri dish, dried (using tissue paper) and weighed to the nearest 0.01 g. The fullness of the individual digestive tract was determined using the stomach fullness (SF) index and expressed as a percentage of fish weight (Otieno *et al.* 2014):

$$\text{SF (\%)} = \frac{\text{SC}}{\text{Fish mass (g)}} \times 100 \qquad [1]$$

Where, SF is stomach fullness and SC is stomach contents in grams.

The stomach contents were analysed using a modified point method originally applied by Hynes (1950) and later modified by Hyslop (1980) as cited in Otieno *et al.* (2014). Each of the stomach contents analysed during the study was awarded an index of fullness from 0 to 20; where 0 = empty; 5 = quarter full; 10 = half full; 15 = three quarters full; 20 = full. When mortalities occurred, fish were dissected and their stomach contents analysed for fullness. Upon completion of the trial, two fish per treatment were euthanised and their stomach contents evaluated for algal consumption. Furthermore, fish that were left over at the end of the trial fish were counted and weighed to calculate the weight gain and specific growth rate. Survival rates were also determined.

Weight gain and specific growth rates (SGR) were determined using the formulas by

Sukri *et al.* (2016) given below:

$$\text{Weight gain (g)} = \text{final BW} - \text{initial BW} \quad [2]$$

and;

$$\text{SGR (\%/ day)} = \frac{[\log \text{final BW} - \log \text{initial BW}]}{\text{Time (days)}} \times 100 \quad [3]$$

where BW is the body weight in grams (g)

The survival rate (SR) was determined using an equation by Tekinay and Davies (2001).

$$\text{SR (\%)} = {}^{N_t}/_{N_0} \times 100 \quad [4]$$

where N_t is the number of organisms alive on termination of the trial and N_o is the number of organisms introduced at the beginning of the trial.

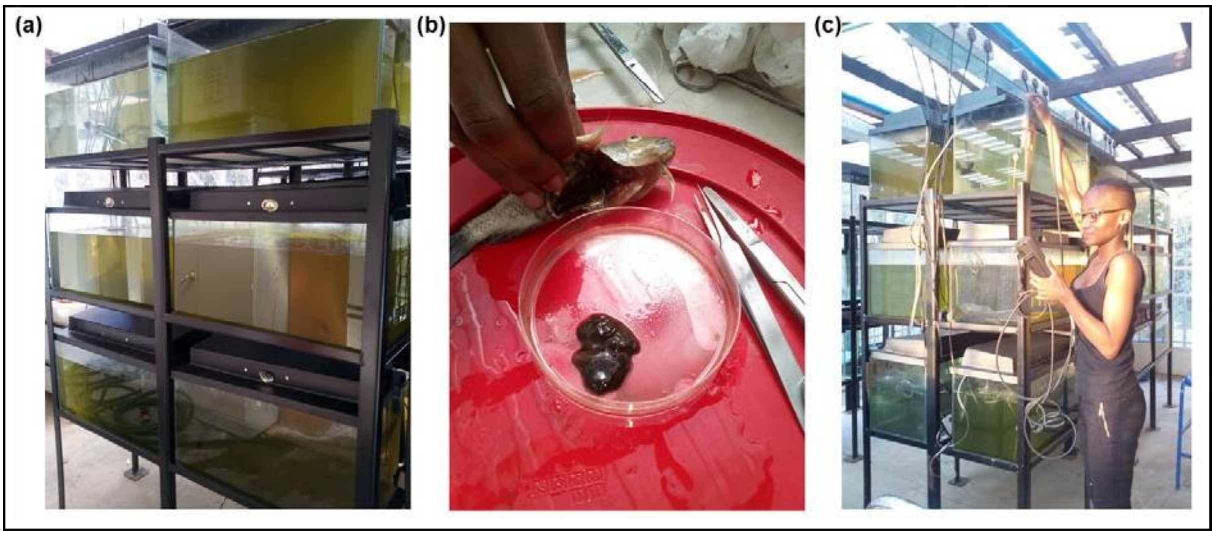

Figure 2.4: Image showing (a) setup of tanks containing algae and wastewater, (b) dissection of fish for stomach contents, (c) measuring water quality parameters using a handheld multiparameter instrument (YSI 5100).

(ii) Establishing the feeding rate (in cells fish $^{-1}$ h^{-1}) of *O. mossambicus* consuming algae

Algal concentrations, as chlorophyll-*a*, was determined in each aquarium using a handheld fluorometer (AquaFluoro ®). Suspended chlorophyll-*a* concentrations were taken three times a day, in the morning (08:00), afternoon (13:00) and in the evening (18:00). To determine the feeding rate (F) in terms of algal cells ingested per fish, the formula by Matveev *et al*. (1994) was used:

$$F = V\,(Co - Ct)/_{Nt}\, x\; 100 \qquad [5]$$

Where V is the volume of the aquarium (ml), t is the length of time (h) the fish were allowed to feed, N is the number of fish in the container, and C_0 and C_t are the initial and final cell concentrations (in cells ml^{-1}), respectively.

Figure 2.5: A handheld fluorometer (AquaFluoro ®) used to measure chlorophyll-*a* concentrations

2.6 Microscopy analysis

A gill arch of the right side of each fish was collected and fixed in Karnovsky's fixative. Gills samples of all the mortalities that occurred during the experiment and from the fish that were euthanised were sent to Onderstepoort Veterinary Institute for histological sections, as there are no histology facilities at the University of Limpopo.

This was done to establish the effects different concentrations of wastewater have on the gill morphology of the fish. Interpretation of histopathology was done at the University of Limpopo using an Olympus microscope.

2.7 Data analysis

Microsoft excel and SPSS (Statistical Package for Social Sciences 2009) were used to analyse data. The mean and standard deviation were used to assess differences in the data. The mean (± SE) and one-way analysis of variance (ANOVA) followed a post hoc multiple comparison was used to calculated to compare mean values of observations on the different treatments. Differences in mean values obtained were considered significant if calculated p-values were <0.05.

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CHAPTER 3

THE SURVIVAL AND HEALTH OF *OREOCHROMIS MOSSAMBICUS* EXPOSED TO VARIOUS CONCENTRATIONS OF TREATED WASTEWATER

3.1 Introduction

The concept of introducing fish in treating wastewater has been practised and studied extensively in certain parts of the world, specially in Asian countrires such as China and India (Allen & Hepher 1976; Cao *et al.* 2007; Bunting *et al.* 2010; Sreenivasa *et al*. 2014). This concept has been applied to control excessive phytoplankton and improve the efficiency of the treatment process (Solamolai *et al.* 2003). Moreover, high fish yields can be produced from these wastewater treatment ponds at a low cost, since little or no additional inputs such as formulated feeds will be administered to fish in the ponds. This is because the nutrient rich effluent will promote the growth of photosynthetic organisms, such as algae, which will serve as food when consumed by the fish. Findings from a study by Okoye *et al.* (1986), reported that the stocking of common carp and tilapia in sewage ponds in New Bussa, Nigeria, resulted in a good production of fish. Furthermore, Solamalai *et al.* (2003) indicated that the yield of fish from ponds supplied with sewage effluent is higher than when cultured only in freshwater.

Although the concept of introducing fish in sewage ponds is common in other parts of the world, published records on the use of wastewater effluent in fish ponds in South Africa dates back to the past two decades (Hey 1955; Mortimer 1963; Hepher & Schroeder 1975; Woynarovich 1976; Gaigher 1982; Gaigher & Toerien 1985; Prinsloo *et al.* 1989; Prinsloo & Scoonbee 1991). Results achieved from these studies showed that good fish yields can be obtained when fish are reared in wastewater treatment ponds. However, most of these studies focused on the production of fish, as opposed to fish being used as a means to control or reduce algal densities. Furthermore, there is little known about the utilisation of *Oreochromis mossambicus* in wastewater treatment ponds and whether conditions at the treatment plant will be suitable for their health and survival. Therefore, this study investigated the feasibility of utilising *O. mossambicus* in wastewater treatment ponds as a means to decrease the high nutrient load. *Oreochromis mossambicus* was selected in this study because it is indigenous to the Olifants River System, furthermore, it is the only fish in southern Africa that is able to ingest phytoplankton to some degree (FAO 1992; Skelton 2001).

3.2 Methods and materials

Prior to initiating the trials, domestic wastewater was collected from maturation ponds at Motetema treatment plant in 200 L containers and transported to the University of Limpopo. A sample of the undiluted wastewater was taken upon arrival at the University of Limpopo and analysed at Capricorn Veterinary Laboratory for nutrients. As described in chapter two, trials comprised of two sets having treatments with wastewater concentrations of 0, 25, 50, 75 and 100%. Control treatments comprised of matured tap water that was left to mature for three days, while other treatments had inclusion concentrations of domestic wastewater and matured tap water. Trials comprised of two sets of experiments, whereby one set had aquariums supplied with compressed air via the use of diffusers while the other set of tanks were void of aeration. Each treatment was replicated three times and aquariums were then stocked with eight fish 20.1g ± 0.4 g (mean ± SD).

3.3 Results

3.3.1 Physico-chemical parameters

Water temperature

Temperature ranged from 19.1 to 27.9 ^{o}C in non-aerated tanks and from 19.0 to 27.6 ^{o}C in aerated tanks (Figure 3.1). Temperature fluctuations were, however, not significant ($p>0.05$) between tanks and between treatments.

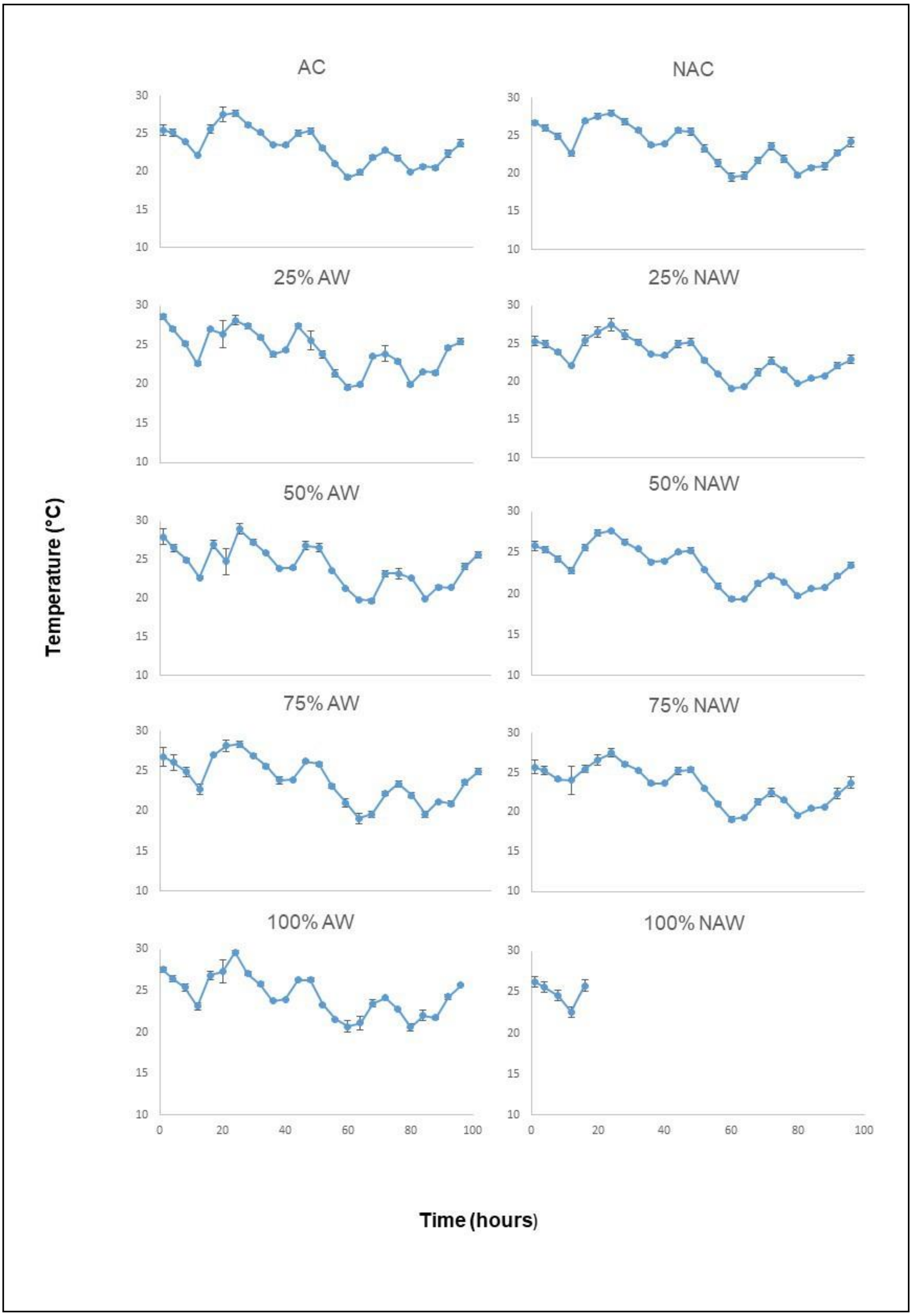

Figure 3.1: The mean temperature (± standard error) recorded in tanks with and without aeration, comprising of 0, 25, 50, 75 and 100% wastewater over a 96-hour period, where **AC** = aerated control; **NAC** = non-aerated control tanks; **AW** = aerated domestic wastewater tanks; **NAW** = non-aerated wastewater tanks. Error bars indicate standard error about the mean value.

Dissolved oxygen

Dissolved oxygen (DO) values from the different treatments ranged from 3.3 mg/l to 12.4 mg/l in aerated treatments and from 1.0 mg/l to 9.1 mg/l in non-aerated treatments (see Figure 3.2). The highest DO levels were recorded in aquaria having 25% domestic wastewater in both aerated and non-aerated tanks. Lowest DO levels were recorded in non-aerated tanks comprising of 75% domestic wastewater and in the control treated with aeration. Overall, DO concentrations gradually increased over the trial period. The DO concentrations fluctuated according to the time of day, slightly increasing during day time and decreasing during night time. However, non-aerated tanks had larger sways in DO concentrations than in aerated tanks, especially in the first 28 hours of the study. Aerated tanks had higher oxygen levels than those without aeration. In most instances, DO values recorded decreased with increasing wastewater concentrations. There were significant differences ($p<0.05$) recorded from treatments over the 96-hour period.

pH

Water pH values of the treatments were slightly neutral to alkaline throughout the trial. The pH values ranged from 7.27 to 9.34 in aerated tanks and from 7.45 to 9.20 in non-aerated tanks (Figure 3.3). High pH values were recorded in tanks treated with 100% domestic wastewater in both aerated and non-aerated tanks. Overall, pH levels increased rapidly in the first 16 hours before stabilising and then increasing gradually for the remainder of the trial period. Tanks provided with aeration were more alkaline than those that were not aerated, with the exception of the control tanks. The pH range over the 96-hour period varied significantly ($p<0.05$) between treatments.

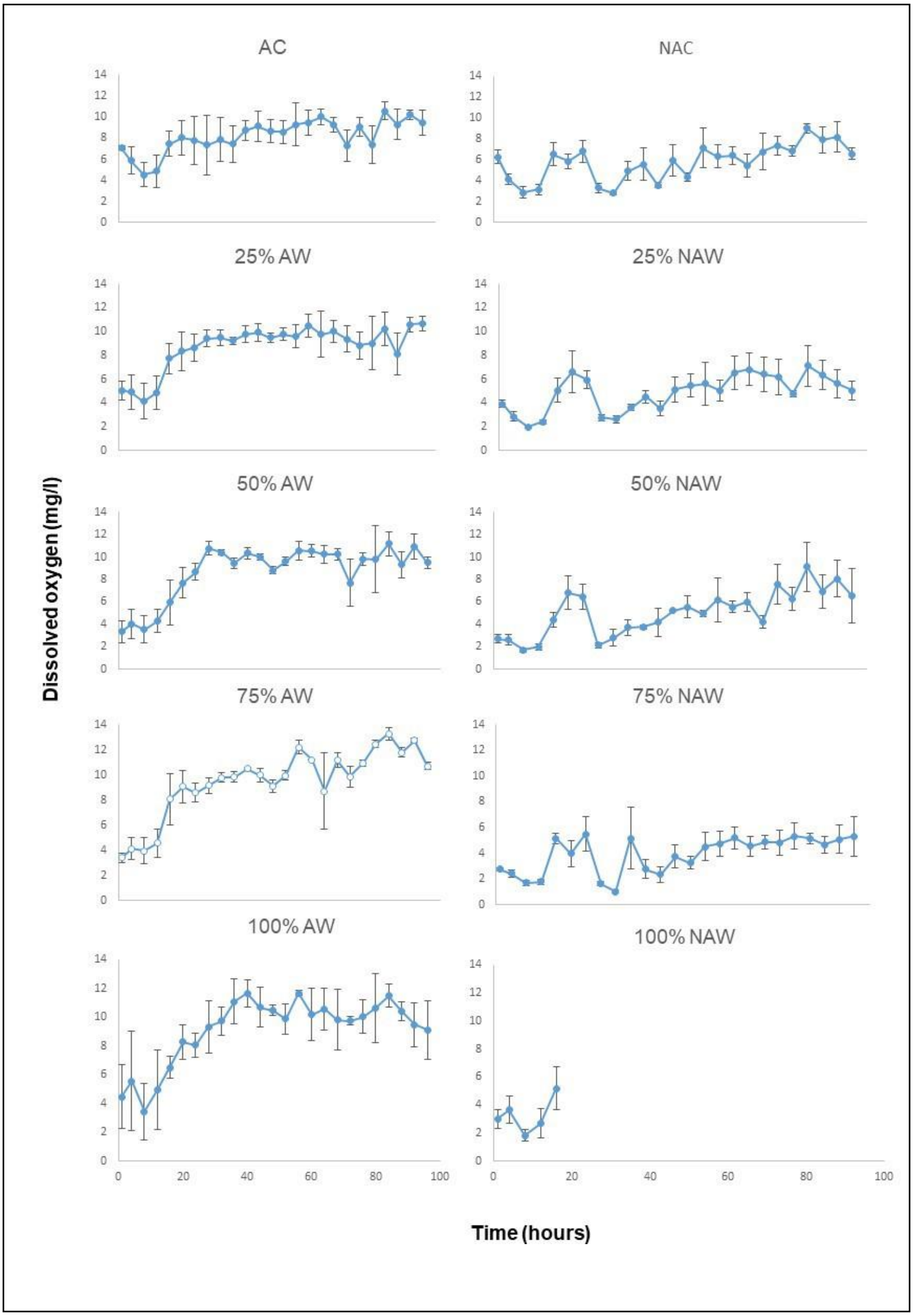

Figure 3.2: Mean dissolved oxygen values (± standard error) recorded in tanks with and without aeration over a 96-hour period containing concentrations of 0, 25, 50, 75 and 100% effluent water, where **AC** = aerated control tanks; **NAC** = non-aerated control tanks; **AW** = aerated domestic wastewater tanks; **NAW** = non-aerated wastewater tanks. Error bars indicate standard error about the mean value.

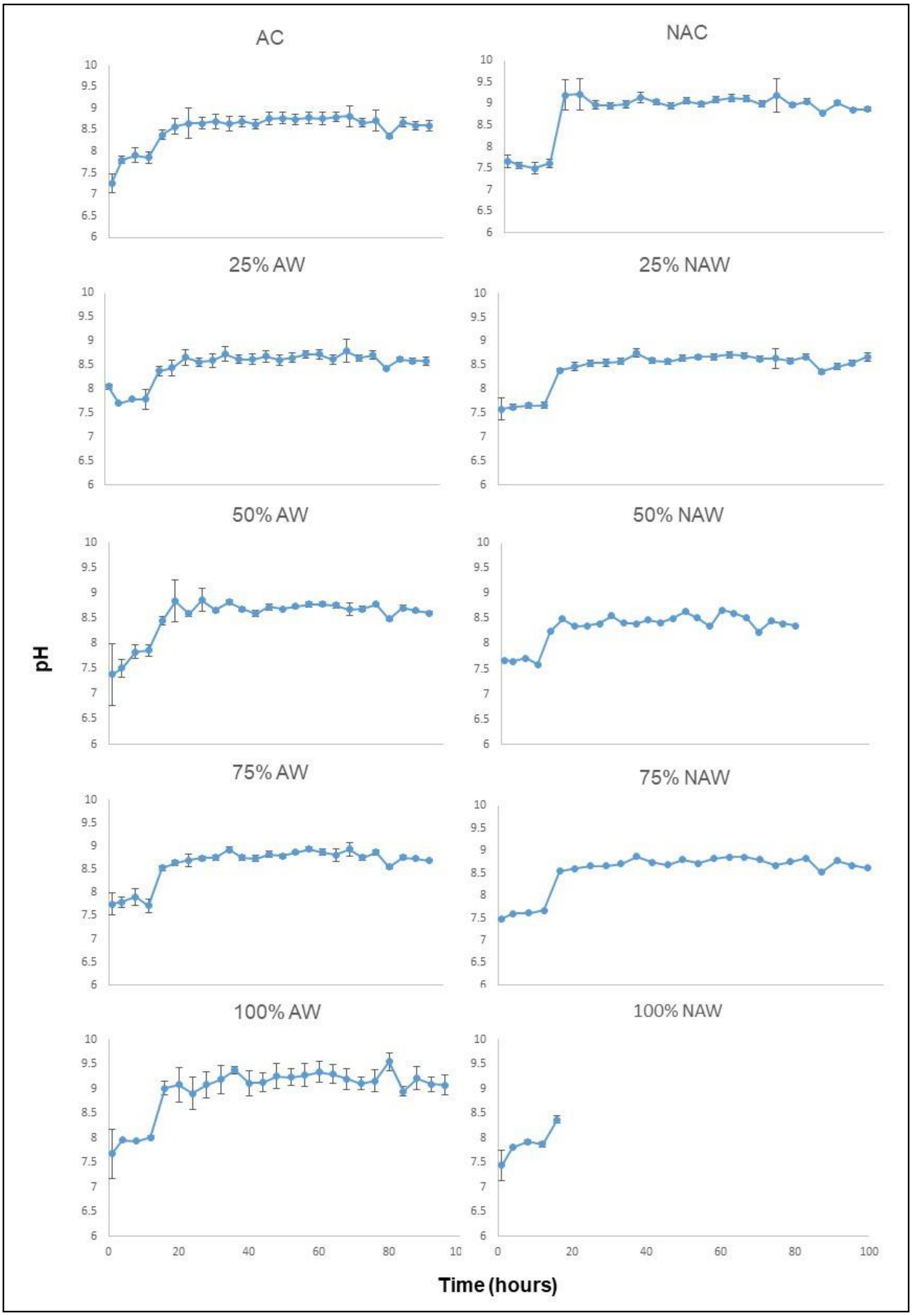

Figure 3.3: The pH recorded over a 96-hour period in tanks with and without aeration containing 0, 25, 50, 75, 100% wastewater, where **AC =** aerated control tanks; **NAC** = non-aerated control tanks; **AW** = aerated domestic wastewater tanks; **NAW** = non-aerated domestic wastewater tanks. Error bars indicate standard error about the mean value.

Total dissolved solids

The range of total dissolved solids (TDS) in the present study was between 35.3 to 1125.0 mg/l in aerated treatments and from 54.9 to 1070.0 mg/l in non-aerated tanks as indicated in Figure 3.4. Highest TDS values were recorded in tanks with 100% domestic wastewater while low values were recorded in the controls of both aerated and non-aerated treatments (Figure 3.4). Total dissolved solids values recorded increased with increasing wastewater concentrations over the study period. There were significant differences ($p<0.05$) in TDS levels between treatments over the 96-hour period.

Salinity

High salinity values were recorded from the 100% wastewater treatment and low salinity values from control tanks for both aerated and non-aerated tanks. Salinity values from aerated treatments ranged from 0.03 to 0.51 ppt and from 0.03 to 0.52 ppt in non-aerated tanks (Figure 3.5), with salinity values increasing 10-fold in tanks with wastewater. Significant differences ($p<0.05$) were recorded in aerated tanks with 25, 75 and 100% wastewater, and in tanks 25% and 100% wastewater in non-aerated tanks over the period of the study.

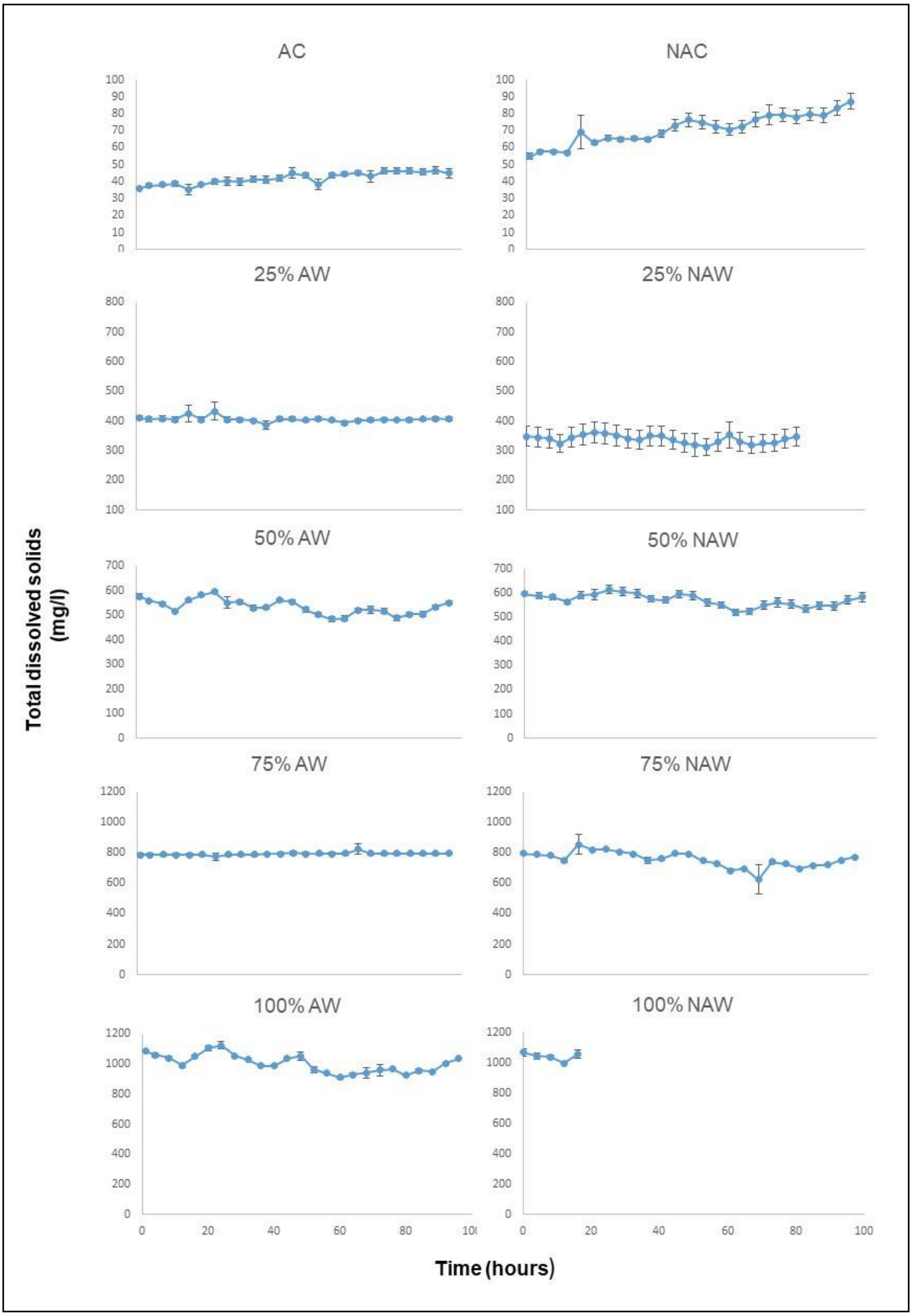

Figure 3.4: Mean total dissolved solids (TDS) recorded over a 96-hour period for tanks containing 0, 25, 50, 75 and 100% effluent water that was either aerated or non-aerated, where **AC** = aerated control tanks; **NAC** = non-aerated control tanks; **AW** = aerated domestic wastewater tanks; **NAW** = non-aerated wastewater tanks. Error bars indicate standard error about the mean.

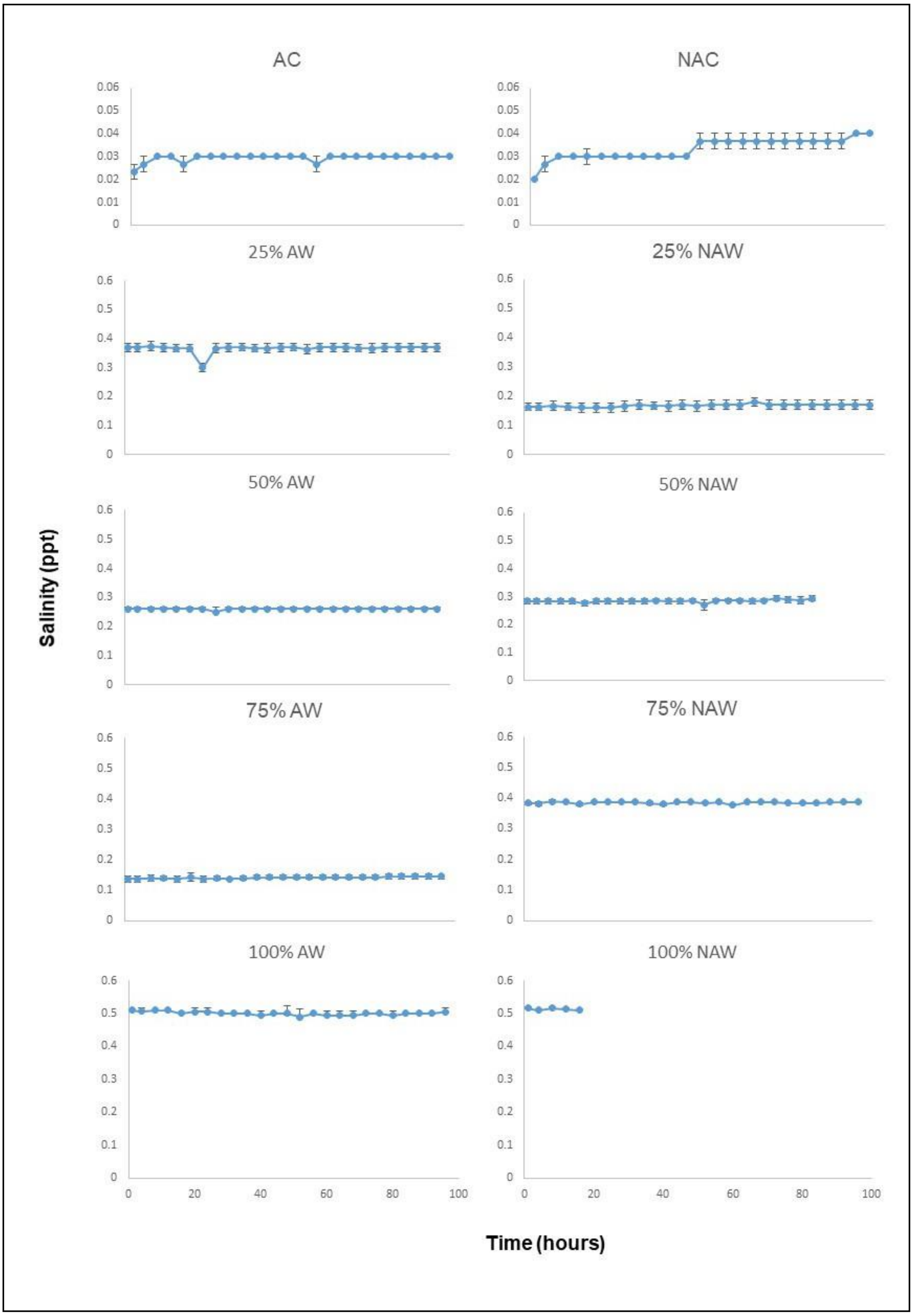

Figure 3.5: The mean salinity (± standard deviation) values of aerated and non-aerated tanks containing 0, 25, 50, 75 and 100% concentrations of wastewater over the 96-hour period, where **AC** = aerated control tanks; **NAC** = non-aerated control tanks; **AW** = aerated domestic wastewater tanks; **NAW** = non-aerated wastewater tanks. Error bars indicate standard error about the mean value.

3.3.2 Nutrients

Ammonia concentrations

Ammonia concentrations taken on site were recorded at 19.2 mg/l. Over the trial period ammonia concentrations ranged from 0.5 to 17.7 mg/l in aerated tanks and from 0.5 to 21.3 mg/l in non-aerated tanks (Figure 3.6). The highest ammonia levels were recorded in tanks treated with 100% wastewater with the lowest concentrations recorded in the control of both aerated and non-aerated tanks. Control tanks treated with aeration had ammonia readings that exceeded 2.0 mg/l after 72 hours, and after 60 hours in tanks void of aeration. Overall, ammonia concentrations increased with increasing wastewater concentrations. Ammonia levels were significantly higher ($p<0.05$) in treatments consisting of wastewater with ammonia concentrations exceeding those considered suitable for the health and survival of *Oreochromis mossambicus*, especially in treatments with wastewater. Significant differences ($p<0.05$) were observed between treatments over the 96-hour period.

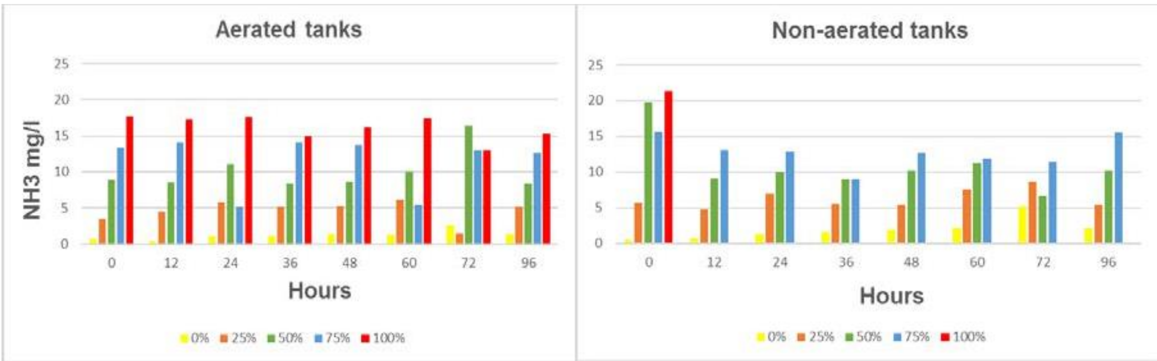

Figure 3.6: Ammonia concentrations (mg/l) of water samples collected from different treatments having concentrations of 0, 25, 50, 75 and 100% effluent water, over a 96-hour period under aerated and non-aerated conditions.

Nitrate concentrations

Mean nitrate concentrations varied from 0.06 to 0.57 mg/l in aerated tanks and 0.06 to 0.64 mg/l in non-aerated tanks (Figure 3.7). Non-aerated tanks with 50% and 75% wastewater had nitrate concentrations mostly below the detectable levels (<0.06 mg/l) over the trial period. Higher nitrate concentrations were recorded in control tanks and from treatments with 25% wastewater in both aerated and non-aerated conditions.

Statistically, there were significant differences (*p>0.05*) in nitrate levels between treatments over the study period.

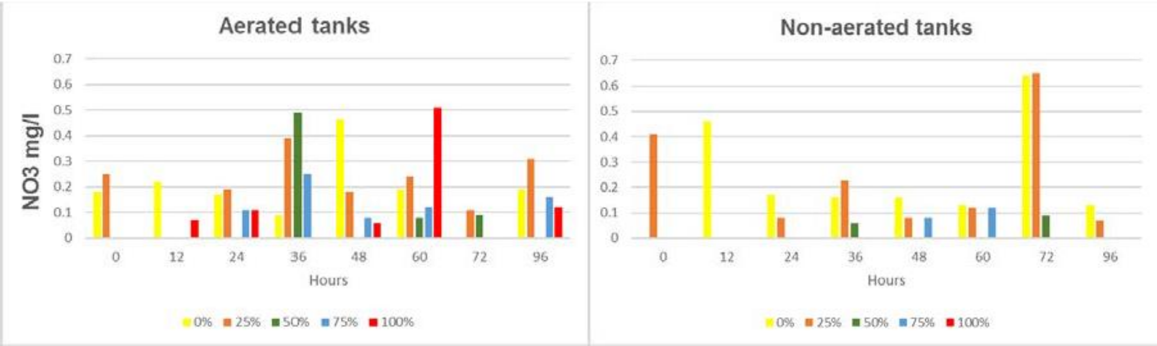

Figure 3.7: Nitrate concentrations (mg/l) of tanks with different concentrations 0, 25, 50, 75 and 100% domestic wastewater under aerated and non-aerated conditions, over a 96-hour period.

Nitrite concentrations

Nitrite concentrations between treatments ranged from 0.01 to 0.19 mg/l in non-aerated tanks and from 0.01 to 0.25 mg/l in aerated treatments (Figure 3.8). Statistically, significant differences (*p<0.05*) of nitrite concentrations were observed between treatments over the 96-hour period.

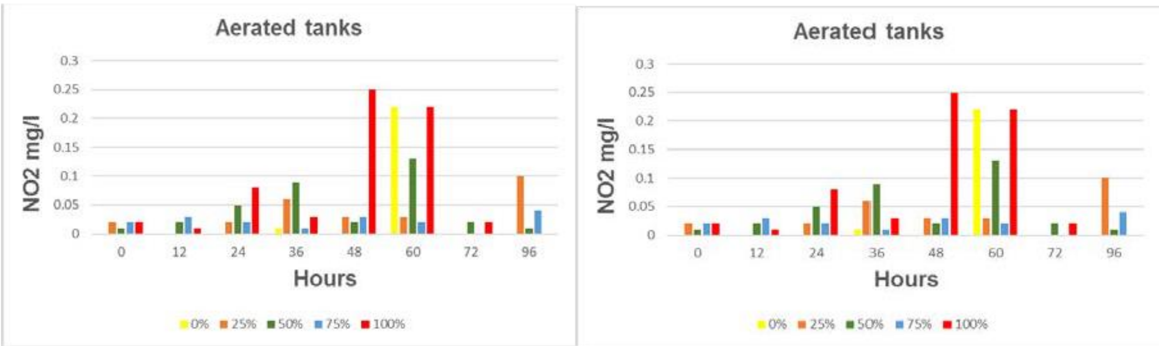

Figure 3.8: Nitrite levels (mg/l) of aerated and non-aerated tanks, containing 0, 25, 50, 75 and 100% concentrations of domestic wastewater over a 96-hour period.

Phosphate concentrations

Phosphate levels were mostly below detectable levels in treatments with 100% wastewater, over the 96-hour period. Phosphate concentrations ranged from 0.10 to 0.35 mg/l in aerated tanks and from 0.07 to 0.54 mg/l in non-aerated tanks (Figure

3.9). Fluctuations were observed in phosphate concentrations over the 96-hour period, with high levels mostly recorded from control groups in non-aerated treatments. Phosphate levels increased drastically after 60 hours for most treatments then remained relatively high for the remainder of the study.

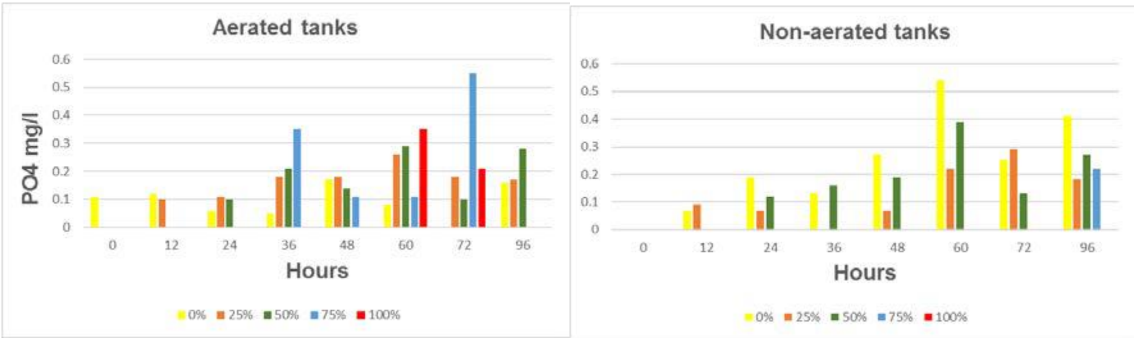

Figure 3. 9: Phosphate concentrations (mg/l) of various treatments tanks containing 0, 25, 50, 75 and 100% domestic wastewater over a 96-hour period, in aerated and non-aerated tanks.

Sulphate concentrations

In this study sulphate concentrations showed fluctuations, ranging from 2.4 to 120.0 mg/l in aerated tanks and 1.7 to 92.5 mg/l in non-aerated tanks (Figure 3.10). Sulphate levels recorded increased with an increase in wastewater concentrations over the 96-hour period. Values for sulphate were below detectable levels in control tanks and highest in treatments with 100% wastewater, from aerated and non-aerated treatments during the experimental period.

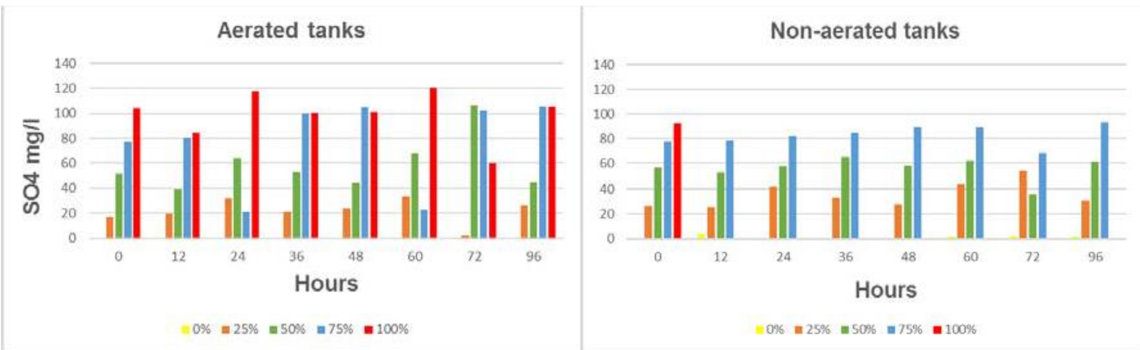

Figure 3. 10: Sulphate levels (mg/l) over a 96-hour period from tanks containing 0, 25, 50, 75 and 100% domestic wastewater that was either with and without aeration.

3.3.3 Survival rates

A 100% mortality occurred in non-aerated tanks with 100% wastewater. In contrast, a 38% survival was observed from fish exposed to 100% domestic wastewater in aerated tanks over the study period. A 100% survival was recorded in treatments diluted with 25% wastewater in aerated and non-aerated (Figure 3.11). Observations from the trial indicate that a lower number of mortalities were recorded when tanks were supplied with aeration, whereas a higher number of mortalities was observed in treatments void of aeration.

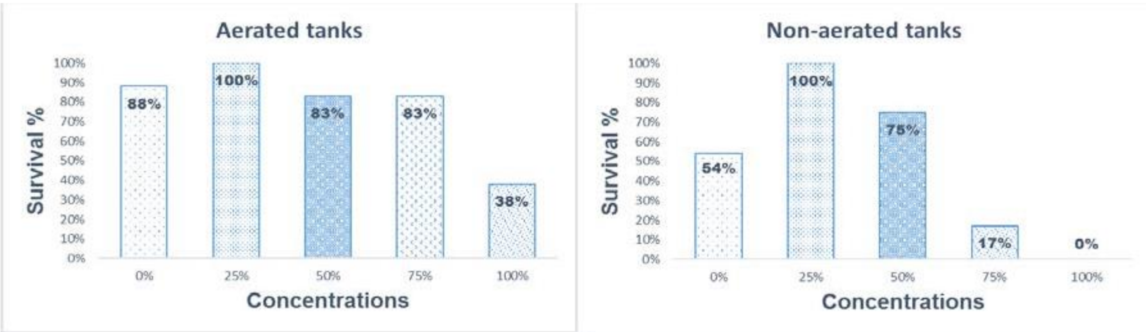

Figure 3.11: The survival rate expressed as a percentage of fish exposed to different concentrations of domestic wastewater over a 96-hour period.

3.3.4 Histological analysis

Gill filaments and gill lamellae of *O. mossambicus* from the control group were normal (Figure 3.12a) and displayed with no histopathological lesions. However, histopathological lesions occurred in fish exposed to wastewater. Severity of histopathological lesions increased with increasing domestic wastewater concentrations and were more pronounced in treatments comprising of 100% domestic wastewater (Figure 3.12b). Histopathological lesions observed include aneurism of the gill lamella, mild epithelial lifting, focal hyperplasia, clubbing of the terminal end of the secondary lamellae, haemorrhage and epitheliocystis (Figure 3.12e, f).

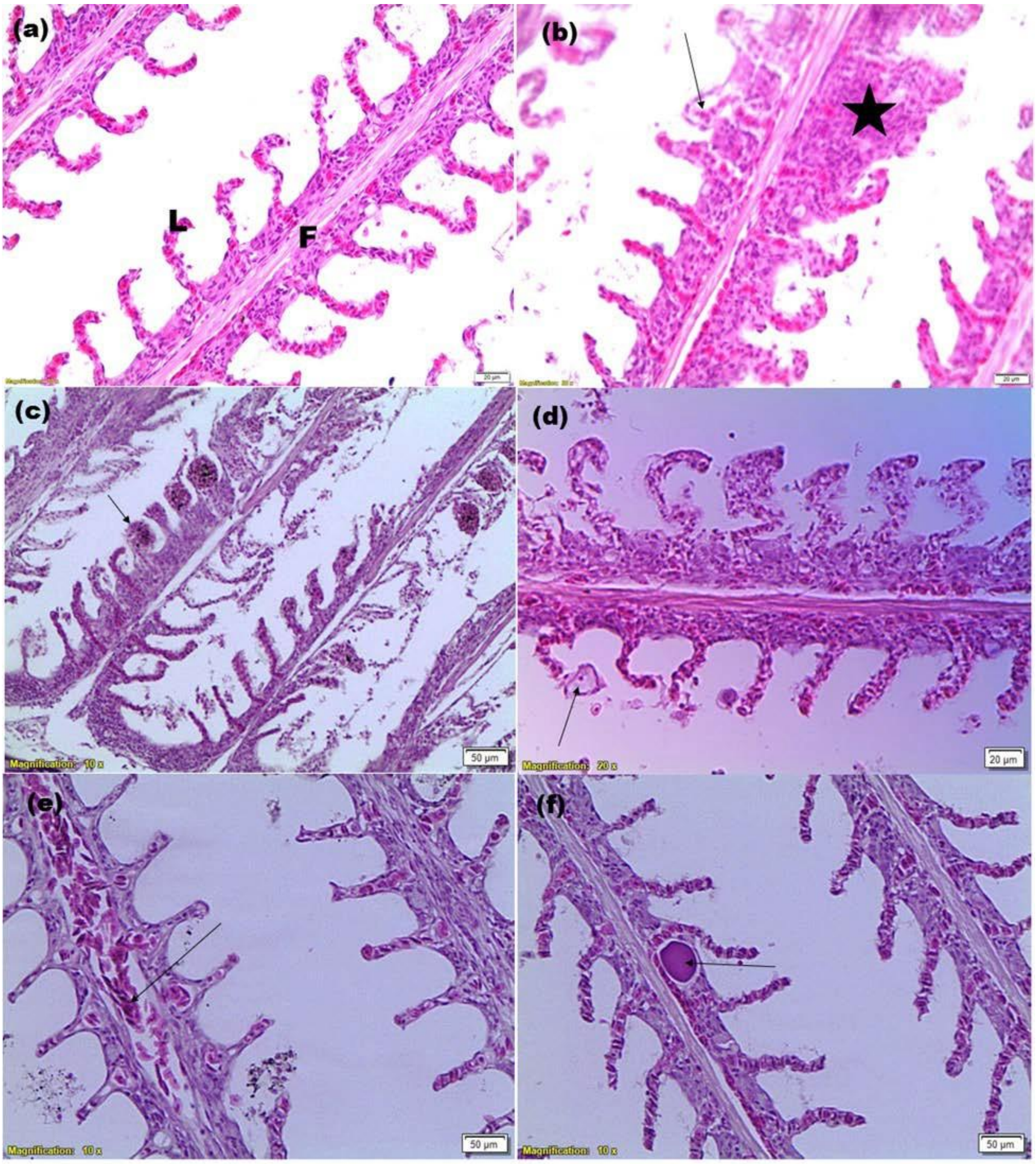

Figure 3.12: Histologic sections of gill of *Oreochromis mossambicus* showing **(a)** Normal appearance of gill filaments (F) and lamellae (L). **(b)** Fusion of the gill lamellae (star) and epithelial lifting (arrow). **(c)** Vascular congestion or lamellar aneurisms (arrow) **(d)** Clubbing of the terminal end of the secondary lamellae **(e)** Haemorrhage of the blood vessels (arrow) **(f)** Epitheliocystis (arrow).

3.5 Discussion

Temperature is a fundamental parameter which influences the chemical and bio-chemical characteristics of water body. Availability of dissolved oxygen is directly affected by water temperature. Moreover, water temperature affects the metabolic rate, feed intake, growth, reproduction, physiological processes, disease immunity, movements and respiration rate of fish. Furthermore, temperature influences the susceptibility of aquatic organisms to potential toxic compounds and ammonia levels (Klontz 1993). Water temperature records for this study remained between 19.0 and 27.9°C (Figure 3.1) over the trial period and were not regulated so as to simulate the conditions on site. Lowest temperatures were recorded 56 hours into the study, fluctuations experienced were attributed to ambient temperature. Boyd (1990) states that water temperature can be affected by ambient temperature. Russel *et al*. (2012) states that adult *O. mossambicus* prefer waters between 22 to 30 °C and have a tolerance of 19 to 32 °C. A study by Allanson *et al*. (1971) indicates that *O. mossambicus* could survive low temperature of 11 °C and not lapse into a chill coma. Temperature results recorded in the study were within the optimum range for the health and survival of *O. mossambicus*.

Dissolved oxygen refers to the level of non-compound oxygen present in water. It is an important parameter when assessing the quality of water because of its influence on the organisms living within a body of water (Wetzel 2001). Dissolved oxygen plays an important role in the aquatic environment and it is essential for the growth and survival of fish. Waters of consistently high DO levels are usually considered healthy and stable aquatic ecosystems, which are capable of supporting many different kinds of aquatic organisms (Kemker 2013). Dissolved oxygen values recorded from aerated tanks were within the optimum values for the survival and growth of fish (tilapia) (>3.0 mg/l), as postulated by Ross (2002). Moreover, results from this study indicated that DO levels in aerated aquaria were higher than those without aeration. This is because, stagnant waters have lower oxygen due to less mixing of the water in the tanks, thus there is no oxygen exchange occurring. The use of aerators resulted in higher dissolved oxygen concentrations due to an increase in the gaseous exchange between air and water, thus adding DO to water. However, DO levels in non-aerated tanks steadily increased after 28 hours of the trial period. This could be due to the high number of mortalities that occurred within the first 16 hours of the experiment, an increase in fish mortalities resulted in an increase in oxygen concentrations.

The presence of algae could have additionally contributed to increases in oxygen concentrations during daytime, although algal concentrations were not measured during the trial (Gaigher & Toerien 1985; Nandini 1999). All plants, including algae produce oxygen during daylight hours as a product of photosynthesis. Photosynthesis is defined as a process by which plants use light and energy to produce food from carbon dioxide and water. Thus, photosynthesis peaks during the day and declines after dark. During night time the fish and algae continue consuming oxygen and producing carbon dioxide, however, no oxygen is produced. Therefore, oxygen levels will slightly increase in the day and decrease at night (Gaigher & Toerien 1985; Haas *et al.* 2013; Misra & Chaturvedi 2016). According to Caulton (1979), *O*. *mossambicus* is able to survive concentrations below 2.0 mg/l for short periods of time. However, several authors quoted by Colt *et al*. (1979) and Russel *et al*. (2012) recorded survival of *O. mossambicus* below oxygen concentrations of 1.0 mg/l. Haas *et al*. (2013) states that critically low oxygen concentrations or hypoxic conditions are most severe at night when all the taxa are consuming oxygen via respiration. In such cases increased biological oxygen demand may outweigh photosynthetic oxygen release during daylight (Haas *et al*. 2013). Similar results were observed in this study DO fluctuations were probably due to algal respiration at night. Furthermore, results from this study indicates that oxygen might have possibly been a limiting factor at times because most mortalities were observed during night time.

Water pH is a measure of the acid balance of a solution. Stone and Thomforde (2003) asserts that, pH values between 7.5 and 9.5 are optimum for the growth and survival of most fish species. *Oreochromis mossambicus* are hardy species with regard to this variable and are able to survive at pH of 3.5 to 11 (Yada & Ito 1997). However, a rapid increase in pH values over the first 16 hours of the study was observed. According to Tucker and D’Abramo (2008), the accumulation of wastes in the form of fish excrements in the water will cause the pH levels to rapidly increase. Therefore, increases in pH could be due to the presence of waste products of the fish introduced in aquaria. Changes in pH, especially sudden changes which have proven to have adverse effects on the fish or cause mortalities. As the pH rises it transforms nitrogen in the water tanks to a more toxic ammonia (Klontz 1993), which can result in fish mortalities when ammonia is found in high concentrations. Although pH levels were alkaline and fell within the suitable range for fish, high pH levels contributed to increased toxicity levels of ammonia causing fish mortalities.

Total dissolved solids are defined as a composite measure of the total amount of soluble materials dissolved in the water. Total dissolved solids are not an aquatic health concern but it can serve an indicator of other water quality. According to WHO (1993), the mineral content needs to stay constant to fish to be able to survive, so it is bad for the total dissolved solid content in water to fluctuate. Total dissolved solids in this study were relatively constant and increased with increasing wastewater concentrations. Total dissolved solids concentrations recorded during the study period were found to be below the permissible limit of <1000 mg/l for drinking water as stated by WHO (1993), except in treatments with 100% domestic wastewater in both aerated and non-aerated tanks were concentrations were above 1000 mg/l (Figure 3.4). High TDS in tanks with 100% wastewater is possibly due to the high concentrations of solids which is caused by the addition of high quantity of sewage (Kiran 2014). Dallas and Day (2004) state that, when TDS concentrations are too low or too high may limit the growth of fish and may lead to the death of many aquatic organisms. High TDS (>1000 mg/l) concentrations may cause toxicity through increases in salinity, changes in the ionic composition of the water and toxicity of individual ions.

Salinity has been highlighted by Jooste *et al.* (2005) to be of vital importance to fish health as it impacts directly on the metabolic and physiological processes. However, the tolerance of fish to variations in salinity depends on their physiological adaption. Fish species capable of tolerating a wide range of salinity are defined as euryhaline while those tolerating limited ranges are referred to as stenohaline (DWAF 1996a; Skelton 2001). All species of tilapia are euryhaline and *O. mossambicus* can tolerate salinity levels of up to 20 ppt (Popma & Lovshin 1994; Dallas & Day 2004; Iqbal *et al.* 2012). Mozambique tilapia has been recorded living in salinity levels even higher than that of sea water (Popma & Lovshin 1994). Salinity values recorded over the trial period were thus well below the optimal range for the health and survival of fish.

Ammonia is a dissolved gas present naturally in surface and wastewaters. It is a major nitrogenous waste product of fish and also results from the decomposition of organic matter. It is soluble in water, especially at low pH values and is removed from water by plants or bacteria, either as a nutrient or energy source. Ammonia in water is present in two forms – un-ionized ammonia (NH_3) and the ionized form (NH_4^+) and the relative proportion of each type depends on pH and temperature. As pH increases, there is an increasing proportion of un-ionized ammonia, which is very toxic to fish. According to Randall and Tsui (2002), ideal water conditions of ammonia levels should be zero as ammonia is toxic to fish. However, the tolerance of ammonia toxicity varies with every fish species (Stone & Thomforde 2003).

Bartone (1985) states good survival and growth is possible in fish ponds fed with tertiary effluent, when the total ammonia concentration is less than 2.0 mg/l and unionized concentration is less than 0.5 mg/l, with the latter only exceeding 2.0 mg/l for short periods of time. Findings by Bartone *et al.* (1985) were in line with those by Prinsloo and Scoonbee (1991), with results by Prinsloo and Scoonbee (1991), showing high survival rates of fish in tertiary treatment ponds when ammonia levels ranged between of 0.42 to 0.92 mg/l. As described in Chapter one, the purpose of tertiary treatment ponds is to provide the final treatment stage to further improve the effluent before it is discharged in the receiving environment or is re-used. The treatment process is responsible for removing remaining inorganic substances such as nitrogen and phosphorus, additional suspended solids, heavy metals and dissolved solids which cannot be removed by secondary treatment.

Gaigher and Toerien (1985) states that toxic concentrations of un-ionized ammonia to *O. mossambicus* can occur in ponds with raw sewage if pH increases over 8 at a total ammonia concentration of 10.0 mg/l. According to Sampath *et al*. (1991), *O. mossambicus* can withstand ammonia concentrations of 3.0 mg/l without any significant adverse impact on food uptake and growth. However, a 0% survival rate was recorded by Sampath *et al*. (1991) when fish were exposed to ammonia concentrations >14 mg/l for a period of 96 hours. Ammonia levels recorded in this study were high, exceeding those suitable for the growth and survival of fish, more especially in treatments with 100% wastewater. Therefore, the high ammonia concentrations recorded were considered to be the most probable cause for the high mortality rates recorded over the trial period. Furthermore, high pH levels may have contributed to increased toxicity levels of ammonia, as water pH influences ammonia toxicity. In an acidic water bodies, ammonia becomes less toxic to fish. However, under alkaline conditions, ammonia is much more toxic (Randall & Tsui 2002). Ammonia levels in excess may have adverse effects on the aquatic life and is thought to be one of the main causes of unexplained fish mortalities. When exposed to ammonia concentrations above the suitable range fish may suffer loss of equilibrium, increased respiratory activity and increased heart rate. Furthermore, higher ammonia levels can lead to skin and gill damage, which will reduce the fish’s ability to breath. At extremely high ammonia levels, fish may ultimately experience convulsions, coma and death (Randall & Tsui 2002). Thus, high pH levels together with ammonia levels could have contributed to high mortalities rates recorded over the trial period.

Nitrate is the end product of two bacterially mediated processes in the nitrification of ammonia, occurring as a result of the oxidation of plants, animal debris and excrements (DWAF 1996b). Nitrates stimulate the growth of algae, of which some are toxic and can cause bad odours in the waters. Nitrate concentrations were consistently <1.0 mg/l throughout the trial period. Nitrate is relatively nontoxic to fish and is not a health hazard except at high levels. Stone and Thomforde (2003) stated that nitrate concentrations above 90 mg/l may have adverse effects on fish. However, according to DWAF (1996b) there are no known adverse effects on fish when nitrate concentrations are <300 mg/l. Nitrate concentrations recorded in this study fell well within the suitable range for the health and survival of fish.

Nitrite is another form of nitrogenous waste product that can be found in water bodies. Stone and Thomforde (2003) suggested that desirable limits of nitrite range of 0 – 1 mg/l, and Santhosh and Singh (2007) recommended that nitrite levels should not exceed 0.5 mg/l for freshwater fish. Moreover, nitrite values of 0.0625 – 0.25 mg/l are considered safe for freshwater fish (DWAF 1996a). Nitrite values for the current study were all below 0.25 mg/l and were within the suitable range for the health and survival of fish. Low concentrations of nitrite may be due to the fact that nitrite is readily oxidised to nitrate or is reduced to ammonia which were above the suitable range for fish over the 96-hour period (Kroupova *et al.* 2005).

Phosphate is an essential plant nutrient and that is often in limited supply in the freshwater bodies. Almost all of the phosphorus available in water is in the form of phosphate (Stone & Thomforde 2003). Much of the phosphorus found in surface water is bound to either living or dead particulate matter. Stone and Thomforde (2003) asserts that a typical range for phosphate in surface waters is 0.005 to 0.5 mg/l. Phosphate values recorded in this study were slightly above the recommended range, with the highest phosphates level of 0.54 mg/l. Phosphate concentrations drastically increased towards the end of the trial period. Phosphates naturally occur in water bodies as wastes are broken down, such as fish excrements. Therefore, the accumulation of fish wastes over the 96-hour period could have possibly attributed to the build-up of phosphate in tanks.

Sulphate is a common constituent of water and arises from the dissolution of mineral sulphates in soil and rocks and other partially soluble sulphate minerals. Sulphate concentrations for this study fell well within the suitable range for the health and survival of fish. Except in aerated tanks with 100% domestic wastewater where values were above the recommended levels of 100 mg/l as suggested by the Canadian guidelines for aquatic ecosystems (CCME 2012). However, Stone & Thomforde (2003) state that levels above 500 mg/l are of concern if the water is used for other purposes, as fish are able to tolerate a wide range of sulphate concentrations. Sulphates are not considered toxic to fish at normal concentrations and the mortalities occurred cannot be attributed to sulphate concentrations. Sulphate concentrations were suitable for the health and survival of fish.

A 100% survival was observed in tanks diluted with 25% wastewater in both aerated and non-aerated aquariums, and a 100% mortality percentage recorded from non-aerated tanks with 100% wastewater (Figure 3.11). The 100% mortality from non-aerated tanks with 100% wastewater was probably due to stress and less resistance of fish to tank conditions of high concentrations of ammonia and low dissolved oxygen levels recorded over the duration of the experiment. Furthermore, observations of this study indicated that *O. mossambicus* tolerated concentrations of 25% domestic wastewater, as no mortalities were recorded from this concentration in both aerated and non- aerated conditions. An indication that fish were able to regulate their body functions within this concentration. Gill samples collected from these treatments showed histopathological alterations, however, these alterations were less severe or pronounced in treatments with 25% wastewater. Moreover, results indicate that effluent levels >25% are detrimental to fish thus fish should be introduced in treatment ponds under less concentrated conditions and mechanical aerators should be deployed to increase oxygen levels. Additionally, another pond can be constructed to further treat the wastewater before the final pond where fish would be introduced.

Literature states that the effects of ammonia poisoning include kidney, tissue and gill damage, reduction in growth, reduced disease resistance, susceptibility to infections, neurologic and behavioural abnormalities such as motionless fish gasping and hovering at the bottom of the tanks, inflamed gills, red streaks or inflammation in the fins, inflamed eyes or anus, lethargy and mortalities (Randall & Tsui 2002; Estim & Mustafa 2014). Fish in this study displayed a normal response, with mortalities in control tanks and 0% mortalities when exposed to 25% wastewater. In this study, exposure of fish to concentrations >25%, *O. mossambicus* exhibited signs of ammonia poisoning, which include lethargy, motionless fish hovering at the bottom of the tanks and fish gasping at the bottom of the tanks. Furthermore, fish expressed increased opercula movements, which is said to be a sign that fish are under respiratory distress. The increased opercula movement has been reported to be an adaptive mechanism of fish to environments that are over the tolerable limit for fish (Iwana *et al.* 1997; Hassan *et al.* 2013). The severity of ammonia poisoning increased with increasing wastewater concentrations and were more severe in tanks with 100% wastewater.

Histopathological observations in the current study revealed alterations in the gill structures of *O. mossambicus* when exposed to domestic wastewater. It is possible that the pathological alterations on the gills of fish could be a direct result of stress due to the exposure of fish to domestic wastewater. This was because histopathological changes were usually observed in the gills of fish exposed to sublethal ammonia concentrations (Ip *et al*. 2001). After exposure to wastewater, fish gills showed moderate signs of hyperplasia, epithelial lifting, clubbing of the gill lamellae and epitheliocystis. Furthermore, aneurysm of the gill lamellae and haemorrhage of the blood vessels were more prevalent and pronounced in fish exposed to wastewater. Signs of epitheliocystis were observed only in gills of fish exposed to 100% wastewater. Epitheliocystis is defined as a gill disease, mainly caused by the presence of pathogenic intracellular bacteria. Infections often lead to respiratory distress and may ultimately result in death of juvenile or cultured fish.

Fusion of the secondary lamellae and hyperplasia results from proliferation of the gill filaments and the secondary lamellae (Camargo & Martinez 2007). Partial or total fusion of the gill lamellae and epithelial lifting are defence mechanism that results from gills trying to increase the distance between the external environment and the blood, thus serving as a barrier to the entrance of the contaminants (Albassam *et al*., 1987; Camargo & Martinez 2007; Alim & Matter 2015). This phenomenon is often observed as a result of intoxication with chloride, phenol and ammonia, initially affecting a limited area before spreading over the entire gill lamella (Satchell 1984; Mallat 1985; Strzyzewska *et al.* 2016).

Reactions of gill hyperplasia reduce oxygen diffusion across gill epithelium which in extreme cases leads to hypoxia (Heath 1987), which is deficiency in the amount of oxygen reaching the tissues. Lamellae aneurysm occur when the pillar cells rupture due to a bigger flow of blood inside the gill lamellae (Rosety-Rodriguez *et al.* 2002; Martinez *et al.* 2004). The appearance of aneurysm of the gill lamellae could cause a reduction in the gaseous exchange capabilities of fish and consequently weakening the affected the fish (Srivastava *et al.* 2014). Although gill lesions are not lethal, aneurysms might retard the growth of fish and affect fish production (Das & Murkherjee 2000).

Gill samples from this study showed signs of hyperplasia, epithelial lifting, clubbing of the gill lamellae, aneurysm, haemorrhage of the blood vessels and epitheliocystis. Similar results were observed in other studies were tilapias where exposed to high levels of ammonia. In a study by Chezhian *et al.* (2012), fish displayed similar histopathological lesions when exposed to high ammonia levels. The histopathological lesions included hyperplasia, deformation of the gill lamellae and lamellar fusion. Osman *et al.* (2009) also noted congestion of the gill arch and lamella blood vessels, together with oedema, haemorrhage and lamellar epithelium hyperplasia. A study by El-sherif *et al.* (2008), on the effects of ammonia on Nile tilapia (*O. niloticus*) performance and some haematological and histological measures, noted mild hyperplasia, congestion of the central vein and aneurisms of the secondary lamella. Several authors have reported similar histopathological alterations on the gills of different fish species exposed to ammonia (Mallat 1985; Aysel & Gulten 2005; Spencer *et al.* 2008). The above mentioned authors further stated that the resulting gill lesions may cause reduced oxygen diffusion across membranes and predispose fishes to bacterial infections.

3.6 Conclusion and recommendations

The physicochemical parameters of the sampled tanks were within the acceptable limit for the growth and survival of *O. mossambicus*, except dissolved oxygen, ammonia concentrations and total dissolved solids in treatments with 100% domestic wastewater. Water quality parameters over the suitable range for fish are known to stress, especially those exposed to high ammonia levels. Although, *O. mossambicus* is a hardy fish species and can tolerate high levels of ammonia, ammonia concentrations exceeding 3.0 mg/l are reported to have adverse effects on fish. In the present study high levels of ammonia resulted in definite histopathological changes in the gills such as aneurism of the gill lamella, mild epithelial lifting, focal hyperplasia and clubbing of the terminal end of the secondary lamellae. The presence of fish mortalities in treatments >25 domestic wastewater shows that conditions at the Motetema WWTP will be unfavourable for fish. Furthermore, results from the study indicate that wastewater would need to be diluted to less concentrated levels to ensure the survival of fish in wastewater treatment ponds and that mechanical aerators needs to be deployed to increase dissolved oxygen levels in the treatment ponds, as better fish survival was recorded from tanks which were aerated. However, when fish were exposed to 25% treated domestic wastewater, better survival (100%) of fish was recorded from both aerated and non-aerated tanks, indicating that levels < 25% are less detrimental to the health of fish. Another possible solution would be to further treat wastewater by constructing an additional pond which will improve pond conditions. However further intensive studies conducted over a longer period of time are needed to determine as to whether the introduction of fish to less concentrated conditions (<25 %) can be implemented.

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CHAPTER 4

ASSESSING THE CONSUMPTION OF ALGAE BY *OREOCHROMIS MOSSAMBICUS* BASED ON ALGAL CELL DENSITY COUNTS IN FISH AQUARIA AND STOMACH FULLNESS

4.1 Introduction

The concept behind the approach of using algae in the treatment of wastewater is that the nutrients available in the treatment ponds are expected to be absorbed and assimilated by the algae, which in turn will be converted into fish biomass when ingested. Consequently, the uptake of nutrients will enhance the quality of water that is discharged into the receiving watershed (Park et al. 2011; Abdel-Raouf et al. 2012; Edmundson and Huesemann 2015). However, the success of such an approach is dependent on the ability of fish to utilise the algae. In Asian countries, phytoplankivorous fish such as silver carp have been used in ponds to control algal densities (Leventer & Teltsch 1990; Starling 1993). Other fish species such as *O. mossambicus* that can consume algae to some degree have also been used to control algal densities in countries like Israel (Leventer & Teltsch 1990). Findings by Gaigher *et al.* (1982) showed that *O. mossambicus* is capable of removing microalgae and is able to filter suspended micro-algae from the water column.

Although algae have shown to be useful in the treatment of wastewater, high concentrations of organic matter in the form of algae pose a major pollution risk to the receiving environment (Tucker & Abramo 2008), as they can alter the physical and chemical properties of water by decreasing oxygen levels and by increasing the water pH and turbidity (Pan *et al.* 2006). Furthermore, high densities of certain algae e.g blue-green algae, may produce toxins that can affect the survival of fish and other taxa (Acarli & Lok 2010). Therefore, conditions in the treatment ponds would need to be favourable for the survival of fish, as water quality parameters above tolerable limits will negatively impact the health of fish and reduce the ability of fish to filter feed (Dempster *et al.* 1995). Therefore, this chapters aims to establish how effective *O. mossambicus* will be in the removing *Chlorella vulgaris* and *Chlorella protothecoides* from treatment ponds at Motetema wastewater treatment works (WWTW).

4.2 Methods and materials

Experiments consisted of 21 glass aquaria (60 L), whereby three aquariums served as a control void of algae. The remaining glass aquaria were divided into two sets comprising of tanks with algae, and tanks with algae and fish. Tanks were dosed with different algal concentrations: 33, 66 and 100%, as described in chapter 2. Algae concentrations comprised of two strains *C*. *vulgaris* and *C*. *protothecoides* were used ad will be collectively referred to as algae in this chapter. Each treatment was replicated thrice. The feeding trial was conducted over a period of 10 days and each aquarium was stocked with twelve fish. The aquariums were well aerated to prevent oxygen deficiencies as a result of algal respiration and temperatures were maintained at 27 ^{o}C. Fish were not fed 24 hours prior to initiating the trial to purge the fish from all the gut content. Before the commencement of the experiment, five fish were dissected and their stomach contents analysed. On the fifth day, two fish from each tank were randomly selected, sacrificed and their stomach contents analysed. Upon completion of the trial, two fish per concentration were euthanized and their stomach contents analysed for algal consumption. The stomach of fish was rated based on the percentage of fullness whereby the stomach fullness index was determined using a method in Otieno *et al*. (2014), as described in chapter two. Water quality measures were measured using a handheld multiparameter instrument (YSI 5100) every eight hours. Subsequently water samples were collected every second day and sent for nutrient analysis to the aforementioned laboratory. Algal concentrations were determined in each aquarium using a handheld fluorometer (AquaFluoro ®). Fish that were left over at the end of the trial were counted and weighed, then the weight gain, survival rates and specific growth rate were determined.

4.3 Results

4.3.1 Physico-chemical parameters

The control group of tanks had temperature values that were relatively constant over the period of the study, ranging from 26.2 to 27.5 ^{o}C (Figure 4.1). The mean dissolved oxygen values recorded in the control group fluctuated between 1.8 to 3.6 mg/l (Figure 4.1), having the mean value of 2.7 mg/l. The pH values were considerably neutral and ranged between 7.1 to 7.7, with a mean value of 7.3. Water pH values gradually increased increased from 7.2 in the beginning of the study to 7.7 on day five thereafter pH values decreased to 7.1 on day eight. Total dissolved solids readings ranged from 35.9 to 83.9 mg/l (Figure 4.1). Mean electrical conductivity (EC) steadily increased over the 10-day period and from 59.1 to 133.7 mS/m.

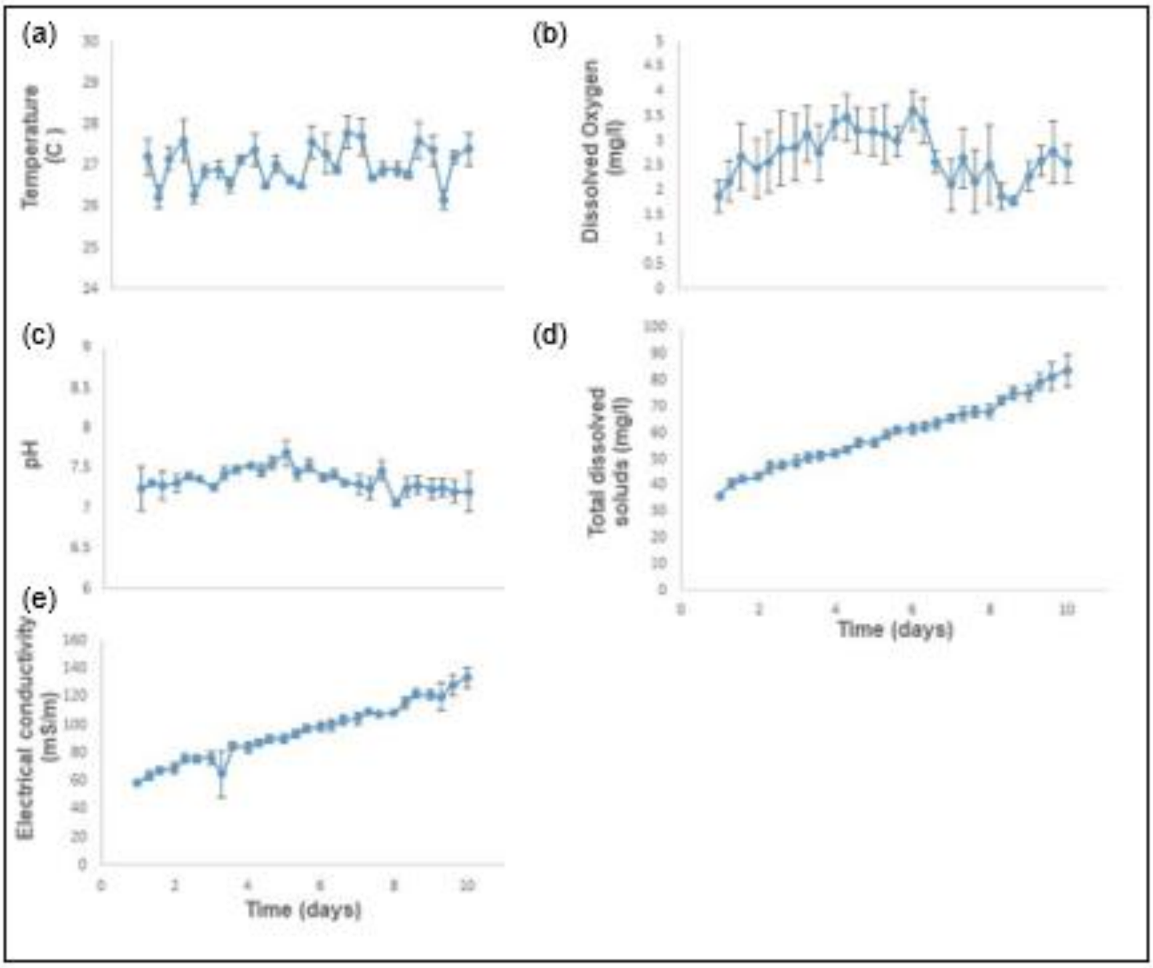

Figure 4.1: Water quality parameters recorded in the control group over a 10-day period (a) indicating the mean daily temperature, (b) the mean daily pH, (c) dissolved oxygen values, (d) mean total dissolved solids, (e) mean electrical conductivity. Bars indicate standard error about the mean.

Water temperature

In tanks with algae and fish, water temperatures fluctuated between 24.9 to 28.4 ºC over the duration of the study, with higher temperatures recorded from treatments with 66% algae and lowest in treatments with 33% algae (Figure 4.2). In tanks without fish, water temperatures ranged from 23.1 to 29.1 ºC (Figure 4.2). Highest values were recorded from treatments with 66% algae and lowest values were recorded from treatments with 33% algae. Between tanks with algae and no fish, and tanks with algae and fish there 33% algae. Between tanks with algae and no fish, and tanks with algae and fish temperature fluctuations of less than 2 ºC would occur at any given time. Statistically, no significant differences *(p>0.05)* were recorded between temperature readings between the various treatments over the duration of the study.

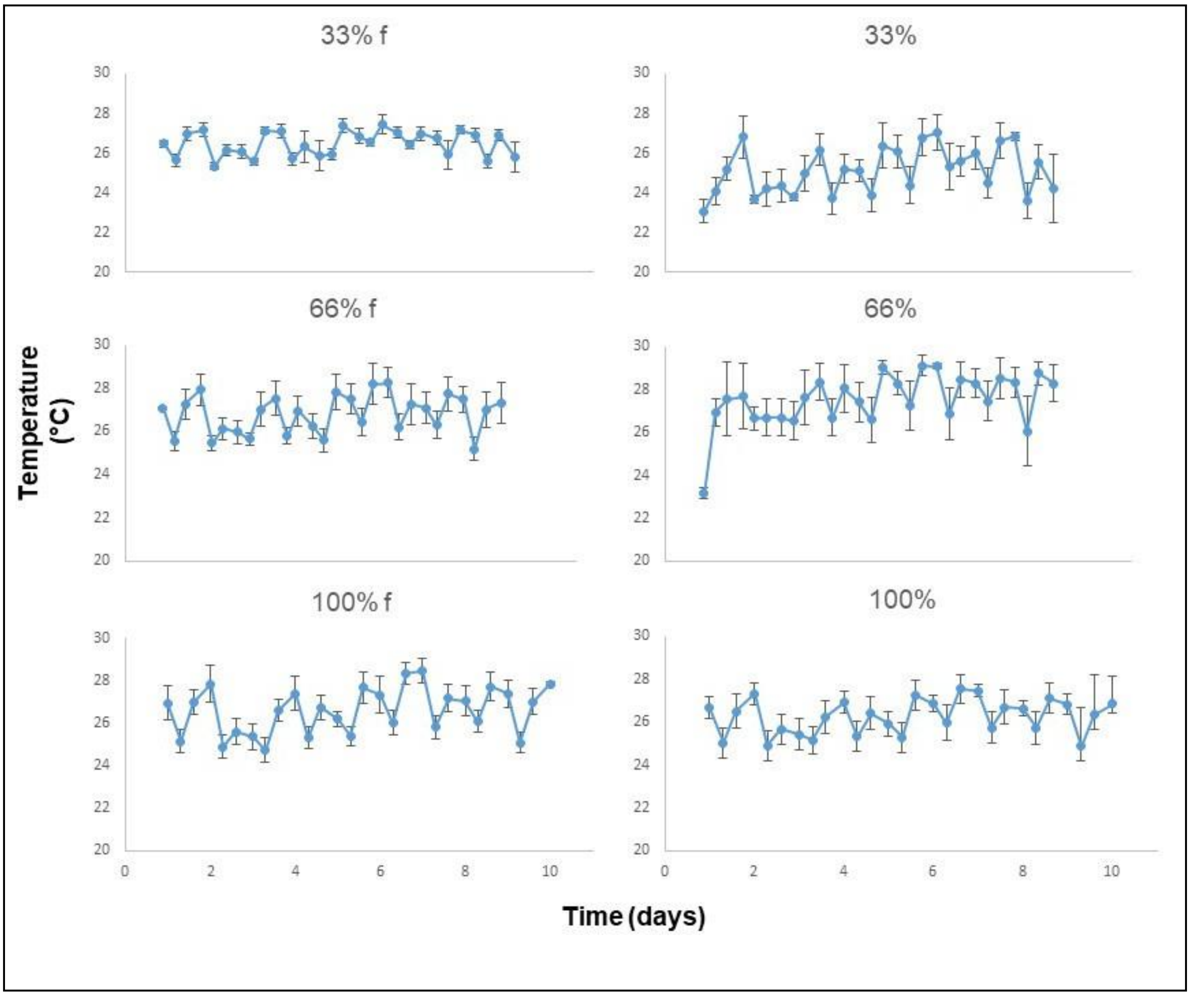

Figure 4.2: The mean temperature ^{o}C values (± standard error) of treatments containing 33, 66 and 100% algae, with (f) or without fish recorded over a 10-day period. Bars indicate standard error about the mean.

Dissolved oxygen

The DO concentrations ranged from 3.4 to 6.0 mg/l in tanks with algae and fish, and from 4.4 to 9.4 mg/l in tanks without fish (Figure 4.3). tanks with algae had an average of 4.68 mg/l over the study period and an average of 6.2 mg/l in tanks without fish. In both treatments with and without fish, higher DO concentrations were recorded in tanks with 100% algae while lower values were recorded in tanks comprising of 33% algae. However, when comparing controls with tanks comprising of 33, 66 and 100% algae, lowest DO concentrations were recorded in the control tanks (Figure 4.1). Dissolved oxygen concentrations fluctuated over the period of the study and varied according to the time of day, slightly increasing during the day and decreasing during night time. Dissolved oxygen levels were significantly different *($p>0.05$)* between treatments.

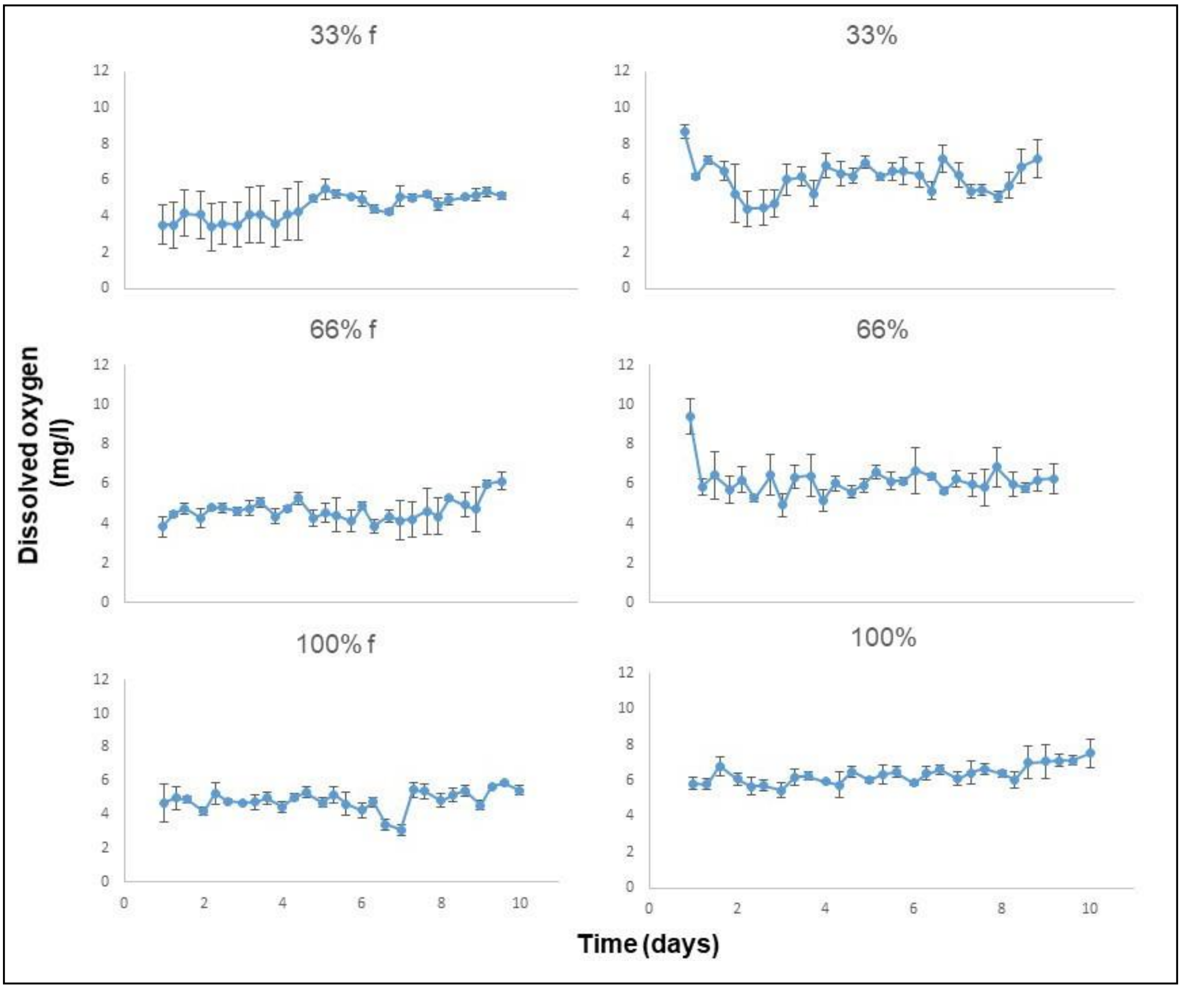

Figure 4.3: The mean dissolved oxygen mg/l (± standard error) concentrations of treatments containing 33, 66 and 100% algal concentrations, either with (f) or without fish over a 10-day period. Bars indicate standard error about the mean value.

Water pH

Water pH showed a narrow range of variation between treatments with and without fish. Water pH values were relatively constant over the period of the study, ranging from 6.8 to 7.6 in tanks with fish and algae, with an average of 7.3. In tanks without fish, water pH ranged between 7.2 to 8.5, with a mean value of 7.7 (Figure 4.4). Similar to the observed trend in control tanks, tanks comprising of algae and fish were considerably neutral. Water pH levels in treatments with fish gradually increased over the trial period. Moreover, pH readings were relatively constant for the first five days of the trial period then decreased slightly for the remainder of the study (Figure 4.4). Water pH values in tanks without fish escalated, increased and decreased over the period of the study. Overall, pH values recorded were slightly acidic to alkaline, moreover, tanks void of fish were generally more alkaline than tanks that contained fish. Significant differences *($p<0.05$)* were observed from tanks with 66% and 100% algae and fish, and from tanks with 66% algae and no fish.

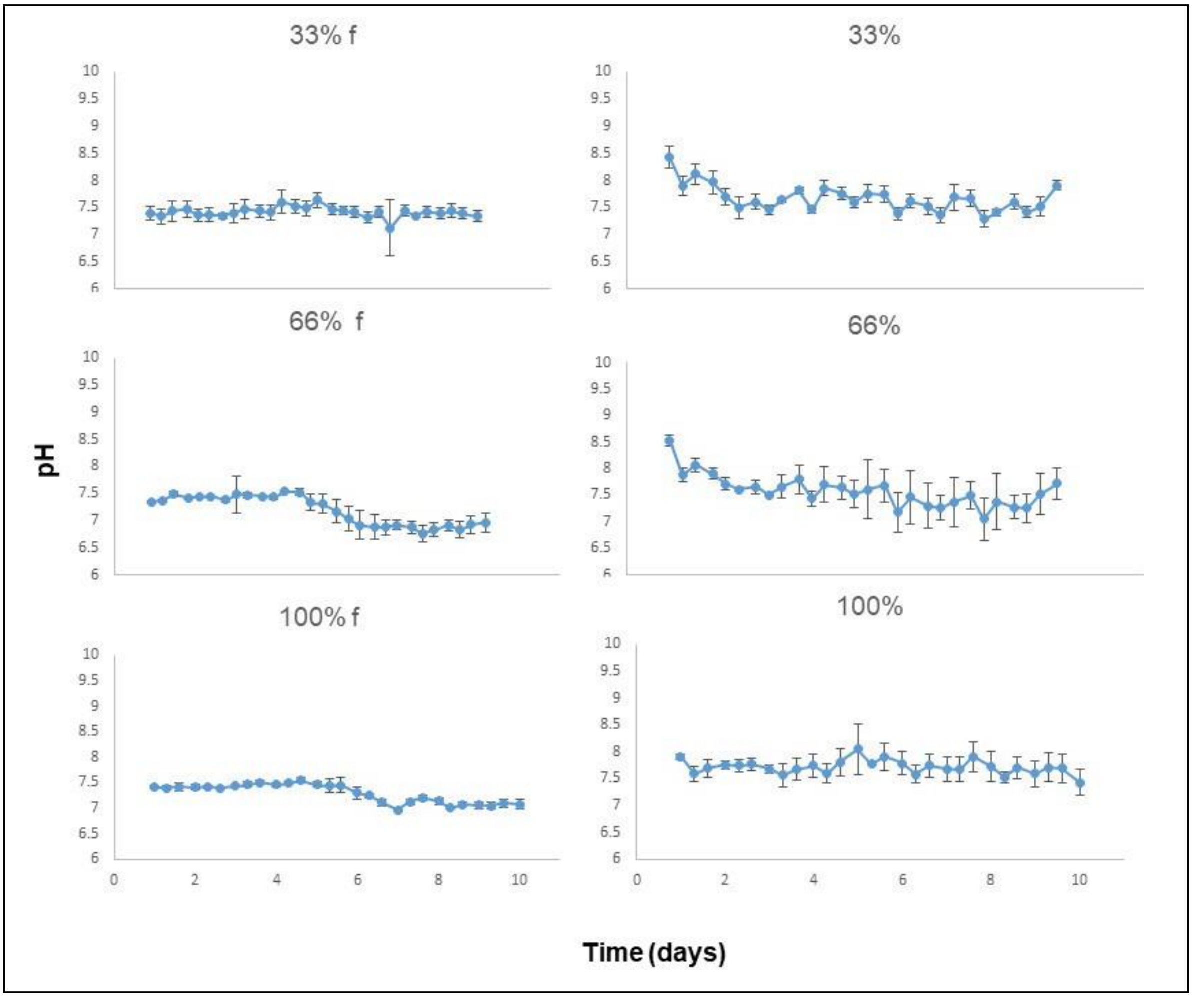

Figure 4.4: The mean pH values (± standard error) of treatments containing algal concentrations of 33, 66 and 100%, either with (f) or without fish over a 10-day period. Bars indicate standard error about the mean.

Total dissolved solids

In this study, mean total dissolved solids readings varied between 156.7 to 463.6 mg/l in tanks with fish, and from 113.8 to 409.5 mg/l in treatments without fish (figure 4.5). Both treatments with and without fish, had lower TDS values recorded from 33% algae and higher values were in tanks with 100% algae. Slight drop in TDS values were observed on certain days over the study period. However, in tanks with 100% algae and no fish, TDS values remained relatively constant with less variation. Total dissolved solids were slightly higher in treatments that contained fish. Moreover, TDS values increased over time and increased with increasing algal concentrations. Overall, lower values were recorded from control tanks (Figure 4.1), whereas higher values were in tanks with 100% algae and fish. Similar to control tanks, TDS readings in tanks with algae and fish increased over the trial period. Total dissolved solids varied significantly *(p>0.05)* between treatments.

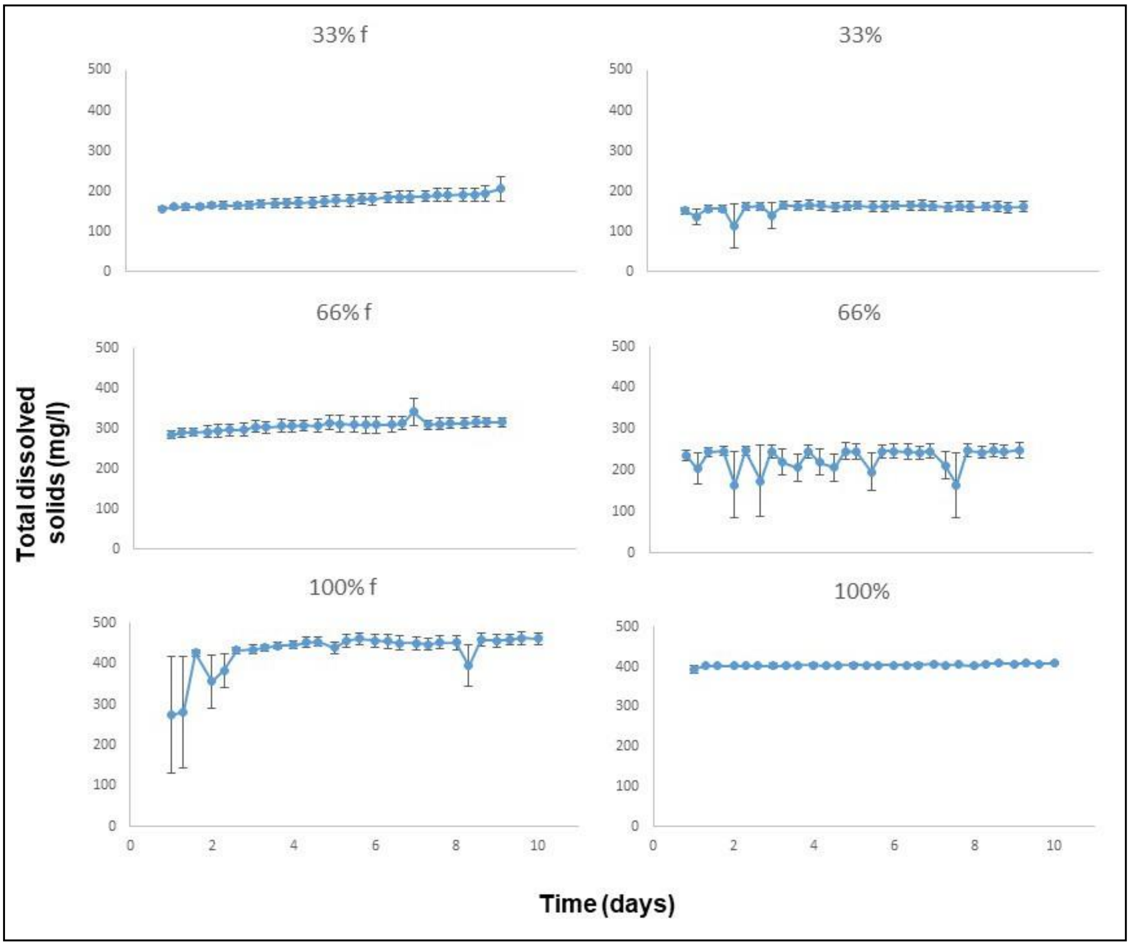

Figure 4.5: Mean total dissolved solids values mg/l (± standard error) of treatments containing algal concentrations of 33, 66 and 100%, either with (f) or without fish over a 10-day period. Bars indicate standard error about the mean value.

Electrical conductivity

Electrical conductivity values increased with increased algal concentrations and were relatively higher in tanks with algae and fish than in tanks without fish. Mean EC values fluctuated from 219.8 to 742.3 mS/m in tanks with algae and fish, and from 173.2 to 652.7 mS/m in tanks without fish. Treatments with or without fish, had higher records of EC values from tanks with 100% algae and lower values from tanks with algae. Overall, lowest EC values were recorded from control tanks (Figure 4.1), and highest values were recorded from tanks with 100% algae and fish. Other treatments however, had slight sways, drastically decreasing and increasing over time. Similarly, EC readings observed in tanks with 100% algae, and from tanks with 66% algae and fish, followed the same trend as with TDS readings gradually increasing over the trial period. Significant differences were observed in EC values between treatments over the period of the study.

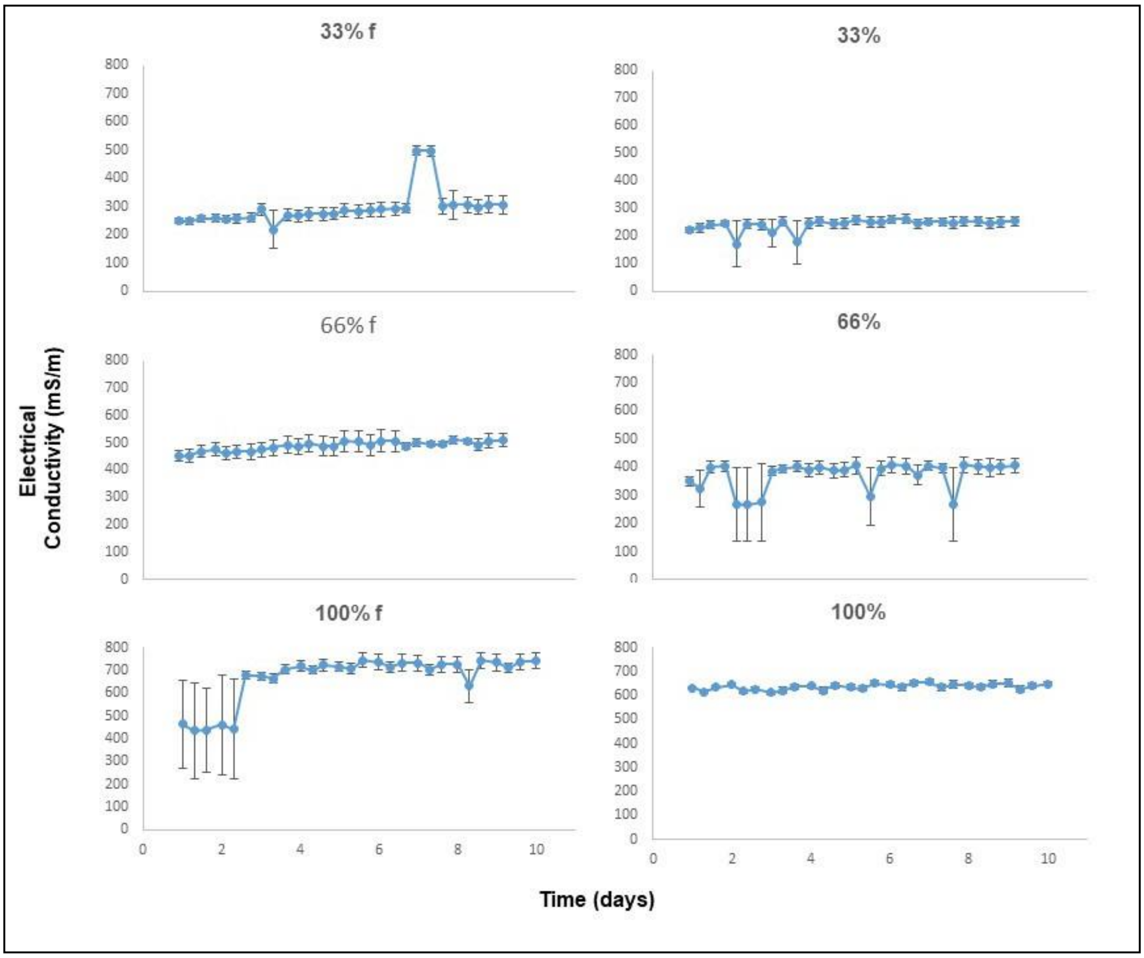

Figure 4.6: Mean electrical conductivity mS/m (± standard error) of treatments containing algal concentrations of 33, 66 and 100%, either with (f) or without fish over a 10-day period. Bars indicate standard error about the mean.

4.3.2 Nutrients

Ammonia concentrations

Ammonia concentrations in the control tanks ranged from 1.17 mg/l on the first day to 7.52 mg/l on the last day. Concentrations in the control tanks increased over the period of the study. In tanks comprising of 33% algae and fish, ammonia levels ranged from 5.51 mg/l on day one to 3.73 mg/l on day nine. Ammonia levels were higher at the beginning of the trial then decreased on day three to 2.74 mg/l. Concentrations increased to 5.66 mg/l on day seven, thereafter decreased once more to 3.73 mg/l on the last day of the experiment. Ammonia levels in treatments with 66% algae and fish varied from 1.61 to 9.43 mg/l. Ammonia levels increased from 2.07 mg/l on day one to 9.43 mg/l on day three. Ammonia concentrations then decreased from 7.16 mg/l on day five to 1.61 mg/l on day seven. On the last day of the study period, ammonia levels were below detectable levels. In tanks with 100% algae and fish, ammonia concentrations ranged from 4.25 to 3.4 mg/l on day six with concentrations being below detectable levels for the remainder of the trial period.

Ammonia concentrations in tanks with 33% algae and no fish, ranged from 1.17 mg/l on day two to 2.39 mg/l on day four. Thereafter, ammonia concentrations were below detectable levels for the remainder of the trial period. In tanks with 66% algae and no fish, ammonia concentrations were high on day two with maximum values of 8.29 mg/l. Ammonia levels then remained below to 2.00 mg/l for period of the study. Lastly, in tanks with 100% algae and no fish, ammonia concentrations were recorded at 0.55 mg/l on day four. However, concentrations were below detectable levels on other days. Ammonia concentrations for controls varied significantly with tanks comprising of 66% and 100% algae and no fish. Furthermore, ammonia concentrations recorded were above the suitable range for the health and survival.

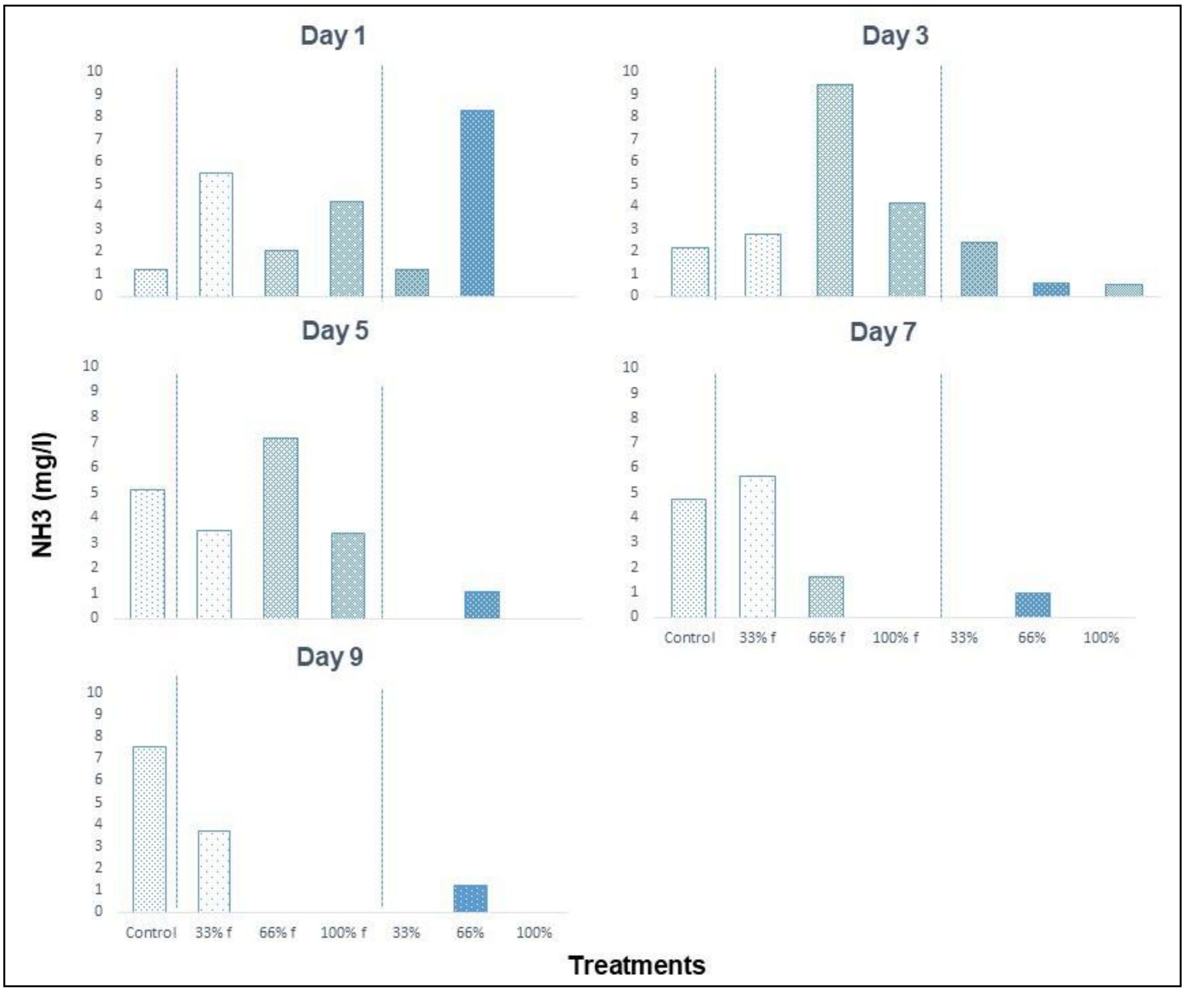

Figure 4.7: Ammonia concentrations (NH_3) of treatments with 33, 66 and 100% algae, either with (f) or without *Oreochromis mossambicus* over a 10-day period.

Nitrite concentrations

Nitrite concentrations in control tanks differed significantly from tanks with 100% algae. In controls nitrite levels were consistently below detectable levels (<0.01), except for day five when values were recorded at 0.03 mg/l (Figure 4.9). Nitrite concentrations in tanks with 33% algae and fish varied from 0.06 mg/l to 1.54 mg/l. In tanks with 66% algae and fish, nitrite concentrations recorded ranged from 0.08 to 4.2 mg/l. Nitrite concentrations were recorded to be 2.97 mg/l on day two, decreasing to 0.32 mg/l on day four. Concentrations decreased to 0.08 mg/l on day five, thereafter increased to 3.96 mg/l on day seven. On day nine, concentrations were recorded at 4.2 mg/l. Nitrite values in tanks with 100% algae and fish ranged from 0.73 to 6.3 mg/l. Concentrations were higher in the beginning of the trial period, then decreased towards the end of the trial period.

In tanks with 33% algae and no fish, nitrite levels ranged from 0.04 to 1.71 mg/l (Figure 4.9). Nitrite values decreased from 1.36 mg/l on day one to 0.04 mg/l on day three. Concentrations for nitrite then increased to 1.71 mg/l on day five thereafter decreased till completion of the trial. In tanks 66% and no fish, nitrite values ranged from 0.05 to 1.02 mg/l (Figure 4.9). Concentrations decreased in tanks with 66% algae and no fish over the period of the study. Tanks with 100% algal concentrations were high on day two, with a maximum reading of 4.80 mg/l. Thereafter, nitrite levels decreased to 0.01 mg/l on day four. Readings then remained low for the remainder of the trial period. Significant differences *(p>0.05)* were observed between the control group and treatments with 100% algae.

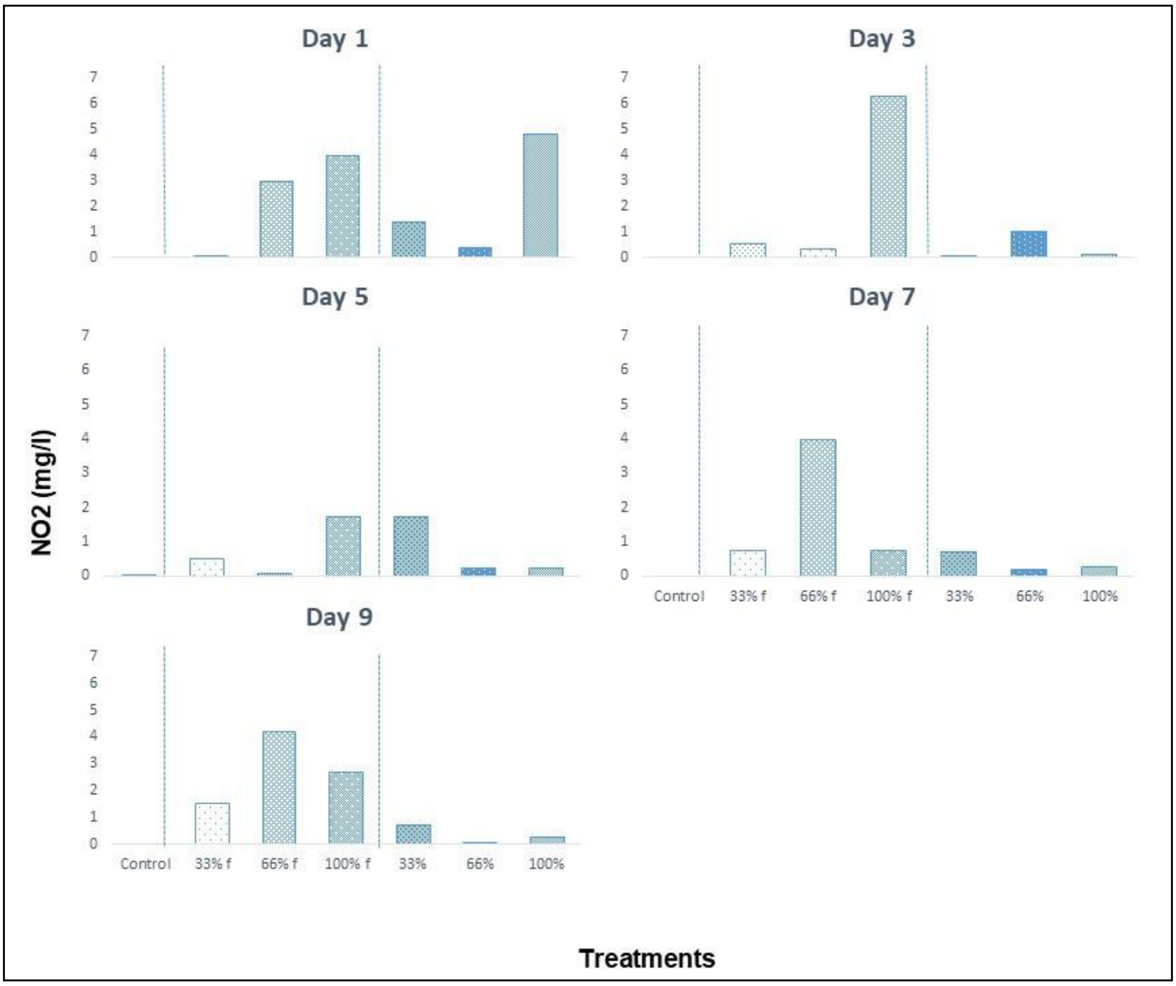

Figure 4.8: Nitrite concentrations (NO_2^-) of treatments containing 33, 66 and 100% algae, either with (f) or without *Oreochromis mossambicus* over a nine-day period.

Nitrate concentrations

In control tanks nitrate values ranged from 0.18 to 0.29 mg/l (Figure 4.9). Overall, nitrate values were relatively low (<0.3 mg/l) in control tanks over the period of the study. In tanks comprising of 33% algae and fish, nitrate levels ranged from 3.57 to 8.01 mg/l. Nitrate concentrations decreased from 5.49 mg/l on day one to 3.57 mg/l on day seven. Thereafter, concentrations increased to 8.01 mg/l on day nine. Nitrate concentrations in tanks comprising of 66% algae and fish, increased over the trial period, ranging between 6.17 to 16.46 mg/l. Tanks with 100% algae and fish, had nitrate concentrations that ranged from 6.66 to 27.72 mg/l (Figure 4.9). Concentrations decreased on day three to 6.65 mg/l then increased to 17.03 mg/l on day five. Nitrate concentrations then remained high for the remainder of the trial. Statistically, there were significant differences *($p>0.05$)* observed in nitrate concentrations between the various treatments over the trial period.

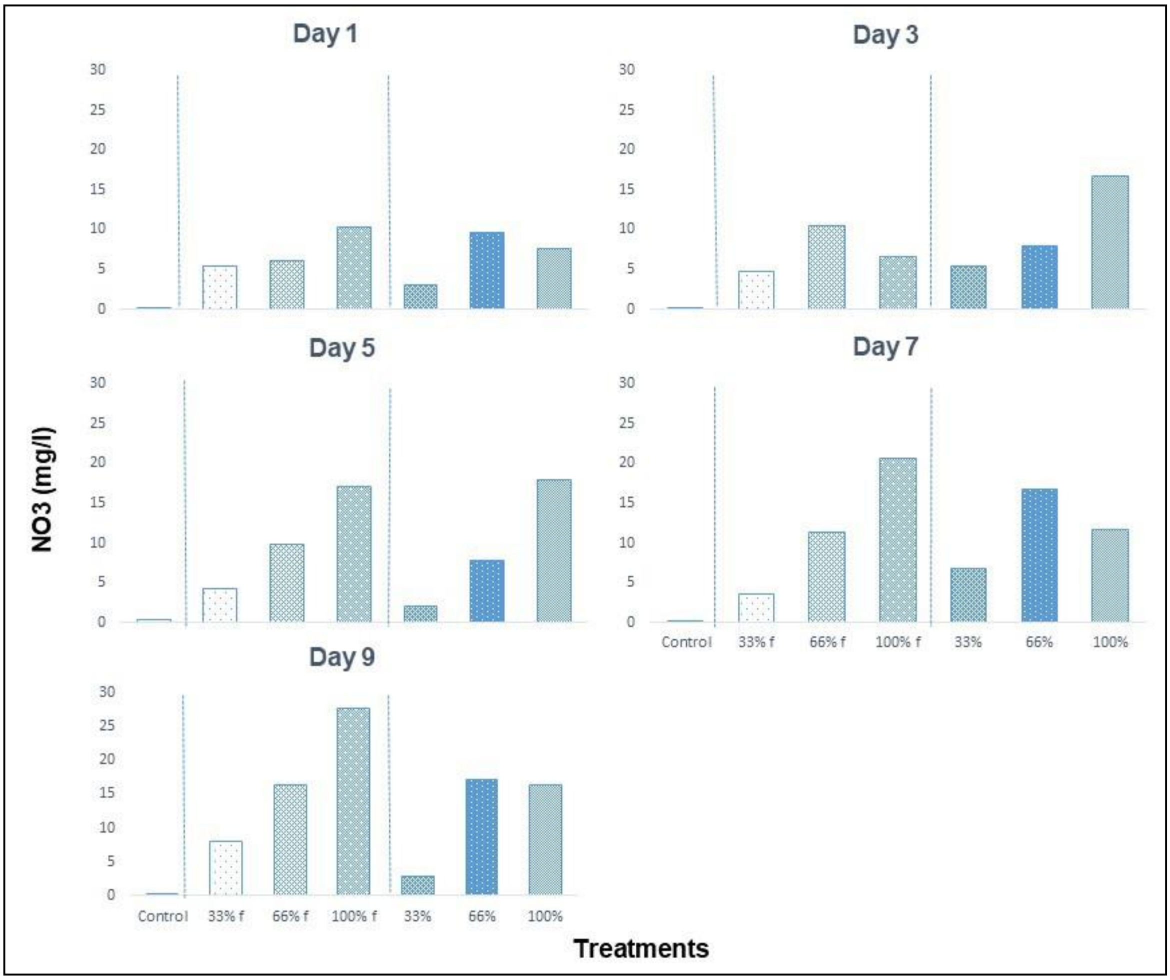

Figure 4.9: Nitrate concentrations (NO_3^-) of treatments containing 33, 66 and 100% algae either with (f) or without *Oreochromis mossambicus* over nine days.

Sulphate concentrations

Sulphate concentration in control tanks ranged from 1.83 mg/l and 80.62 mg/l in tanks with 100% algae from all treatments (Figure 4.11). Sulphate levels for the control group were below detectable levels (<1.47 mg/l) for the first four days of the study and remained below 3.5 mg/l for the reminder of the study. Sulphate levels increased with increasing algal concentrations over the study period. Statistically, significant differences were observed in treatments comprising of 66% algae and fish; and from treatment with 100% algae with other treatments over the period of the study.

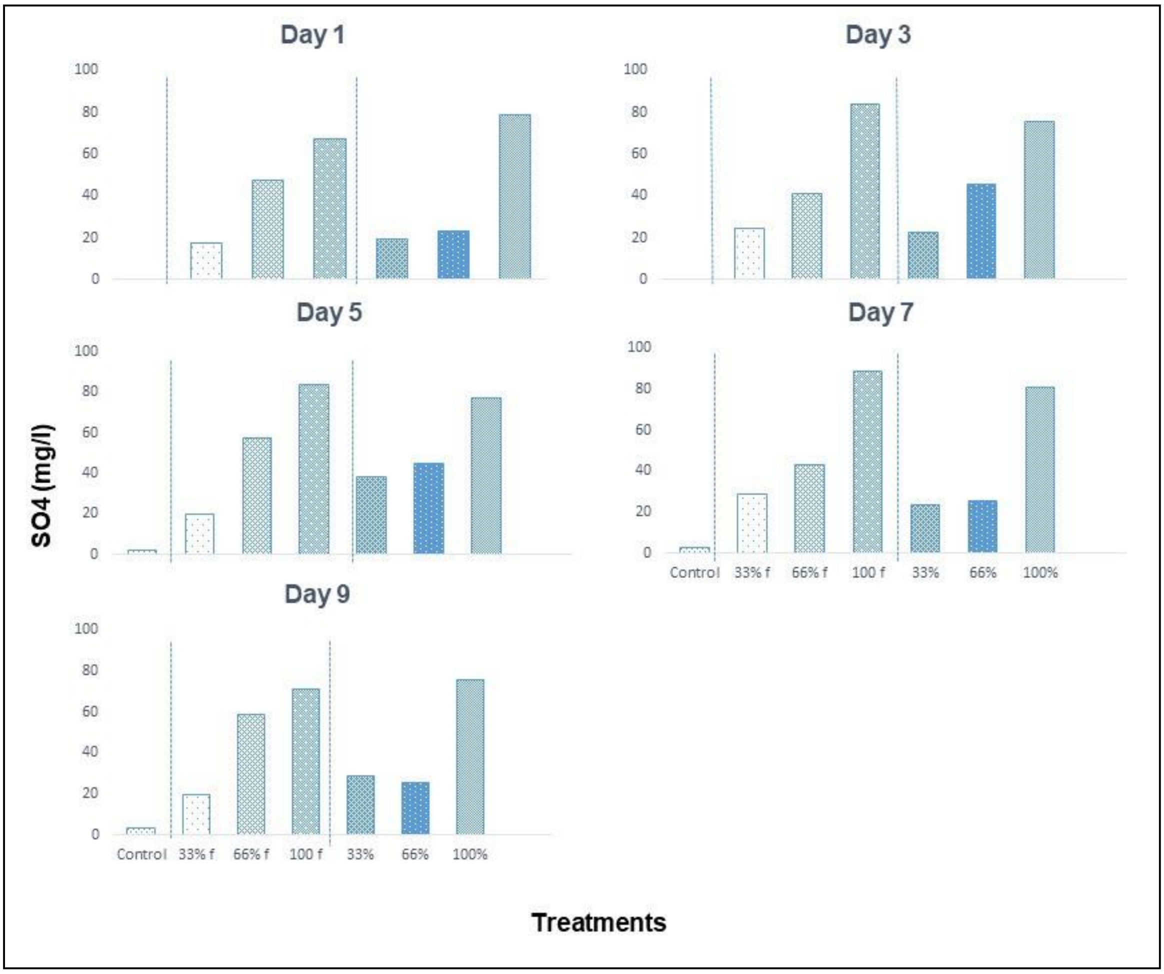

Figure 4.10: Sulphate concentrations ($SO_4{}^{2-}$) of treatments containing 33, 66 and 100% algae, either with (f) or without *Oreochromis mossambicus* over nine days.

Phosphate concentrations

Values for phosphate in the control group gradually increased and ranged between 0.16 to 1.13 mg/l (Figure 4.11). In tanks with 33% algae and fish, phosphate concentrations ranged from 2.63 to 3.06 mg/l. Phosphate levels strictly increased from

2.63 mg/l on day one to 3.04 mg/l on day three. Concentrations remained relatively constant before slightly decreasing on the last day. Phosphate levels in tanks comprising of 66% algae and fish increased over the period of the study, ranging from 6.33 to 9.48 mg/l. In tanks with 100% algae and fish, phosphate concentrations increased from 7.65 mg/l on day one to 12.65 mg/l on day three (Figure 4.11). Thereafter, phosphate concentrations decreased until the end of the trial.

Phosphate concentrations of tanks comprising of algae and no fish, generally increased over the trial period. In tanks with 33% algae, concentrations ranged from 2.27 to 4.86 mg/l (Figure 4.11). Concentrations for phosphate in tanks with 66% algae ranged from 4.36 to 7.88 mg/l. Phosphate levels decreased from 7.88 mg/l on day five to 5.04 mg/l on day seven, thereafter concentrations remained low. Concentrations in tanks without fish and 100% algae ranged from 8.13 to 9.30 mg/l. Phosphate levels differed significantly (*p>0.005*) and increased over the period of the study.

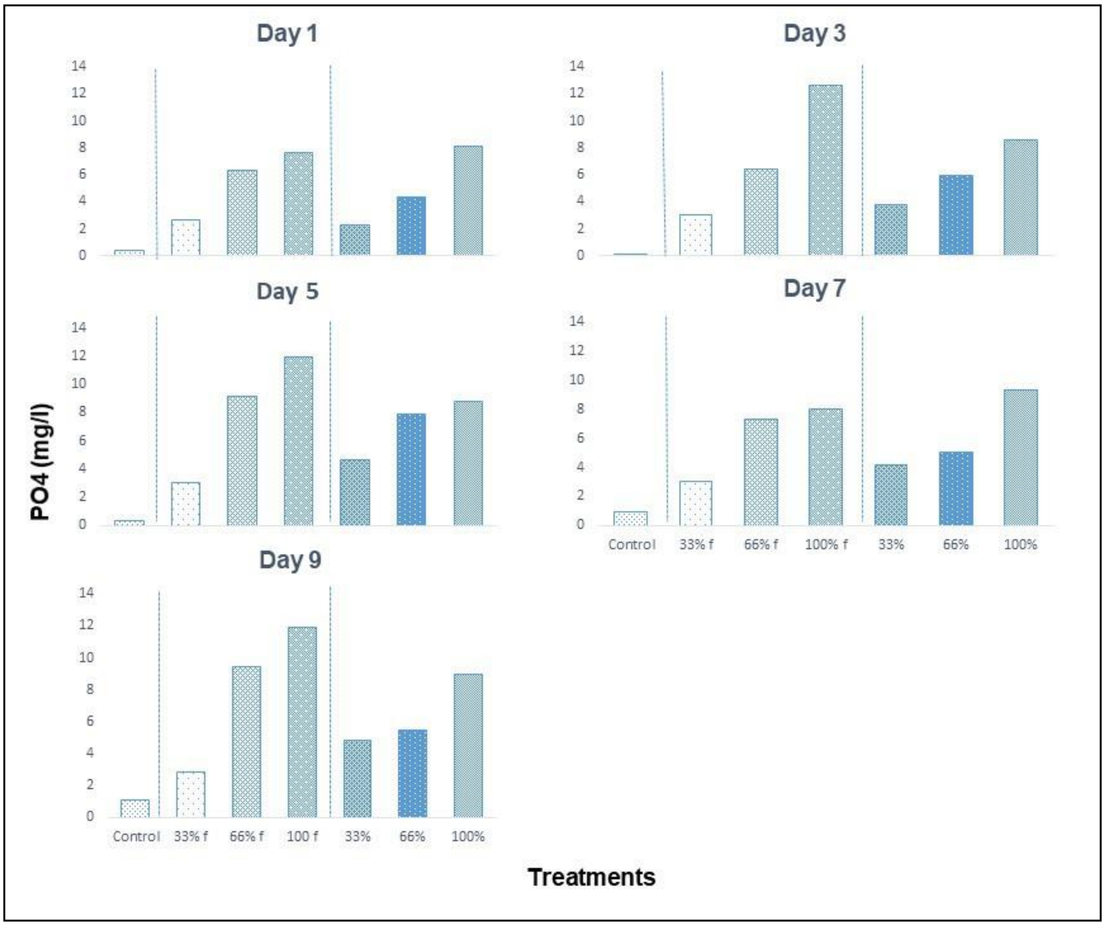

Figure 4.11: Phosphate concentrations (PO_4^{3-}) of treatments containing 33, 66 and 100% algae, either with (f) or without *O. mossambicus* over a nine-day period.

4.3.3 Suspended Chlorophyll-*a*

Suspended chlorophyll-*a* concentrations showed variations throughout the duration of the experiment. In tanks with fish chlorophyll-*a* ranged from 93.9 to 240.6 ug/l in and from 33.7 to 225.8 ug/L tanks without fish (Figure 4.13). Overall, suspended chlorophyll-*a* concentrations were higher in treatments with fish and decreased over the trial period. All treatments were seen to have algae that had settled at the bottom of the tanks.

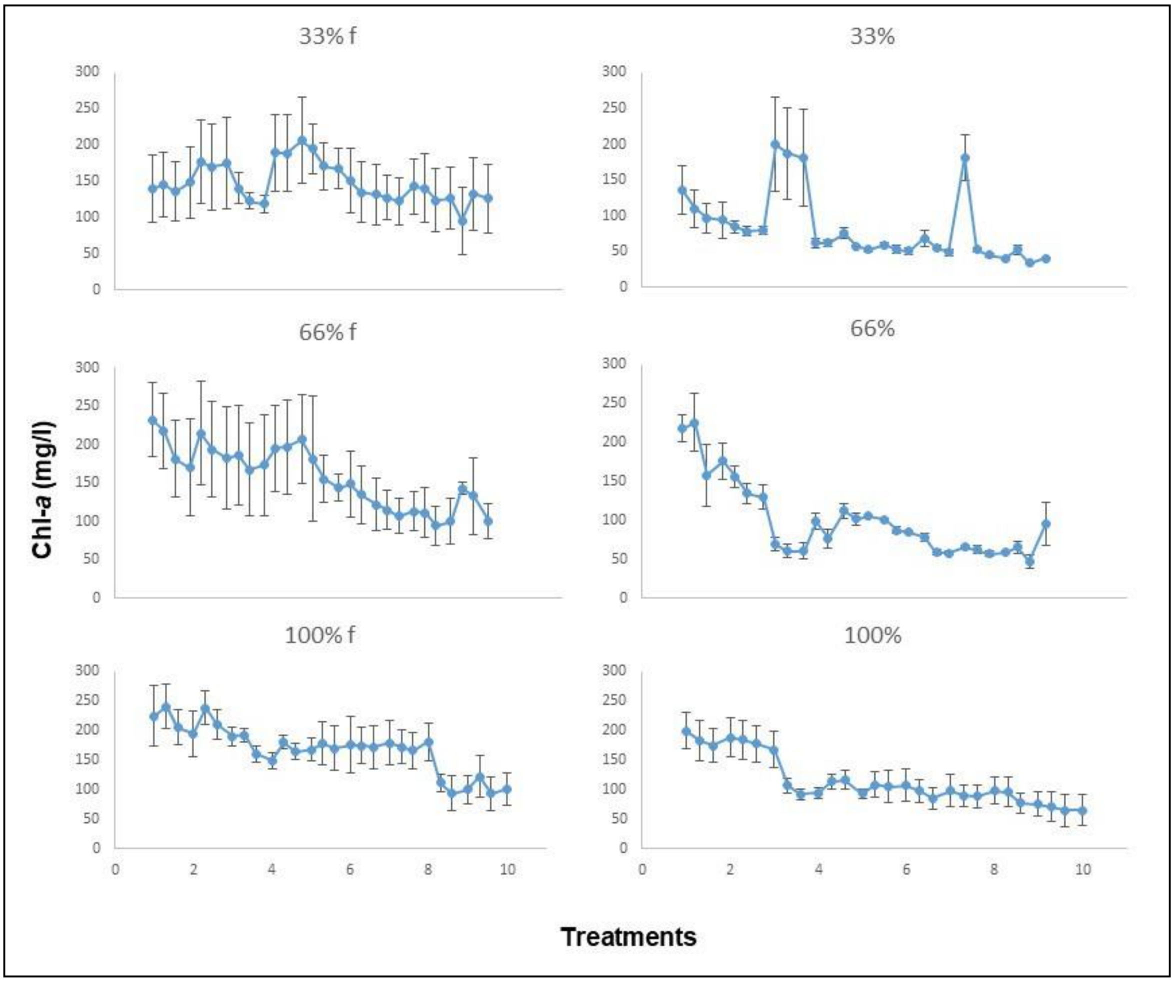

Figure 4.12: Mean suspended chlorophyll-*a* concentrations of treatments containing 33, 66 and 100% algae, either with (f) or without fish over a 10-day period. Bars indicate standard error about the mean.

4.3.4 Stomach content analysis

A total of 87 stomachs of *O. mossambicus* were analysed. Of the stomachs examined 83.9% were a quarter full, 12.6% were half full, 1.1% were three quarters full and none were found to be completely full (Table 4.1). Statistically, significant differences in stomach fullness (*p<0.005*) were observed between treatments.

Table 4.1: Categorisation of stomach fullness based on percentage fullness of *Oreochromis mossambicus* in treatments containing 33, 66 and 100% algal concentrations sampled over a 10-day period.

Stomach fullness	Number of samples per treatment			
	Control	**33% algae and fish**	**66% algae and fish**	**100% algae and fish**
0.00	-	-	1	1
0.25	11	16	24	22
0.50	-	2	3	6
0.75	-	-	1	-
1.00	-	-	-	-
Total	**11**	**18**	**29**	**29**

4.3.5 Growth and survival rates

Results revealed a decrease in body mass (negative growth rate) of fish in the control group and in tanks with 33% algae. However, there was a slight increase in the final fish mass in treatments with 66% and 100% algae. Better growth was recorded in tanks comprising of 66% and 100% algal concentrations (Table 4.2). The specific growth rate (SGR) attained at the end of the study ranged from -0.06%/ day in the control group and 0.11%/ day in tanks comprising of 66% algae. The highest SGR was recorded from fish in treatments with 66% algae and lowest from those in the control group. Mortalities were observed from all treatments over the duration of the trial. Control treatments had higher survival rates of 61%, whilst lower survival rates of 19.4% were observed in treatments comprising of tanks with fish in 66% algae (Table 4.3).

Table 4.2 Growth and survival rates of *Oreochromis mossambicus* fed with different algal concentrations (33%, 66% and 100%) for a 10-day period. Values are presented as mean values.

Growth Rate parameters	Control	Tanks with 33% algae and fish	Tanks with 66% algae and fish	Tanks with 100% algae and fish
Initial Weight (g)	22.2	22.1	24.0	28.0
Final Weight (g)	21.9	21.7	24.6	28.6
Mass gain (g)	-0.3	-0.4	0.6	0.6
Growth rate (%. day^{-1})	-0.06	-0.08	0.11	0.09
Survival rates (%)	**61%**	**50%**	**19%**	**25%**

4.3.6 Feeding rate

The estimation of the feeding rate was based on the decline in suspended chlorophyll-*a* concentration. Treatments with 33% algae and fish had starting concentrations of 143.4 cells fish^{-1}h^{-1} and final concentrations to 118.2 cells/fish/h. In tanks with 66% algae and fish, suspended chlorophyll-*a* concentrations were 189.9 cells/fish/h in the beginning of the trial to 125.3 cells/ fish/h at the end of the trial. In tanks with 100% algae and fish, starting concentrations were 213.8 cells/fish/h and final concentrations were recorded at 106.1 cells/fish/h. Highest feeding rates were recorded from treatments with 100% algal concentrations and lowest rate from treatments with 33% algal concentrations as shown in Figure 4.15.

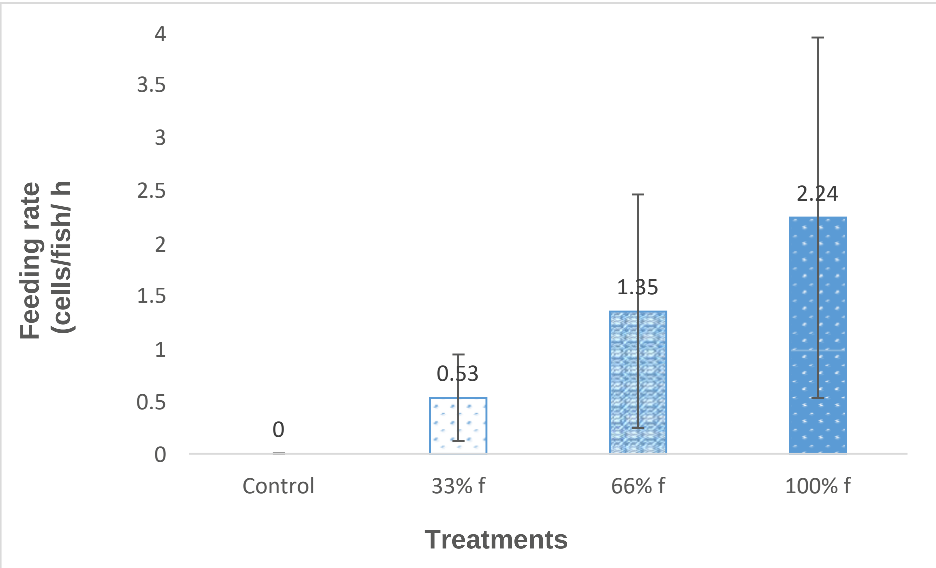

Figure 4.13: Feeding rate of treatments with algae and fish (f) for the duration of the study.

4.4 Discussion

In aquatic ecosystems temperature plays a major role in the water quality, affecting the rates of chemical reactions, metabolic rates, feed intake, growth, reproduction and distributions of aquatic organisms. For example, aquatic organisms have a specific temperature at which optimal growth and reproduction is met (Palmer *et al.* 2004). *Oreochromis mossambicus* require temperatures of 22–30°C for optimal growth and reproduction but can tolerate temperatures of 19–32°C to survive. Water temperature values recorded during the study period were between 23.0 and 29.1°C (Figures 4.1a; 4.2) and fell within the suitable range for the health and survival of *O. mossambicus* (DWAF 1996).

According to Suzuku and Takahashi (1995), favourable high temperatures stimulate the growth of algae but once optimal temperatures are reached, any further increase will prevent algae growth due to stress. Suzuki and Takahashi (1995) further states that *Chlorella vulgaris* and *Chlorella protothecoides* are able to grow effectively at 26°C. However, another study by Converti *et al.* (2009) has shown that *Chorella* spp. can also grow at extreme temperatures of 30–35°C and at a pH of 3 (Mayo 1997). Temperature values in this study fell within the recommended range for the growth of *C. vulgaris* and *C. protothecoides*

Dissolved oxygen concentrations between 1.4 and 5.0 mg/l are known to have adverse effects on the performance and survival of fish (DWAF 1996a). Moreover, when fish are exposed to low DO levels it reduces their feed intake and growth rate, whereas values below 0.75 mg/l may result in fish mortalities. However, findings by Ross (2002) and Riche and Garling (2003) suggest that DO concentrations of 3.0 mg/l should be the minimum to allow optimum growth of *O. mossambicus*. In this study low DO levels were recorded from the control group, with minimum values of 1.8 mg/l and maximum concentrations of 3.6 mg/l (Figure 4.1b). Dissolved oxygen concentrations reported in this study were mostly below the recommended range (<3.0 mg/l) indicated by Ross (2002) and Riche and Garling (2003). However, it was observed by Colt *et al.* (1979) and Russel *et al.* (2012) that *O. mossambicus* are able to survive at oxygen concentrations below 1.0 mg/l. Although lowest DO levels were recorded from the control group, higher survival rates were observed in this treatment. Thus, results in this study indicate that mortalities recorded over the study period cannot be attributed to DO concentrations.

Dissolved oxygen concentrations varied according to the time of day, with readings being slightly higher during the day and lower at night however these differences were not significant. Thus, higher oxygen concentrations in tanks with algae together with daily oxygen fluctuations could be attributed to the presence of algae in treatments since, algae play an extremely vital role in the dynamics of DO in water bodies (Haas *et al*. 2013). In the presence of sunlight, algae produce oxygen through photosynthesis thus providing more oxygen for the fish. Conversely at night and on cloudy days, algae remove oxygen from the water for respiration (Misra & Chatarvedi 2016). This phenomenon was observed in the daily cycle as oxygen levels fluctuate with light levels throughout the day, causing wide swings in DO concentrations in water between night and day readings. Moreover, treatments with algae only had higher DO concentrations than treatments with algae and fish. This could be attributed to the presence of fish in tanks. Misra and Chatarvedi (2016) states if sunlight is minimal or unavailable for prolonged periods of time, fish will utilise dissolved oxygen quicker than phytoplankton can restore it. Algae will then compete with fish for the available oxygen, resulting in less oxygen being available for fish and may be an explanation as to why tanks without fish had higher DO values than those with fish.

Water pH is a measure of the acidic or basic character of a solution. The target water quality range (TWQR) for pH set for aquatic ecosystems is 6.5–9 (DWAF 1996a). Highest pH values were recorded in tanks with 66% algae at 8.5, and low values in tanks with 66% algae and fish at pH of 6.77 over the trial period. DWAF (1996a) states that pH values between 6.5–9 pH are tolerable for most freshwater fish. Additionally, Yada and Ito (1997) states that *O. mossambicus* can survive a pH of 3.5 to 11. Therefore, pH values recorded in this study to be within the suitable range for the survival and growth of *O. mossambicus*. Leavitt *et al.* (1999), states that algal abundance increases when pH levels are slightly acidic ± 5.0 and growth is reduced when pH levels exceed 9.5 (Pedersen & Hansen 2003). Work by Converti *et al*. (2009) has shown that *Chlorella* spp. can grow well under acidic conditions with pH of 3. All recorded pH values for this study were within the desired range for growth and reproduction of *C. vulgaris* and *C. protothecoides*.

Total dissolved solids are defined as a composite measure of the total amount of soluble materials dissolved in the water. Total dissolved solids comprise of inorganic salts such as calcium, magnesium and potassium, and organic matter such as algae

(EPA 2012). There is no TWQR for TDS in South African for aquatic ecosystems, however, WHO guidelines stipulate that the acceptable limit should be 1000 mg/l. Total dissolved solid values were <1000 mg/l and fell well within the acceptable limit stipulated by the WHO (1993) guideline during the study. Furthermore, values for TDS were slightly higher in tanks with fish and increased with increasing algal concentrations. According to Boyd (2004), the presence of fish excrements increases TDS values. Thus, the presence of TDS values could be attributed to the presence of fish excrements in fish tanks.

Electric conductivity is the numerical expression of the ability of water to conduct an electric current, which is expressed in millisiemens per meter (mS/m) (DWAF 1996a; Dallas & Day 2004). Similar to TDS, EC concentrations increased with increasing algal concentrations. Rusell *et al*. (2011) states that conductivity levels between 150 and 500 mS/m are ideal for fish culture. However, Stone *et al*. (2013), put the desirable range of conductivity for fish at between 100 and 2000 mS/m. Electrical conductivity levels recorded in this study were below 2000 mS/m and thus were suitable range for the health and survival of fish.

Ammonia is present in small amounts in air, soil, water and in large amounts of decomposing organic matter. Ammonia is a major metabolic waste product from fish (Francis-Floyd and Watson 1990). Results from a study by Nyanti *et al*. (2012) indicate that fish excrements increased ammonia levels. Ammonia concentrations recorded during the study period where above the target water quality range (TWQR) for un-ionized ammonia as stipulated by DWAF (1996a). Control tanks and tanks comprising of algae and fish had higher ammonia concentrations than treatments without fish. Thus, it can be inferred that high concentrations could be attributed to excretory products of fish that accumulated in fish tanks. This is because fish were consuming the algae and producing by-products which is excreted in the water thereby increasing ammonia levels. Work by Francis-Floyd *et al.* (1996) and Nyanti *et al.* (2012) showed that organic matter in the form of fish excretory products increases ammonia concentrations thus explaining the increase in ammonia levels over period of the study. However, ammonia levels dropped on day 8 and 10, in tanks comprising of 66% and 100% algal concentrations and fish, when there was less fish in tanks. Resulting in, less excretory products in this tanks. Furthermore, a decline in ammonia levels seemed to affect chlorophyll-*a* concentrations in tanks with algae and fish. When ammonia concentrations declined, a decrease in chlorophyll-*a* was recorded. Results in this study indicate that ammonia concentrations could have been a limiting factor in the growth of algae. As ammonia is a preferred nutrient for the growth of algae (Sugiyama & Kawai 1979).

Ammonia concentrations were high in tanks with 66% algae and no fish, as high ammonia levels were recorded on day two from these tanks. Nitrogen occurs in many forms and is continuously cycled among these forms by a variety of bacteria. Gaseous nitrogen (N_2) can be converted to ammonia (NH_3 or NH_4^+) via biological fixation by a process called nitrogen fixation. Although not conclusive as to why ammonia levels were high in tanks with 66% algae and no fish It can be assumed that some nitro-bacteria interaction exchanged that could have taken place therefore sustaining those high levels. However, to be sure, in future nitrogen materials or concentrations should be determined.

Nitrite is an invisible killer in fish and levels between 0.06–0.25 mg/l are considered safe for freshwater fish (DWAF 1996a). Santosh and Signh (2005) recommended that nitrite concentrations should not exceed 0.5 mg/l, however Stone and Thomforde (2004) suggested that the desirable nitrite levels are between 0–1.00 mg/l. High levels of nitrite are not commonly found in water bodies (Nyanthi *et al.* 2012). Nitrite oxidizes haemoglobin to methaemoglobin in the blood, turning blood and gills brown, hindering with respiration, this accounts for the gasping behaviour often observed in fish (Lawson 1995). Furthermore, it causes damage to the nervous system, liver, spleen and kidneys of the fish. Nitrite problems are typically more likely in closed systems due to insufficient, inefficient, or malfunctioning filtration systems. Fish in this study were observed to be gasping for air at the bottom of the tanks. Furthermore, some of the fish gills had changed color and turned brown especially in tanks with 100% domestic wastewater. Thus, nitrite and ammonia levels recorded in this study could have attributed to the high mortality rates in treatments with algal concentrations of 66% f and 100%, as higher concentrations of ammonia and nitrite were recorded from these treatments.

Nitrate are abundant in surface waters (<0.1 mg/l) because of the photosynthetic activity of the plants in the water. Nitrates stimulate the growth of algal blooms and some are toxic to man, animals and cause bad odours in waters (DWAF 1996b). There are no guidelines for nitrate in aquatic ecosystems. However, for aquaculture purposes nitrate levels below 300 mg/l are known to have no adverse health effects on fish

(DWAF 1996a). Stone and Thomforde (2003) suggest that nitrate concentrations above 90 mg/l may have adverse effects on fish. Nitrate values in this study ranged between 0.18 to 20.5 mg/l and were thus below the recommended range for *O. mossambicus.*

There are no South African water quality guidelines (SAWQG) of sulphate available for aquatic ecosystems (Dallas & Day 2004), because sulphates are not considered toxic for fish at normal concentrations. However, when found in high concentrations sulphate levels form sulphuric acid thus affecting aquatic life (CCME 2012). Canadian guidelines for aquatic ecosystems (2012), proposed sulphate values of ≥100 mg/l. However, Stone and Thomforde (2003) state that levels above 500 mg/l are of concern if the water is used for other purposes, as fish are able to tolerate a wide range of sulphate concentrations. highest sulphate values were recorded at 80.62 mg/l. Thus, sulphate concentrations were within the suitable range for fish.

Phosphates naturally occur as wastes broken down. Phosphates are also found in waste products of animals and is released during decomposition of organic matter (Boyd 1971), and as phytoplankton. High levels of phosphates are considered to sufficiently increase algal or plant growth. However, serious concerns arise when nutrients are found in high concentrations because high nutrient concentrations may cause DO levels within the water body to decrease. The depletion of DO may result in death and elimination of aerobic benthic organisms, in severe cases, fish kills (Rabalais *et al*. 1996). Stone and Thomforde (2003) suggests phosphate levels of 0.005 to 0.5 mg/l in surface waters. Highest phosphate concentrations were recorded at 11.9 mg/l. Therefore, phosphate concentrations were above the desired range. This could be attributed to the presence of fish excrements in fish tanks.

Suspended chlorophyll-*a* concentrations are often used as an indicator of algal biomass. As a general principle, algae require a supply of inorganic nutrients, sufficient light and favourable temperatures to grow. Three primary nitrogen molecules for algal growth are ammonia, nitrate and nitrogen gas (Bold & Wynn 1978). All algae take up nitrate and ammonia, and ammonia is the preferred nutrient for algal growth. Algae incorporate ammonia very rapidly (Sugiyama & Kawai 1979). Algae will continue to grow as long as the above-mentioned requirements are met. When one or more of the stated requirements are not available for growth, then algal productivity is said to be limited by those limitations. Limitation of algal growth is best described by Leibig's Law of the Minimum, which states that if one of the essential plants nutrients is deficient, plant growth will be poor (Sugiyama & Kawai 1979). Ammonia levels recorded over the study period were higher in tanks with fish. This could be linked to by-products of fish released into the water, thereby sustaining algal growth in the beginning of the trial till day eight where ammonia concentrations declined resulting in a decline in suspended chlorophyll-*a* concentrations. Moreover, ammonia concentrations decreased over the period of the study, especially in tanks without fish. Thus, explaining the decline in suspended chlorophyll-*a* concentrations in tanks with or without fish as low ammonia levels could have been the limiting factor in the growth of algae over the period of the study.

Tanks without fish had a decrease in suspended chlorophyll-*a* concentrations. This was probably due to the system being static because there was no fish in these tanks therefore no by-products were produced. Furthermore, this could have resulted in algal die-offs as tanks with and without fish had algae settled at the bottom of the aquarium. An indication that the algae in the aquariums were dying-off which in turn will decrease oxygen levels. Moreover, Acarli and Lok (2010) stated that *Chlorella* spp. can produce toxic products when concentrations are high. Plankton die offs together with high concentrations of algae may explain the occurrence of mortalities in treatments which fish were introduced. As organic matter, in the form of dead algae, decreases oxygen levels in the water body therefore decreasing oxygen concentrations available for fish. A decrease in suspended chlorophyll-*a* concentrations in tanks without fish could possibly be due to algal die offs in the treatments due to algal limitation. Furthermore, decreases in suspended chlorophyll–*a* concentration in treatments with algae and fish could be linked to the consumption of algae by the fish. This was verified by the presence of algae in the stomach of fish over the 10-day period.

The well-being and the growth of fish is not only determined by the quality of water, the quality and quantity of the food the fish takes partly plays a role (Jobling *et al*. 1993). The inclusion of algae in aquaculture feeds has shown to have significant improvements to growth rates, overall animal health and disease resistance. Results from the study indicate that there was consumption of algae by fish recorded over the trial period, however, none of the stomachs examined over the study period were 100% full. The majority of the stomachs analysed were a quarter full and only 1% of the stomachs were three quarters full. Results from the study are in line with literature by FAO (1992) and Skelton (2001), which state that *O. mossambicus* is able consume algae to a certain degree, indicating that algae used in this study might not be the preferred food for *O. mossambicus.* Being omnivore *O. mossambicus* it prefers a variety of food items, thus explaining why none of the stomachs were full over the trial period and the slow growth rates. Furthermore, tank conditions could have additionally attributed to less consumption of algae by fish. As high nutrient values in the beginning of the trial could have resulted in fish feeding less due to stress. As previously mentioned in Chapter one, gastric secretions which are vital for the digestion of algal cell walls can be compromised in stressed fish, reducing the rate of algal removal in ponds treated with algae (Jones *et al.* 2016).

Results of the feeding experiment indicate that growth performances of *O. mossambicus* were significantly affected by the different concentrations of *Chlorella* spp. The negative growth rate value from treatments with 33% algae could have been due to the feeding concentration having insufficient nutritional value for fish in these treatments. Findings from a study conducted by Dempster *et al.* (1995) on juvenile cichlids (*Oreochromis* spp.) corresponds with results from this study, showing that sometimes algae may provide insufficient nutritional value for a sustained growth, explaining the loss in fish weight that occurred over the trial period. Conversely another study by Sultana *et al.* (2001), observed that an increase in feeding rates does not always produce an increase in fish growth, which could explain the results obtained in tanks with 100% algae and fish. As feeding rates from tanks with 100% algae did not result in highest growth rates as expected. However, higher growth rates were recorded in tanks with 66% algae supporting literature by Sultana *et al.* (2001).

Survival rates at the end of the experiment showed that there were significant differences among the different treatments over the study period. Survival percentages ranged from 19% to 61%. Overall survival results indicate that better survival was recorded from control tanks, comprising of matured tap water only. In treatments with algae, best survival percentage was recorded from treatments with 33% algal concentration which had less amount of *Chlorella* spp. followed by treatments with 100% algae and lastly treatments with 66% algae. In this study *Chlorella* spp. improved the growth rates of *O. mossambicus* but increased the mortality of fish in tanks with 66% and 100% algal concentrations. Acarli and Lok (2010), states that although *Chlorella* spp. are usable as food, it produces toxic products when their concentrations are too high. Toxic secretions of *Chlorella* spp. could have also negatively affected the survival of fish especially given the confined space in which these fish were housed. Results from Acarli and Lok (2010), showed that when *Chlorella* spp. is used together with other species, negative influences could be neutralised. Whereby, adding other species such as *Scenedesmus* spp. for the treatment of wastewater can possibly neutralise toxic secretions by *Chlorella* spp. so as to make the environment suitable for the survival of fish. Furthermore, higher ammonia concentrations in tanks 66% and 100% algal concentrations could have played a role in fish mortalities.

Feeding rates from tanks with 33% algal concentrations did not result in any fish growth, however, growth of fish was observed in tanks with 66% and 100% algal concentrations. According to Deyab and Hussein (2015), feeding rates are known to highly influence specific growth rates, as specific growth rates increase with increasing feeding rates. Thus, greater growth rates are expected when fish are fed with higher feeding rates than when fed with smaller feeding rates (El-Ebiary & Zaki 1995; Fontaine *et al.* 1997). Highest feeding rates in this study were observed from tanks with 100% algal concentrations, however, did not result in high growth rates as expected. Results from this study were partly in line with those by El-Ebiary and Zaki (1995) and Fontaine *et al.* (1997). As lower growth rates from this study were recorded from tanks with the lowest feeding rate in tanks with 33% algal concentrations, and higher growth rates were observed from tanks with 66% algal concentrations, which had a feeding rate of 1.35×10^3 cells fish $^{-1}h^{-1}$ as opposed to tanks with 100% algal concentrations which had a feeding rate of (2.24×10^3 cells fish $^{-1}h^{-1}$).

4.5 Conclusion and recommendations

Water quality parameters such as temperature, dissolved oxygen, pH, total dissolved solids, electrical conductivity and nutrients such as phosphates, sulphates and nitrates were found to be within the desired range, however, ammonia and nitrites were found to be above the acceptable range for *O. mossambicus*. Better survival in this study were observed from treatments with 33% algal concentrations. It is possible that toxic secretions from algae could have attributed to the high mortality rates or low survival rates recorded during the study period in tanks with 66% and 100% algal concentrations. In addition to this ammonia and nitrite values that were above tolerable limits for fish could have also contributed to the high mortality rates. Growth performances of *O. mossambicus* were significantly affected by different levels *Chlorella* spp. As higher growth rates were observed from tanks with 66% algal concentrations with a feeding rate of 1.35 x 10^3 cells/fish/h, as opposed to tanks with 100% algal concentrations which had a feeding rate of 2.24 x 10^3 cells/fish/h. However, no growth was recorded from tanks with 33% algal concentrations which had feeding rates of 0.53 x10^3 cells/fish/h. Findings from this study indicate that *O. mossambicus* is able to consume algae, which resulted in chlorophyll-*a* concentrations decreasing. However, further experiments would need to be conducted so as to establish whether negative influences would be neutralised, when other species such as *Scenedesmus* spp. are used together with *Chlorella* spp. for the treatment of wastewater, so as to make the environment suitable for the survival of fish.

There were slight decreases in suspended chlorophyll-*a* concentrations in tanks with and tanks void of fish. Decreases in suspended chlorophyll –*a* concentration in tanks with fish could be linked to consumption of algae by the fish. This was verified by the presence of algae in the stomach of fish euthanised during and at the end of the experiment. Although fish were able to consume algae it was not in high or desired quantities as there were no stomachs observed to be full over the duration of the trial. Therefore, in a practical situation fish would need to be stocked in high densities so as to reduce the algae to desirable levels. However, this can result in low dissolved oxygen levels in ponds and high nutrient levels, which are detrimental to fish and causes mortalities. In this study high mortalities were recorded from a system where wastewater was not introduced. Therefore, in a practical situation it will be high unlikely that fish would survive the conditions.

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CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The aim of the study was to assess the health and survival of *O. mossambicus* exposed to domestic wastewater from the Sekhukhune Municipality treatment plant using specific algae for treatment and to establish the extent by which these species consume micro-algae within the water column. This was achieved through; monitoring the survival rate of *O. mossambicus* exposed to various concentrations of sewage water under aerated and non-aerated conditions over a 96-hour period and by assessing the consumption of algae by *O. mossambicus* based on algal cell density, feeding rate and stomach fullness.

The survival and health of *O. mossambicus* exposed to various concentrations of treated wastewater

The physicochemical parameters of the sampled tanks were all within the acceptable levels for the growth and survival of fish. However, ammonia levels over the study period were above the recommended range. Ammonia levels exceeding concentrations of 2. 0 mg/l are known to have adverse effects on the health of fish, by causing changes in the structures of the gills and ultimately leading to mortalities in wastewater concentrations >25%. Moderate signs of hyperplasia, lifting of the epithelium, fusion of the secondary lamella and aneurysm of the gill lamellae, may have resulted from the effects of ammonia toxicity. Results from this study indicated that the treated wastewater would need to be diluted to less concentrated levels for the approach to be viable. Moreover, mechanical aerators would have to be deployed to increase the dissolved oxygen levels in treatment ponds, as the introduction of fish to 100% wastewater under non-aerated conditions resulted in 0% survival rates. However, when fish were exposed to 25% algae treated wastewater, better survival (100%) of fish was recorded from both aerated and non-aerated tanks, indicating that levels <25% are less detrimental to the health of fish. However further intensive studies are needed to establish whether the introduction of fish to less concentrated conditions (<25 %) can be implemented. Alternatively, it is suggested that the effluent be further treated to decrease ammonia levels after the algae is removed by the fish so as to ensure the survival of fish and the successful implementation of these treatment process maintainable.

Assessing the consumption of algae by *Oreochromis mossambicus* based on algal cell density counts in fish aquaria and stomach fullness

Water quality parameters in tanks with algae were all within recommended ranges for the growth and survival of fish. However, ammonia and nitrite values recorded over the duration of the study were above recommended levels for the survival of fish which could be attributed to the high mortality rates recorded over the study period. Furthermore, toxic secretions by *Chlorella* spp. could have also attributed to the low survival rates of fish, as *Chlorella* spp. in some instances give low survival rates when found in high concentrations. However, to be sure further experiments would need to be conducted to establish whether negative influences by *Chlorella* spp. can be neutralised, when other algal species such as *Scenedesmus* spp. can be used together with *Chlorella* spp. for the treatment of wastewater, towards making the environment suitable for fish survival.

Of the fish examined none of the stomachs were found to be completely full. However, majority of the stomachs were found to be half full. However, consumption was minimal as there were no stomachs observed to be full. Given the low growth rates and that no stomachs were observed to be full, this could be an indication that being omnivore, *O. mossambicus* cannot sorely survive on algae strains used in this study. Therefore, in a practical situation fish would need to be introduced in high densities in order to reduce algal concentrations to desired levels. This will have a repercussion of decreasing oxygen levels in treatment ponds and increasing nutrient levels. Which will be a challenge as high mortalities were already recorded in a system where wastewater was not introduced. Thus, it is highly unlikely that the fish would survive conditions at the treatment plant nonetheless reduce algal concentrations to desired levels. Therefore, future research should focus on minimising the impact of deteriorating the quality of water when high stock of fish is used to remove algae.