# The influence of Aquahatch on the growth performance of larval and juvenile African catfish (*Clarias gariepinus*, Burchell 1822)

by
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# Declaration

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# Abstract

Aquaculture is one of the fastest growing food production sectors in the world. However, the limited supply of quality seed stock is bottlenecking its growth. This is especially evident in the African catfish (*Clarias gariepinus*) subsector that is characterized by poor survival during the juvenile stage. During early development, fish are especially prone to nutrient deficiencies that affect development and overall growth.

This study evaluated the potential of Aquahatch, a nutrient solution consisting of L-carnitine, B vitamins and nucleotides, to improve early development, yolk sac utilization and overall growth in African catfish. The product was administered once to the culture water at four inclusion levels, i.e. 0.0mL/L (control), 0.25mL/L, 0.5mL/L, and 1.5mL/L, and the duration of exposure was seven days. Three separate trials were conducted, with Trial 1 and Trial 3 conducted in a system of 100 L tanks, with a high but unknown number of African catfish larvae. Additional tanks were also allocated in Trial 1 that were absent of fish, to determine the stability/behaviour of the product in water over time. Trial 2 was carried out in a system consisting of 14L tanks, with 30 African catfish larvae allocated to each tank. Trials 1 and 3 were replicated nine times, and Trial 2 replicated four times.

Trial 1 and 2 ran for seven days, both focussing on fish production performance during the treatment period. Fish parameters recorded during Trial 1 included larva body length, yolk sac dimensions (height, length and volume), yolk sac volume rate of change, body length:yolk sac volume ratio, and final individual fish weight at the end of the treatment period. The parameters analysed for Aquahatch behaviour in the culture water in Trial 1 include a comparison of treatment concentrations in tanks, with and without fish, as well as the change in concentration over time. Trial 2 reports on fish survival. Trial 3 ran for 104 days and focused on post-treatment growth performance which included individual weight and length, biomass, absolute growth rate, relative growth rate, specific growth rate, feed conversion ratio, mortalities, body condition, proximate analysis, haematocrit, cephalosomatic index and viscerosomatic index. In Trial 3, three different sampling methods, in combination with image analysis, were also evaluated as a tool for optimising data collection and fish wellbeing during sampling.

In this study, no beneficial effects of Aquahatch, when supplemented in the culture water of early development African catfish, were found. Increasing Aquahatch inclusion resulted in a deterioration of water quality, as evident in the higher total ammonia nitrogen levels and decreased survival rate. An unfavourable short larvae body length was also observed with the 0.25mL/L inclusion level.

The combination of the three sampling methods and image analysis used in Trial 3, proved to be of benefit during sampling. However, the image analysis procedure requires refinement to reduce analysis time.

Observations that were not conclusive and require further investigation include a tendency for improved body condition, a decreased cephalosomatic index, and larger yolk sac volume and shorter body length during the period associated with the development of oral ingestion capabilities of the fish.

# Dedication

This thesis is dedicated to my dad, Bruce Joscelyne.

Your spirit will live on in everything I do.

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I would like to express my greatest appreciation to all the people and institutions that helped me along this journey. Your help has got me to where I am now.

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# Preface

This thesis is presented as a compilation of 6 chapters.

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# List of abbreviations

| | |
|---|---|
| µm | Micrometre |
| AGR | Absolute growth rates |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| BBD | Gamma-butyrobetaine hydroxylase |
| BL:YSV | Individual total body length of larvae: Yolk sac volume ratio |
| C | Celsius |
| CoA | Coenzyme A |
| CPT1 | Carnitine palmitoyl transferase 1 |
| CPT2 | Carnitine palmitoyl transferase 2 |
| DNA | Deoxyribonucleic acid |
| DO | Dissolved oxygen |
| FFA | Free fatty acids |
| FRC | Feed conversion ratio |
| HTML | 3-hydroxy-6-N- trimethyllysine |
| HTMLA | 3-hydroxy-trimethyllysine aldolase |
| Kg | Kilograms |
| kW | Kilowatt |
| L | Liter |
| m | Meters |
| mg | Milligrams |
| mL | Millilitres |
| mm | Milometers |
| NAD | Nicotinamide adenine dinucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate |

| | |
|---|---|
| Ng | Nanogram |
| nm | Nanometer |
| PE:NPE | Protein energy:non-protein energy ratio |
| PLP | Pyridoxal phosphate |
| RAS | Recirculating aquaculture systems |
| RGR | Relative growth rates |
| RIS | Ruptured intestine syndrome |
| RNA | Ribonucleic acid |
| SE | Standard error |
| SGR | Specific growth rate |
| SM1 | Sampling method 1 |
| SM2 | Sampling method 2 |
| SM3 | Sampling method 3 |
| T | Treatment |
| TAN | Total ammonia nitrogen |
| TMABA | 4-N-trimethylaminobutyraldehyde |
| TMABADH | Trimethylaminobutyraldehyde dehydrogenase |
| TML | Trimethyllysine |
| TMLD | Trimethyllysine hydroxylase |
| W | Watt |

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# Chapter 1

# Introduction

The increase in the consumption of fish protein is estimated to be twice the increase in the human population (FAO, 2018a). Aquaculture, the farming of aquatic organisms, has the potential to supply the demand of the world's growing population with a healthy protein source. Aquaculture is one of the fastest growing food production sectors, with a lower ecological footprint than any other animal protein production system (Ye *et al.,* 2017).

In terms of biomass produced, finfish contributed 67.6% of the total global aquaculture production (FAO, 2018b). In 2016, production from the freshwater species carp, tilapia, and catfish amounted to 58%, with this contribution expected to increase to 62% in 2030 (FAO, 2018a).

Catfish is a popular choice in aquaculture production systems, with African catfish being the preferred species in African countries (Anetekhai, 2013). Characteristics of catfish that contribute to this popularity include fast growth rates, tolerance of a large range of environmental variables, good performance under high stocking rates, the ability to breathe air, and a successful artificial breeding protocol (Richter & Huisman, 1987; Hecht *et al.*, 1988; Olaniyi & Omitogun, 2014; Dauda *et al.*, 2018). However, sustainable and cost-efficient African catfish production is hampered by poor quality and limited availability of seed stock. Major catfish producing countries, such as Nigeria, have been limited in expanding their industries due to minimal access to seedling supply (Anetekhai, 2013).

African catfish seedling production is characterized by high mortality rates, which can be attributed to numerous factors (Ponzoni & Nguyen, 2008). A contributing factor to the poor survival rate is diets that do not meet the developmental and nutritional needs of these juvenile fish. African catfish larvae and fry have a high developmental rate and are capable of 100% growth per day (Conceicao *et al.,* 1998a). African catfish are also prone to high rates of cannibalism (Kubitza *et al.*, 1999), which could possibly be reduced through proper nutrition.

African Catfish make use of various nutrient sources during early development. Once hatched, a residual yolk is their first source of nutrients, which represents an endogenous feeding strategy. At the end of the endogenous feeding period, first feeding fry have great success, in terms of growth and survival, when they are first transitioned to a diet that consists of live feed, such as *Artemia nauplii* (Nyang *et al.,* 2018). The use of decapsulated artemia cysts is a common alternative to live feed as it is less expensive and technically easy to use (Bardocz *et al.*, 1999). For the remainder of production, fry are then weaned onto a specially formulated artificial diet.

An important aspect for improving seedling production and quality is a better understanding of the nutritional requirements during the early stages of development. Optimizing diets and improving feeding protocols will allow nutrients to be utilized and delivered more efficiently to the animal. Problems associated with inadequate and nutrient-deficient diets rapidly manifest in juvenile fish due to their small weight and high rate of growth (Dabrowski, 1984). This can ultimately lead to the fish becoming underdeveloped and not achieving their full production potential. Research aimed at improving nutrient availability during the early developmental stages of African catfish will help to improve seedling operations (hatcheries and nurseries) and ensure the growth of the African catfish industry.

Alternative methods that enable fish to have increased access to nutrients need to be researched further. One possible method is the addition of supplemental nutrients directly into the cultured water. Fish commonly obtain certain nutrients, such as minerals, from the water (Terech-Majewska *et al.*, 2016). Immersion of fish in treated water is also a common method used in treating diseases and delivering vaccinations, especially for small fish (Huising *et al.,* 2003). However, it is less common practice for nutrients to be supplemented directly into the water. Using the cultured water as a means of delivering additional nutrients to fish potentially has added benefits over other nutrient supplementation methods.

To allow water to form the delivery mechanism of the nutrient to the fish, the type of nutrients supplemented in this manner would have to be highly water-soluble. The nutrients would also have to be identified as beneficial during the early developmental stage of the fish. Since these nutrients would be supplemented together with the normal diet of the fish, it would be beneficial to provide supplemental nutrients that allow fish to make better use of the already available nutrients. L-carnitine is one such nutrient. It is thought to be most beneficial during the juvenile phase of fish, but due to a large amount of conflicting studies additional research on this nutrient is required (Harpaz, 2005). Some studies have shown L-carnitine to work well when supplemented in conjunction with other nutrients (Celik *et al*., 2003; Tekeli *et al.*, 2006; Sharifzadeh *et al.*, 2017), therefore a multi-nutrient supplement might be beneficial. B vitamins

and nucleotides have also been shown as beneficial on their own as nutrient supplements in aquaculture (Waagbo, 2010; Li & Gatlin, 2006). Aquahatch is a commercially available nutrient supplement that contains a mixture of these three nutrients. No publication could be found that investigates the combined effect of these three nutrients, but due to their association with L-carnitine in physiological process, these nutrients have the potential to work together to produce an enhanced effect on fish development.

## 1.1 Study Aim

This study aimed at evaluating the addition of Aquahatch, a nutrient solution consisting of L-carnitine, nucleotides and B vitamins, into the culture water of African catfish as a method of providing additional nutrients to the fish during early development and therefore, improving post-treatment performance.

## 1.2 Study objectives

To achieve the aim of the study, objectives were set out which include:

- Establishing an effective supplementation level of Aquahatch.
- Investigating selected production performance parameter during the Aquahatch treatment period and post-treatment period.
- Evaluating altered yolk sac utilization due to Aquahatch.
- Evaluating the method of culture water as a means of delivering the nutrients contained in Aquahatch to the fish.
- Determining behaviour of Aquahatch in water.
- Setting up recommendations for future studies and use of Aquahatch.
- Evaluating procedures used to collect data for juvenile African catfish.

# Chapter 2

# Literature review

## 2.1 Global and African Aquaculture

Fisheries and aquaculture can be divided into two sectors namely, the freshwater sector and the marine sector. China and Asia dominate all sectors of aquaculture production as fish form an integral part of Asian diets. Globally, excluding aquatic plants, the majority of aquaculture production is contributed by the freshwater sector (FAO, 2018a). On the African continent, freshwater fish is a vital source of protein, with 25% of global freshwater catches contributed by several African countries. However, only 17-18% is from aquaculture. Even though this percentage is low, African counties are experiencing higher annual growth rates in aquaculture compared to the global annual average of 5.8%. This rise in aquaculture operations can be attributed to increased culturing capacity, rising local demands, and local policies promoting aquaculture in African countries (FAO, 2018b).

Aquaculture plays an important role in food security by representing a high-quality protein source for the expanding global population (Finegold, 2009). Globally, the consumption of fish has increased annually by an average of 3.2% in the period from 1961 and 2016. This is higher than the 2.8% reported for the consumption of meat from all terrestrial animals (FAO, 2018b). This increase in consumption of fish, in conjunction with the increasing human population, lead to a higher demand for our natural fish resources, resulting in over-fishing. Aquaculture operations thus presents an approach to potentially alleviate the over-utilization of natural fish resources. The global reliance on where fish is sourced for human consumption has started to shift from fisheries to aquaculture. In 2016, global fish production peaked at 171 million tonnes, of which 47% was contributed by aquaculture. From these figures, if non-food uses, such as fishmeal and fish oil are excluded, global reliance on aquaculture as a fish resource increased to 53 percent (FAO, 2018b).

A total of 598 species of fish are being produced under aquaculture, of which finned fish represent the majority with 369 species (FAO, 2018b). Although there is such great diversity in the number of species produced, production is dominated by a handful of species, with 20 finfish species accounting for 84.2% of the total finned fish produced. The most cultured group of finned fish includes freshwater species, such as carp, tilapia, salmonids and catfish (FAO, 2014; Dauda *et al.*, 2018). It is estimated that these species will represent about 62% of total world aquaculture production by 2030, as compared with 58% in 2016 (FAO, 2018b). In many countries around the world, and especially in Africa and Asia, catfish contribute significantly to annual fish production (Olaniyi & Omitogun, 2014).The popularity of farming with African

catfish stems from their fast growth rate and the ability to thrive at high stocking densities. The species can tolerate a wide range of environmental conditions, and their ability to breathe air is greatly beneficial, especially in African countries where electricity supply is unstable. All these factors, combined with well-established artificial propagation protocols, make African catfish an ideal aquaculture species (Richter & Huisman, 1987; Hecht *et al.*, 1988; Olaniyi & Omitogun, 2013; Dauda *et al.*, 2018). Among the catfish species, the most cultured are Amur catfish (*Silurus asotus*), Channel catfish (*Ictalurus puncuatus*), Striped catfish (*Pangasius hypophthalmus*), and African catfish (*Clarias gariepinus*) (FAO, 2014; Dauda *et al.*, 2018). In Sub-Saharan Africa, 75% of aquaculture production comes from Nigeria and Uganda, with the majority being contributed by Nigeria (Gordon *et al*., 2013). Catfish accounts for 90.8% of fish production in Nigeria, with *C. gariepinus* being the dominate species (Anetekhai, 2013). In Nigeria, the fingerling demand to stock grow-out facilities is far higher than that which can be supplied (Adewumi & Olaleye, 2011), this is also observed in many other developing countries (Olaniyi & Omitogun, 2013). The inability to meet the demand for fingerlings can be accredited to poor survival, nutrition and quality of the fish during the fingerling production stage (Aruho *et al.*, 2017). To help the catfish industry grow, it is vital to improve these early stages of production to combat a bottleneck in the industry's growth.

The South African aquaculture sector is developing slowly, with 4% growth from 2014 to 2015. The marine sector is the major contributor to the total South African aquaculture production, with 66.29% of production in 2015. The freshwater sector that contributed 33.71% of aquaculture production in 2015 includes farmed species such as Rainbow trout and brown trout (*Onchorynchus mykiss* and *Salmo trutta*), crocodiles (*Crocodylus niloticus*), Ornamental fish (e.g., *Koi carp*), African sharptooth catfish (*C. gariepinus*), Mozambique and Nile tilapia (*Oreochromis mossambicus* and *O. niloticus*), Marron Crayfish (*Cherax tenuimanus*) and Common carp (*Cyprinus Carpio* and *Ctenopharygodon idella*). The trout subsector is the highest contributor to the freshwater sector, with 82.43% in 2015, although there are catfish farms operating in South Africa, the subsector did not record any production for 2015 and has not since 2011 (DAFF, 2017). This can be accredited to an initial interest in farming with African catfish due to its worldwide popularity and its strengths as an aquaculture species but poor local acceptance by local consumers (Oellermann, 1995). With the local acceptance of catfish being poor, the majority of local producers focused on the production of high quality fingerling for export (Urban-Econ & DAFF, 2018). With African catfish being one of our limited indigenous big freshwater fish, it would be of great value to continue to try and develop the industry (Stander, 2007). The ever-increasing price of wild caught fish has caused South Africa to re-address the production of African catfish for the local market. Small growth and continued interest in the South Africa catfish industry can be seen through the involvement of catfish in

several aquaculture development projects. One project is the China-South Africa Agricultural Technology Demonstration Centre at Gariep Dam in the Free State, which is aimed at conducting research on freshwater fish and the production of African catfish fingerlings. Another project is Camdeboo Satellite Aquaculture Project (CSAP) by the Blue Karroo Trust. This project aims at producing 20 tons of catfish per month, with the main components of the project being the growing and processing of African catfish (DAFF, 2017).

## 2.2 Life stages of African catfish

African catfish, *Clarias gariepinus* Burchell 1822, also known sharptooth catfish, belong to the Clariidae family, most of which, including the African catfish, are freshwater fish except for two (Olaniyi & Omitogun, 2013). In the wild, adult African catfish reach sexual maturity at 1 or 2 years of age, but in closed aquaculture systems this is reduced to 6-9 months. This is a result of constant ideal high temperature, which results in increased growth and development (Kurbanov & Kamilov, 2017). The large quantity of egg that a mature female can produce attributes to the attractiveness of the species for aquaculture (Natea *et al.*, 2017). During the natural spawning process, the female release eggs into the water to be externally fertilized by the male. Once fertilized, the development from egg to larvae to fry is rapid, and largely temperature dependent. At ideal temperatures, 28-32 hours after fertilization, eggs start to hatch (Peteri *et al.*, 1992).

The terminology used to describe the early life stages of the fish after hatch is often confusing. Akankali *et al.* (2011) who looked at fish hatchery management in Nigeria, gave some indication of the appropriate terminology used to describe the fish at these various early life stages marked by specific events. After hatch, the fish emerge as yolk-sac larvae. They are referred to as yolk-sac larvae, as they utilize residual yolk contained in a yolk sac for nutrition during this period. When about 70% of the yolk has been absorbed, the larvae will start to vigorously swim in search of feed, with this occurring around 3 days after hatch. At this stage they are known as early fry or feeding fry, however some literature may still refer to them as first feeding larvae. The next stage is the advanced fry stage, which is marked by the completed metamorphosis and development of major organs, usually occurring 10-18 days after hatch. By this time the air breathing organs of the fry have developed, and they start rising to the surface frequently to breathe air. Fry are called fingerlings at four weeks of age. Fingerling is the final juvenile stage of fish development. After the juvenile stage they grow until their gametes first start to mature. At this point they start reaching sexual maturity and are considered adults (Haylor, 1992).

## 2.3 Production facilities

Like all aquaculture species, there are a variety of production systems used to produce African catfish, each with their own advantages and disadvantages. The choice of the systems used is mainly based on location, set-up costs and desired market qualities. Commonly used catfish production systems include stagnant ponds, flow through systems and recirculating systems (FAO, 2010). In Africa, the majority of catfish grow-out production takes place in ponds, as they require relatively low start-up and running costs (Alawode *et al*., 2016; Dauda *et al.,* 2018; Hecht, 2013). However, with this type of production system, the environmental conditions need to be ideal. In areas where water is scarce or natural environmental conditions are not suited for the fast growth of African catfish, the fish are raised in indoor recirculating aquaculture systems (RAS) for the entire life cycle, instead of being moved to outdoor earthen ponds. This is the case for African catfish production in South Africa (James, 2016).

There are three sections in a typical catfish production facility, i.e. a hatchery, a nursery and a grow-out section, with the size of the section becoming bigger with an increase in the size of the fish. The production of African catfish larvae usually takes place in a hatchery which consist of tanks (e.g. plastic or concrete) for holding broodstock, egg incubation and larval rearing (Haylor, 1993). Production of fertilized eggs is usually done by artificially inducing broodstock fish to spawn, by the use of fish pituitary gland or a synthetic hormone (Brzuska, 2003). The collected eggs are then placed into an incubation tank and maintained until hatching. At the end of the hatching period, the dead eggs are removed and the yolk-sac larvae remain in tank until they become advanced fry. This helps for improved care during this sensitive time which increases their rate of survival (Peteri *et al.*, 1992). The tanks for incubating eggs and rearing larvae are also relatively small, which allows better management of the fish (Rakocy, 1989).

From the hatchery, the advanced fry are moved to the nursery. Nurseries can consist of larger ponds or tanks, but are still small enough to make management easy. Fry are kept in the nursery where they are grown to fingerlings. The fingerlings are then stocked in larger grow-out ponds where the fish are raised until harvested. Seed fish are the fish that are used to initially stock an aquaculture production system. They are usually eggs, fry or juveniles. Food fish are fish that are grown until market size. In Africa, grow-out facilities for food fish production is the most popular option (Alawode *et al.*, 2016). This results in many farms buying in their seed stock from hatcheries and nurseries. It is important that hatcheries and nurseries are managed correctly and can produce the needed quantity and quality, as this will not only affect their own production but also the production of other farms.

## 2.4 Water quality

Water quality is a vital factor to consider in aquaculture production as it play a determining role in the health and growth of the cultured organism (Mallya, 2007). Aquaculture aims to achieve optimal water quality parameters for efficient growth of the cultured species, but due to the artificial nature of production, this is often a difficult feat. Thus, management of the water quality is important and can only be done by regular monitoring and knowing what the optimal water quality parameters are (Boyd, 2012).

Some of the most important water quality parameters in aquaculture include temperature, dissolved oxygen, ammonia, and pH (Buttner *et al.*, 1993). Even though African catfish can generally tolerate a wider water quality range, compared to other fish species used in aquaculture, striving to get as close to optimal will be extremely beneficial for production. This is especially important when the fish are still young and undergoing a fast rate of development. Poor conditions in the water quality during the early developmental stages has the potential to affect future production potential. Once the high rate of development has decreased, the fish become more tolerant to poor water quality conditions, but it is still good practise to keep the water condition as close to optimal as possible (Hecht *et al.*, 1988; Boyd, 2012) .

### 2.4.1 Temperature

Temperature is considered the most important water quality parameter as it has both the biggest direct and indirect influence on other water quality parameters and performance of the cultured species (Boyd, 2012). African catfish are a warm-water species and can experience good growth rates over a relative wide temperature range of 22-33°C, with optimal growth occurring at 30°C (Britz & Hecht, 1987). In the hatchery, temperature plays a major role in the rate of morphogenesis (Haylor & Mollah, 1995).

### 2.4.2 Dissolved oxygen (DO)

Low dissolved oxygen levels can lead to poor appetite, slow growth and increased susceptibility to disease (Boyd, 2012). Dissolved oxygen content of the water is greatly dependent on the temperature, with the solubility of oxygen decreasing with high water temperatures and *vice versa* (Ernest, 2011). Even though African catfish can breathe air, dissolved oxygen levels should be maintained above 6mg/L in the hatchery when they are still developing their air breathing organs, and 5mg/L in the following production phases for optimal performance (Peteri *et al.*, 1992; Masser *et al.*, 2016).

### 2.4.3 pH

The measure of the hydrogen ion concentration of a solution is known as pH. It describes how acidic or alkaline a solution is on a scale of 0 to 14, with 7 being neutral, less than 7 being acidic, and greater than 7 being alkaline. Low pH values primarily affect the respiration ability of the fish by causing gill damage (Boyd, 2012). The optimal pH for African catfish is at a pH of 7 (Ndubuisi *et al.*, 2015), but freshwater fish can have good growth between a pH range of 6.5 and 9 (Boyd, 2012).

### 2.4.4 Ammonia

Ammonia is the main nitrogenous waste product released by fish as a result of protein metabolism (Masser *et al.*, 2016). It exists in two forms, unionized $NH_3$ which is the toxic form, and ionised $NH_4^+$ which is the non-toxic form (Parker, 2012). Ammonia is commonly measured as total ammonia nitrogen (TAN) which includes both forms (Boyd, 2012). The proportion of the two forms is dependent mainly on pH and to a lesser extent on temperature (Boyd, 2012). An increase in pH and temperature results in a shift to the more toxic form and a decrease in pH results in a shift to the less toxic from. With pH levels lower than 7, there is very little toxic ammonia present (Francis-Floyd *et al.*, 2012). African catfish are able to tolerate relatively high levels of ammonia due to various defence mechanisms (Ip *et al.*, 2004). During the hatchery phase, unionized ammonia levels should not exceed 0.05mg/l and 15mg/L ionized ammonia (Tucker, 1991; Peteri *et al.*, 1992). After the early developmental phase, catfish are able to tolerate higher ammonia levels but they should not exceed 0.34mg/L unionized ammonia (Roques *et al.*, 2015).

## 2.5 Stocking density

Commercial aquaculture production systems often operate on very high stocking densities to make their system more productive. Some fish species are not able to handle these high stocking densities and experience adverse effects as a result. In catfish, higher stocking densities have shown to have positive effects, such as increased growth performance and decreased aggression (Hossain *et al.*, 1998). The stocking density of catfish varies with growth, but for the juvenile stages a stocking density of 200kg/$m^3$ for African catfish, in a recirculating Aquaculture system, is acceptable (Van De Nieuwegiessen *et al.*, 2008).

## 2.6 Factors limiting African Catfish production

Proper management in Aquaculture is important. The two main factors limiting the expansion of African catfish production in Africa are the availability, quality, and affordability of feed and fish seed stock (Adewumi & Olaleye, 2011; Gordon *et al.*, 2013; Patrick & Kagiri, 2016; Amenyogbe *et al.*, 2018). A great deal of research in recent years has been on the improvement of feed quality and a reduction in the costs of feeding. However, by not being able to start with a good quality seed stock or desired quantity, this will put production systems at a disadvantage from the start. The main reason for the inadequate supply of seed stock for African catfish is due to the poor survival rates experienced in the first few weeks of hatching (Ansa, 2014). These poor survival rates can be due to several factors but a major cause is poor nutrition (Aruho *et al.*, 2017).

## 2.7 Larvae and fry nutrition

When compared to adult and juvenile fish, the nutritional requirements of fish larvae and fry are less understood (Hamre *et al.*, 2013). During this stage, fish undergo rapid morphological and physiological changes causing nutritional requirements to differ greatly to that of more mature fish. African catfish larvae and fry are able to grow at 100% per day (Conceicao *et al.*, 1998a). This makes it important to provide a well-balanced supply of nutrients which help facilitate these high growth rates. If the nutrient demand is not met during early ontogenesis, it can lead to the fish becoming underdeveloped, ultimately affecting their future growth potential (Zambonino-Infante & Cahu, 2010).

An essential and much studied component of larviculture nutrition is the various feeding strategies used and their effectiveness in providing nutrients to the fish (Fotedar, 2017). During early ontogenesis, fish commonly utilize and require a variety of feeding strategies. Their ability to utilize these various feeding mechanisms as a source of nutrients plays an important role in their success or failure. The first feeding strategy that African catfish utilize, after hatch, is endogenous feeding via their residual yolk sac. By having a better understanding of the larvae's yolk sac one can potentially help improve its use. It can also provide valuable knowledge on the nutrient requirements for first feeding fry (Hamre *et al.*, 2013). The yolk-sac larval phase in African catfish usually last for a period of 3 days at 28°C (Ing and Chew, 2015). This is mainly dependent on temperature, as colder temperatures slow down the rate of utilization. Other popular aquaculture species in South Africa also go through this endogenous yolk-sac larval phase. For trout, a cold-water species, this phase can last up to 4 weeks (Hoitsy *et al.*, 2012) and for tilapia, a warm-water species, this phase can be between 5-13 days, but they will start accepting feed after 2 days (Terpstra, 2015).

The yolk sac provides the larvae with a rich source of energy needed for growth. Lipids, proteins and carbohydrates all act as energy substrates during early development, with their proportional energy contribution changing throughout development (Tong *et al.*, 2017). Lipids and fatty acids provided by the yolk sac constitute the major energy source during the yolk-sac larval stage (Polat *et al.*, 1995). They also play a major role in important developmental processes such as pigmentation, immunity, and most importantly, cell membrane structure and functioning (Tocher, 2010; Kurbanov & Kamilov, 2017). Lipids, fatty acids and proteins are first used to satisfy maintenance energy requirements, and what is left is used for growth (Conceicao, 1997). Growth, which is an energetically expensive process, is the net result of protein synthesis and degradation (Conceicao *et al.*, 1997). Effective utilization of the limited lipids and fatty acids from the yolk sac is therefore, critical in order to provide the utmost amount of energy to achieve maximum growth, development and a successful transition into exogenous feeding. When the nutrient demand is not met the larvae become starved. During starvation tissues start to be catabolised, with the energy reliance shifting from lipids to proteins, which is a more expensive source of energy. Starvation reduces growth, causes morphological deformities, increases vulnerability to predators and affects survival (Gwak & Tanaka, 2001; Dou *et al.*, 2002; Fotedar, 2017; Lima *et al.*, 2017) . Starvation is common during the transitional period from exogenous to endogenous feeding (Polat *et al.*, 1995). At this point there has been a substantial reduction in nutrient and energy reserves (Ronnestad *et al.*, 1998; Hastey *et al.*, 2010). Further research is required into limiting starvation and improving the success of this transition.

Starvation is due to delayed feeding caused by ineffective feed and feeding of the larvae during the transition stage (Lima *et al.*, 2017). The complete absorption of the yolk sac is commonly used to determine the shift from endogenous to exogenous feeding and when feed should be given. It has been demonstrated that this transition is not clear and a period of mixed feeding between endogenous and exogenous feeding exists (Jaroszewska & Dabrowski, 2011). In African catfish, complete yolk sac absorption occurs around 72 hours post-hatch at typical hatchery conditions but larvae have been shown to start accepting feed (endogenous feeding) as early as 32 hours post-hatch (Ing & Chew, 2015). Although this early acceptance of feed has been demonstrated, poor acquisition and utilization of feed by the larvae is displayed during this period of mixed feeding and even past the complete absorption of the yolk sac leading to starvation. Fish larvae possess poor feed detection capability due their developing sensory organs and therefore, are not highly responsive to feed given.

The limited swimming ability of the fish larvae results in initial feeding being highly dependent on chance encounters as they are outcompeted for feed by stronger, faster growing larvae. Even when the larvae are able to gain access to the available feed, they can still be restricted

by the size of the feed particle that they can fit into their small mouth gape. Therefore, the nutrients in the feed that is ingested by the larvae is poorly utilized due to their underdeveloped digestive system (Paulet, 2003; Ronnestad *et al.*, 2013; Fotedar, 2018). Evidence of poor nutrient accessibility and starvation in African catfish larvae cannot only be seen in survival, but also in size variation and cannibalistic behaviour which can occur as early as 96 hours post-hatch (Ing & Chew, 2015).

After the yolk sac of the African catfish have been absorbed, first feeding African catfish fry have shown best results when fed with a live feed (Delince *et al.,* 1987; Arimoro, 2007; Robinson *et al.*, 2001). A common live feed source for catfish larvae is zooplankton of which artemia is the most popular (Schoonbee *et al.*, 1988). The use of live artemia is however, expensive and also increases the complexity of operations (Kerdchuen & Legendre, 1994; Sales, 2011). Decapsulated artemia cysts is another first feed used for African catfish fry. Although it is relatively expensive, it has shown to be superior to that of formulated feed with a cheaper cost and lower complexity than that of live artemia (Olurin & Oluwo, 2010).

Despite live artemia and decapsulated artemia cysts being a superior first feed options for African catfish fry when compared to other options, they still have sub-optimal and fluctuating dietary levels of certain nutrients needed for good fish growth and development (Sorgeloos *et al.*, 2001). To provide additional nutrients deemed to be lacking or of benefit to the fish, the artemia are enriched with these nutrients. Live artemia are filter feeders and non-selectively ingest particular matter after moulting into the second larval stage, allowing for enrichment (Sorgeloos *et al.,* 1993). Dry decapsulated artemia cysts are able to absorb a large amount of water and therefore, can be enriched by water soluble nutrients (Bardocz *et al.*, 1999). A large variety of nutrients have been used to enrich artemia, most commonly fatty acids. They are crucial for marine larvae (Sorgeloos *et al.,* 1993) and offer improved performance for freshwater fish (Kolkovski *et al.*, 2000). The numerous amount of research conducted on enrichment of artemia with fatty acids illustrates the importance of this type of nutrient during the larvae stage (Tizol-Correa *et al.*, 2006). Therefore, the improved utilization of fatty acids during the larval stage is also critical, especially when additional enrichment of the diet with fatty acids is not practised.

Other common nutrients used for enrichment of artemia include phospholipids, amino acids, plant and algae extracts, vitamins, minerals, carotenoids and other pigments, antioxidants, proteolytic enzymes, immunostimulants and bacteriostatic additives (Yufera *et al.*, 2010). Water soluble vitamins, such as vitamin C, are commonly used to enriched decapsulated artemia cysts to improve fish performance (Bardocz *et al.*, 1999). A combination of supplemented nutrients can also be of added benefit, with the combined nutrients providing

additional support for each other to enhance their beneficial effect. Akbary *et al.* (2011)'s study demonstrated this, where the combined enrichment of artemia with fatty acids and vitamin C was found to improved growth performance, survival, body protein content and temperature stress in rainbow trout larvae. Even with only 1 week of supplementation, improvements in growth continued to be found 3 weeks after changing to an unsupplemented commercial diet.

Despite the benefits of artemia enrichment, there are some downfalls as well. Freshly hatched artemia provide the small size needed for fish larvae with small mouth gapes. The enrichment process requires additional time after hatch, which leads to the artemia increasing in size potentially becoming too big for the small fry to utilize (Sorgeloos *et al.,* 2001; Prusinska *et al.,* 2015). The enrichment process can also cause higher mortalities among the artemia (Harel *et al.,* 2002). This causes the fry to feed on dead artemia therefore, decreasing the value of the feed and preventing the motion triggered feed reaction of the larvae. Understanding which nutrients are commonly used for artemia enrichment and the success of these nutrients to improve larvae production, may help to develop other nutrient supplements and supplementation methods. These developments can either be of direct benefit or help to improve the use of other important nutrients that are limited during the early larval and fry stages.

## 2.8 Aquahatch

Specially formulated commercial supplements are available to provide fish with additional nutrients. These supplements can either consist of a single specific nutrient or a mixture of nutrients deemed to be beneficial to the fish. One such supplement is Aquahatch (Nutrition Hub, South Africa) which contains water soluble nutrients. The main components of Aquahatch are L-carnitine, B vitamins, and nucleotides. No published papers were found that investigated this combination of nutrients on fish. Based on literature involving the individual nutrients, this commercial product has potential to be most beneficial as a nutrient supplement in the aquaculture industry. Aquahatch may have the biggest impact during the juvenile phase of production due to the high nutrient demand of the fish during this phase. The nutrients in isolation have a potential beneficial effect on fish, however, when supplemented as a mixture there is also potential for these nutrients to work together and be of even greater benefit.

Aquahatch, which contains only water-soluble nutrients, may provide an alternative method of nutrient supplementation *via* the water compared to feed. Very little research has been conducted on providing these types of nutrients to fish by means of the water. Further research into this mode of supplementation may present alternative benefits over supplementation on the feed. This mode of supplementation may be especially beneficial in providing additional nutrients to fish during endogenous feeding, before they are able to take in solid feeds, and during early exogenous feeding, where they have limited capacity to process solid feeds.

## 2.9 L-carnitine

### 2.9.1 Chemical properties of L-carnitine

L-carnitine (l-3-hydroxy-4-N,N,N-trimethylaminobutyrate) (Vaz & Wanders, 2002)is a non-essential, water-soluble organic nutrient (Harpaz, 2005). There are two isomers of carnitine, L- and D-, with the L- form being physiologically active and D- form being inactive (Gross & Henderso, 1984).

### 2.9.2 Biological function of L-carnitine

The main function of L-carnitine is in fatty acid metabolism. Carnitine aids in the transportation of activated long-chain fatty acids (acetyl-CoA) into the mitochondria where they are used to produce energy through beta-oxidation (Longo *et al.*, 2016). The mechanism in which carnitine transports activated long-chain fatty acids is called the carnitine shuttle (Figure 2.1), which involves various enzymatic actions (Vaz & Wanders, 2002). At the end of the process acetyl Coenzyme A is also produced. This goes on to produce additional energy through the Kreb's cycle (Williams, 2002).

Fats are a major source of energy in the body. The body contains adipose tissue, stored fat, which consists of triglycerides. Triglycerides contain fatty acids and glycerol molecules. Triglycerides are broken down into these components by hormone-sensitive lipases. The free fatty acids (FFA) enter the blood stream, bind to albumin and are then transported to cells that can metabolize fatty acids. The mitochondrial membrane of these cells is impermeable to long-chain fatty acids. Once in the cytosol of the cell, long-chain fatty acids can be acted on by the enzyme Acyl-CoA synthetase, which is located at the outer mitochondrial membrane. Acyl-CoA synthetase utilizes adenosine triphosphate (ATP) and CoASH to activate the long chain fatty acid, transforming it into fatty Acyl-CoA by adding a coenzyme A group. Fatty Acyl-CoA can be acted on by carnitine palmitoyl transferase 1 (CPT1) which is located within the outer mitochondrial membrane. Here, CPT1 removes the CoASH and adds a carnitine to the fatty Acyl chain to form fatty Acyl-carnitine, this allows it to enter the intermembrane space. Once in the intermembrane space, the carnitine portion allows the fatty Acyl chain to cross the inner mitochondrial membrane and enter the mitochondrial matrix. This is achieved by carnitine acylcarnitine translocase which is located within the inner mitochondrial membrane. Once in the mitochondrial matrix, fatty Acyl-carnitine can be acted on by the enzyme carnitine palmitoyl transferase 2 (CPT2).

This enzyme takes a CoASH, removes the carnitine portion and adds a coenzyme A to the fatty Acyl portion of the chain. This produces a fatty Acyl-CoA and regenerates carnitine. Fatty Acyl-CoA within the mitochondrial matrix can undergo beta-oxidation, which ultimately produces energy. The carnitine that is reformed can then be pumped back into the cytosol where it can be used again. Circulating carnitine levels are highly conserved and can be maintained within a narrow range due to the highly efficient active transport mechanism, allowing for reabsorption. This allows for increased reabsorption when carnitine levels decreases, and increased excretion when circulating carnitine levels increase (Longo *et al.*, 2016).

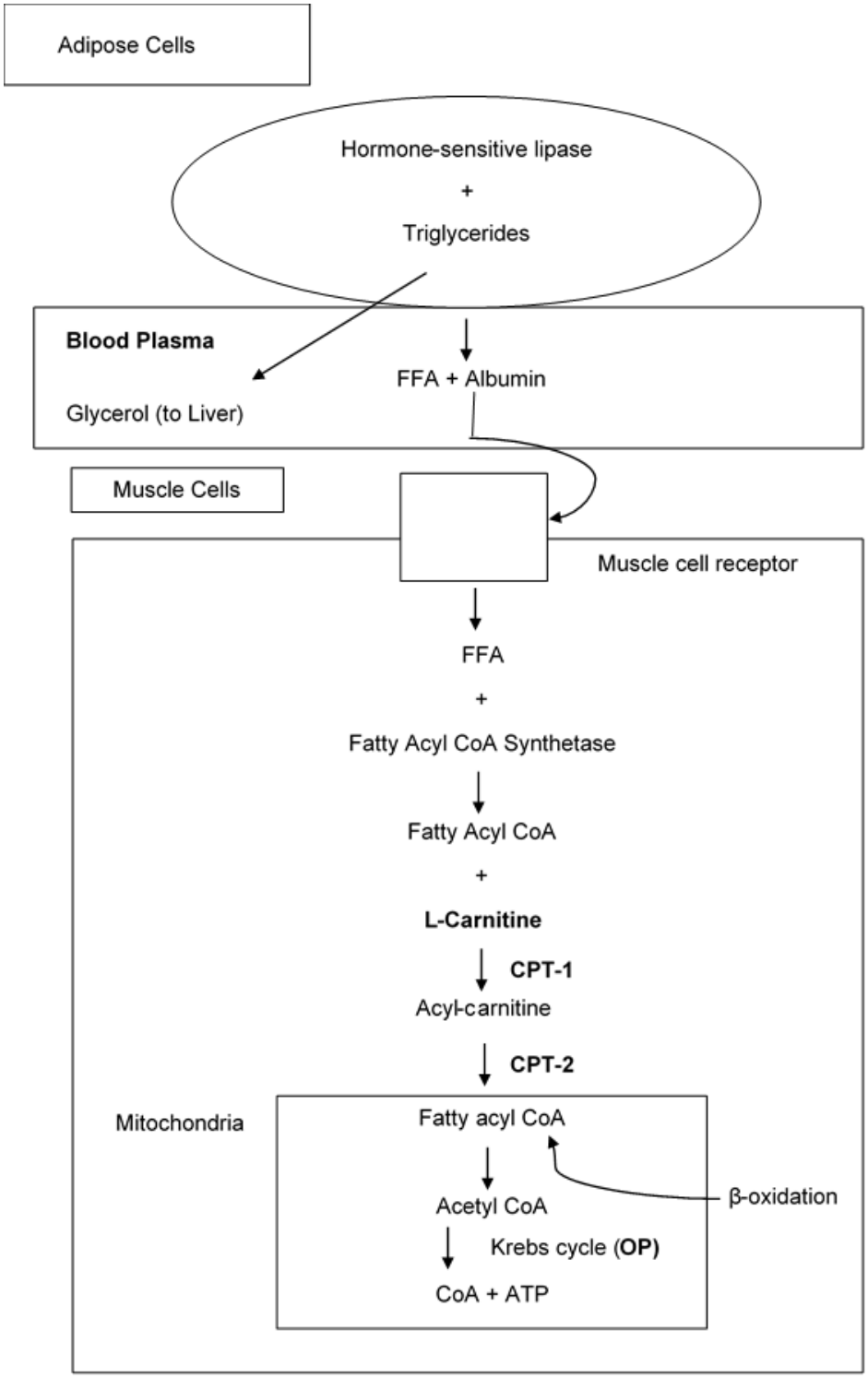


**Figure 2.1** Transport of fatty acids into the mitochondria by the carnitine shuttle (Tekle *et al.*, 2004).

Carnitine's major role is fatty acid transportation into the mitochondria for beta-oxidation. However, Vaz & Wanders (2002) summarized the other functions of carnitine which includes its involvement in the transfer of the products of peroxisomal β-oxidation, including acetyl-CoA to the mitochondria for oxidation to $CO_2$ and $H_2O$in the Krebs cycle, modulation of the acyl-CoA/CoA ratio, storage of energy as acetyl-carnitine and the modulation of toxic effects of poorly metabolized acyl groups by excreting them as carnitine esters.

### 2.9.3 Sources of L-carnitine

L-carnitine can be biosynthesized in the body or derived from the diet (El-hattab & Scaglia, 2015).

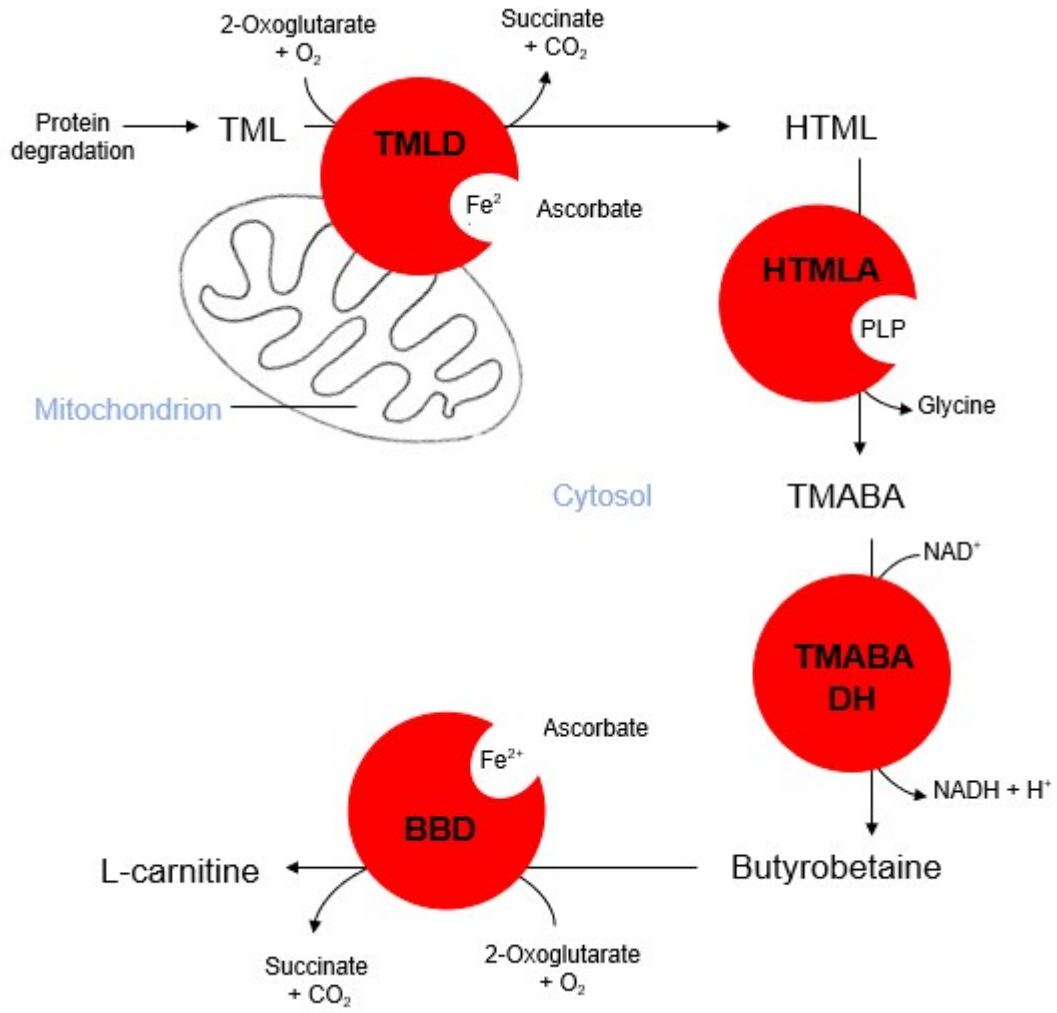


**Figure 2.2** The carnitine biosynthesis pathway (Vaz & Wanders, 2002).

In vertebrates, L-carnitine is synthesized mainly in the liver, kidney and brain and is transported to tissues that use fatty acids as their primary dietary fuel, such as skeletal and

cardiac muscle (Li *et al.*, 2018). The biosynthesis process (Figure 2.2) requires lysine and methionine, which are derived from protein degradation and respectively provide the carbon backbone and 4-N-methyl groups of carnitine (Vaz & Wanders, 2002). Lysine and methionine are two limiting amino acids which are often found in short supply in feed and therefore dictate growth rates (Fagbenro *et al.*, 1998, 1999). The biosynthesis process begins with trimethyllysine (TML) which is a lysine derived residue obtained from protein degradation. The methyl group in TML is derived from methionine through protein degradation. The biosynthesis process is catalysed by four different enzymes. Trimethyllysine hydroxylase (TMLD), which requires iron ($Fe^{2+}$) and ascorbate (vitamin C) as cofactors and hydroxylates TML to produce 3-hydroxy-6-N- trimethyllysine (HTML). 3-hydroxy-trimethyllysine aldolase (HTMLA) which requires pyridoxal phosphate, the active form of vitamin $B_6$, cleaves HTML to 4-N-trimethylaminobutyraldehyde (TMABA) and glycine. Trimethylaminobutyraldehyde dehydrogenase (TMABADH) which utilizes niacin in the form of nicotinamide adenine dinucleotide ($NAD^+$) to produce 4-N-trimethylaminobutyrate (butyrobetaine) from TMABA. Then finally butyrobetaine is hydroxylated by gamma-butyrobetaine hydroxylase (BBD) to yield carnitine (El-hattab and Scaglia, 2015)

In the diet, meats, especially red meats (500 to 1200mg/kg), followed by fish, chicken and dairy products (16 to 64mg/kg) are the best source of L-carnitine, while plant-based foods contain very little carnitine (<0.5mg/kg) (Harpaz, 2005). In aquaculture, fish and animal products have been the main source of protein in feed. However, there are increasing efforts aimed at shifting the reliance of aquaculture feed away from animal-based proteins to a more sustainable, plant-based protein source or a higher energy diet in the form of lipids (Yang *et al.*, 2012). With these changes, L-carnitine, which was previously supplied in sufficient quantities, could possibly become under supplied. A deficiency or increased demand for L-carnitine is also likely to occur during the juvenile phases (Harpaz, 2005; Li *et al.*, 2018) when fish are experiencing rapid growth with a high energy demand (Pedersen, 1997) and when L-carnitine's biological pathways, such as the biosynthesis and conservation pathway, are still poorly developed (Yamaguchi *et al.*, 1994) . By supplementing L-carnitine in the diet, these deficiencies can be overcome with the potential added benefit of improved fish growth performance and feed utilization. The need for the body to biosynthesize L-carnitine is also reduced. This allows for important nutrients involved in the biosynthesis process, such as lysine and methionine, to be utilized elsewhere, such as in protein production (Li *et al.*, 2018).

### 2.9.4 Supplementation of L-carnitine

Harpaz (2005) and Li *et al.* (2018), who reviewed L-carnitine supplementation in fish culture and nutrition, found that L-carnitine was supplemented for multi-functional purposes. The main uses included: growth promotion, specifically by aiding the utilization of fats in the diet

(commonly referred to as a “protein sparing effect”), changes in body composition to reduce body fat, protection against toxic levels of ammonia and xenobiotics, temperature stress alleviation and improved reproduction.

#### 2.9.4.1 Growth, feed intake and FCR

Growth, in terms of weight gain, is one of the most important aspects of aquaculture. Efficient fish growth needs to be fast and economical, therefore, further investigation into supplements, such as L-carnitine, that can potentially help to improve the efficiency of growth is of great benefit to the aquaculture industry. L-carnitine is believed to improve growth by allowing for improved energy utilization through increased fatty acid oxidation by the mitochondria (Bilinski & Jonas, 1970). The feed conversion ratio (FCR) is one measure that is important in determining the economic viability of growth in the fish. Lower FCRs illustrate a better utilization of the feed, leading to less feed being needed for similar growth (Hasan & Soto, 2017). Torreele *et al.* (1993) demonstrated the benefits of L-carnitine supplementation with 5g African catfish fingerlings, where the supplementation was shown to improve growth rates and FCR when supplemented at an inclusion levels of 500mg/kg. Improved growth parameters like this have also been demonstrated in other popular aquaculture species. In 2g male Mozambique tilapia, Jayaprakas *et al.* (1996) found success in improving growth and FCR with increasing levels of dietary L-carnitine up to 900mg/kg. At the lower supplementation rates of 150mg/kg, Becker *et al.* (1999) continued to show the benefit L-carnitine had on the growth and FCR of 32g hybrid tilapia. However, in a few cases, only high levels of L-carnitine supplementation have shown to be effective in improving growth and FCR performance. This was the case for Asgharimoghadam *et al.* (2012), who only found success in common carp with a high L-carnitine inclusion level of 2500mg/kg. Schlechtriem *et al.* (2004) failed to show a positive growth effect of L-carnitine supplemented tilapia hybrids. Despite showing improvement to some fish performance at high inclusion levels, one must question the economic viability of L-carnitine supplementation.

#### 2.9.4.2 High fat diets

The use of a high fat diet in aquaculture is a common practise. In terms of feed ingredients, fats are a less expensive form of energy compared to proteins. By incorporating more fats into the diet, the dietary energy levels can be met and the reliance on proteins as a source of energy can be reduced.(Li *et al.*, 2012). However, there are negative effects associated with high fat diets due to lipid accumulation which can result in poor quality meat or fish health problems (Du *et al.*, 2006; Tao *et al.*, 2018; Cao & Liu, 2019). This has warranted the investigation into supplements, such as L-carnitine, which have the potential to help fish better utilize fats in the diet and reduce lipid accumulation due to its close association with fat metabolism.

Santulli *et al.* (1988) found that 100mg/kg of L-carnitine supplementation helped to reduce fat accumulation when sea bass weighing 219g were given a high fat diet. This allowed for the elimination of fasting periods which was used to reduce high levels of tissue fat. However, results between studies tend to vary. While L-carnitine may change lipid metabolism, it does not always result in better fish growth. At 96 and 155g/kg, low and high dietary lipid levels, respectively, Torreele *et al.* (1993) found L-carnitine supplementation of 236.7mg/kg and 448.8mg/kg to be effective in improving feed utilization in African catfish.

Similar results were also seen in common carp by Soltan *et al.* (2016). However, Ozorio *et al.* (2001a) did not find L-carnitine to effect any growth parameters with African catfish fed a high fat diet, but did find evidence that it effected non-protein energy metabolism. Again, there are also studies that find little to no positive results when looking at improved utilization of high fat diets due to L-carnitine, such is the case for hybrid striped bass seen by Gaylord and Gatlin (2000a,b), as well as in rainbow trout by Chatzifotis *et al.* (1997), where a wide variety of L-carnitine (230, 500, 1000, 2000, 4000 mg/kg diet) and lipid (50, 100, 150, 258 g/kg diet) levels were used.

### 2.9.4.3 Body composition

A common statement is that carnitine supplementation may have a “protein-sparing effect” due to it facilitating an increased use of fatty acids as an energy source and therefore, preventing proteins from being catabolized (Ozorio *et al.*, 2005). This has led to many studies that investigate the changes in body composition of fish supplemented with L-carnitine, with the expectance for L-carnitine to reduce the lipid content of the fish and increase the protein content. Chen *et al.* (2010) was successful at demonstrating this with 5g genetically improved Nile tilapia, a strain characterised by fast growth. It was shown with L-carnitine supplementation that the protein content in the whole body and muscles increased and the lipid content in the whole body, muscle tissues and liver decreased.

An increase in protein and decrease in lipid content, due to L-carnitine supplementation, was also found in rainbow trout (Sharifzadeh *et al.*, 2017) and common carp (Soltan *et al.*, 2016). With 5g African catfish, Torreele *et al.* (1993) was successful in reducing the body lipid content but without increase in protein. Becker *et al.* (1999) who found improved growth for tilapia hybrids, due to L-carnitine supplementation, failed to find differences in whole body protein and lipid composition.

### 2.9.4.4 Ammonia and xenobiotics protection

Stress is a major problem in an aquaculture environment as results in poor performance and even death. One common cause of stress is high levels of ammonia due to the intensive nature of aquaculture. L-carnitine supplementation has shown to both help prevent and reduce stress

cause by ammonia. Ozorio *et al.* (2001b) found a reduction in the total ammonia nitrogen production by African catfish and Becker and Focken (1995) found a 15% reduction in nitrogen excretion in common carp. This would help reduce the rate of ammonia accumulation in an aquaculture system, making it easier to prevent stressful, toxic levels. Tremblay & Bradley (1992) found that L-carnitine administered through injection helped improve the survival of Chinook salmon after ammonium acetate injection. However, Harpaz (2005) expressed that administration of L-carnitine through different means, such as injection and the diet, is bound to have different success rates. Therefore, the effectiveness of L-carnitine in preventing and reducing ammonia stress needs further investigation when utilizing various administration techniques.

Another stressor in aquaculture are xenobiotics. These are biologically active substances that are not naturally found in an organism or environment and can be harmful at elevated concentrations (Godheja *et al.*, 2016). Due to the nature of aquaculture systems, especially in recirculating systems where the water is reused, this can be a common problem. Schreiber *et al.* (1997) found that when supplemented with L-carnitine, the gill epithelia of guppies were less permeable to anionic xenobiotics and more efficient at eliminating these substances by the cell membranes due to the improved membrane integrity. A similar protective function to xenobiotics was found by Schlechtriem *et al.* (2004) with tilapia hybrids.

#### 2.9.4.5 Temperature stress

A further common stressor in aquaculture is temperature shock caused by a sudden change in temperature. Due to the important role lipid metabolism plays in conserving metabolic and physiological functions, when fish are exposed to stressors (i.e. sudden temperature change), L-carnitine and its potential for reducing temperature stress has been investigated (Harpaz, 2005). Harpaz *et al.* (1999) demonstrated that warm water ornamental cichlid's supplemented with L-carnitine at varying levels from 500-2000mg/kg showed improved survival when exposed to cold shock, as well as a better reconditioning rate to normal temperatures.

#### 2.9.4.6 Reproduction

L-carnitine is believed to improve the reproductive performance of fish. Jayaprakas *et al.* (1996) found improved reproduction potential in male Mozambique tilapia supplemented with L-carnitine in terms of high gonadosomatic index, sperm cell concentration, motility and percentage viability of the spermatozoa. However, this is thought to possibly be attributed to the improved growth performance experienced by the L-carnitine supplementation. A contradicting result was found by Dzikowski *et al.* (2001) who failed to see an overall effect in guppies on their reproductive performance in terms of brood interval or fry production efficiency when supplemented with 1100mg/kg of L-carnitine.

### 2.9.4.7 Contradicting studies of L-carnitine

With the great number of contradicting L-carnitine studies in aquaculture, further investigation is required to help better answer the question of where, when and how L-carnitine supplementation can be of benefit in aquaculture. Differences in the effect of L-carnitine supplementation, within a study, such as that done by Ozorio (2001) with African catfish, demonstrates that the efficiency of L-carnitine supplementation could be affected by the nutrient source being utilized by the fish. In one experiment L-carnitine was shown to have a positive effect on growth rate and FCR at an inclusion level of 660mg/kg, when dietary protein energy:non-protein energy ratio (PE:NPE) was low (0.7), but in another experiment no differences in growth and FCR of African catfish were observed between high and low carnitine supplementation (200 and 1000mg/kg, respectively) when fed a diet varying in lysine and fat content. The age and size at which supplementation is most effective, is also highlighted by the differences in results in studies done by Ozorio (2001) who found L-carnitine supplementation to be less beneficial to 23g African catfish than that found by Torreele *et al.* (1993) in 5g African catfish. This showed the potential of L-carnitine to have a greater effect in smaller younger fish. Alternative techniques of L-carnitine supplementation, as opposed to feed, may potentially help to obtain a better understanding of L-carnitine supplementation in aquaculture.

### 2.9.4.8 L-carnitine supplementation during the juvenile stage of fish

Most L-carnitine studies in aquaculture look at supplementation with an inert formulated feed. When using a formulated diet, it is easy to estimate the L-carnitine content since the natural content in the ingredients used are generally known (Arslan, 2006). This makes it easier to determine whether supplementation is required. While older fish mainly utilize formulated diets, juvenile fish utilize multiple other forms of diets, where less is known about the L-carnitine content. As previously discussed, live feed is a commonly utilized at the start of exogenous feeding for fish fry. The L-carnitine content of live feed is unreliable as it is not commonly determined and varies with the source and the environmental conditions that the live feed is cultured in (Zhang *et al.*, 2006). Ghosh (2015) demonstrated the beneficial effects of early L-carnitine supplementation by enriching the live feed for clownfish fry, which lead to improved growth, metamorphosis and survival rates. This study is one of the limited few that look at L-carnitine enrichment of live feed for fish fry.

Another feeding phase where L-carnitine supplementation is neglected in aquaculture is the endogenous yolk-sac phase. No aquaculture studies were found that investigate L-carnitine supplementation during this phase. With the yolk being an important source of lipids, the supplementation of L-carnitine can potentially be of great benefit for improved utilization of the yolk by the fish larvae. Poultry studies have shown great promise in L-carnitine

supplementation during the yolk-sac phase. They also present an alternative way of supplementing L-carnitine, which has not yet been investigated in aquaculture, *via* the drinking water. Nouboukpo *et al.* (2010) provides evidence that indicates the potential of L-carnitine benefits during the yolk-sac stage, where it was found that newly hatched layer-type chicks utilize their residual yolk at a faster rate when supplemented with L-carnitine in their drinking water. However, this faster rate of yolk utilization failed to translate into better growth. Improved yolk utilization, and the extent of which L-carnitine might be of benefit, warrants further research within aquaculture.

#### 2.9.4.9 L-carnitine supplementation in water

Supplementation of L-carnitine in the feed is the most common administration technique used in aquaculture, with only a few studies looking at injection administration. Since L-carnitine is water soluble, the poultry industry has investigated an alternative method of supplementation, which is through the drinking water. This is also often the preferred method of L-carnitine supplementation in the poultry industry, as it results in a more homogenous mixture (Arslan, 2006). Oladele *et al.* (2011) showed that the addition of L-carnitine into the drinking water had similar improved production performance for broiler chickens compared to when supplanted onto the feed. Other poultry studies also reported on the benefit of supplementing L-carnitine in the drinking water (Celik *et al.*, 2004; Hrncar *et al.,* 2015; Abouzed *et al.*, 2019). Despite the success of L-carnitine supplementation in the drinking water for the poultry industry, this method of supplementation has not been investigated in the aquaculture finned fish industry, and therefore requires investigation as an alternative method of supplementation for L-carnitine.

#### 2.9.4.10 L-carnitine and other nutrients

The supplementation of L-carnitine can possibly benefit from being supplemented in conjunction with other closely linked nutrients. These nutrients may potentially work in a synergistic manner with L-carnitine but also exert their own effect. Vitamin C, a water-soluble vitamin that acts as a cofactor in the L-carnitine biosynthesis process, has been demonstrated by Sharifzadeh *et al.* (2017) to help improve growth performance in rainbow trout when supplemented in conjunction with L-carnitine. Vitamin B is another water-soluble vitamin which plays an important role as a cofactor in the mitochondria as well as in the carnitine biosynthesis process (Rausch et al., 2019). With no fish studies that demonstrate the combined effect of L-carnitine and vitamin B, the combination of these nutrients as supplements in aquaculture requires further research. The success in the combination of L-carnitine with B vitamins has however, been demonstrated in poultry studies. Tekeli *et al.*, (2006) illustrated that the

combined supplementation of L-carnitine and vitamin $B_6$, which participates in L-carnitine biosynthesis, was of benefit to broiler chicken's production performance, as well as reducing heat stress. Celik *et al.* (2003) found that the supplementation in the drinking water of L-carnitine and niacin (vitamin $B_3$), another cofactor in L-carnitine biosynthesis, had positive effects on weight gain and feed intake for broiler chicks, but only during the early stages of growth.

## 2.10 B vitamins

In aquaculture nutrition, vitamins are a crucial but expensive ingredient used in a complete diet. Vitamins primarily act as cofactors for enzymes, with a shortage in vitamins leading to reduced enzyme activity (Yossa *et al.*, 2015). Therefore, to maintain normal metabolic functions and optimal heath, it is vital that the vitamin requirements are met.

There are two types of vitamins - fat soluble and water soluble. Fat soluble vitamins dissolve in fat, which also acts as a store for these vitamins before they are absorbed into the bloodstream. Water soluble vitamins dissolve in water and cannot be stored by the body (Paul *et al.*, 2010). This has two meaningful implications. The one implication is that the oversupply of fat-soluble vitamins can be toxic whereas, the oversupply of water soluble vitamins is unlikely to be harmful as it is not stored in the body (Delince *et al.*, 1987). The other is that the body is more susceptible to deficiencies in water soluble vitamins if not adequately supplied in the diet (Waagbo, 2010).

Thiamine, niacin, vitamin $B_{12}$, folate, vitamin C, riboflavin, vitamin $B_6$, pantothenic acid and biotin are classified as water soluble vitamins (Yossa *et al.,* 2015). B vitamins, which include thiamine ($B_1$), riboflavin ($B_2$), niacin ($B_3$), pantothenic acid ($B_5$), pyridoxine ($B_6$), biotin ($B_7$), folate ($B_9$) and cobalamin ($B_{12}$) are important cofactors or precursors in biochemical reactions during intermediate energy metabolism. This is evident by high concentrations being found in metabolically active fish tissues (Waagbo, 2010). Due to their role in energy metabolism, the requirements for B vitamins are greater during larval stage of fish, when the metabolic rate is high, causing younger fish to be more prone to vitamin deficiencies than older fish (Dabrowski, 1986).

During the larval stage of development, vitamins are transferred from the yolk sac to larval body, with a net decline of vitamins during this period (Sato *et al.*, 1987). At the start of exogenous feeding, this net decline continues (Ronnestad *et al*, 1997) due to the early fry not being able to efficiently utilize their new feed source yet. If the feed source is lacking in these now depleted vitamins, the fry will be unable to replenish their reserves. At this sensitive stage, where fish are greatly affected by nutrient deficiencies, this can result in serious deficiency symptoms. B vitamin deficiencies are however, not obvious, especially at the early stages of

development of farmed fish where these high mortality rates are usually experienced (Waagbo, 2010). The B vitamins that are commonly found to be deficient in fish include thiamine (vtamin $B_1$), nicotinic acid (vitamin $B_3$), folic acid (vitamin $B_9$), Cyanocobalamin (vitamin $B_{12}$) (Hertrampf & Piedad-Pascual, 2012). Generally B vitamin deficiency symptoms include reduced growth and feed intake, with a few also leading to anaemia and changes in body colouration (Hansen *et al*, 2015). With the inability to store B vitamins, a net decline during the larval stage and the difficulty in identifying deficiency symptoms, it becomes important to supplement B vitamins during the early stage of development in fish. The additional supply of vitamins will also have the potential added benefit of improved production.

### 2.10.1 B vitamin biochemistry and supplementation

#### 2.10.1.1 Thiamine ($B_1$)

Thiamine is a very active molecule which has various roles in energy metabolism (Lonsdale, 2006). The active form of thiamine is thiamine pyrophosphate (TPP). It plays an important role as a cofactor for enzymes, such as pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and transketolase, which are involved in various carbohydrate metabolism processes (Hansen *et al.*, 2015). In the pyruvate dehydrogenase complex, TPP activates decarboxylation of pyruvate to form acetyl CoA. This together with oxaloacetate forms citrate, the first component of the citric acid cycle. Since TPP is a cofactor in the decarboxylating component of alpha-ketoglutarate dehydrogenase, it is another important link in the citric acid cycle. Transketolase, an enzyme that occurs twice in the pentose shunt requires TPP. The functions of the pentose shunt pathway are to provide pentose phosphate for nucleotide synthesis and to supply reduced Nicotinamide adenine dinucleotide phosphate (NADP) for various synthetic pathways (Lonsdale, 2006).

In fish, salmonid yolk-sac larvae have been shown to suffer sever mortalities due to thiamine deficiencies. This syndrome has been named M-74 syndrome and supplementation of thiamine has been successful in preventing it (Koski *et al.*, 2001; Tja *et al*, 2002). In a review of vitamin requirements for fish, Woodward (1994) reported finding improved weight gain in common carp, turbot and channel catfish with thiamine concentration of 0.5, 0.6 and 1.0mg/kg, respectively in their diet. In channel catfish, a deficiency in thiamine has been seen to result in neurological disorders, anorexia, poor growth, dark colouration, and increased mortalities (Wilson, 1991).

#### 2.10.1.2 Riboflavin ($B_2$)

Riboflavin is a precursor of flavin mononucleotide and flavin adenine dinucleotide, which are both important coenzymes for oxidation-reduction reactions in energy metabolism of all macronutrients (Hansen *et al.*, 2015). Riboflavin is also involved in the metabolism of vitamin

$B_6$, folate, vitamin $B_{12}$, and other vitamins (Thakur *et al.*, 2017). Rat models have indicated that riboflavin is important in the early development of the brain (Ogunleye & Odutuga 1989) and the gastrointestinal tract (Yates *et al.*, 2003). Riboflavin deficiencies in fish can lead to poor growth, anorexia, altered pigmentation, abnormal swimming behaviour, cataracts, photophobia and haemorrhages (Soliman & Wilson, 1992a; Paul *et al.*, 2010). It also the only vitamin to cause body dwarfism as reported by Serrini *et al.*, (1996) in a study on channel catfish. In a review study, Woodward (1994) found catfish to have a higher requirement of riboflavin, 9.0mg/kg diet, than other freshwater species. The requirement for riboflavin, like many other nutrients, is greater in younger fish (Amezaga & Knox, 1990). The supplementation of riboflavin in the diet of salmon parr has been show by Waagbo *et al.*, (2002) to increase lipid utilization. Li *et al.*, (2010) has demonstrated with Jian carp that riboflavin helped to improve growth performance, gross protein retention efficiency, lipid production values and intestinal development.

#### 2.10.1.3 D-pantothenic acid ($B_5$)

Pantothenic acid has a D- and L- isomer, with the D- isomer being the biologically active one (Sampedro *et al.*, 2015). Pantothenic acid is used in the formation of CoA and acyl carrier protein. These carry and transfer acetyl and acyl groups, respectively (Kelly, 2011). CoA is a vital cofactor functioning in over 70 enzymatic pathways (Sampedro *et al.*, 2015), some of which include fatty acid oxidation, lipid elongation and fatty acid synthesis (Kelly, 2011). Due to its importance in energy metabolism, tissues rich in mitochondria and tissues that experience rapid cell division, like those found in the gills, are especially vulnerable to pantothenic acid deficiencies (Hansen *et al.,* 2015). Clubbed gills, which is hyperplasia of the epithelial cells of the gill lamellae, is the most distinct symptom caused by a pantothenic acid deficiency (Soliman & Wilson, 1992b; Olsvik *et al.*, 2013).

The addition of calcium pantothenic acid into the diet of channel catfish fingerlings was found to be beneficial by Wilson *et al*, (1983) in improving growth performance and feed efficiency and at a dietary level of 15mg/kg it also helped to prevent gill lesions. Due to varying requirements, based on factors such as fish size, age, water temperature, fish density and oxygen availability, a recommendation of 30mg/kg diet is suggested. Similar results were found in green catfish by Hien & Doolgindachbaporn (2011a) where deficiency signs, such as anorexia, dark body colour and sluggishness were noticed at low levels of 5mg/kg but at high levels of 40mg/kg it improved growth and feeding performance.

#### 2.10.1.4 Pyridoxine ($B_6$)

Pyridoxine, pridoxal, and pyridoxamine are all forms of vitamin $B_6$ with equivalent activity in animals (Tucker, 2013). All forms are phosphorylated into the functional coenzyme pyridoxal

phosphate (PLP) which plays a major role in many enzymatic reactions, involving amino acids, such as transamination, decarboxylation, and dehydration (Zambonino-Infante & Cahu, 2010). In lipid metabolism, PLP is a cofactor for serine palmitoyltransferase which is involved in the biosynthesis of sphingolipids (Hanada, 2003). It participates in degradation of glycogen and biosynthesis of porphyrins (Halver, 2002). As previously mentioned, PLP also acts as a cofactor in the synthesis of L-carnitine, where it is required by the enzyme HTMLA (El-hattab & Scaglia, 2015). Pyridoxal phosphate is also a cofactor in the synthesis of important neurotransmitters (serotonin, dopamine, noradrenaline and adrenaline) which causes deficiencies in pyridoxine that commonly result in nervous disorders (Hansen *et al.*, 2015). Other vitamin $B_6$ deficiency symptoms in fish include anorexia, anaemia, changes in skin colouration, changed behaviour and damage to organs (Hansen *et al.*, 2015).

In channel catfish, Andrews & Murai (1979) found that pyridoxine hydrochloride supplemented at 2.2mg/kg diet alleviated deficiency signs such as anorexia, nervous disorders, tetany, greenish-blue body coloration and eventual mortality. A dietary level of 3mg/kg was found to the best for optimal growth. Similar results were found by Mohamed (2001) in Indian catfish fed on semi-purified diets containing varying levels of pyridoxine. It was also demonstrated that levels above 3.4mg/kg resulted in less weight gain but still performed better than the control. In Nile tilapia Teixeira *et al.* (2012) found pyridoxal HCl at a dietary level of 10mg/kg to be sufficient for normal growth. These fish were also subjected to heat stress and from various haematological parameters analysed it was determined that pyridoxine could help fish deal with heat stress. The importance of vitamin $B_6$ during the larval phase was illustrated by Ronnestad *et al.* (1997) who showed a net decline during the larval stage of halibut which continued into first feeding until they were developed enough to make efficient use of their feed source. This decline is attributed to vitamin $B_6$ being needed for protein synthesis and growth, which is characteristically high during the larval stage (Waagbo, 2010).

#### 2.10.1.5 Folic acid ($B_9$)

Tetrahydrofolate is the active form of folic acid (vitamin $B_9$) (Hansen *et al.*, 2015). Folic acid has several coenzymes derived from tetrahydrofolate, whose main function is to facilitate the transfer of one-carbon in a number of biological reactions (Luban, 1971). This includes important biosynthetic pathways that lead to the production of methionine, purine and pyrimidine. It also play a role in the interconversion of serine and glycine and catabolism of histidine (Lucock, 2000). Folic acid deficiencies are similar to that of other B vitamin deficiencies. A deficiency in folic acid leads to megaloblastic anaemia which is characterised by large immature erythrocytes (Hansen *et al.*, 2015).

It was demonstrated by Duncan & Lovell (1994) that the incorporation of folic acid into the diet could reduce the mortalities in channel catfish due to *Flexibacter columnaris*. In the same study, an interaction between folic acid and ascorbic acid was seen where the combination of the two led to further decreases in mortalities and increased antibody production. Hien & Doolgindachbaporn (2011b) also showed improved survival in green catfish, as well as growth and feeding with the addition of folic acid at 2mg/kg diet.

#### 2.10.1.6 Cyanocobalamin ($B_{12}$)

Vitamin $B_{12}$ is supplemented in fish feed as cyanocobalamin (Hansen *et al.,* 2015). It is only synthesised by microorganisms and is therefore, not found in plant feedstuff (Tucker, 2013). Like other B vitamins, vitamin $B_{12}$ acts as a coenzyme in several metabolic reactions. One important function of vitamin $B_{12}$ is to act together with folic acid, in the transfer of single-carbon units during biochemical processes such as methylation. Here it is needed for the conversion of tetrahydrofolic acid into its coenzyme form (Lovell, 2012). Therefore, a deficiency in vitamin $B_{12}$ can result in a deficiency in folic acid which can again lead to megaloblastic anaemia.

Limsuwan & Lovell (1981) found that the intestinal microorganisms of channel catfish have a limited ability to produce vitamin $B_{12}$, with a production of approximately 1.4ng/g of body weight. The ability of channel catfish microorganisms to produce vitamin $B_{12}$ was further seen to be reduced by the removal of cobalt from the diet or with the use of antibiotics. This is because cobalt forms the nucleus of vitamin $B_{12}$ (Lovell, 2012) and antibiotics destroy the intestinal microorganism population. Poor growth, appetite and reduced haematocrit can be seen when channel catfish are fed a vitamin $B_{12}$ deficient diet (Sugita *et al.*, 1991; Tucker, 2013). Some fish species, as demonstrated by Lovell & Limsuwan (1982) with Nile tilapia, show no deficiency symptoms when vitamin $B_{12}$ is excluded from their diet. This is due to their intestinal microorganism population being able to produce sufficient quantities of vitamin $B_{12}$ for normal growth (Sugita *et al.,* 1991). However, other fish species, whose intestinal microorganism population are not able to produce sufficient quantities of vitamin $B_{12}$, warrant the inclusion of it in their diet. As the production of vitamin $B_{12}$ is so reliant on microorganisms it becomes especially important to supplement this vitamin in the diet of fish larvae and fry who have yet to establish a developed intestinal microorganism population.

#### 2.10.1.7 Nicotinic Acid ($B_3$)

Nicotinic acid, also called niacin and vitamin $B_3$, is active in the form of niacinamide, which is a component of the coenzymes NAD and NADP (Lovell, 2012). These play an important role in enzyme systems which involve the transfer of $H^+$ and $e^-$ in a series of redox reactions in the metabolism of carbohydrates, lipids and amino acids (Hansen *et al.*, 2015). Niacin in the form

of $NAD^+$ also acts as a cofactor in the synthesis of L-carnitine, where it acts on the enzyme TMABADH (El-hattab & Scaglia, 2015). Niacin, like other B vitamins, is also involved in the synthesis of fatty acids, protein and deoxyribonucleic acid (DNA) (Tucker & Hargreaves, 2004). Deficiencies in Niacin mainly involve skin and fin damage, such as haemorrhages, lesions, erosion and dermopathies (Hansen *et al.*, 2015).

The dietary requirement for niacin varies between fish and even catfish species. In African Catfish, Morris *et al.* (1998) showed that a diet deficient in niacin resulted in feed refusal, listlessness, weight loss, poor feed utilization, skin haemorrhage, anaemia and high mortalities. Skin conditions could still be observed in a diet containing niacin at 17mg/kg but were not influential on fish health. This led to a recommended dietary level of 33.1mg/kg. This is higher than that recommended by Ng *et al.* (1997) of 7.4mg/kg for channel catfish. Ng *et al.* (1997) found similar deficiency symptoms in channel catfish fed a nicotinic free diet. Dietary niacin levels of 10mg/kg were suggested by Hien & Doolgindachbaporn (2011b) for green catfish, which resulted in improved growth and feeding performances. For Indian catfish, Mohamed & Ibrahim (2001) found growth performance to be best at a niacin level of 25mg/kg diet, with similar deficiency symptoms in the niacin free diet as other studies. It was also noticed that both the protein and lipid content was high with higher levels of niacin (20 and 40mg/kg).

#### 2.10.1.8 Biotin ($B_7$)

Biotin is required in several carboxylation and decarboxylation reactions, where it acts as a coenzyme for any one of five carboxylase enzymes, namely acetyl-CoA carboxylase 1 and 2, pyruvate carboxylase, propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase (Zempleni *et al.*, 2009). By covalently bonding with its specific carboxylase, biotin plays an important role in the transfer of $CO_2$ for several steps in intermediary metabolism (Yossa *et al.*, 2015). Acetyl-CoA carboxylase 1 is important in fatty acid synthesis while acetyl-CoA carboxylase 2 helps regulate fatty acid oxidation (Zempleni *et al.*, 2009). Pyruvate carboxylase is a key enzyme in gluconeogenesis while propionyl-CoA carboxylase is essential for amino acid metabolism (Zempleni & Mock, 1999; Hansen *et al.*, 2015). 3-methylcrotonyl-CoA carboxylase catalyses an essential step in leucine metabolism (Zempleni & Mock, 1999). Recently, biotin has been shown to be involved in cell signalling, epigenetic regulation of genes and chromatin structure (Zempleni *et al.,* 2009). Due to the enzymes that require biotin being involved in a range of metabolic systems, deficiency symptoms are quite wide and include organ damage, altered behaviour and skin discoloration (Hansen *et al.*, 2015)

Shaik Mohamed *et al.* (2000) who looked at the quantitative dietary biotin requirements of Asian catfish found deficiency signs, such as higher mortality and lower weight gain, specific

growth rate, feed efficiency ratio and protein efficiency ratio when fed a diet containing no biotin. From this study, they recommended a dietary biotin level of 2.49mg/kg. Similar deficiency symptoms were also experienced by Shaik Mohamed (2001a) with Indian catfish, however a lower dietary biotin level of 0.25mg/kg was suggested. Both these studies showed that a biotin supplemented diet has a positive effect on growth and feeding performance, with Shaik Mohamed (2001a) also finding high body protein and lipid content with biotin supplementation. When comparing purified diets supplemented with biotin at 1mg/kg compared to diets containing biotin antagonist (freeze-dried egg whites), Lovell & Buston (1984) found similar deficiency symptoms in channel catfish, as seen in other studies, when given diets containing biotin antagonist. However, these deficiencies were not observed when a practical diet was used (one containing soybean meal and corn, and another containing soybean meal, corn and fishmeal). This indicates that biotin supplementation is not needed for normal growth when using a conventional diet. However, Robinson & Lovell (1978) suggested that biotin supplemented at 1mg/kg helped to improve maximum growth and lipid utilization rate of channel catfish.

## 2.11 Nucleotides

### 2.11.1 Functions of nucleotides

Nucleotides are made up of a nitrogenous base (purine or pyrimidine), a pentose sugar (ribose or 2'-deoxyribose) and one or more phosphate groups. Nucleosides consist of just the nitrogenous base and pentose sugar portion (Uribe *et al.*, 2011). Nucleotides are important precursors for nucleic acids and therefore, play a variety of significant roles in the body which include precursors of nucleic acids, energy metabolism (ATP being the main form of cellular chemical energy), cellular signalling, constituents of coenzymes, activated intermediates, allosteric effectors and cellular agonist (Carver & Walker, 1995).

### 2.11.2 Source of nucleotides

Nucleotides are supplied to the body by *de novo* synthesis, salvage pathways and in the diet (Boza & Martinez, 2002). In the *de nova* synthesis process, purine rings are synthesized from derivatives of glycine, aspartate, glutamine, tetrahydrofolate and $CO_2$ in the cytosol, while pyrimidines are synthesized from aspartate, glutamine and $CO_2$ in cytosol and mitochondria (Li & Gatlin, 2006). This process however, uses valuable raw materials, requires a lot of energy and takes time (Barness, 1994). The salvage pathway is a more energy efficient process of obtaining nucleotides than *de novo* synthesis. It also allows for maintenance of nucleotides in cells that are incapable of performing *de novo* synthesis and for conservation of energy, by recovering bases resulting from endogenous nucleotide and nucleic acid breakdown (Boza & Martinez, 2002). When nucleotides are provided in the diet, there is a shift toward salvage

pathway to preserve energy (Low *et al.,* 2003). This is important, especially during early development where energy reserves are limited, and many biological pathways, such as the *de novo* synthesis of nucleotides, are still underdeveloped. By supplying nucleotides in the diet, it takes the body's reliance off the energy costly *de nova* synthesis pathway and promotes the salvage pathway by providing it with preformed nucleosides and nitrogenous basses (Boza & Martinez, 2002).

### 2.11.3 Nucleotide supplementation

The presence of nucleotides in human breast milk has led to nucleotides being supplemented in formula given to babies with great success in growth and improved immunity (Grimble & Westwood, 2001). The supplementation of nucleotides to babies has been so successful that it was recommend to the United States Food and Drug Administration for approval (Aggett *et al.*, 2003). Research into nucleotide supplementation in diets has led them to be considered as conditionally essential nutrients (Madalla *et al.*, 2013). These conditions would be during period of rapid growth and stress, which is characteristic of the early developmental stages and high intensity stages in aquaculture. In aquaculture the use of nucleotides has shown some success in improved growth, immunity, stress resistance, intestine morphology and as a feed chemoattractant (Huu, 2016).

#### 2.11.3.1 Feed Chemoattractant

In aquaculture, early interests in nucleotides was due to them serving as a possible feed attractant (Li & Gatlin, 2006). This ability has been successfully demonstrated for a variety of species. Kiyohara *et al.*, (1975) found the presence of a chemoreceptor on the lips of puffer fish that responded to some nucleotides. Inosine and inosine monophosphate were found to have the greatest stimulatory effect for turbot when 47 nucleosides and nucleotides were compared by Mackie & Adron (1978). For some marine fish species, Ishida & Hidaka (1987) found that uridine monophosphate was the most effective chemoattractant, followed by adenosine diphosphate and inosine monophosphate. The responses of fish to nucleotides as a chemoattractant differs between species (Li & Gatlin, 2006). The ability of nucleotides to act as a chemoattractant for feed can translate into improved growth as a result of increased feed intake, less feed wastage and decreased nutrient seepage due to an improved feeding reaction.

#### 2.11.3.2 Growth

Research has recently started to focus on other possible benefits of nucleotide dietary supplementation, such as health and related growth benefits. Enhanced growth performance, due to nucleotide supplementation, has been reported in many species. It was found by Ruyet *et al.,* (1983) that the nucleotide inosine was an effective attractant for turbot larvae.

Supplementation during the weaning period also helped to improve growth and survival. With only 10 days of supplementation in the diet there was already noticeable increases in weight gain, while at 5 days improved survival and FCR was seen. Yeast has been a good source of ribonucleic acid (RNA) and free nucleotides, and when used whole as a supplement itself, it is also high in vitamin B complex, amino acids and enzymes (Hossein *et al.*, 2011). For this reason, various forms of yeast are commonly used in nucleotide supplement studies where they are used whole or nucleotides are extracted from the yeast. In hybrid tilapia (*O. niloticus* × *O. aureus*), Xu *et al.*, (2015) found improved weight gain and feed efficiency due to a supplemented nucleotide mixture, originating from yeast at a 0.60% dietary inclusion level. This could be attributed to improved intestinal growth which is important for the utilization of nutrients provided. This is supported by the work of Burrells *et al.* (2001a) on Atlantic salmon, who found improved growth and intestinal surface area when supplemented with a commercial nucleotide mixture (Optimun) at 0.03% inclusion level. With the same commercial nucleotide mixture at 1.5-2g/kg diet, Tahmasebi-Kohyani *et al.* (2011) found that rainbow trout exhibited better growth and FCR. Yellow catfish supplemented with a nucleotide mixture in their diet at a rate of 1.5g/kg, were shown to have a better specific growth rate (SGR) and feed efficiency with a higher protein efficiency ratio and whole-body lipid content (Zhao *et al.*, 2017). Improved growth performance with nucleotide supplementation has also been seen in other species like grouper at 1.5g/kg diet (Lin *et al.*, 2009), during the first week of supplementation in red drum at 0.03-0.3% inclusion in the diet (Li & Gatlin, 2007), and in Beluga sturgeon at 0.35% inclusion level (Abtahi *et al.*, 2013). Although dietary supplementation of nucleotides was seen to improve the intestine of striped catfish, through increased mid-intestine folds and enterocyte heights, Yaghobi *et al.* (2015) failed to see this translate into improved growth performance. From early studies with striped catfish and nucleotide supplementation, Yaghobi *et al.* (2014) also failed to see improvement in the growth parameter except for the condition factor at an inclusion level of 0.75 and 1%.

#### 2.11.3.3 Immunity

Improved immunity and stress tolerance from nucleotide supplementation in the diet has been very successful in aquaculture. With regards to the innate immune response, the stimulation of this due to nucleotide supplementation has been seen mainly in terms of elevated serum lysozyme activity, as seen by Xu *et al.* (2015) in hybrid tilapia and Ozluer-Hunt *et al.* (2016) in rainbow trout. However, where they failed to see improvement in the alternative complement activity, another important part of the innate immune system, Tahmasebi-kohyani *et al.* (2011) and Sakai *et al.* (2001) were able to demonstrate this together with increased lysozyme activity in rainbow trout and common carp, respectively. Sakai *et al.* (2001) was also able to demonstrate this stimulatory immune response with a supplementation period of only 3 days,

with the beneficial effect being experienced 10 days post treatment. Welker *et al.* (2011) failed to find any innate immune stimulatory response in channel catfish.

#### 2.11.3.4 Pathogens

By improving the immune system with nucleotide supplementation it has the potential to help fish become more resistant to harmful pathogens commonly experienced in the aquaculture industry. Burrells *et al.* (2001b) was successful in demonstrating this, as salmonids supplemented with nucleotide were more resistant to infection with *Vibrio anguillarum*, *Piscirickettsia salmonis*, salmon anaemia virus and infesting sea lice. Tahmasebi-kohyani *et al.* (2011) added to this with improved survival of rainbow trout when challenged with *Streptococcus iniae*. Other fish species that have found success with improved survival after being challenged with pathogens include hybrid striped bass challenged with *S. iniae* (Li et al., 2004) and red drum with *V. harveyi* (Li & Gatlin, 2007).

#### 2.11.3.5 Stress tolerance

Stress can greatly affect the health and growth of fish. However, owing to the nature of intensive aquaculture, stressors are plentiful. Common stressors include aspects such a normal aquaculture practices (i.e. crowding and handling) or altered water quality. Periods of high growth and development, which is characteristic of the juvenile phase, also put a large amount of stress on the fish. Improved nutrition is an effective way to help fish better cope with stress, and nucleotides are one such nutrient that have been seen to be beneficial to improve stress resistance (Li & Gatlin, 2006). Burrells *et al.* (2001a) was one of the first aquaculture studies to test improved stress tolerance due to nucleotide supplementation. This was done by seeing the response of Atlantic salmon after being transferred from fresh water to saltwater in one trial, and in another trial their response after vaccination. With stressors like these a decreased growth rate would usually be found. The nucleotide supplemented diet was found to prevent this suppression in growth, giving a good indication of improved stress tolerance. Improved osmoregulation, which would help with saltwater transfer stress, was also noticed by lower plasma cortisol levels. High plasma cortisol levels is a general indication of stress in fish. Leonardi *et al.* (2003) was able to give further evidence of improved stress tolerance by evaluating cortisol level of rainbow trout subjected to infectious pancreatic necrosis virus. In the study they found that fish which received nucleotides in their diet showed decreased plasma cortisol levels and were able to survive until the end of the study 60 days later. Whereas, without nucleotide supplementation, all fish were dead 8 days after infection. Lower cortisol levels due to nucleotide supplementation was supported by Welker *et al.* (2011) in channel catfish after being subjected to lower water levels. Another sign of stress linked to cortisol levels would be plasma glucose levels, as elevated cortisol levels stimulates gluconeogenesis (Barton & Iwama, 1991). Kenari *et al.* (2013) found both reduced cortisol and

glucose levels in Caspian brown trout fed a nucleotide supplemented diet after being subjected to a confinement stress test and salinity stress test.

#### 2.11.3.6 Live feed nucleotide enrichment

In juvenile fish, where there is a low cell death rate, the ability to obtain nucleotides by the salvage pathway is reduced. With the possibility of the *de novo* synthesis pathway also not being fully developed yet, it becomes vital to supplement nucleotides in the diet of young fish in order to obtain optimal growth and good health. Despite this understanding, research into nucleotide supplementation during the early developmental stages is limited (Huu, 2016). Lanes *et al.*, (2012) investigated nucleotide supplementation for Atlantic cod larvae and found great success by enriching artemia found to have a poor nucleotide profile, with two commercially available nucleotide products. Once the artemia was enriched with nucleotides, the nucleotide profile of the artemia improved. This improved nucleotide profile of the live feed led to a high dry weight of Atlantic cod larvae fed with it. Studies on live yeast, which is a good source of nucleotides and a common fish larval feed, can give further indication of the importance that a good source of nucleotides can have on fish larvae. Infante & Cahu (2004) who researched the inclusion of live yeast (*Debaryomyces hansenii*) in the diet of European sea bass found multiple benefits including improved survival, reduced deformation, improved development and a greater final weight. With the limited amount of research done in nucleotide supplementation during the early developmental stages and the success of studies that have looked at it during this stage, further investigation is called for.

### 2.12 Nutrients in water

Water plays a big role in all aspects of fish's life as it forms their living environment. For this reason, fish not only have the ability to utilize nutrients in their feed but also in their surrounding waters. In most fish species, the digestive tract accounts for the majority of nutrient absorbed from the water, with the gills and skin of fish having some capability (Glover *et al.*, 2013). The uptake of minerals by the gill due to a concentration gradient is well documented in fish (Terech-Majewska *et al.*, 2016). This may indicate the ability of the gills to utilize other soluble nutrients at high concentration in the water, although it is not well documented, it is commonly suggested (Limsuwan & Lovell, 1981).The skin of fish has limited permeability but this may be increased during the larvae phase to assist the still underdeveloped gut with nutrient absorption (Glover *et al.*, 2013). It was previously thought that the main route of uptake for immersion vaccinations was through the gills, but it has been shown that when vaccines are given in the water, a significant amount of antigen is located in the intestinal tract, indicating that fish are drinking the vaccine they are immersed in (Robohm & Koch, 1995). With fish gaining the ability to drink water at an early age, before they are able to ingest large feed

particles, water provides a way of introducing substances to the fish at an earlier age (Dalmo *et al.*, 2000). Drinking is a more significant feature during the juvenile phase than the adult phase. Tytler *et al.*, (1990) noticed higher drinking rates in juvenile rainbow trout than that of adults, with the rate of drinking increasing over the course of the juvenile phase. A notable increase in drinking was also found during the transition from endogenous to exogenous feeding and during feeding periods. Successful implementation of water-soluble nutrient supplementation has been demonstrated with salmonids where yolk-sac larvae, with low levels of thiamine resulting in early mortality syndrome (EMS), have been supplemented through bath immersion with improved survival (Koskil *et al.*, 1999; Lee *et al.*, 2009). The main route of uptake of water-soluble nutrients like vitamins, L-carnitine and nucleotides from the environment is however, still unknown (Harder *et al.,* 2018). Success in vaccination and probiotic immersion technique helps to illustrate the benefits of the immersion method for administering substances over other methods, such as feed an injection (Villumsen & Raida, 2013). The possible benefits of providing nutrients in the water, rather than in the feed, could include earlier access to nutrients without being limited by mouth gape, equal access to nutrients due to less competition and uniform dispersion in the water, continual nutrient uptake during periods of stress, and increased nutrient availability above that which is provided in the feed (Jahangiri & Esteban, 2018). The regular ingestion of water also resulting in the unselective uptake of substances contained in the water, which prevents fish from actively not consuming the supplement (Robohm & Koch, 1995; Nursaliza *et al.*, 2016). The success of currently used water administration methods suggests that further investigation is required to determine the effectiveness and capability of water as an alternative mode of administrating other substances, such as water-soluble nutrients, especially during the larval and fry stages.

# Chapter 3

# Methodologies

This section explains the way in which the study was conducted. It includes the experimental design, the facility and rearing system used, the husbandry of the African catfish, and the parameters recorded and analysis of them throughout.

Ethical approval for the study was obtained from the Research Ethics Committee: Animal Care and Use (REC: ACU) from the University of Stellenbosch (protocol number: 1087).

## 3.1 Experimental design

A commercially available liquid nutrient supplement, Aquahatch (Nutrition Hub, Stellenbosch, South Africa), which consisted of a mixture of L-carnitine, nucleotides and B vitamins (Table 3.1) was used as the treatment. This supplement had never been tested on fish before. Aquahatch was added at three concentrations to the rearing tanks of African catfish immediately after hatch and remained in the tanks for a period of 7 days (treatment period). A control, where no Aquahatch was added to the rearing tanks of fish, was included for comparison. The different treatment concentrations were 0mL/L (T0), 0.25mL/L (T0.25), 0.5mL/L (T0.5) and 1.5mL/L (T1.5) with T0 considered as the control.

**Table 3.1** Nutrient composition of Aquahatch.

| Content | Concentration (mg/L) |
|---|---|
| Thiamine ($B_1$) | 4000 |
| Riboflavin ($B_2$) | 5000 |
| D-pantothenic acids ($B_5$) | 4000 |
| Pyridoxine ($B_6$) | 2000 |
| Folic acid ($B_9$) | 400 |
| Cyanocobalamin ($B_{12}$) | 20 |
| Nicotinic acid ($B_3$) | 10000 |
| Biotin ($B_7$) | 50 |
| L-carnitine | 30000 |
| Nucleotides | 10000 |

The study consisted of 3 trials. Trial 1 investigated the effect of Aquahatch on the yolk sac utilization and body growth during larval development and how Aquahatch behaved in the water. Trial 2 looked at the effect of Aquahatch on the survival during the yolk-sac larvae and early fry developmental phase. Trial 3 was a growth trial that looked at the influence Aquahatch had on the post treatment performance of African catfish when introduced during the larval and early fry developmental stage. All trials were run separately. Trial 1 and 2 were run at the same time period and used fish from the same spawning batch, whereas Trial 3 was run at a separate time period and used fish from a different spawn batch. Due to trials being run

separately, and potential differences that could have occurred between them (e.g. time, tank size, environmental, and fish stock), individual trials were seen as separate entities.

For Trial 1 and 3, each rearing tank contained 50L of water during the treatment period. For Trial 2, smaller tanks were used which contained 14L of water each. Each treatment was replicated nine times in Trial 1 and 3, and four times in Trial 2. Due to an initial complication in Trial 3, one of the replicates from T0.25 was discarded. In Trial 1, an 800mL sealed bottle was also floated in each tank. This bottle was filled with treated tank water at the beginning of the trial and represented a treated tank without the effect of fish. This was done in order to monitor the behaviour of the treatment in the water, with and without fish.

Aquahatch was added to the tanks soon after the yolk-sac larvae had been allocated to the tanks. The addition of Aquahatch was tempered by adding 0.25mL/L every 15 minutes until the final concentration was reached for each respective treatment. After the initial application, Aquahatch was not administered again for the remainder of the trials. The African catfish remained in the treated tanks for a period of 7 days, during which no flow or water exchange was provided. This was done to prevent dilution and contamination with other treatments. During the treatment period, tanks were thoroughly aerated to ensure a high dissolved oxygen level was maintained. Aeration was also continued after the treatment period. After the 7-day treatment period, Trial 1 and 2 were concluded. After the treatment period, Trial 3 continued but the tanks were first emptied, and new water added (i.e. devoid of Aquahatch). The system was then allowed to circulate and proceeded as normal for the remainder of Trial 3, which concluded on Day 104.

### 3.2 Experimental facilities

The trials were conducted at the Aquaculture Research Section of the Department of Animal Sciences which is located on the Welgevallen Experimental Farm of Stellenbosch University. The geographical coordinates of the facilities are: 33°56' 33.95" S and 18°51'56.15" E, Stellenbosch, Western Cape province, South Africa.

Trial 3 was conducted in a recirculating aquaculture system (RAS), the design of which is illustrated in Figure 3.1. The RAS system consisted of four rows of nine glass tanks (36 glass tanks in total), a 500L sump, 250L swirl filter, two trickle biological filters (0.8m wide, 0.7m wide, 3m high, with a 500L collection tank on the bottom), a 450W pump, 750W pump, 1100W pump and a 1.1kW blower. The 36 tanks had a volume of 100L each, set by the outflow pipe at the top of the tank. Water inflow into each tank was controlled by means of an adjustable inflow valve. Each tank was supplied with aeration by using air stones and a 1.1 kW blower (FPZ Effepizeta, SRL, Model SCL V4, Incorezzo, Milano, Italy). Trial 1 was conducted in the same system using the same tanks however, only made use of the aeration and not the

recirculating water. Trial 2 was conducted in the same facility but used 16 smaller 14L glass tanks with the same aeration source as the other trials and no recirculation of water between tanks. Trial 2 used smaller tanks to easily observe the lower larvae densities used. In all trials the water column height was the same. Before and between trials, tanks were cleaned by flushing them with clean water and drying them with a towel. The tanks were left to dry until a week before the start of the next trial.

Water temperature was dependant on the ambient temperature in the greenhouse. The air temperature inside the greenhouse was controlled by an air-conditioning unit that allowed for heating (when ambient temperature fell below the set point) and cooling (when the ambient temperature went above the set point) of the air, so that a set room temperature could be maintained within desired limits. Water temperature was recorded twice daily and if the water temperature was outside the desired range, the air-conditioning unit was adjusted accordingly to bring it back into the desired range. Tanks were covered with a lid to prevent fish from escaping. A black plastic sheet was draped over each row to prevent excessive light penetration during early fish development (Mukai & Lim, 2011). This sheeting remained on for the entire period of Trial 1 and 2 and was removed after day 21 for Trial 3.

Water flowed from the tanks to a 500L sump that contained filter mats that trapped solid particles. The mats were cleaned weekly using a pressure hose to ensure removal of solids that built up during the trial. At the same time the sump was drained, and clean water was added. The sump was fitted with two float valves connected to municipal water taps which were used to maintain the water level in the sump. Water was pumped, using a 750W pump, from the sump to a 250L swirl filter, there after the water was returned to the sump. The bottom of the swirl filter was partially drained every day to remove solids that had settled at the bottom. Water was pumped from the sump using an 1100W pump to two trickle biological filters (0.8m wide, 0.7m wide, 3m high, with a 500L collection tank on the bottom). These biological filters helped reduce the total ammonia nitrogen (TAN) load in the system. Water was pumped from the collection tank using a 750W pump to the glass tanks.

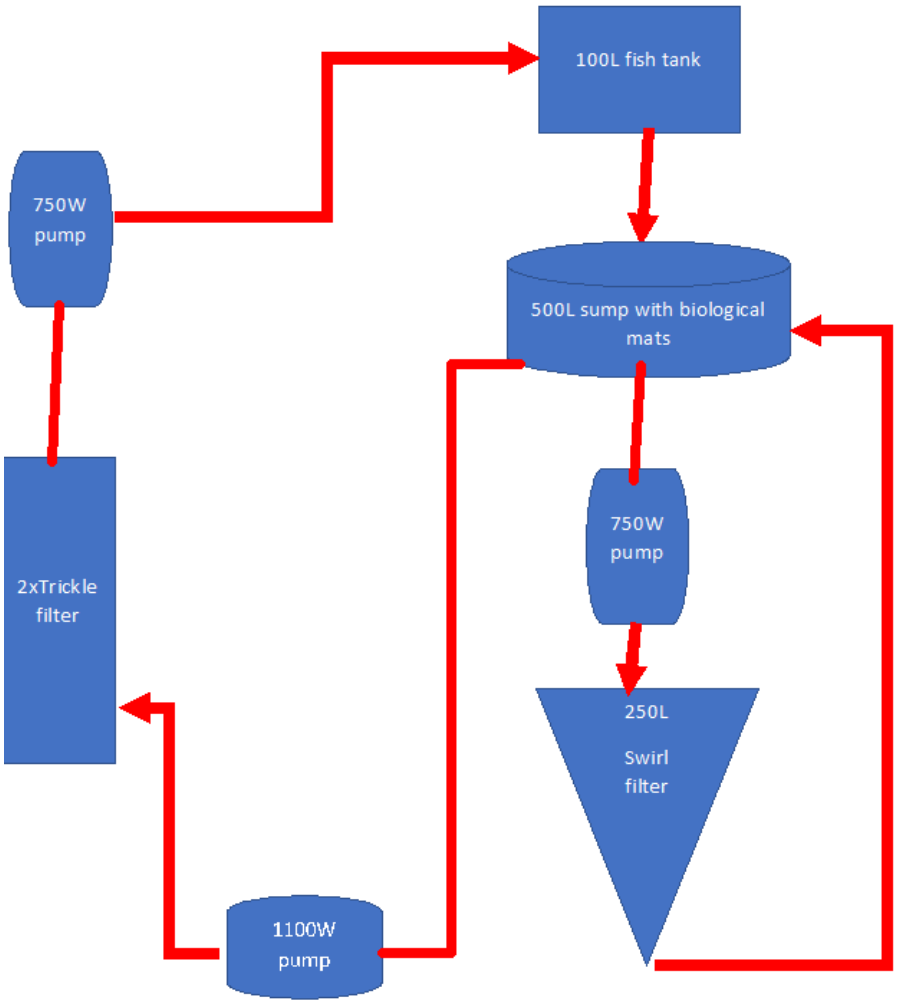


**Figure 3.1** A flow diagram indicating the design of the recirculating aquaculture system.

### 3.3 Experimental animals and husbandry

The animals used in the study, African catfish, were obtained from artificial spawning of African catfish broodstock which were maintained at the Aquaculture Research Section of the Welgevallen Experimental Farm of Stellenbosch University. Trial 3 used larvae obtained from a different spawning than that of Trial 1 and 2, which both used larvae obtained from the same spawning. The artificial spawning procedure, along with the collection and incubation of eggs to obtain the yolk-sac larvae, is described under experimental procedures.

Once 90% of the fertilized eggs had hatched to produce yolk-sac larvae, the larvae were carefully siphoned out of the incubation tanks and randomly divided into 36 2L containers. This was done to obtain densities of larvae in each container that were visually judged to be similar. Visual determination of densities was carried out to avoid prolonged handling, netting and removal of the fragile yolk-sac larvae from the water (Viveen *et al.*, 1977). It also allowed for a relatively short time period between hatching, placing and the administration of the

treatment to the fish. High fish densities were required for fish samples to be taken throughout Trial 1 and 3. Trial 2 did not require high fish densities, therefore, only 30 fish were used. These were counted and placed using a siphon. Each container was then floated in their respective glass tank to acclimatise the fish. Since the incubation tanks were housed in the same room as the glass tanks, both water temperatures were effectively equal, allowing for the fish to be released into the tanks after 30 minutes. On Day 21 of Trial 3, once the necessary fish samples had been taken, the number of fish was reduced to 200 fish per tank to allow for growth during the remainder of the trial. Fish growth in Trial 1 and 2 lead to the fish developing into early fry by the end of the trials. Fish growth in Trial 3 led to the fish developing into advanced fingerlings by the end of the trial.

The fish were maintained according to guidelines for African catfish production (Delince *et al.*, 1987). Fish were checked daily for signs of diseases and stress, such as reduced feed intake, change in behaviour and sudden mass mortalities. If mortalities were found, they were removed when seen.

### 3.3.1 Feeding

The larvae relied on endogenous feeding from their residual yolk until the beginning of Day 3, at which point they had used up the majority of their yolk sac nutrient reserves and developed into early fry. At this stage the early fry started swimming up to the water surface which signalled the start of exogenous feeding. From the beginning of Day 3 to the end of Day 7, the fry were fed *ad lib* four times a day (Verreth & Denbieman, 1987) to saturation using decapsulated artemia cysts (Great Salt Lake Brine Shrimp Cooperative, Utah, USA). The fry were judged to be satiated by monitoring them for reduced swim-up behaviour and by observing their translucent gut (which went bright orange from eating the decapsulated artemia cysts). Since Trial 3 continued after Day 7, the fish were continued to be fed *ad lib* with guidance from fish behaviour, biomass from previous sampling and water quality conditions. After day 7 the fish were weaned onto a formulated diet. The fish were weaned from the beginning of Day 8 to the end of Day 12 onto a commercial trout diet (Specialized Aquatic Feeds, Hermanus, South Africa) by feeding 50% decapsulated artemia cysts and 50% trout diet (New, 1987). At the beginning of Day 13 they received only the trout diet which the fish continued to be fed on until the end of Day 66. From the beginning of Day 67 until the end of the trial, Day 104, a formulated commercial catfish diet (Nutrition Hub, Stellenbosch, South Africa) was fed to the fish. From Day 21 onwards, fish were fed equal portions between two feeding sessions a day, i.e. in the mornings between 08:00-09:00 and in the afternoons between 16:00-17:00. The feed containers were weighed (UWE DM-11000) before each sampling period to calculate the amount of feed given.

### 3.3.2 Water quality

Throughout each trial temperature, dissolved oxygen (DO), TAN and pH were monitored.

#### 3.3.2.1 Trial 1

Temperature, DO and pH were measured in 6-hour intervals at the same time other samples were collected. After 72-hours, at the end of the yolk-sac period, temperature and DO were measured twice daily before morning and afternoon feeding using an Oxyguard probe. pH measurements were also reduced to once a day in the morning using a Sega waterproof pH pen. Due to technical difficulties, no pH readings were recorded for Hour 36 and 42, and no water quality reading were recorded for Hour 66. Total ammonia nitrogen was measured in every tank before the start of the treatment period, at the end of the 72-hour yolk-sac period, and at the end of the treatment period, using a Hach DR850 colorimeter. Total ammonia nitrogenwas measured according to the Nitrogen, Ammonia (0 to 0.50mg/L $NH_3$-N) Salicylate method (Hach Company, 2013a). Due to high TAN levels on Day 3 and 7, the sample water was diluted 50x with deionized water in order to record a reading, values were adjusted accordingly (Hach Company, 2013a).

#### 3.3.2.2 Trial 2

Water temperature and DO were measured twice daily before morning and afternoon feeding using an Oxyguard. The pH was also measured once a day in the morning before feeding using a Sega waterproof pH pen. Total ammonia nitrogen was measured at the start of the trial, on Day 3 and at the end of the trial using a Hach DR850 colorimeter. Total ammonia nitrogen was measured according to the Nitrogen, Ammonia (0 to 0.50mg/L NH3-N) Salicylate method. High TAN levels in the sample were diluted 50x with deionized water in order to record a reading, values were adjusted accordingly (Hach Company, 2013a).

#### 3.3.2.2 Trial 3

Temperature and DO of the tanks were monitored twice daily before feeding, using an Oxyguard probe. When TAN was low during the trial, it was read using a Hach DR850 colorimeter according to the Nitrogen, Ammonia (0 to 0.50mg/L $NH_3$-N) Salicylate method (Hach Company, 2013a). When TAN was high (in a range that the DR850 could not read) during the trial, an ammonia probe (Intellical™ ISENH318101 Ammonia Ion Selective Electrode) connected to HQ40D Portable Multi Meter was used to take readings, according to operational manual (Hach Company, 2013b). Total ammonia nitrogen was measured at the start of the trial, at the end of the treatment period and then weekly onwards until the end of the trial. The pH was measured at the same time as TAN, using a Hanna pH 211 microprocessor bench meter.

## 3.4 Experimental procedures

### 3.4.1 Artificial spawning, collection of eggs and incubation protocol

Three female (Trial 1 and 2: individual live weights = 1192g, 1260g, 1314g, Trial 3: individual live weights = 709g, 747g, and 661g) and two male (Trial 1 and 2: individual live weights = 1322g and 1256g, Trial 3: individual live weights = 950g and 853g) African catfish broodstock were sourced from the Aquaculture Research Section of the Welgevallen Experimental Farm. The fish were induced to spawn to obtain oocytes (from the females) and spermatozoa (from the males), which were then subsequently fertilized. The females were induced to spawn using an adapted technique of De Graaf & Janssen (1996), which involved a hormonal treatment that made use of the synthetic hormone Ovaprim (Syndel, USA).

To determine the hormone treatment dosage, the broodstock females were anaesthetised in a bath of clove oil at a dosage of 2.5mL/L to reduce handling stress (Diyaware *et al.*, 2015), and then individually weighed (UWE DM-11000). Once weighed, each fish was placed on a damp towel to keep them moist. Their head and eyes were covered with the towel to further reduce stress. The dosage of hormone, as calculated according to the instructions of the manufacturer (0.5mL hormone/kg fish weight), was injected into the dorsal muscle of the fish. Once injected, each female fish was transferred to their own individual plastic cage, which were floated in the broodstock rearing tank. Each cage was fitted with a lid to prevent the fish from escaping. Water temperature was monitored and controlled a week before injecting the fish to ensure that it remained stable. At the time of broodstock fish injection and placement, the average water temperature was 26°C for all trials. The table of time intervals between injections and stripping of female catfish in relation to water temperature (Table 3.2) was used to determine when the female fish would be ready to release their eggs.

**Table 3.2** The time interval (hours) between hormone injection and stripping of the eggs from the female African catfish in relation to water temperature (°C) (De Graaf & Janssen, 1996).

| Water temperature (°C) | Time between hormone injection and egg stripping (hours) |
|---|---|
| 20 | 21 |
| 21 | 18 |
| 22 | 15.5 |
| 23 | 13.5 |
| 24 | 12 |
| 25 | 11 |
| 26 | 10 |
| 27 | 9 |
| 28 | 8 |
| 29 | 7.5 |
| 30 | 7 |

The females were inspected for egg release an hour before the expected release of the eggs given by Table 3.2. Inspection was carried out by lifting each female out of the water with a

net to see whether the weight of the net on the body, as caused by the live weight of the fish, with additional application of a slight amount of pressure, would cause eggs to be released. Once the females started releasing some eggs, they were returned to their cages, and the males were prepared for testes removal.

The two males were caught using a net, and anaesthetised in a bath of clove oil at a dosage of 2.5mL/L (Diyaware *et al.*, 2015). They were then sacrificed by cervical dislocation with a sharp knife to collect the testes. Each male was then dissected and opened by cutting from its vent towards the gills along the belly line, and then both testes were removed. The testes were tapped dry with a paper towel to remove excess blood. The respective males' testes were then transferred to a sterile glass beaker and 0.9% saline solution (Adcock Ingram Critical Car Pty Ltd, Midrand, South Africa) was added to completely cover the testes. One pulse of a 300W hand blender (Braun, South Africa) was then used to slice through the testes tissue to release the sperm. The resulting mixture of sperm, saline solution and teste tissue was sieved using a plastic 1mm strainer to remove testes tissue and yield a solution containing only spermatozoa. The creation of this solution allowed for the sperm to be easily mixed with the eggs for fertilization. The solution was stored for a short time at room temperature while the females were prepared for egg collection.

To allow for egg collection, each female was immersed in a bath containing clove oil at a dosage of 2.5mL/L (Diyaware *et al.*, 2015) until completely anesthetized. Each female was then removed, dried off and pressure was gently applied to the abdominal area to allow for the assisted release of the eggs. These eggs were collected in a dry bowl. In Trial 1 and 2 all three females produced 507g of eggs, in Trial 3 they produced 209g of eggs.

The eggs were immediately fertilized by adding the sperm solution slowly over the eggs and gently mixing them together using a clean paint brush. Clean water from the incubation tank was then added into the bowl and mixed using a circular motion for one minute. The addition of the water naturally caused the eggs to become sticky, however, the circular motion of mixing prevented the eggs from sticking to the bowl for a short period. The eggs were then poured in a single layer over a mosquito net with 1mm pores. The stickiness of the eggs allowed them to adhere to the netting. The mosquito net was placed into four 700L incubation tanks that were thoroughly aerated in a manner that submerged all the eggs under the water but prevented them from touching the bottom of the tank. This enabled the nearly hatched yolk-sac larvae to fall through the pores while the mosquito netting, with left-over egg casings, could be removed from the incubation tank.

The water temperature of the incubation tank was monitored and controlled a week leading up to placement of the eggs to ensure that it remained stable. At the time of the placement of

the eggs, the water temperature of the incubation tanks averaged 26°C for all trials. The table of time intervals between fertilization and hatching of African catfish eggs in relation to water temperature (Table 3.3) was used to determine when the eggs would start hatching.

**Table 3.3** The time interval (hours) between fertilization and hatching of African catfish eggs in relation to water temperature (°C) (De Graaf and Janssen, 1996).

| Water temperature (°C) | Time interval (hours) between fertilization and hatching of eggs |
|---|---|
| 20 | 57 |
| 21 | 46 |
| 22 | 38 |
| 23 | 33 |
| 24 | 29 |
| 25 | 27 |
| 26 | 25 |
| 27 | 23 |
| 28 | 22 |
| 29 | 21 |
| 30 | 20 |

### 3.4.2 Trial 1: Yolk sac sampling and image analysis

Once the yolk-sac larvae had been placed into their respective tanks, 45 yolk-sac larvae from the left-over fish, which had not been exposed to the treatment, were randomly selected and preserved in 10% formalin for analysis of body morphology at a later stage. These 45 yolk-sac larvae served as a starting point for the trial. Six hours after placing the yolk-sac larvae and initial administration of the treatment, 20 fish from each tank were randomly siphoned out of the tank and caught on a grid to drain the water. They were then carefully placed, using a paint brush, into a 10mL tube containing 10% formalin solution for analysis of body morphology at a later stage. This was repeated in 6-hour intervals for 72 hours. Yolk-sac larvae were not collected at Hour 71 due to technical issues. A Zeiss Stereo Discovery V8 microscope with Zeiss Axiocam 105 color camera at Sea Point Research Aquarium was used to capture images of five yolk-sac larvae from each tank for each sampling period. The images were then exported to ImageJ for analysis of the total body length (mm), yolk sac length (mm) and yolk sac height (mm). Total body length was measured from the tip of the snout to the end of the caudal fin, following the lateral line. Yolk sac length and yolk sac height were respectively the horizontal and vertical measurements of the yolk sac relative to the lateral line of the fish (Figure 3.2). The yolk sac length and height were used to calculate the estimated volume of the yolk sac, using the formula for a prolate spheroid (Ing & Chew, 2015).

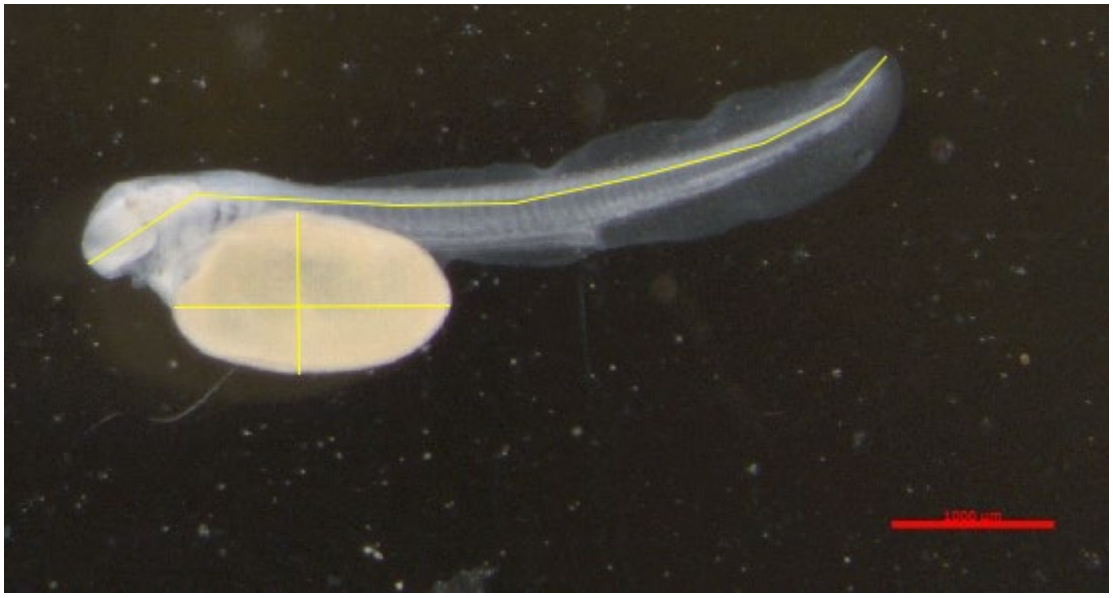

**Figure 3.2** Image analysis of yolk-sac larvae body morphology (body length, yolk sac length, yolk sac height) (bar =1000µm).

### 3.4.3 Trial 1: Spectrophotometry sampling for treatment behaviour in water

Spectrophotometry was used as a novel method of determining the concentration of Aquahatch in the water. The approach was adapted from guidelines given by Barwick (2003).

Before the start of Trial 1, a standard curve equation was generated to determine the concentration of Aquahatch in the water. This was based on the light absorbance values (nm) recorded for the water by a spectrophotometer (SPECTROstar Nano, BMG LABTECH). This was done by creating a series of known concentrations of Aquahatch with distilled water (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.6, 1.7, 1.8mL/L), and recording each light absorbance value standardized at a wavelength of 450nm, done in duplicates.

To determine the concentration of Aquahatch in the water during Trial 1, for the first 72 hours starting at Hour 6 and then every 12 hours after that, water samples were collected in sealable test tubes from both tanks containing fish and not containing fish. The water was filtered through a 125µm net to remove any large solid particles. They were then immediately analysed for treatment concentrations using a spectrophotometer. After 72 hours, water samples were analysed once a day in the morning until the end of the trial at 168 hours.

The water samples were analysed by pipetting 2mL of sample water into a cuvette and analysing its light absorbance value at a wavelength of 450nm, these tests were done in duplicates. The absorbance value of the sample water was then put through the previously created standard curve equation for light absorbance value vs concentration, in order to calculate the concentration of Aquahatch in the water.

### 3.4.4 Trial 3: Sampling

During Trial 3, three sampling methods were used to calculate the mean individual weight and growth parameters. Sampling method 1 (SM1) made use of the individual weight of 10 fish (5% of the total number of fish placed on Day 21). At the same time the individual length was recorded by means of image analysis described below. Sampling method 2 (SM2) made use of the combined weight of 20 fish (10% of the total number of fish placed on Day 21) and sampling method 3 (SM3) made use of the total biomass of the fish (with a fish count done through image analysis also described below). The days at which sampling was conducted can be seen under data collection and calculations. When weighing the fish, an EJ-300 scale (A&D Weighing) was used for weights less than 300g and a DM-11000 scale (UWE) was used for weights greater than 300g, unless stipulated.

At the end of the treatment period (beginning of Day 8) , before the tanks were replaced with clean, untreated water, 20 fish were collected (SM2) and preserved in 10% formalin solution (SANParks Animal Use and Care Committee, 2009) for the duration of transport to the lab, and were then weighed (PS 750.R2, RADWAG). On Day 21, the number of fish in each tank was reduced. 200 fish were randomly selected from each tank (SM3), weighed (EJ-300, A&D Weighing) in groups of 20 (SM2) and returned to their respective tanks. The remaining biomass of the fish was weighed (EJ-300, A&D Weighing) and then sacrificed by placing them in a bath of clove oil at a concentration of 1mL/L (10x the recommended aesthetic concentration) for longer than 10 minutes or until all signs of motion ceased (Neiffer & Stamper, 2009; Leary *et al.*, 2013; Adeshina *et al.*, 2016). Once the fish had been sacrificed, they were placed and sealed in an air-tight plastic bag, which was then placed in a freezer at -20°C for preservation until later analysis for proximate body composition (Oluwaseyi, 2016).

Shooters were removed at every sampling period starting from Day 21. “Shooters” is a common term for juvenile catfish which grow at a much faster rate than others of the same age (Biu *et al.*, 2015). In this study, shooters were classified as African catfish that were visually much larger than other fish in the same tanks. They were removed in an effort to reduce cannibalism. Not all tanks contained shooters at every sampling period. From Day 77 onwards it became difficult to identify shooters as individual size variation was too numerous. Therefore, the removal of shooters was discontinued for the remainder of the trial.

On sampling days, fish were sampled in the mornings and only fed in the afternoon. The tanks were drained, and the fish were netted into a large bucket of water. For SM1, 10 fish were anesthetised and then weighed individually. The fish were then placed, in the order they were weighed, onto a damp towel next to a ruler, so that a picture of all the fish together could be taken using a smartphone. At a later stage, the picture was analysed for total individual length

using image analysis described below. After the picture of the fish was taken, they were placed into a large bucket with aeration until they recovered from the anaesthetic. Once recovered, they were then returned to their original tank. For SM2, 20 fish were netted out of the bucket, weighed together and then placed into their original tanks. For SM3, the remaining fish biomass of the tank was netted out of the bucket and weighed. They were then placed into a large flat container, containing a damp towel, and spread out. A picture was then taken of the fish, to be analysed for fish count at a later stage, using image analysis described below. The fish were then placed back into their original tank.

At the end of the trial, Day 104, the normal sampling procedure was carried out including additional procedures. Five fish from each tank were used to collect at least 0.5mL of blood for haematocrit analysis. This was done by anesthetising the fish in a bath of clove oil, at a dosage of 0.1mL/L (Adeshina *et al.*, 2016) and then using a 27-gauge needle and 1mL syringe to withdraw blood. The withdrawal site was located at the base of the anal fin, towards the lateral line (Canada Department of Fisheries and Oceans Animal, 2004). The blood was immediately transferred to a micro haematocrit capillary tube, which was filled to the three-quarter mark. The tubes were then sealed on the one end with Critoseal and placed into a micro haemocytometer to be centrifuged, with the sealed end facing outwards. The micro haemocytometer was set to run for 5 minutes. Once done, the haematocrit value was determined using a graphic reader (Oluwaseyi, 2016). The 10 fish used for SM1 were also used to determine the viscera somatic and cephalosomatic index. The visceral organs of the fish were removed from the abdominal cavity by using a dissection kit to cut the digestive tract at the oesophagus and anus. The visceral organ cluster that was removed which included the gallbladder, liver, spleen, digestive system and abdominal fat deposits. The visceral organ cluster was then weighed. The cephalosomatic index was determined by removing the head of the fish, using a sharp knife, at the base of the skull. The head was then weighed (Oluwaseyi, 2016).

Three fish from each tank were also removed, sacrificed by cervical dislocation with a sharp knife, sealed in an air-tight plastic bag and frozen at -20°C to preserve them for later analysis of proximate body composition.

### 3.4.5 Trial 3: Image analysis

The image analysis procedure used in SM1 and SM3 made use of ImageJ image analysis software on a computer, which was free to download at the time of the study. The photos taken were downloaded onto a computer and uploaded onto ImageJ. In order to measure the total body length of the individual fish for SM1, each image was calibrated on ImageJ by using the ruler contained within the image. A segmented line tool was used to measure the distance

(mm) from the tip of the fish's snout, following the dorsal fin line, towards the tip of the caudal fin. In order to measure the fish count in SM3, a count tool was used to count the fish and keep track of which fish had already been counted.

### 3.4.6 Trial 3: Proximate composition analysis

Proximate composition analysis was carried out on experimental fish samples at the Department of Animal Sciences (Stellenbosch University, South Africa). The samples were analysed for crude fat, moisture, ash and crude protein, following the standard methods of AOAC (2002). All measurements were carried out in duplicate.

#### 3.4.6.1 Sample preparation

The frozen fish samples were thawed overnight in a 4°C walk-in fridge. For the fish samples taken on Day 21, the whole fish body was homogenised. For the fish sample taken on Day 104, the heads were removed as the skulls were too hard to be put through the homogenizer. The remaining fish body and visceral organs were cut into smaller pieces using a sharp knife and then homogenised using a homogenizer and a hand bender. The homogenised samples were then placed into a plastic vacuum-sealed bag and frozen at -20°C. The homogenised samples were thawed overnight in the 4°C walk-in fridge before analysis.

#### 3.4.6.2 Moisture

Moisture analysis was conducted according to the AOAC (934.01) International method for moisture analysis. Dry and clean crucibles of a known weight with a weighed-out homogenised sample of 2.5g were placed in an oven at 100-105 °C for 24 hours. After 24 hours they were cooled down in a desiccator for 30 minutes and then the weight of the moist free sample was taken.

#### 3.4.6.3 Ash

Ash analysis was conducted according to the AOAC International method for ash analysis (942.05). The 2.5g sample from the moisture analysis, that was now moist free, was placed in a furnace at 500°C for 6 hours. The furnace was then allowed to cool overnight. The samples were then placed in a desiccator for 30 minutes and then weighed.

#### 3.4.6.4 Crude lipid

Crude lipid was determined by Chloroform/methanol extraction method according to Lee *et al.* (1995). 3g of homogenised sample was weighed out and placed into a glass tube with 30mL of chloroform/methanol (2:1). The sample was then mixed with a blender and filtered through Whatman no. 1 filter paper into a separation funnel. 12mL of 0.5 % NaCl was added and the funnel gently shaken. The funnel was allowed to stand for 1 hour to give time for the solution to separate. The bottom layer of the solution was collected in a 100mL Erlenmeyer flask and

3mL was pipetted, using a 5mL pipette, into a previously weighed beaker. The beaker was placed onto a hot sand bed for 45 minutes until all the chloroform/methanol had evaporated, after which it was allowed to cool in a desiccator for 30 minutes and then weighed.

#### 3.4.6.5 Crude protein

Crude protein analysis was conducted according to the Dumas method using a LECO FP 528. The fish sample residue, left on the filter paper after total fat determination, was collected in a plastic vile and placed in an oven at 60°C for longer than 4-hours to dry out the sample. The sample was then ground using a pestle and mortar and placed back into the oven to remove any moisture gathered. The ground up sample was weighed on a foil cup, which was folded and combusted at ± 900 °C in the presence of oxygen using a LECO FP 528 (LECO FP 528, USA) connected to a computer to record % nitrogen. The LECO was calibrated using EDTA. The % nitrogen results were used to calculate the crude protein content of the fish sample using a conversion factor of 6.25.

### 3.5 Statistical analysis

A completely randomised experimental design was used with treatment as the main effect. Data was analysed by means of SAS Enterprise Guide (2002) for windows. PROC GLM was used for all the ANOVA for analysis of variance and PROC REG for regression analysis. Bonferroni post hoc tests were used to determine significant differences between the least-squares means with alpha of 0.05. Normality and homogeneity were tested using the Shapiro-Wilk's test and Levene's tests. Where data was not homoscedastic, Welch's ANOVA was calculated. Two tailed t-test were also performed in order to compare calculated gradients to a set value. Pearson's correlation coefficient (r) was used to determine correlation strength between certain parameters. Prior to all analysis, influential observations identified as outliers were removed. Data is presented as means ± standard error (SE). Differences were regarded as significant when $p \leq 0.05$. The regressions confidence was used indicate the change of certain parameters over time.

## 3.6 Sample collection and calculations

### 3.6.1 Trial 1

*Where numbers are related to the hour at which the data was collected

#### 3.6.1.2 Sample collection

- Individual total body length of larvae (mm): BL0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72
- Yolk sac length (mm): YSL 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72
- Yolk sac height (mm): YSH 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72
- Combined weight of 20 fish (g) (SM2): CW7
- Light Absorbance values (nm): A6, 18, 30, 42, 54, 72, 96, 120, 144, 168

#### 3.6.1.3 Calculations

$$\text{Equation 1: } VYS = \frac{\pi}{6} \times ((YSL)(YSH^2))$$

Where:

VYS = volume of yolk sac ($mm^3$)

YSL = yolk sac length (mm)

YSH = yolk sac height (mm)

$$\text{Equation 2: } BL{:}YSV = \frac{BL}{YSV}$$

Where:

BL:YSV = Individual total body length of larvae: Yolk sac volume ratio ($mm/mm^3$)

BL = Individual total body length of larvae (mm)

YSV = yolk sac volume ($mm^3$)

### 3.6.2 Trial 2

*Where numbers are related to the day at which the data was collected

#### 3.6.2.1 Sample collection

- Fish number: Nf1, 7

#### 3.6.2.2 Calculations

$$\text{Equation 3: Survival}(\%) = \frac{Nfa}{Nfb} \times 100$$

Where:

Nfa = number of fish before

Nfb = Number of fish after

### 3.6.3 Trial 3

*Where numbers are related to the day at which the data was collected

#### 3.6.3.1 Sample Collection

- Individual Weight of 10 fish (g) (SM1): IW43, 49, 57, 64, 71, 77, 84, 91, 98, 104
- Individual Total body Length of 10 fish (mm) (SM1): L49, 64, 77,91, 104
- Combined weight of 20 fish (g) (SM2): CW8, 21, 36, 43, 49, 57, 64, 71, 77, 84, 91, 98, 104
- Fish biomass in tank (g) (SM3): B21, 36, 43, 49, 57, 64, 71, 77, 84, 91, 98, 104
- Head Weight (g): head(wt) 104
- Viscera Weight (g): viscera(wt) 104
- Feed Weight (g): FW 21, 36, 43, 49, 57, 64, 71, 77, 84, 91, 98, 104
- Blood: B104
- Fish Number: Nf 21, 36, 43, 64, 71, 77, 84, 91, 98, 104
- Proximate Analysis: PA21, 104

#### 3.6.3.2 Calculations

$$\text{Equation 4: IW(SM2)} = \frac{CW}{Nf}$$

Where:

IW (SM2) = mean individual weight of fish obtained from sampling method 2 (g)

CW = combined weight of fish (g)

Nf = Number of fish.

$$\text{Equation 5: } IW(SM3) = \frac{B}{Nf}$$

Where:

IW (SM3) = mean individual weight of fish obtained from sampling method 3 (g)

B = biomass of fish (g)

Nf = Number of fish.

$$\text{Equation 6: } AGR_{Biomass} = Bf - Bi$$

Where:

$AGR_{Biomass}$ = Absolute Growth Rate for biomass (g)

Bf = final biomass (g)

Bi = initial biomass (g)

$$\text{Equation 7: } RGR_{Biomass} = 100\% - \left(\frac{Bi}{Bf}\right)$$

Where:

$RGR_{Biomass}$ = Relative Growth Rate for biomass (%)

Bf = final biomass (g)

Bi = initial biomass (g)

$$\text{Equation 8: } SGR_{Biomass} = \frac{\ln(Bf) - \ln(Bi)}{nt} \times 100$$

Where:

$SGR_{Biomass}$ = Specific Growth Rate of biomass (%/d)

ln(Bf) = natural logarithm of final biomass

ln(Bi) = natural logarithm of initial biomass

nt = number of rearing days

$$\text{Equation 9: } AGR_{Individual} = IWf - IWi$$

Where:

$AGR_{Individual}$ = Absolute Growth Rate for average individual weight(g)

IWf = final average individual weight (g)

IWi = initial average individual weight (g)

$$\text{Equation 10: } RGR_{Individual} = 100\% - \left(\frac{IWi}{IWf}\right)$$

Where:

$RGR_{Individual}$ (%) = Relative Growth Rate for average individual weight (%)

IWf = final average individual weight (g)

IWi = initial average individual weight (g)

$$\text{Equation 11: } SGR_{Individual} = \frac{\ln(IWf) - \ln(IWi)}{nt} \times 100$$

Where:

$SGR_{Biomass}$ = Specific Growth Rate of average individual weight (%/d)

ln(IWf) = natural logarithm of final average individual weight

ln(IWi) = natural logarithm of initial average individual weight

nt = number of rearing days

$$\text{Equation 12: } AGR_{Length} = Lf - Li$$

Where:

$AGR_{Length}$ = Absolute Growth Rate for average individual length (mm)

Lf = final average individual length (mm)

Li = initial average individual length (mm)

$$\text{Equation 13: } RGR_{Length}(\%) = 100\% - \left(\frac{Li}{Lf}\right)$$

Where:

$RGR_{Length}$ (%) = Relative Growth Rate for average individual length (%)

Bf = final average individual length (mm)

Bi = initial average individual length (g)

$$\text{Equation 14: } SGR_{Length} = \frac{\ln(Lf) - \ln(Li)}{nt} \times 100$$

Where:

$SGR_{Length}$ = Specific Growth Rate of average individual length (%/d)

ln(Lf) = natural logarithm of final average individual length

ln(Li) = natural logarithm of initial average individual length

nt = number of rearing days

$$\text{Equation 15: } CSI(\%) = \frac{head(wt)}{IW} \times 100$$

Where:

CSI = Cephalosomatic index (%)

head (wt) = head weight of fish (g)

IW = Individual weight of whole fish (g)

$$\text{Equation 16: } VSI(\%) = \frac{viscera(wt)}{IW} \times 100$$

Where:

VSI = viscerosomatic index (%)

viscera (wt) = viscera weight from fish (g)

IW = individual weight of whole fish (g)

$$\text{Equation 17: } CF = \frac{IW \times 100}{L^{b}} \times 100$$

Where:

CF = condition factor

IW = individual weight of fish (g)

L = individual length of fish (mm)

b = regression coefficient for the natural logarithm of weight and length

$$\text{Equation 18: } FCR = \frac{TFG}{AGR_{Biomass}}$$

Where:

FCR = feed conversion ratio (g/g)

TFG=total feed given (g)

$AGR_{biomass}$ = Absolute growth rate for biomass (g)

$$\text{Equation 19: } FR = \frac{TFG}{B}$$

Where:

FR = Feeding rate (%)

TFG=total feed given (g)

B = Biomass (g)

$$\text{Equation 20: } Hct(\%) = \frac{PRBV}{TBV} \times 100$$

Where:

Hct = haematocrit (%)

PRBV = packed red blood cell volume (%)

TBV= total blood volume (%)

$$\text{Equation 21: Cumulative mortalities}(\%) = 100 - \frac{Nfa}{Nfs}$$

Where:

Nfa = number of fish after

Nfs Number of fish at start

$$\text{Equation 22: Daily Mortalities}(\%) = \left(100 - \frac{Nfa}{Nfb}\right)/nt$$

Where:

Nfa = number of fish after

Nfb Number of fish before

nt = number of rearing days

$$\text{Equation 23: \% moisture} = \frac{(Wc+Ws)-Wmfs}{Ws} \times 100$$

Where:

Wc = Weight of crucible (g)

Ws = weight of sample (g)

Wmfs = weight of moist free sample (g)

$$\text{Equation 24: \% ash} = \frac{Wa-Wc}{Ws} \times 100$$

Where:

Wa = weight of ashed sample and crucible (g)

Wc = weight of crucible (g)

Ws = weight of sample (g)

$$\text{Equation 25: \% Crude lipid} = \frac{(Wb+Wfb)-(Wb)}{Ws} \times \frac{Vc}{Vp} \times 100$$

Where:

Wb = weight of beaker (g)

Wfb = weight of beaker with fat residue (g)

Ws = weight of sample

Vc = volume of chloroform (mL)

Vp = volume of solution pipetted (mL)

Equation 26: % crude protein=% nitrogen ×protein conversion factor of 6.25

# Chapter 4

# African catfish growth performance during Aquahatch supplementation

This chapter presents the results obtained from Trial 1 and Trial 2 of the study in the form of tables and graphs, with major findings highlighted. These results are then discussed and contextualized with what was observed while conducting the study and in other studies. A conclusion was then drawn from the results and discussion.

Trial 1 focused on the potential growth effects in terms of yolk sac utilization and body length during the Aquahatch treatment period. Trial 2 focussed on the survival of the African catfish during the Aquahatch treatment period. Water quality parameters were assessed in both trials.

## 4.1 Results

### 4.1.1 Trial 1

#### 4.1.1.1 Water quality

The water quality parameters measured from Hour 6 to Hour 72 were temperature, dissolved oxygen (DO), pH and total ammonia nitrogen (TAN) (Table 4.1-4.4). DO did not differ between treatments throughout the 72-hour yolk-sac period, with a mean of 7.69 ± 0.024mg/L for the entire period. Hour 12 and Hour 36 saw the water temperature of T0.5 (25.79 ± 0.14°C and 27.52 ± 0.09°C) differ ($p \leq 0.05$) to that of T0 (24.98 ± 0.21°C) and T0.25 (26.79 ± 0.39°C) therefore, in both cases T0.5 experienced higher temperatures. The mean temperature for the entire period was 25.45 ± 0.08°C. Hour 60 saw pH of T0.25 (5.97 ± 0.06) differ ($p \leq 0.05$) to T0 (6.16 ± 0.10) and T1.5 (6.2 ± 0.02), with T0.25 having a lower pH. The mean pH for all treatments for the entire 72-hours was 6.45 ± 0.03. Both Hour 72, the end of yolk-sac period, and Hour 168, the end of treatment period, saw higher TAN in T1.5 (7.44 ± 0.28 and 9.61 ± 0.77, respectively) than in all other treatments. TAN was seen to increase with increasing concentrations of Aquahatch.

**Table 4.1** Water temperature (mean °C ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 25.76±0.31 | 26.18±0.18 | 26.33±0.35 | 26.08±0.22 | 0.503 |
| 12 | $24.98^{b}$±0.21 | $25.18^{ab}$±0.19 | $25.79^{a}$±0.14 | $25.24^{ab}$±0.23 | 0.040 |
| 18 | 22.78±0.22 | 22.86±0.49 | 23.43±0.13 | 23.12±0.24 | 0.421 |
| 24 | 23.23±0.25 | 22.91±0.26 | 23.11±0.17 | 23.07±0.13 | 0.748 |
| 30 | 28.38±0.22 | 28.21±0.20 | 28.44±0.13 | 28.18±0.19 | 0.700 |
| 36 | $26.92^{ab}$±0.22 | $26.79^{b}$±0.39 | $27.52^{a}$±0.09 | $27.13^{ab}$±0.20 | 0.049 |
| 42 | 24.88±0.21 | 24.91±0.23 | 25.30±0.14 | 25.12±0.18 | 0.385 |
| 48 | 24.77±0.12 | 24.72±0.14 | 24.76±0.10 | 24.7±0.09 | 0.974 |
| 54 | 26.27±0.11 | 26.39±0.10 | 26.48±0.08 | 26.27±0.10 | 0.375 |
| 60 | 26.18±0.14 | 26.16±0.12 | 26.34±0.05 | 26.13±0.14 | 0.589 |
| 72 | 24.72±0.17 | 24.86±0.16 | 24.76±0.12 | 24.59±0.11 | 0.619 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.2** Dissolved oxygen (mean mg/L ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 7.88±0.13 | 7.74±0.11 | 7.71±0.04 | 7.89±0.04 | 0.361 |
| 12 | 7.89±0.11 | 8.09±0.07 | 7.77±0.12 | 7.91±0.09 | 0.165 |
| 18 | 8.22±0.08 | 8.27±0.06 | 8.12±0.09 | 8.02±0.12 | 0.234 |
| 24 | 8.20±0.08 | 8.29±0.08 | 8.18±0.04 | 8.2±0.06 | 0.650 |
| 30 | 7.41±0.09 | 7.54±0.08 | 7.33±0.05 | 7.18±0.15 | 0.094 |
| 36 | 7.73±0.12 | 7.49±0.29 | 7.67±0.03 | 7.29±0.18 | 0.317 |
| 42 | 7.78±0.16 | 7.92±0.10 | 7.87±0.11 | 7.71±0.07 | 0.581 |
| 48 | 7.88±0.18 | 8.08±0.05 | 8.08±0.05 | 7.81±0.11 | 0.208 |
| 54 | 7.50±0.08 | 7.36±0.08 | 7.52±0.04 | 7.43±0.07 | 0.328 |
| 60 | 7.11±0.12 | 7.09±0.12 | 7.04±0.05 | 6.97±0.11 | 0.759 |
| 72 | 7.32±0.12 | 7.31±0.12 | 7.31±0.05 | 7.24±0.11 | 0.947 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.3** pH (mean ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **6** | 6.19±0.05 | 6.19±0.05 | 6.13±0.04 | 6.07±0.03 | 0.129 |
| **12** | 6.42±0.06 | 6.37±0.06 | 6.29±0.05 | 6.29±0.06 | 0.272 |
| **18** | 6.53±0.05 | 6.44±0.08 | 6.44±0.06 | 6.47±0.06 | 0.705 |
| **24** | 7.04±0.08 | 6.89±0.12 | 6.92±0.07 | 6.97±0.08 | 0.651 |
| **30** | 7.28±0.10 | 7.23±0.12 | 7.01±0.10 | 7.11±0.11 | 0.299 |
| **48** | 6.36±0.08 | 6.52±0.19 | 6.66±0.18 | 6.59±0.09 | 0.496 |
| **54** | 5.86±0.07 | 6.04±0.12 | 6.02±0.09 | 5.93±0.07 | 0.447 |
| **60** | $6.16^{a}$±0.10 | $5.97^{b}$±0.06 | $6.03^{ab}$±0.05 | $6.20^{a}$±0.02 | 0.044 |
| **72** | 6.53±0.09 | 6.36±0.06 | 6.30±0.04 | 6.43±0.04 | 0.071 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.4** Total ammonia nitrogen (mean mg/L ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **0** | 0.173±0.026 | 0.127±0.019 | 0.113±0.008 | 0.166±0.015 | 0.068 |
| **72** | $2.06^{b}$±0.26 | $3.06^{b}$±0.56 | $3.17^{b}$±0.28 | $7.44^{a}$±0.28 | <0.0001 |
| **168** | $3.50^{b}$±0.72 | $4.06^{b}$±0.35 | $4.63^{b}$±0.25 | $9.61^{a}$±0.77 | <0.0001 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

#### 4.1.1.2 Yolk sac parameters

The morphological parameters measured, between Hour 6 and Hour 72, for the yolk-sac larvae treated with Aquahatch include yolk sac length (mm) (Table 4.5), yolk sac height (mm) (Table 4.5) and total body length (mm) (Table 4.9). Yolk sac length and height were then used to calculated yolk sac volume ($mm^3$) (Table 4.7).

The yolk sac volume was used to determine the rate of utilization of the yolk sac given by the slope of the regression for yolk sac volume and time (Table 4.8). The yolk sac volume was also compared to total body length in the body length:yolk sac volume ratio (BL:YSV) (Table 4.10). Before treatment with Aquahatch, and before the yolk-sac larvae was placed into their respective tanks, the initial yolk sac length, height and volume was 1.53 ± 0.01mm, 0.959 ± 0.006mm, and 0.747 ± 0.013$mm^{3,}$ respectively.

Hour 42 saw yolk sac length differ in larvae from T0 being longer compared to that of larvae from T1.5 (1.31 ± 0.02mm vs 1.21 ± 0.03mm; $p \leq 0.05$). Differences ($p \leq 0.05$) between treatments for yolk sac height, length and volume occurred at Hour 48 with T1.5 (1.19 ± 0.02mm, 0.693 ± 0.016mm, and 0.314 ± 0.018$mm^3$, respectively) being greater than T0.5 in all aspects (1.08 ± 0.02mm, 0.605 ± 0.017mm, and 0.220 ± 0.014$mm^3$) and with regards to T0 in height and volume (0.627 ± 0.016mm and 0.241 ± 0.013 $mm^3$, respectively). The rate of utilization of the yolk sac did not differ ($p \geq 0.05$) between treatments.

Uncharacteristic increases in the yolk sac dimensions from previous sampling intervals were seen at certain points. For yolk sac length this includes T0 at Hour 6, T0.25 at Hour 18 and 24, and T1.5 at Hour 18. For yolk sac height this includes T0.25 and T0.5 at Hour 42, and T1.5 at Hour 30 and 48. For yolk sac volume this includes T0.25 at Hour 42 and T1.5 at Hour 48.

At the end of the yolk-sac period the mean yolk sac height, length and volume was 0.344 ± 0.007mm, 0.596 ± 0.015mm, and 0.040 ± 0.002, respectively.

**Table 4.5** Yolk sac length (mean mm ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 1.48±0.02 | 1.51±0.02 | 1.48±0.02 | 1.51±0.02 | 0.363 |
| 12 | 1.49±0.02 | 1.44±0.02 | 1.46±0.02 | 1.44±0.02 | 0.185 |
| 18 | 1.47±0.02 | 1.45±0.02 | 1.45±0.02 | 1.50±0.01 | 0.154 |
| 24 | 1.45±0.02 | 1.47±0.02 | 1.44±0.02 | 1.42±0.03 | 0.529 |
| 30 | 1.45±0.02 | 1.43±0.02 | 1.42±0.02 | 1.41±0.02 | 0.565 |
| 36 | 1.34±0.02 | 1.29±0.03 | 1.32±0.02 | 1.32±0.03 | 0.416 |
| 42 | $1.31^{a}$±0.02 | $1.28^{ab}$±0.02 | $1.23^{ab}$±0.02 | $1.21^{b}$±0.03 | 0.006 |
| 48 | $1.13^{ab}$±0.02 | $1.12^{ab}$±0.03 | $1.08^{b}$±0.02 | $1.19^{a}$±0.02 | 0.012 |
| 54 | 1.08±0.02 | 1.07±0.04 | 1.00±0.026 | 1.05±0.03 | 0.170 |
| 60 | 0.970±0.036 | 0.924±0.038 | 0.946±0.028 | 0.883±0.039 | 0.351 |
| 72 | 0.603±0.031 | 0.606±0.035 | 0.575±0.023 | 0.602±0.030 | 0.874 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.6** Yolk sac height (mean mm ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 0.965±0.016 | 0.967±0.012 | 0.945±0.013 | 0.966±0.011 | 0.579 |
| 12 | 0.916±0.015 | 0.906±0.015 | 0.899±0.013 | 0.883±0.015 | 0.423 |
| 18 | 0.839±0.011 | 0.856±0.018 | 0.831±0.014 | 0.849±0.012 | 0.605 |
| 24 | 0.799±0.015 | 0.817±0.014 | 0.796±0.012 | 0.774±0.019 | 0.249 |
| 30 | 0.793±0.011 | 0.791±0.014 | 0.786±0.012 | 0.784±0.013 | 0.953 |
| 36 | 0.731±0.016 | 0.699±0.017 | 0.698±0.012 | 0.723±0.016 | 0.315 |
| 42 | 0.701±0.015 | 0.717±0.018 | 0.720±0.020 | 0.684±0.017 | 0.433 |
| 48 | $0.627^{b}$±0.016 | $0.645^{ab}$±0.016 | $0.605^{b}$±0.017 | $0.693^{a}$±0.016 | 0.002 |
| 54 | 0.575±0.017 | 0.543±0.024 | 0.561±0.018 | 0.563±0.019 | 0.702 |
| 60 | 0.511±0.016 | 0.524±0.020 | 0.527±0.017 | 0.519±0.018 | 0.924 |
| 72 | 0.341±0.013 | 0.355±0.017 | 0.341±0.014 | 0.339±0.016 | 0.864 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.7** Yolk sac volume (mean $mm^3$ ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 0.717±0.023 | 0.748±0.022 | 0.700±0.024 | 0.743±0.021 | 0.401 |
| 12 | 0.663±0.023 | 0.627±0.024 | 0.630±0.022 | 0.602±0.025 | 0.330 |
| 18 | 0.549±0.017 | 0.571±0.025 | 0.533±0.020 | 0.573±0.018 | 0.457 |
| 24 | 0.499±0.022 | 0.522±0.021 | 0.485±0.018 | 0.469±0.026 | 0.368 |
| 30 | 0.483±0.016 | 0.482±0.024 | 0.466±0.017 | 0.461±0.016 | 0.777 |
| 36 | 0.388±0.018 | 0.342±0.018 | 0.346±0.016 | 0.371±0.018 | 0.211 |
| 42 | 0.351±0.018 | 0.358±0.020 | 0.343±0.019 | 0.308±0.017 | 0.235 |
| 48 | $0.241^{b}$±0.013 | $0.258^{ab}$±0.016 | $0.220^{b}$±0.014 | $0.314^{a}$±0.018 | 0.0002 |
| 54 | 0.203±0.015 | 0.190±0.018 | 0.178±0.014 | 0.196±0.016 | 0.725 |
| 60 | 0.148±0.014 | 0.154±0.015 | 0.149±0.011 | 0.144±0.013 | 0.960 |
| 72 | 0.043±0.005 | 0.039±0.004 | 0.040±0.004 | 0.039±0.005 | 0.905 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.8** Yolk sac volume regression coefficient (mean ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Period | Treatment inclusion level | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6-72 | -0.0100±0.0002 | -0.0101±0002 | -0.0103±0.0002 | -0.0102±0.0002 | 0.856 |

The initial total body length for the larvae before the application of Aquahatch was 5.04 ± 0.02mm. Larvae from T0.25 were consistently found to have a shorter total body length during the yolk-sac period. At Hour 18 they differed to T1.5 (5.99 ± 0.05mm vs 6.13±0.03; $p \leq 0.1$). They differed to T0.5 at Hour 30 (6.35 ± 0.05mm vs 6.50 ± 0.04mm; $p \leq 0.1$), Hour 36 (6.48 ± 0.07mm vs 6.75 ± 0.05mm; $p \leq 0.05$) and Hour 54 (7.16 ± 0.06mm vs 7.39 ± 0.04mm; $p \leq 0.05$). T0 (7.37 ± 0.05mm and 7.71 ± 0.05mm) was seen to differ ($p \leq 0.05$) to that of T0.25 (7.16 ± 0.06mm and 7.43 ± 0.07mm) at Hour 54 and Hour 60. Whereas, at Hour 42 the larvae from T0 had a longer total body length (7.10 ± 0.04mm; $p \leq 0.05$) than all other treatments. An uncharacteristic decrease in total body length from the previous sampling interval was seen for T0 at Hour 48. At Hour 72, the end of the yolk-sac period, the mean total body length was 7.81± 0.03 for all treatments ($p \geq 0.05$).

The ratio of larvae body length to yolk sac volume (BL:YSV) was found to be greater in T1.5 at Hour 12 compared to T0 (10.51 ± 0.48 vs 8.83 ± 0.28; $p \leq 0.05$). At Hour 48, both T0.25 (33.27 ± 2.51) and T0.5 (33.18 ± 1.85) were seen to have a greater BL:YSV ($p \leq 0.05$) than that of T1.5 (25.63 ± 1.59). At the end of the yolk-sac period the BL:YSV did not differ between treatments ($p \geq 0.05$).

**Table 4.9** Body length (mean mm ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment Inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 5.59±0.05 | 5.66±0.03 | 5.63±0.04 | 5.66±0.04 | 0.470 |
| 12 | 5.82±0.05 | 5.79±0.05 | 5.91±0.04 | 5.83±0.03 | 0.248 |
| 18 | 6.06±0.05 | 5.99±0.05 | 6.11±0.04 | 6.13±0.03 | 0.088 |
| 24 | 6.21±0.04 | 6.12±0.06 | 6.15±0.06 | 6.13±0.05 | 0.615 |
| 30 | 6.44±0.04 | 6.35±0.05 | 6.50±0.04 | 6.47±0.04 | 0.078 |
| 36 | $6.68^{ab}$±0.05 | $6.48^{b}$±0.07 | $6.75^{a}$±0.05 | $6.66^{ab}$±0.05 | 0.007 |
| 42 | $7.10^{a}$±0.04 | $6.82^{b}$±0.06 | $6.88^{b}$±0.05 | $6.89^{b}$±0.05 | 0.001 |
| 48 | 7.09±0.05 | 7.00±0.05 | 7.08±0.05 | 7.13±0.07 | 0.391 |
| 54 | $7.37^{a}$±0.05 | $7.16^{b}$±0.06 | $7.39^{a}$±0.04 | $7.33^{ab}$±0.04 | 0.005 |
| 60 | $7.71^{a}$±0.05 | $7.43^{b}$±0.07 | $7.62^{ab}$±0.05 | $7.51^{ab}$±0.06 | 0.004 |
| 72 | 7.87±0.05 | 7.75±0.06 | 7.80±0.05 | 7.81±0.05 | 0.416 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 4.10** Body length:yolk sac volume ratio (mean ± SE) recorded from Hour 6 to Hour 72 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 6 | 7.75±0.22 | 7.87±0.24 | 8.44±0.28 | 7.86±0.21 | 0.1660 |
| 12 | $8.83^{b}$±0.28 | $9.92^{ab}$±0.44 | $9.93^{ab}$±0.39 | $10.51^{a}$±0.48 | 0.0365 |
| 18 | 11.56±0.40 | 10.50±0.40 | 12.02±0.50 | 11.2±0.36 | 0.0805 |
| 24 | 13.67±0.68 | 12.65±0.57 | 13.48±0.52 | 13.86±0.78 | 0.5581 |
| 30 | 13.95±0.43 | 14.45±0.65 | 14.52±0.55 | 13.70±0.44 | 0.6469 |
| 36 | 18.72±1.01 | 22.03±1.46 | 21.37±1.00 | 19.62±1.05 | 0.1522 |
| 42 | 21.51±0.96 | 21.35±1.21 | 21.68±1.15 | 24.41±1.43 | 0.2247 |
| 48 | $31.13^{ab}$±1.57 | $33.27^{a}$±2.51 | $33.18^{a}$±1.85 | $25.63^{b}$±1.59 | 0.0170 |
| 54 | 47.52±4.03 | 47.27±4.38 | 56.55±5.30 | 50.19±5.26 | 0.4798 |
| 60 | 71.05±6.32 | 75.96±8.76 | 64.65±6.19 | 69.97±7.87 | 0.7517 |
| 72 | 271.35±28.62 | 283.35±32.3 | 290.54±27.81 | 327.92±35.91 | 0.6113 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

Table 4.11 presents the correlation between water quality (temperature, DO, pH and TAN) and body length and between water quality and yolk sac volume for African catfish larvae, given by Pearson's correlation coefficient (r). Dissolved oxygen was the water quality parameter seen to have the highest correlation for body length and yolk sac volume for all treatments however, the correlation was only to a moderate extent. Dissolved oxygen had a negative correlation with body length and a positive correlation with yolk sac volume. Total ammonia nitrogen had a strong, negative correlation for yolk sac volume for T0.5 and T1.5, and a moderate correlation for T0.25. For T0, the TAN had a moderate, negative correlation with body length.

**Table 4.11** Correlation between water quality parameters and yolk sac parameters for African catfish larvae from Hour 6 to Hour 72.

| Treatment inclusion level (mL/L) | Body morphology parameter | Water quality parameters | | | |
|---|---|---|---|---|---|
| | | Temperature (°C) | Dissolved oxygen (mg/L) | pH | Total Ammonia nitrogen (mg/L) |
| **0** | **Yolk sac volume ($mm^3$)** | -0.119 | 0.459 | 0.236 | 0.055 |
| | **Body length (mm)** | 0.175 | -0.539 | -0.249 | -0.519 |
| **0.25** | **Yolk sac volume ($mm^3$)** | -0.137 | 0.456 | 0.183 | -0.513 |
| | **Body length (mm)** | 0.173 | -0.525 | -0.229 | -0.389 |
| **0.5** | **Yolk sac volume ($mm^3$)** | -0.019 | 0.357 | 0.152 | -0.729 |
| | **Body length (mm)** | 0.100 | -0.496 | -0.253 | 0.305 |
| **1.5** | **Yolk sac volume ($mm^3$)** | -0.080 | 0.437 | 0.055 | -0.727 |
| | **Body length (mm)** | 0.121 | -0.524 | -0.131 | 0.096 |
| **Period** | | **6-72** | **6-72** | **6-72** | **72** |

Table 4.12 presents the weight of the fry at the end of the treatment period. No differences ($p \geq 0.05$) were experienced between treatments, with a mean individual weight for all treatments of 0.0037 ± 0.0000g.

**Table 4.12** Individual fry weight (mean g ± SE) at Hour 168 for African catfish larvae that received Aquahatch.

| Hour | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| **168** | 0.0038±0.0001 | 0.0038±0.0001 | 0.0036±0.0001 | 0.0036±0.0001 | 0.344 |

### 4.1.1.3 Spectrophotometry analysis of treatment concentration in the water

Figure 4.1 illustrates the standard calibration curve for the spectrophotometer of light absorbance value (nm) vs know Aquahatch concentration. The equation generated from this calibration curve was Equation 26: Y = 0.161999491 × -0.003881221X with $R^2$ = 0.999, where Y = light absorbance value (nm) and X = Aquahatch concentration (mL/L), at a wavelength of 450nm. This equation was used to determine unknown Aquahatch concentrations in the water for Absorbance value readings taken with the spectrophotometer.

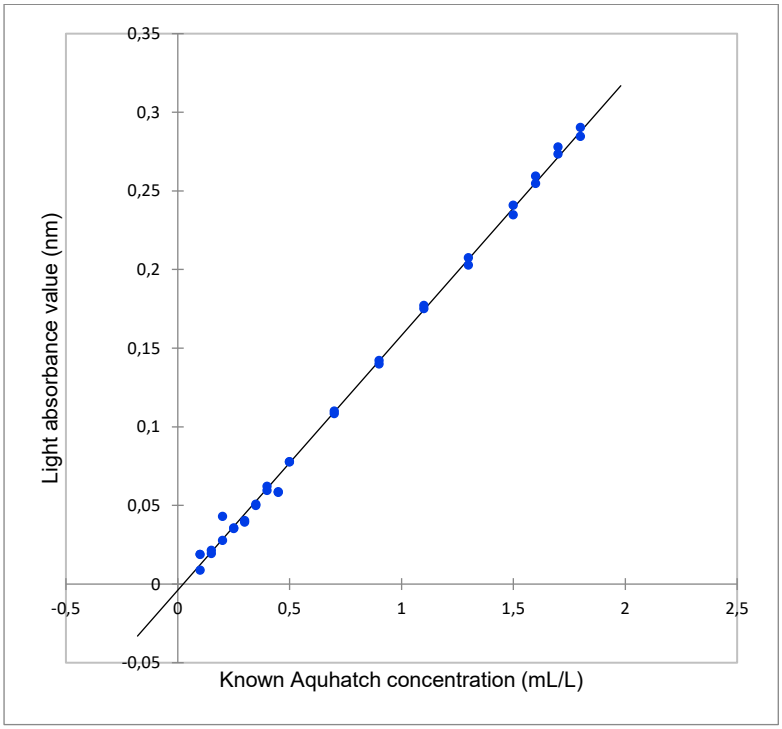


**Figure 4.1** Spectrophotometer calibration curve for the light absorbance value (nm) of known Aquahatch concentration (mL/L) at wavelength 450nm.

Table 4.13 presents changes in Aquahatch concentration. This is given by the regression coefficient for the slope from Hour 6 to Hour 168, and the effect the status of fish in the tank had on the regression coefficient.

The regression coefficient shows that the concentration of Aquahatch decreased in all the tanks between Hour 6 and Hour 168 of the trial. Only T1.5 saw the presence of fish to influence the rate at which the concentration changed, with the fish causing a more pronounced decrease in Aquahatch concentration over the treatment period ($p \leq 0.05$).

**Table 4.13** Change in Aquahatch concentration (mL/L), given by the regression coefficient for the slope (mean ± SE), from Hour 6 to Hour 168 for tanks with and without African catfish.

| Aquahatch Inclusion level (mL/L) | Fish status | Treatment concentration Regression coefficient |
|---|---|---|
| **0** | **No Fish** | $-0.0024^{cd} \pm 0.0003$ |
| **0** | **Fish** | $-0.0024^{d} \pm 0.0003$ |
| **0.25** | **No Fish** | $-0.0022^{cd} \pm 0.0002$ |
| **0.25** | **Fish** | $-0.0021^{cd} \pm 0.0002$ |
| **0.5** | **No Fish** | $-0.0021^{cd} \pm 0.0002$ |
| **0.5** | **Fish** | $-0.0019^{ac} \pm 0.0002$ |
| **1.5** | **No Fish** | $-0.0007^{a} \pm 0.0006$ |
| **1.5** | **Fish** | $-0.0016^{b} \pm 0.0006$ |

Different superscripts (e.g. $^{a, b, c, d}$) in the same column indicate significant differences ($p \leq 0.05$).

Figure 4.2 illustrates the concentration of Aquahatch at regular intervals from Hour 6 to Hour 168, given by spectrophotometry analysis, for both tanks with and without African catfish. A difference in concentration was seen between treatments, and within treatments over time ($p \leq 0.05$). When compared to the control, T1.5 differed throughout the entire period, T0.5 differed until Hour 120, and T0.25 only differed from Hour 18 to Hour 54 ($p \leq 0.05$). For T1.5, Hour 42 to Hour 96 saw a sharp decline in the concentration to a point where it did not differ ($p \geq 0.05$) to that of the next highest treatment concentration, T0.5.

Since no additional Aquahatch was supplemented after its initial inclusion, it was expected that Aquahatch concentrations would either remain the same or would decrease over time. It was also expected that the fish would make use of Aquahatch therefore, decreasing its concentration in the water. However, multiple periods saw changes in Aquahatch concentration in the water that went against what was expected. A higher ($p \leq 0.05$) Aquahatch concentration to that of the previous sample period was seen for T0 at Hour 18, 54 and 144, for T0.5 at Hour 96, and T1.5 at Hour 18 and 54. A higher ($p \leq 0.05$) Aquahatch concentration was also seen in tanks absent of fish for T0 at Hour 18, 30 and 42, T0.5 at Hour 30, 96 and 120, and T1.5 at Hour 72.

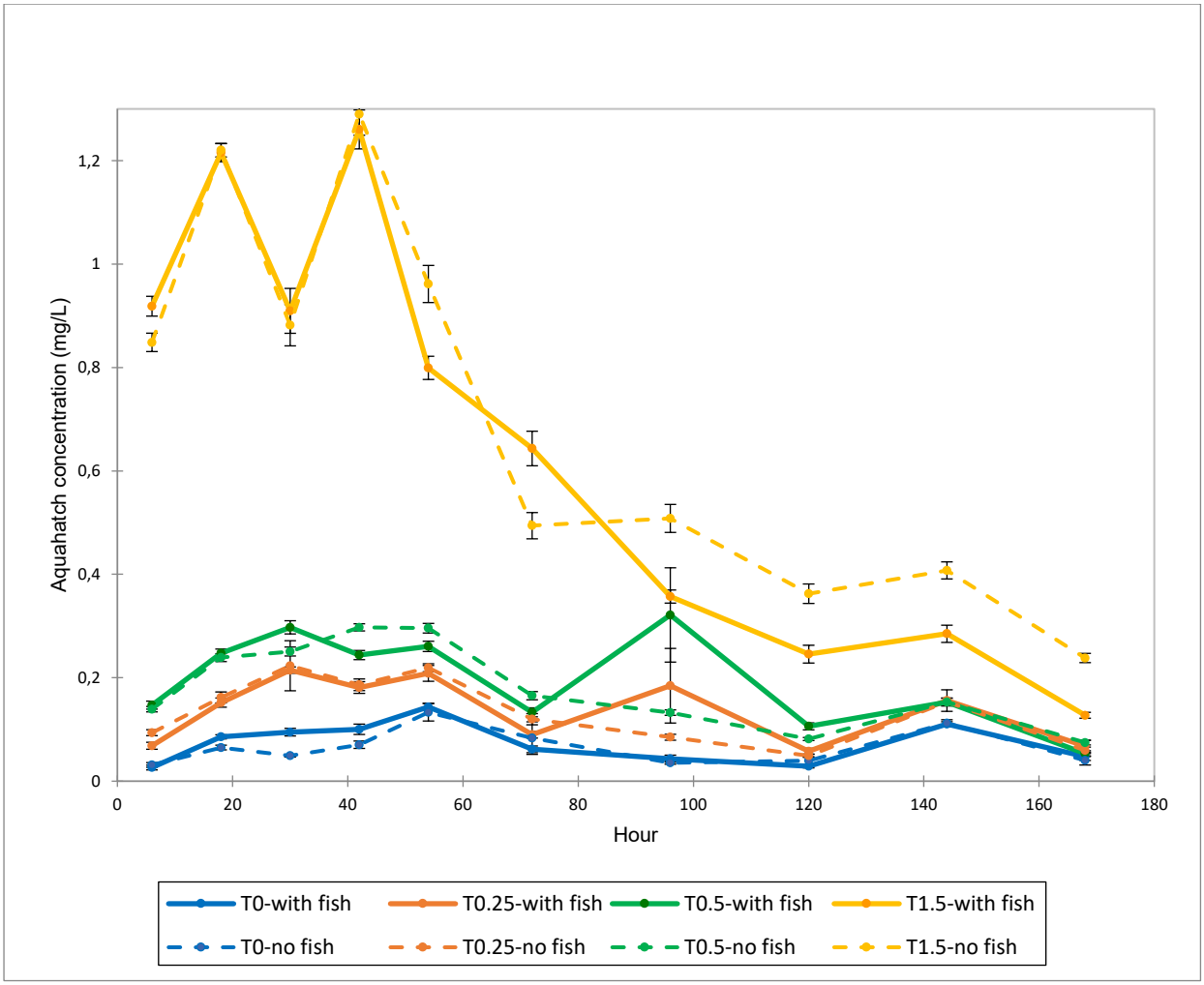


**Figure 4.2** Concentration of Aquahatch (mL/L) at regular intervals from Hour 6 to Hour 168, given by spectrophotometry analysis, for both tanks with and without African catfish.

### 4.1.2 Trial 2

#### 4.1.2.1 Survival

Table 4.14 presents the survival of the African catfish at the end of the treatment period. The survival of the African catfish did not differ ($p \geq 0.05$) between treatments, with an average survival of 26.88 ± 4.07%.

**Table 4.14** Survival (mean % ± SE) for African catfish during the 7-day Aquahatch treatment period.

| | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **Survival (%)** | 33.33±9.53 | 30.00±5.93 | 16.67±4.71 | 27.50±11.42 | 0.545 |

#### 4.1.2.2 Water Quality

Tables 4.15 and 5.16 present the water quality in terms of temperature, DO, pH and TAN for the tanks. The only water quality parameter to differ ($p \leq 0.05$) between treatments was TAN. Tanks of T1.5 (5.5 ± 0.289mg/L) had higher levels compared to T0 (1.38 ± 0.52mg/L) and T0.25 (1.88 ± 0.32mg/L) on Day 3. On Day 7, the TAN levels of T1.5 (7.13 ± 0.43mg/L) continued to be the highest, but now differed ($p \leq 0.05$) to all treatments. The mean tank temperature, DO and pH for all treatments was 25.27 ± 0.09°C, 7.30 ± 0.03mg/L and 6.54 ± 0.03.

**Table 4.15** Water quality (mean ± SE) in terms of temperature (°C), DO (mg/L) and pH for African catfish tanks during the 7-day Aquahatch treatment period.

| **Water quality parameters** | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **Temperature** | 25.39±0.18 | 25.24±0.18 | 25.23±0.18 | 25.21±0.17 | 0.872 |
| **DO** | 7.32±0.06 | 7.35±0.06 | 7.29±0.06 | 7.26±0.06 | 0.723 |
| **pH** | 6.60±0.05 | 6.51±0.05 | 6.50±0.05 | 6.55±0.05 | 0.494 |

**Table 4.16** Total ammonia nitrogen (mean mg/L ± SE) for African catfish tanks recorded at intervals during the 7-day Aquahatch treatment period.

| **Day** | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **1** | 0.228±0.046 | 0.218±0.042 | 0.253±0.063 | 0.295±0.117 | 0.879 |
| **3** | $1.38^{b}$±0.52 | $1.88^{b}$±0.32 | $3.50^{ab}$±0.82 | $5.50^{a}$±0.29 | 0.002 |
| **7** | $1.09^{b}$±0.66 | $0.65^{b}$±0.222 | $2.00^{b}$±0.54 | $7.13^{a}$±0.43 | <0.0001 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

## 4.2 Discussion

### 4.2.1 Trial 1

Throughout the current trial, the recorded R-squared value for the larvae's morphological characteristics were seen to be very low. This can be attributed to a high variation within the groups. The high variation within the groups can be accredited to the high inherent variation within an African catfish population (Martins *et al.*, 2005). This variation is especially prominent during the juvenile stages of African catfish that is characterized by high growth and development rates, which result a large degree of variation within a population. Despite the low R-squared value, significant differences in the data recorded were present, these differences will be discussed below.

#### 4.2.1.1 Body morphology

With fish larvae, total length is a common method used to determine growth in the absence of live weight records (Pepin, 1995), while changes in yolk sac volume can be used to determine yolk sac nutrient utilization (Fotedar, 2017). Yolk sac utilization is commonly discussed in terms of rate, how fast it is utilized, and effectiveness, how well the nutrients provided are used by the larvae for growth and development. Dumas *et al.* (2014) and Prokesova *et al.* (2015) demonstrated the importance of differentiating between the two with Pacific red snapper larvae and African catfish, respectively.

In these studies, it was found that at higher temperatures the yolk sac was absorbed faster however, at lower temperatures, which still fell within a range for good growth, the absorption of the yolk sac resulted in larger larvae at the end of endogenous feeding.

The abovementioned studies help to illustrate the importance of balancing the relationship between fast and effective utilization of nutrients provided by the yolk sac. The current trial aimed at improving utilization of the yolk sac contents by African catfish larvae through nutrient supplementation. The rate of yolk sac utilization was given by the slope of the regression for yolk sac volume and time, while the BL:YSV gave an indication of how well the nutrients provided by the yolk sac were utilized.

Temperature, DO and pH all fell within a range that allows for good African catfish larvae growth (Britz & Hecht, 1987; Tucker, 1991; Ndubuisi *et al.*, 2015). The toxic effect of the high TAN levels found in the current trial was managed by keeping the average pH below 7. This would have led to a greater proportion of the TAN being in the non-toxic form of $NH_3$ (Durborow *et al.*, 1997; Schram *et al.*, 2010).

Water quality, especially temperature, is known to affect the utilization of the yolk sac and growth of the larvae (Conceicao *et al.*, 1998b). In the current trial, the aim was to keep the

water quality parameters, between treatments, as constant as possible. However, periods were experienced where differences in water quality parameters did occur. For temperature and pH, these differences were not considered to be influential due to a poor correlation with body length and yolk sac volume. The moderate correlation seen in DO did not have an influence since DO levels in the tanks did not differ between treatments. Higher TAN levels in the water were seen with increasing concentration levels, which is not a favourable result.

No studies were found which tested the effect of nutrient supplementation on yolk sac utilization by fish larvae. However, a similar concept was tested in layer-type chicks by Nouboukpo *et al.* (2010), who investigated the potential of L-carnitine to improve yolk sac utilization through improving fatty acid metabolism.

Nouboukpo *et al.* (2010) reported that L-carnitine supplementation resulted in a smaller yolk sac weight after a 7-day supplementation period, however, they did not see this translate into an improved weight gain. In the current trial, supplementation of Aquahatch was not seen to consistently alter the utilization of the yolk sac in terms of yolk sac volume and its rate of change, as well as the BL:YSV. Aquahatch supplementation also did not result in larger larvae in terms of body length, at the end of the yolk sac period, and in terms of weight, at the end of the treatment period. However, it was seen that the larvae from T0.25 consistently had the shortest body length but demonstrated potential compensatory growth, evident in the lack of differences at the end of the yolk-sac period. Changes in fish growth during the yolk-sac period should be in favour of a larger average larvae size at the end of endogenous feeding. This helps facilitate a more successful transition from endogenous to exogenous feeding, which results in improved survival and growth (Ing & Chew, 2015).

In the current trial, the larvae body length was comparable to that of Ing & Chew (2015). A smaller initial yolk sac volume was found which may be accredited to a difference in yolk sac reserves initially contributed by the broodstock. Yolk sac volume was also seen to decrease at a slower rate to that observed by Ing & Chew (2015), this is most likely due to the lower average temperature in the current trial of 25.45 ± 0.08°C as opposed to that of 27.5-28.6°C experienced by Ing & Chew (2015).

The uncharacteristic increase in yolk sac dimensions and decrease in body length, observed at particular sampling intervals, can be accredited to the short sampling intervals and morphological characteristics not being sampled from the same fish at each sampling period. It is also worth noting that the differences for yolk sac parameters and body length that occurred between treatments, were experienced around the time where Ing & Chew (2015) noticed first feeding during the mixed feeding period in African catfish, 36 hours after hatch. Discrepancies between results is likely due to differences in average temperatures. At the start

of the trial, the main route of nutrient uptake by the fish was unclear when Aquahatch was added to the water, as no studies were found to report on this. Studies however, indicated that fish can take up water and certain solutes in the water through their gills, epithelial lining and through oral ingestion (Tytler *et al.*, 1990; Robohm & Koch 1995; Terech-Majewska *et al.*, 2016; Harder *et al.*, 2018). These results could indicate that the main route of nutrient uptake for the African catfish larvae was through oral ingestion, and that the nutrients need only be added once the fish are capable of oral ingestion. This period should be further investigated to determine the cause of these results.

Conflicting results for the ability of L-carnitine and nucleotide supplementation to improve growth are often seen in aquaculture and other animal nutrition studies (Harpaz, 2005; Arslan, 2006; Li & Gatlin 2006; Ozorio, 2009; Ringo *et al.*, 2012). Some B vitamin studies show that exceeding recommended levels has little effect on improving growth (Mohamed *et al.*, 2000; Mohamed, 2001; Li *et al.*, 2010; Hien & Doolgindachbaporn 2011a,b). The results found in this study tend to side with the studies that found supplementation of the nutrients contained in Aquahatch to have negligible effect on growth.

#### 4.2.1.2 Spectrophotometry analysis of Aquahatch concentration and behaviour in the water

A spectrophotometric approach was used to analyse the concentration of Aquahatch and its stability in the water. This was a novel and rudimental method. Concentrations of Aquahatch in tanks with fish were compared to that of tanks without fish, as well as results obtained from tanks without Aquahatch. Only the concentration of Aquahatch in T1.5 was seen to be considerably affected by fish, in terms of the regression coefficient for the slope, which represented the change in Aquahatch concentration over time. Aquahatch concentrations were seen to decrease for all treatments over the course of the treatment period.

The active life span of Aquahatch in the water could be indicated by two results found in the current trial. One possible indication is the time at which no difference in Aquahatch concentration was seen between the control tanks (no Aquahatch) and tanks where Aquahatch was added to the water. The point at which treated tanks did not differ from the control saw higher treatment concentrations having a longer active life span in the water, with Aquahatch concentrations of 0.25mL/L, 0.5mL/L and 1.5mL/L lasting 54, 120 and greater than 168 hours, respectively. The other result in the trial that may indicate the active life span of Aquahatch in the water is where a sharp decline in the highest Aquahatch concentration was seen. If the concentration meeting point of T1.5 and T0.5, the next highest concentration, is taken as the active life span of Aquahatch, then these results indicate that Aquahatch remains active in the water for 96 hours. Consequently, additional Aquahatch supplementation might

be required when the treatment period is increased beyond these points, which is likely to make this method of Aquahatch supplementation not economically feasible.

The spectrophotometric analysis values reported in this study did not correspond to the expected decrease in the concentration and lifespan of the product. Results that contribute to this irregular pattern of decline include Aquahatch concentrations that increased at sampling intervals where a decline was expected, a lower concentration in tanks that did not contain fish, and levels recorded for control tanks where no Aquahatch was added to the water. These unexpected results give an indication that this method was affected by external factors. It is suspected that the greatest influencing factor was organic matter in the water created by fish and other living organisms (e.g. phytoplankton, zooplankton, bacterioplankton). Although the samples were filtered, in an effort to remove this organic matter, small organic matter not visible by the eye was able to be detected by the sensitive spectrophotometer.

Therefore, it can be said that this spectrophotometric method of analysing Aquahatch concentration in the water can be used for basic determination of levels, but due to its sensitivity, refinement of this method needs to be done for future use.

### 4.2.2 Trial 2

In Trial 2, the water quality parameters measured for temperature, DO and pH were within the ideal range for African catfish (Britz & Hecht, 1987; Tucker, 1991; Ndubuisi *et al.*, 2015). TAN was the only water quality parameter that fell outside the optimal range for African catfish however, by maintaining a pH less than 7 the ammonia was mainly present in the less toxic form (Durborow *et al.*, 1997; Schram *et al.*, 2010).

During the Aquahatch treatment no statistical differences were experienced in the survival however, differences that were seen are of economic importance. Even a small percentage survival difference has a large effect on the profitability of a hatchery operation. A potential reason for no statistical differences being experienced is due to the low number of treatment replicates and the high variation within treatments. When compared to the control, Aquahatch treatments were seen to negatively affect survival. This would contradict the majority of studies done on the nutrients that are contained in Aquahatch (Halver, 2002; Tja *et al.*, 2002; Harpaz, 2005; Li & Gatlin, 2006; Ozorio, 2009; Waagbo, 2010; Ringo *et al.*, 2012).

The result also indicates that Aquahatch caused an increase in TAN levels of the water. The L-carnitine component of Aquahatch is believed to have a protective function against ammonia toxicity. Costell *et al.* (1984) hypothesised that this is due to the role of L-carnitine in the urea cycle, as an increase urea production was found in L-carnitine supplemented mice. However, with no evidence for stimulated urea synthesis by Costell *et al.* (1984), Kloiber *et al.*, (1988)

suggested that the main protective role of L-carnitine is as a osmotic protector to help facilitate normal functioning of enzymes and proteins under elevated ammonia levels for mice. This theory is supported by Tremblay & Bradley (1992) for fish. In fish studies, Ozorio *et al.* (2001b) and Goncalves *et al.* (2010) found that supplementation of L-carnitine in the diet lowered the TAN level in the water, and suggested it was due to improved utilization of feed resulting in less nitrogen being excreted into the water. Results from this study are contradictory, as treatments with higher supplementation concentrations tended to have high levels of TAN in the water, which is in agreement with that found in Trial 1.

Increase excretion due to an oversupply of nutrients is a potential cause for the higher TAN levels seen with increasing Aquahatch concentrations. A difference in the contribution of urea to nitrogen excretion is seen during the various life stages of African catfish. For African catfish, 20% of their nitrogen is contributed in the form of urea during the yolk-sac larvae stage and increases to 44% during the fry stage. In contrast, urea only constitutes 13% of adult African catfish nitrogen excretion (Eddy *et al.*, 1980; Terjesen *et al.*, 1997, 2001). Since limited differences in the growth parameters measured in Trial 1 were observed with Aquahatch supplementation, there was a possible oversupply of these nutrients. This would have led to an increased excretion of urea in order to get rid of the excess nutrients taken in. Since urea contributes to a greater proportion of nitrogen excreted during the early developmental stages, this is one possible cause for the higher total ammonia levels seen with increasing Aquahatch concentration and the difference seen with other fish studies that use older fish.

Despite higher Aquahatch concentrations possibly resulting in TAN levels in the water, the highest Aquahatch supplementation level of T1.5 did not have the lowest survival, which would support the finding of the abovementioned studies where L-carnitine supplementation assisted in reducing the ammonia stress in fish. However, since Aquahatch might be the cause of the ammonia stress in the current study, future studies need to investigate the Inclusion of Aquahatch at different concentrations that may not contribute to ammonia stress.

## 4.3 Conclusion

The current trials report on a novel method involving the supplementation of the culture water of African catfish during the larval and early fry developmental period, with Aquahatch to potentially improve yolk sac utilization and ultimately fish growth and survival. Aquahatch supplementation did not result in a consistent beneficial effect in terms of yolk sac utilization, growth or survival. Results from these studies suggest that the larvae were only capable of utilizing Aquahatch once oral ingestion capabilities were developed. A potential adverse effect of Aquahatch was seen in higher TAN levels. An Aquahatch inclusion level of 0.25mL/L resulted in a shorter body length during the larval stage. This is, however, unfavourable as the transition to exogenous feeding is facilitated by larvae having a larger body size. Spectrophotometric analysis as a method to measure decay and/or utilization of Aquahatch did not yield reliable results.

In Trial 2, Aquahatch supplementation did not result in an improved survival rate, with findings indicative of a decrease in survival rate. The decline in survival rate linked to higher Aquahatch inclusion levels can potentially be ascribed to the oversupply of nutrients and thus a deterioration of water quality cause by higher ammonia levels. With the highest Aquahatch concentration being characterized by the highest total ammonia levels and not the lowest survival rate, this may indicate a potential protective effect against ammonia stress. These results, however, need to be verified in future studies.

These two trials provide a benchmark study for Aquahatch as a nutrient supplement and its potential to improve yolk sac utilization, growth and the wellbeing of freshwater fish. Future studies need to further refine the Aquahatch supplementation method and inclusion level, and the effects thereof on water quality parameters and when the critical point of supplementation need to commence, i.e. when the fish are capable of benefitting from the supplementation.

# Chapter 5

# African catfish growth performance post Aquahatch supplementation

This chapter presents the results obtained from Trial 3 of the study in the form of tables and graphs, with major findings highlighted. These results are then discussed and contextualize what was observed while running the study and in other studies. A conclusion was then drawn from the results and discussion.

## 5.1 Results

### 5.1.1 Water quality

The water quality parameters recorded throughout the trial fluctuated (Figures 5.1- 5.3) but remained within an acceptable range for African catfish. A summary of the water quality parameters is presented in Table 5.1.

**Table 5.1** A summary of the water quality parameters (mean ± SE) recorded for the system from Day 1 to Day 102.

| Parameter | Mean ± SE | Range |
|---|---|---|
| Temperature (°C) | 28.9 ± 0.2 | 23.2 - 36.7 |
| DO (mg/L) | 7.0 ± 0.1 | 5.0 - 8.5 |
| Total ammonia (mg/L) | 20.43 ± 4.51 | 0.08 - 56.00 |
| pH | 6.21 ± 0.13 | 5.09 – 6.81 |
| Flow rate (L/min) | 3.39 ± 0.13 | 2.03-5.20 |

The water temperature ranged from 23.2-36.7°C, with an average of 28.9 ± 0.2°C. The system heated up over the course of the day and cooled down during the night, as higher temperatures were generally experienced in the afternoon with lower temperatures in the evening and morning.

Dissolved oxygen (DO) ranged from 5.0 – 8.5mg/L with a mean of 7.0 ± 0.1mg/L. The total ammonia levels generally increased over the course of the trial. The total ammonia levels ranged from 0.08mg/L to 56mg/L. The pH ranged from 5.09 – 6.81 with a mean of 6.21 ± 0.13. The mean flow rate of water through each tank was 3.39 ± 0.13 L/min.

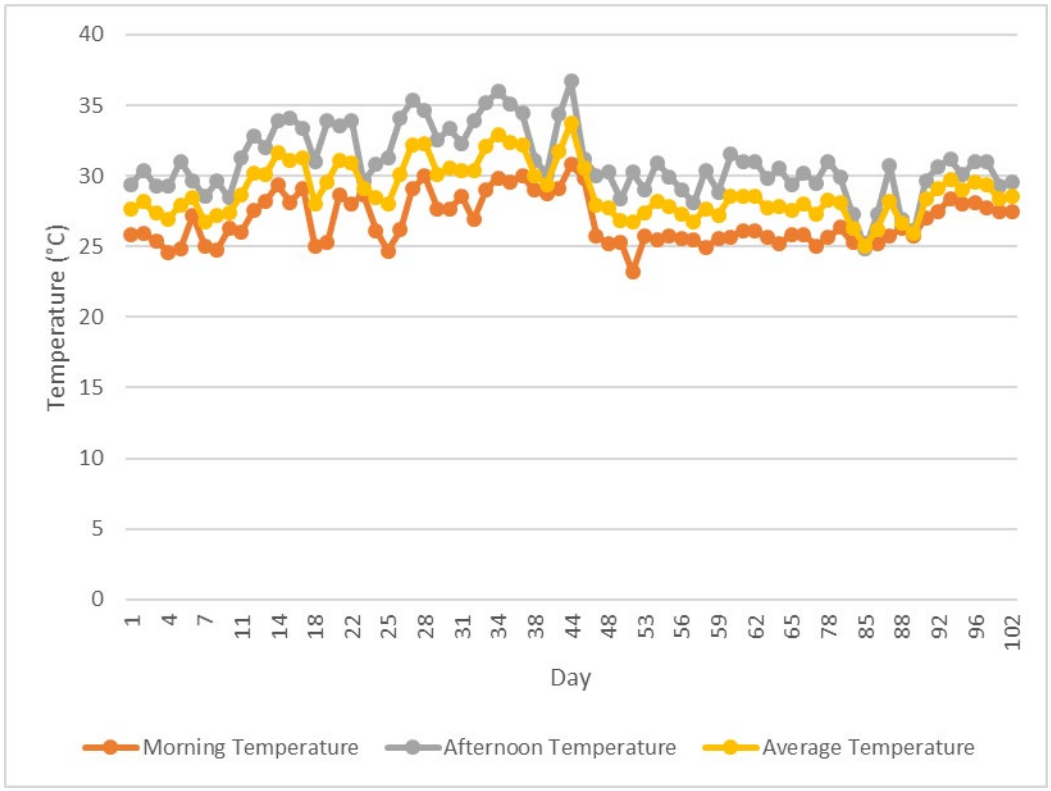


**Figure 5.1** Morning (08:00-09:00), afternoon (16:00-17:00) and mean temperature (°C) of the system recorded from Day 1 to Day 102.

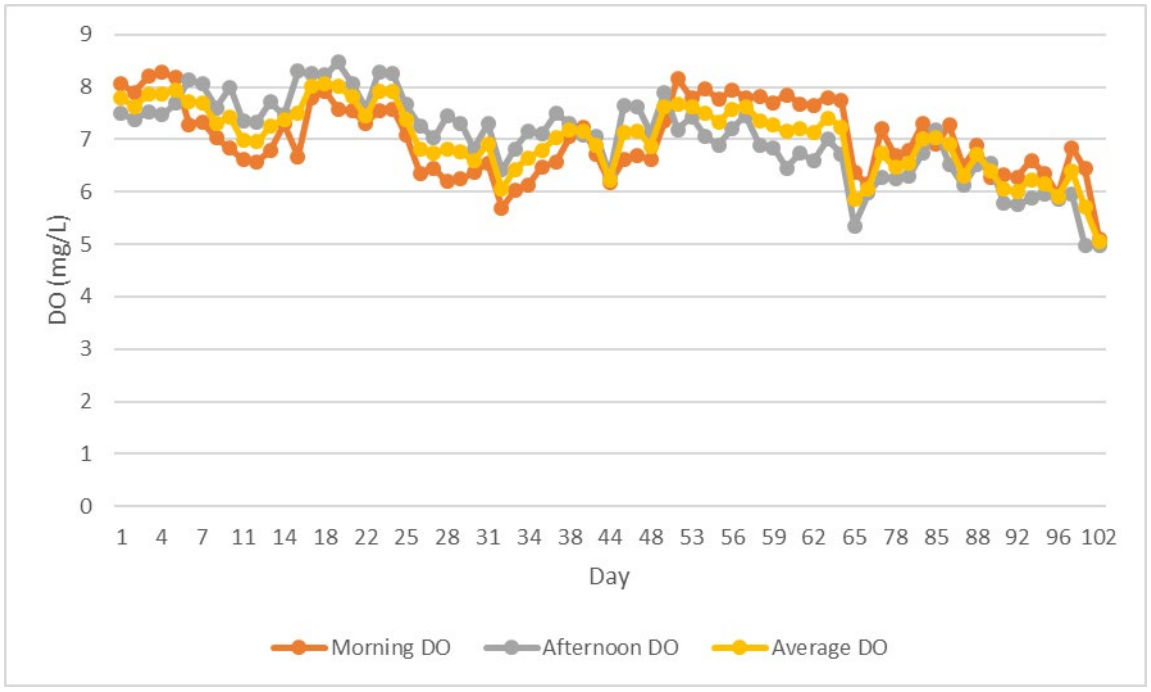


**Figure 5.2** Morning (08:00-09:00), Afternoon (16:00-17:00) and mean DO (mg/L) recorded in the tanks from Day 1 to Day 102.

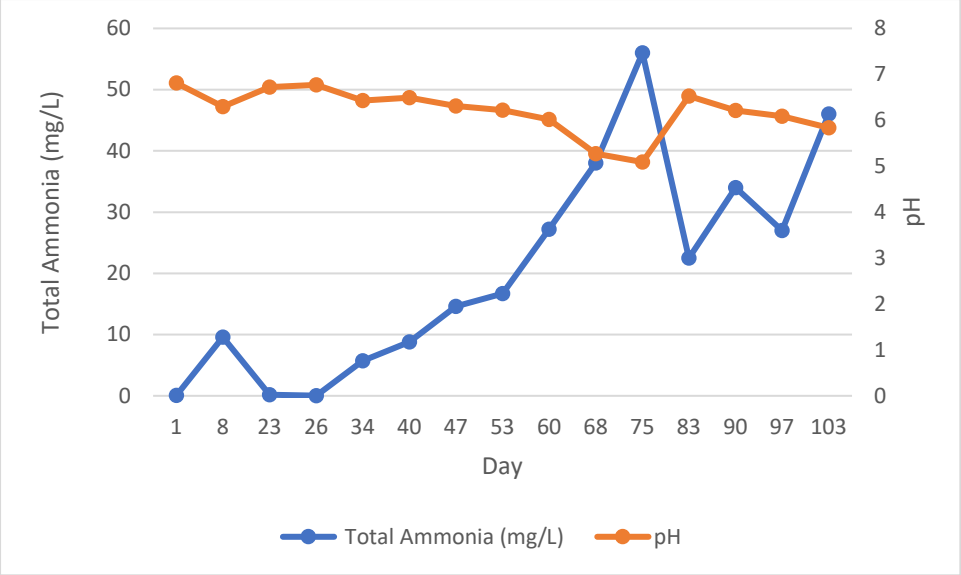


**Figure 5.3** Total ammonia (mg/L) and pH recorded from Day 0 to Day 103.

### 5.1.2 Haematocrit

Figure 5.4 displays the haematocrit values (%) for the African catfish from each treatment on Day 104. There were no differences ($p = 0.106$) between treatments. The mean haematocrit value was 30.86 ± 0.34%.

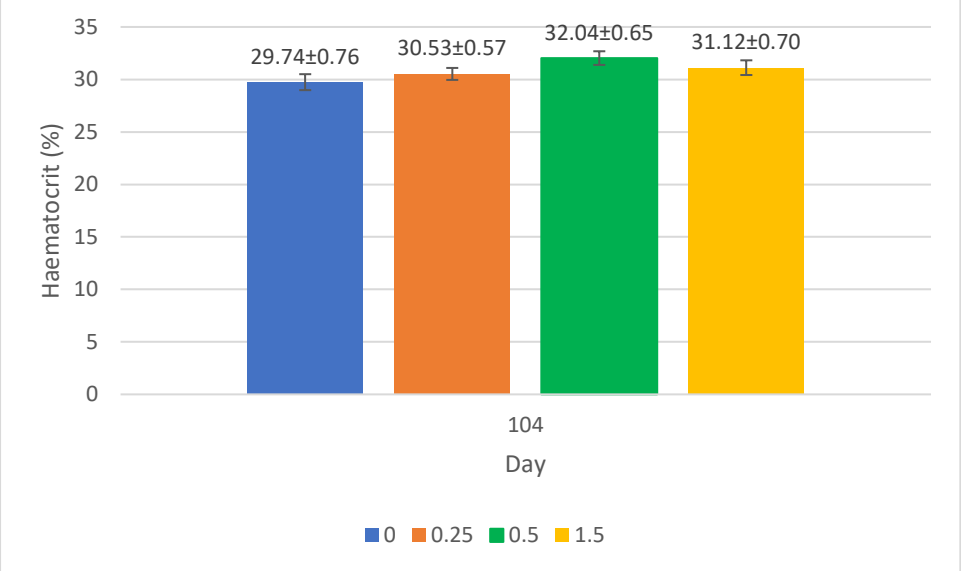


**Figure 5.4** Haematocrit values recorded on Day 104 for African catfish that received Aquahatch during early development.

### 5.1.3 Mortalities

The daily mortalities (%) for each treatment are presented in Table 5.2. Due to the number of African catfish being unknown at the start of the trial, mortalities were only recorded from Day 21 onwards, when the number of African catfish in each tank was adjusted to 200 fish per tank.

**Table 5.2** Daily mortalities (mean % ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **21-36** | 2.27±0.19 | 2.09±0.33 | 2.50±0.27 | 2.89±0.20 | 0.140 |
| **36-43** | 1.250±0.35 | 0.836±0.161 | 0.580±0.081 | 1.050±0.24 | 0.223 |
| **43-64** | 0.593±0.059 | 0.495±0.069 | 0.625±0.104 | 0.543±0.051 | 0.629 |
| **64-71** | 0.378±0.068 | 0.337±0.083 | 0.450±0.145 | 0.553±0.090 | 0.476 |
| **71-77** | $0.605^{a}$±0.081 | $0.299^{ab}$±0.096 | $0.405^{ab}$±0.077 | $0.158^{b}$±0.055 | 0.002 |
| **77-84** | $0.446^{b}$±0.094 | $0.833^{a}$±0.097 | $0.842^{a}$±0.143 | $0.888^{a}$±0.118 | 0.037 |
| **84-91** | 0.433±0.104 | 0.480±0.118 | 0.276±0.031 | 0.471±0.103 | 0.443 |
| **91-98** | 0.539±0.088 | 0.709±0.108 | 0.689±0.121 | 0.665±0.105 | 0.671 |
| **98-104** | $0.436^{a}$±0.078 | $0.406^{ab}$±0.127 | $0.524^{a}$±0.076 | $0.177^{b}$±0.062 | 0.048 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

Differences ($p \leq 0.05$) in the daily mortalities between treatments were experienced during Period 71-77, where T0 (0.605 ± 0.081%) had a higher daily mortality rate than that of T1.5 (0.158 ± 0.055%), Period 77-84 where T0 (0.446 ± 0.094%) had a lower daily mortality rate than all other treatments and Period 98-104 where T0 (0.436 ± 0.078%) and T0.5 (0.524 ± 0.076%) experienced a higher daily mortality rate than that of T1.5 (0.177 ± 0.062).

Figure 5.5 illustrates the cumulative mortalities (%) experienced from Day 21 to Day 104. Only at the end of the trial did the cumulative mortalities differ ($p = 0.029$) between treatments, with T0.25 (52.81 ± 3.90%) having less mortalities than that of T1.5 (64.19±1.56 %).

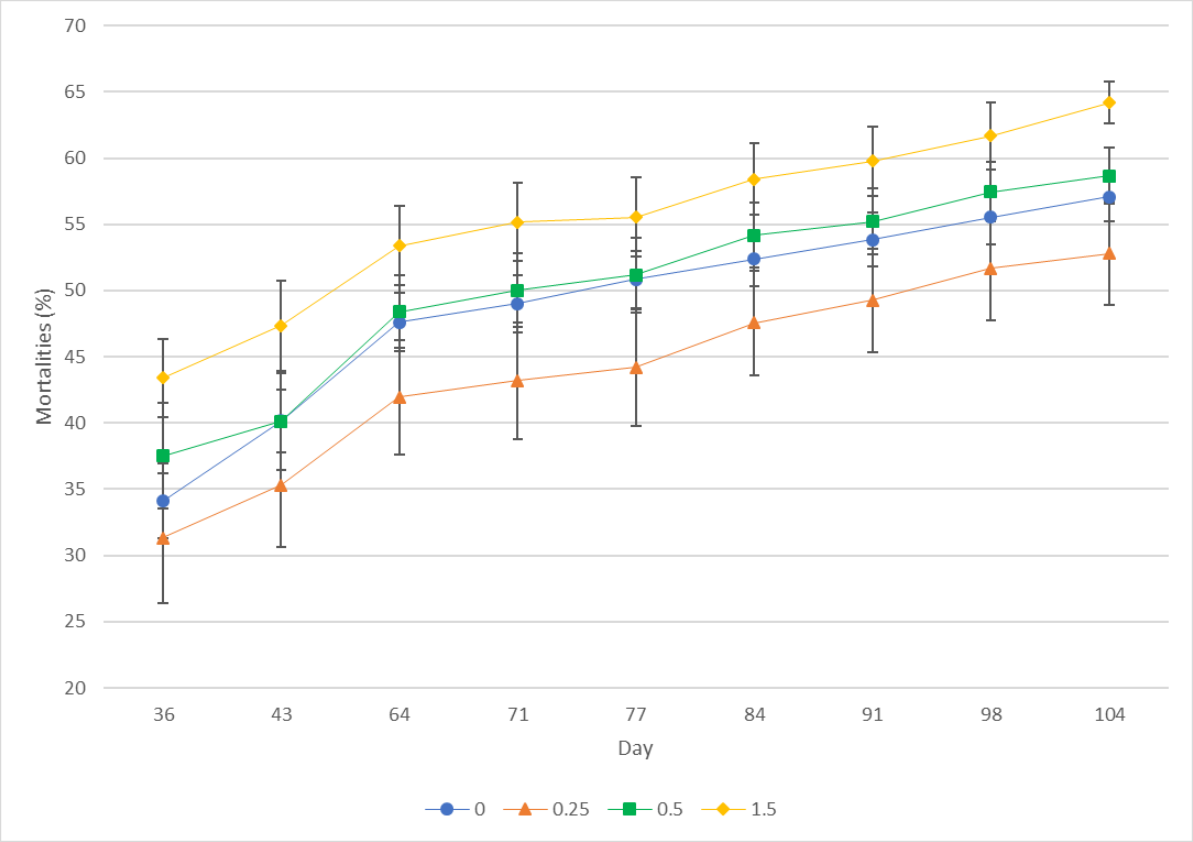


**Figure 5.5** Cumulative mortalities recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

### 5.1.4 Feeding

Table 5.3 presents the feeding rate (%) of the African catfish from Day 21 to Day 104. Only at the end of the trial, during Period 98-104, did a difference ($p \leq 0.05$) in the feeding rate occur. This was between T0.25 (2.92 ± 0.06%) which was given relatively less feed than that of T1.5 (3.16±0.05 %).

Table 5.4 presents the FCR experienced for the African catfish from Day 21 to Day 104. The mean FCR was 0.870 ± 0.013 with no differences ($p \geq 0.05$) between treatments for the cumulative FCR. Results for the FCR between each sampling periods, presented in Table 5.5 did however, see the FCR differing ($p \leq 0.05$) between treatments, but only during two periods. These were Period 43-49, where the FCR for T1.5 (0.655 ± 0.018) was improved over that of T0 (0.770 ± 0.022), and in Period 77-84 where T0 (1.02 ± 0.01) and T1.5 (1.05 ± 0.05) were found to have an improved FCR compared to that of T0.5 (1.22 ± 0.10).

**Table 5.3** Feeding rate (mean % ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **21-36** | 41.11±2.60 | 43.52±3.34 | 37.44±3.09 | 41.16±2.33 | 0.521 |
| **36-43** | 8.70±0.24 | 8.78±0.23 | 8.71±0.35 | 8.37±0.27 | 0.729 |
| **43-49** | 11.89±0.18 | 12.20±0.20 | 12.08±0.23 | 11.66±0.13 | 0.224 |
| **49-57** | 5.18±0.14 | 5.50±0.14 | 5.47±0.18 | 5.18±0.18 | 0.333 |
| **57-64** | 5.46±0.13 | 5.48±0.13 | 5.55±0.11 | 5.26±0.14 | 0.408 |
| **64-71** | 4.87±0.13 | 4.96±0.10 | 4.77±0.11 | 5.18±0.15 | 0.128 |
| **71-77** | 3.80±0.09 | 3.81±0.11 | 3.75±0.09 | 3.72±0.08 | 0.868 |
| **77-84** | 3.03±0.04 | 3.00±0.05 | 3.02±0.03 | 3.00±0.04 | 0.959 |
| **84-91** | 3.20±0.03 | 3.13±0.04 | 3.14±0.05 | 3.19±0.05 | 0.688 |
| **91-98** | 2.83±0.06 | 2.83±0.06 | 2.80±0.14 | 2.83±0.06 | 0.992 |
| **98-104** | $3.05^{ab}$±0.05 | $2.92^{b}$±0.06 | $3.09^{ab}$±0.07 | $3.16^{a}$±0.05 | 0.042 |
| **21-104** | 8.52±1.11 | 8.74±1.25 | 8.16±1.01 | 8.43±1.10 | 0.988 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 5.4** FCR (mean ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **21-36** | 1.20±0.03 | 1.43±0.11 | 1.38±0.09 | 1.24±0.04 | 0.151 |
| **21-43** | 1.02±0.03 | 1.10±0.06 | 1.07±0.05 | 1.08±0.04 | 0.629 |
| **21-49** | 0.893±0.018 | 0.934±0.046 | 0.876±0.028 | 0.852±0.020 | 0.271 |
| **21-57** | 0.727±0.010 | 0.730±0.014 | 0.709±0.015 | 0.711±0.013 | 0.548 |
| **21-64** | 0.750±0.015 | 0.735±0.019 | 0.722±0.014 | 0.728±0.013 | 0.563 |
| **21-71** | 0.725±0.013 | 0.713±0.015 | 0.711±0.015 | 0.713±0.012 | 0.861 |
| **21-77** | 0.739±0.003 | 0.725±0.010 | 0.731±0.002 | 0.738±0.007 | 0.398 |
| **21-84** | 0.790±0.005 | 0.795±0.008 | 0.802±0.008 | 0.802±0.006 | 0.512 |
| **21-91** | 0.822±0.005 | 0.829±0.005 | 0.830±0.008 | 0.828±0.008 | 0.831 |
| **21-98** | 0.853±0.007 | 0.858±0.008 | 0.861±0.012 | 0.858±0.008 | 0.942 |
| **21-104** | 0.872±0.006 | 0.867±0.012 | 0.870±0.013 | 0.862±0.011 | 0.918 |

**Table 5.5** intermediate FCR (mean ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **21-36** | 1.20±0.03 | 1.43±0.11 | 1.38±0.09 | 1.24±0.04 | 0.151 |
| **36-43** | 0.722±0.025 | 0.756±0.034 | 0.731±0.012 | 0.761±0.022 | 0.604 |
| **43-49** | $0.770^{a}$±0.022 | $0.728^{ab}$±0.024 | $0.727^{ab}$±0.056 | $0.655^{b}$±0.018 | 0.010 |
| **49-57** | 0.520±0.007 | 0.529±0.010 | 0.518±0.010 | 0.534±0.008 | 0.546 |
| **57-64** | 0.822±0.053 | 0.761±0.048 | 0.719±0.011 | 0.732±0.009 | 0.227 |
| **64-71** | 0.681±0.020 | 0.674±0.018 | 0.690±0.018 | 0.685±0.019 | 0.936 |
| **71-77** | 0.819±0.051 | 0.793±0.053 | 0.877±0.030 | 0.856±0.043 | 0.592 |
| **77-84** | $1.02^{b}$±0.01 | $1.22^{a}$±0.10 | $1.19^{ab}$±0.05 | $1.05^{b}$±0.05 | 0.019 |
| **84-91** | 0.980±0.046 | 0.985±0.026 | 0.953±0.016 | 0.950±0.036 | 0.8346 |
| **91-98** | 1.06±0.08 | 1.01±0.04 | 1.04±0.05 | 1.01±0.01 | 0.8869 |
| **98-104** | 0.979±0.033 | 0.933±0.053 | 0.921±0.036 | 0.850±0.029 | 0.1497 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

### 5.1.5 Growth and body condition parameters

Table 5.6 presents the biomass in grams (g) of the African catfish for each treatment from Day 21 to Day 104. On Day 21, before the number of fish was reduced to 200 per tank, the mean biomass was 138.71 ± 2.72g, with no difference ($p \geq 0.05$) observed between treatments. After the number of fish was reduced the mean biomass was 31.94 ± 1.08g with no difference ($p \geq 0.05$) between treatments. Throughout the trial no difference ($p \geq 0.05$) in biomass between treatments was recorded. At the end of the trial, on Day 104, the mean biomass was 6316.41 ± 140.89g.

**Table 5.6** Biomass (mean g ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Day | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **21$^{Before}$** | 140.24±3.65 | 144.91±9.04 | 132.50±4.61 | 137.88±4.79 | 0.499 |
| **21** | 32.21±2.23 | 30.37±2.08 | 34.06±2.69 | 31.11±1.69 | 0.670 |
| **36** | 186.91±5.93 | 160.57±11.20 | 158.66±10.74 | 160.75±8.77 | 0.142 |
| **43** | 319.96±15.21 | 290.28±17.92 | 292.09±21.7 | 281.75±13.93 | 0.440 |
| **49** | 617.36±29.36 | 569.26±39.08 | 587.93±38.02 | 580.22±28.32 | 0.776 |
| **57** | 1098.28±44.37 | 1056.78±56.74 | 1076.46±60.29 | 1031.77±50.99 | 0.833 |
| **64** | 1603.63±73.56 | 1606.27±91.11 | 1618.69±95.84 | 1485.20±63.77 | 0.621 |
| **71** | 2385.85±102.08 | 2413.52±128.77 | 2385.65±139.01 | 2247.33±92.27 | 0.742 |
| **77** | 3073.39±115.25 | 3137.34±173.09 | 3029.36±158.29 | 2853.77±125.51 | 0.551 |
| **84** | 3699.39±139.25 | 3686.67±195.99 | 3577.84±201.1 | 3403.57±144.11 | 0.592 |
| **91** | 4558.90±183.31 | 4510.36±239.52 | 4401.28±239.13 | 4181.31±182.08 | 0.595 |

| | | | | | |
|---|---|---|---|---|---|
| **98** | 5439.48±202.75 | 5405.29±294.04 | 5233.40±266.6 | 4992.16±201.59 | 0.545 |
| **104** | 6457.95±224.61 | 6443.84±363.32 | 6294.01±314.27 | 6069.84±253.44 | 0.760 |

$^{Before}$ – Biomass before reduction in fish number to 200

From the three sampling methods used to calculate individual growth, sampling method 3 (SM 3) was deemed the most accurate as it took all fish in each tank into account. Sampling method 1 (SM 1) and sampling method 2 (SM 2) only took a proportion of the fish in the tank into account when calculating mean individual live weight. After comparing SM 1 and SM 2 to SM 3, using a correlation test in XLSTAT, both SM 1 and SM 2 were considered to be good estimates of the mean individual weight of the African catfish in this trial. This was based on a strong positive correlation coefficient (r) between both SM 1 and SM2 with SM 3, $r_{SM1\&3}$ = 0.977 and $r_{SM2\&3}$ = 0.992 respectively.

Table 5.8 presents the individual live weight of the African catfish for each treatment. Means were calculated from sampling method 3, except on days indicated with the superscript $^{SM2}$ (Day 8, 49 and 57), which indicates sampling method 2 was used to calculate the means since it had a stronger correlation with SM 3 than SM 1.

The individual weight on Day 8, the end of the treatment period, did not differ ($p \geq 0.05$) between treatments, with a mean individual fish weight of 0.013 ± 0.000g. On Day 21 when the number of fish were reduced to 200 by random selection, the individual weight did also not differ ($p \geq 0.05$) between treatments, with a mean of 0.159 ± 0.007g. The individual weight on Day 21 was used, in conjunction with biomass, to estimate the number of fish before the tanks were reduced to 200 fish. This resulted in a mean of 939.72 ± 43.04 fish, with no difference ($p \geq 0.05$) between treatments.

There were a few days where a difference ($p \leq 0.05$) in individual fish weight between treatments were found. These include Day 36 and Day 43 where, on both days, the individual weight of T1.5 (1.43 ± 0 .08g and 2.72 ± 0.14g) was heavier than that of T0.25 (1.19 ± 0.06g and 2.28 ± 0.10g), with T0 (2.68 ± 0.12) also being heavier than that of the fish in T0.25 on Day 43.

On days when individual fish weight could not be calculated by SM 3, both SM 1 and SM 2 yielded a difference ($p \leq 0.05$) for individual weight. This difference was between T1.5 (SM2 = 5.29 ± 0.25g) which were heavier than that of T0.25 (SM2 = 4.31 ± 0.19g) on Day 49. There was also a tendency for the individual weight of T1.5 to be heavier than that of T0.25 on Day 64 ($p = 0.090$), 84 ($p = 0.058$), 91 ($p = 0.081$), 98 ($p = 0.085$), and 104 ($p = 0.053$). The mean individual weight of the African catfish at the end of the trial was 75.78 ± 1.46g.

**Table 5.8** Individual weight (mean g ± SE) recorded from Day 8 to Day 104 for African catfish that received Aquahatch during early development.

| Day | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **8**[SM2] | 0.013±0.000 | 0.013±0.001 | 0.014±0.001 | 0.013±0.000 | 0.507 |
| **21** | 0.161±0.011 | 0.152±0.010 | 0.170±0.013 | 0.156±0.008 | 0.670 |
| **36** | 1.36[ab]±0.07 | 1.19[b]±0.06 | 1.27[ab]±0.03 | 1.43[a]±0.08 | 0.047 |
| **43** | 2.68[a]±0.12 | 2.28[b]±0.10 | 2.43[ab]±0.08 | 2.72[a]±0.14 | 0.026 |
| **49**[SM2] | 4.88[ab]±0.24 | 4.31[b]±0.19 | 4.75[ab]±0.17 | 5.29[a]±0.25 | 0.030 |
| **57**[SM2] | 9.13±0.42 | 8.73±0.36 | 9.46±0.19 | 10.02±0.55 | 0.172 |
| **64** | 15.39±0.66 | 14.07±0.64 | 15.69±0.35 | 16.14±0.56 | 0.090 |
| **71** | 23.59±1.14 | 21.75±1.18 | 23.91±0.58 | 25.48±1.05 | 0.104 |
| **77** | 31.52±1.32 | 28.67±1.24 | 31.19±0.77 | 32.58±1.14 | 0.128 |
| **84** | 39.13±1.50 | 35.80±1.52 | 39.08±0.80 | 41.49±1.57 | 0.058 |
| **91** | 49.78±2.19 | 45.43±2.24 | 49.29±1.06 | 52.65±1.77 | 0.081 |
| **98** | 61.72±2.41 | 57.29±2.89 | 61.66±1.20 | 66.07±2.39 | 0.085 |
| **104** | 75.76±2.86 | 69.86±3.20 | 76.28±1.17 | 81.22±3.16 | 0.053 |

Different superscripts (e.g. [a, b]) in the same row indicate significant differences ($p \leq 0.05$).

[SM2] – Sampling method 2

Specific, relative and absolute growth rates for African catfish biomass (SGRB, RGRB, and AGRB, respectively) and individual weight (SGRI, RGRI, and AGRI, respectively) recorded throughout the trial are presented in Tables 5.9-5.11. The means for the growth rates of individual weight were calculated from sampling method 3, except during periods indicated with the superscript $^{SM2}$ (Period 8-21, 43-49, 49-57 and 57-64), which indicates sampling method 2 was used to calculate the means since it had a stronger correlation with SM 3 than SM 1.

There were multiple periods where the growth rate of the fish differed ($p \leq 0.05$) for each of the various growth rate calculations.

For SGRB, T0 (2.73 ± 0.06%) differed ($p \leq 0.05$) from T0.25 (2.32 ± 0.16%) and T0.5 (2.32 ± 0.09%) during Period 77-84, whereas T1.5 (3.39 ± 0.08%) differed ($p \leq 0.05$) to T0 (2.87 ± 0.10%) and T0.25 (2.91 ± 0.14%) during Period 98-104.

For RGRB, during Period 43-49 and Period 98-104 it was found that T1.5 (107.57 ± 3.30% and 22.55 ± 0.61%) showed a higher growth rate over T0 (93.13 ± 2.62 and 18.84 ± 0.69%). This changed during Period 77-84 where T0 (21.02 ± 0.47%) RGRB was higher than that of T0.25 (17.65 ± 1.34%).

During Period 21-36, Period 36-43, and Period 77-84, T1.5 (1.28 ± 0.07%, 1.29 ± 0.08%, and 8.92 ± 0.63%) differed ($p \leq 0.05$) to that of T0.25 (1.03 ± 0.05%,1.09 ± 0.06%, and 7.13 ± 0.41%) in terms of AGRI. A higher AGRI for T1.5 (9.34 ± 0.51%) was also seen during Period 64-71, but this was over that T0 (7.46 ± 0.29%).

**Table 5.9** Specific growth rate for biomass and individual weight (mean % ± SE) recorded from Day 8 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Biomass | | | | | Individual weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **8-21**[SM2] | - | - | - | - | - | 19.31±0.42 | 19.10±0.38 | 19.09±0.42 | 19.13±0.48 | 0.981 |
| **21-36** | 11.58±0.20 | 11.11±0.54 | 10.28±0.33 | 10.94±0.25 | 0.088 | 14.31±0.35 | 13.74±0.24 | 13.54±0.51 | 14.81±0.33 | 0.092 |
| **36-43** | 8.36±0.39 | 8.49±0.31 | 8.65±0.23 | 7.89±0.08 | 0.298 | 9.71±0.21 | 9.36±0.38 | 9.24±0.26 | 9.16±0.35 | 0.578 |
| **43-49**[SM2] | 10.96±0.23 | 11.67±0.23 | 11.79±0.59 | 12.05±0.27 | 0.205 | 10.34±0.32 | 10.96±0.76 | 11.28±0.64 | 10.80±0.43 | 0.671 |
| **49-57**[SM2] | 7.23±0.17 | 7.81±0.27 | 7.62±0.24 | 7.19±0.24 | 0.185 | 8.35±0.49 | 8.84±0.60 | 8.28±0.60 | 7.94±0.57 | 0.745 |
| **57-64**[SM2] | 5.39±0.31 | 5.95±0.35 | 5.82±0.25 | 5.24±0.33 | 0.339 | 8.17±0.56 | 7.64±1.09 | 8.63±0.58 | 7.68±0.54 | 0.696 |
| **64-71** | 5.69±0.19 | 5.84±0.11 | 5.55±0.15 | 5.93±0.10 | 0.291 | 6.08±0.21 | 6.18±0.17 | 6.02±0.21 | 6.50±0.13 | 0.260 |
| **71-77** | 4.24±0.28 | 4.36±0.30 | 4.02±0.25 | 3.97±0.22 | 0.686 | 4.86±0.27 | 4.67±0.31 | 4.43±0.25 | 4.13±0.20 | 0.226 |
| **77-84** | 2.73[a]±0.06 | 2.32[b]±0.16 | 2.32[b]±0.09 | 2.53[ab]±0.05 | 0.023 | 3.11±0.07 | 3.18±0.14 | 3.23±0.13 | 3.45±0.17 | 0.293 |
| **84-91** | 2.84±0.09 | 2.88±0.06 | 2.97±0.07 | 2.94±0.12 | 0.738 | 3.41±0.18 | 3.37±0.16 | 3.31±0.07 | 3.42±0.12 | 0.943 |
| 91-98 | 2.54±0.19 | 2.58±0.08 | 2.39±0.10 | 2.55±0.15 | 0.794 | 2.95±0.12 | 3.31±0.16 | 3.21±0.08 | 3.23±0.08 | 0.143 |
| 98-104 | 2.87[b]±0.10 | 2.91[b]±0.14 | 3.08[ab]±0.13 | 3.39[a]±0.08 | 0.016 | 3.43±0.18 | 3.33±0.18 | 3.56±0.17 | 3.57±0.07 | 0.684 |

Different superscripts (e.g. [a, b]) in the same row indicate significant differences ($p \leq 0.05$).

[SM2] – Sampling method 2

**Table 5.11** Relative growth rate for biomass and individual weight (mean % ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Biomass | | | | | Individual weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** | **0** | **0.25** | **0.5** | **1.5** | **P-value** |
| **8-21[SM2]** | - | - | - | - | | 1065.80±54.37 | 1141.54±46.63 | 1166.66±81.92 | 1126.72±73.72 | 0.739 |
| **21-36** | 490.97±19.69 | 469.13±40.43 | 408.03±22.93 | 454.61±20.46 | 0.163 | 764.27±44.74 | 689.19±29.2 | 679.57±56.58 | 831.65±47.29 | 0.089 |
| **36-43** | 84.73±2.46 | 82.16±3.61 | 83.39±3.01 | 77.24±2.45 | 0.293 | 97.54±2.90 | 93.07±5.15 | 91.22±3.48 | 90.30±4.88 | 0.608 |
| **43-49[SM2]** | 93.13$^{b}$±2.62 | 101.48$^{ab}$±2.84 | 103.88$^{ab}$±6.96 | 107.57$^{a}$±3.30 | 0.028 | 85.72±5.34 | 91.78±8.15 | 99.74±6.41 | 92.86±5.30 | 0.474 |
| **49-57[SM2]** | 78.96±2.58 | 87.12±3.95 | 84.68±3.31 | 77.99±3.40 | 0.178 | 100.49±7.65 | 114.48±6.92 | 90.91±7.63 | 84.04±9.52 | 0.071 |
| **57-64[SM2]** | 48.49±3.85 | 52.00±3.59 | 54.64±1.18 | 48.57±2.83 | 0.447 | 86.44±10.99 | 62.58±9.10 | 83.95±7.19 | 72.57±5.50 | 0.175 |
| **64-71** | 50.61±2.59 | 51.80±2.07 | 48.69±1.83 | 53.08±1.41 | 0.464 | 53.16±2.21 | 54.23±1.85 | 52.50±2.22 | 57.61±1.43 | 0.268 |
| **71-77** | 29.15±2.22 | 30.05±2.29 | 25.66±1.03 | 26.95±1.63 | 0.362 | 34.02±2.21 | 32.45±2.40 | 30.58±2.04 | 28.16±1.57 | 0.226 |
| **77-84** | 21.02$^{a}$±0.47 | 17.65$^{b}$±1.34 | 17.95$^{b}$±0.75 | 19.34$^{ab}$±0.42 | 0.032 | 24.29±0.61 | 24.95±1.22 | 25.42±1.12 | 27.35±1.52 | 0.290 |
| **84-91** | 21.98±0.74 | 22.35±0.55 | 23.11±0.60 | 22.84±1.02 | 0.730 | 27.06±1.60 | 26.69±1.40 | 26.11±0.63 | 27.06±1.06 | 0.937 |
| **91-98** | 19.54±1.64 | 19.79±0.66 | 18.22±0.83 | 19.57±1.20 | 0.790 | 22.94±1.03 | 26.12±1.38 | 25.17±0.69 | 25.41±0.67 | 0.141 |
| **98-104** | 18.84$^{b}$±0.69 | 19.13$^{b}$±1.03 | 20.33$^{ab}$±0.93 | 22.55$^{a}$±0.61 | 0.017 | 22.88±1.31 | 22.15±1.33 | 23.83±1.23 | 23.85±0.49 | 0695 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

[SM2] – Sampling method 2

**Table 5.12** Absolute growth rate for biomass and individual weight (mean g ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Biomass | | | | | Individual weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value | 0 | 0.25 | 0.5 | 1.5 | p-value |
| **8-21[SM2]** | - | - | - | - | - | 0.148±0.011 | 0.139±0.010 | 0.156±0.013 | 0.143±0.008 | 0.694 |
| **21-36** | 162.48±4.32 | 138.85±10.38 | 136.10±8.49 | 140.70±8.21 | 0.121 | $1.20^{ab}$±0.06 | $1.03^{b}$±0.05 | $1.10^{ab}$±0.03 | $1.28^{a}$±0.07 | 0.027 |
| **36-43** | 142.49±8.34 | 131.14±8.87 | 133.43±11.43 | 122.74±4.27 | 0.446 | $1.32^{a}$±0.05 | $1.09^{b}$±0.06 | $1.16^{ab}$±0.05 | $1.29^{a}$±0.08 | 0.044 |
| **43-49[SM2]** | 297.39±15.42 | 278.98±23.95 | 295.84±21.5 | 302.13±15.83 | 0.853 | 2.26±0.14 | 2.07±0.17 | 2.33±0.14 | 2.52±0.16 | 0.239 |
| **49-57[SM2]** | 500.50±10.67 | 487.52±21.51 | 491.68±26.37 | 451.55±26.84 | 0.449 | 4.67±0.41 | 4.42±0.33 | 4.46±0.35 | 4.73±0.46 | 0.931 |
| **57-64[SM2]** | 529.38±44.38 | 549.49±46.25 | 562.89±42.98 | 494.22±26.88 | 0.651 | 7.10±0.73 | 6.19±1.02 | 7.52±0.55 | 6.95±0.42 | 0.568 |
| **64-71** | 809.14±52.26 | 827.99±49.45 | 783.96±47.61 | 786.45±35.22 | 0.900 | $7.46^{b}$±0.29 | $7.68^{ab}$±0.57 | $8.22^{ab}$±0.36 | $9.34^{a}$±0.56 | 0.042 |
| **71-77** | 687.54±46.00 | 723.82±66.81 | 643.71±40.34 | 606.44±46.92 | 0.396 | 8.27±0.31 | 6.92±0.37 | 7.29±0.45 | 7.09±0.32 | 0.077 |
| **77-84** | 626.00±31.57 | 549.33±46.65 | 548.47±47.00 | 549.80±48.79 | 0.515 | $7.61^{ab}$±0.22 | $7.13^{b}$±0.48 | $7.87^{ab}$±0.29 | $8.92^{a}$±0.63 | 0.036 |
| **84-91** | 859.51±59.33 | 823.69±47.38 | 823.44±42.84 | 777.74±50.58 | 0.719 | 10.65±0.87 | 9.63±0.81 | 10.21±0.34 | 11.16±0.41 | 0.404 |
| **91-98** | 880.58±65.79 | 894.92±61.73 | 832.12±47.30 | 810.84±45.75 | 0.682 | 11.94±0.70 | 11.86±0.82 | 12.37±0.31 | 13.42±0.67 | 0.311 |
| **98-104** | 1018.47±35.90 | 1038.55±85.84 | 1060.61±63.55 | 1077.68±74.99 | 0.926 | 14.04±0.82 | 12.57±0.69 | 14.62±0.60 | 15.16±0.97 | 0.150 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

[SM2] – Sampling method 2

Table 5.13 presents the total body length of the African catfish fish in millimetres (mm) for each treatment from Day 49 to Day 104. Table 5.14 presents the various growth rates (absolute, relative and specific) in terms of total body length of the African catfish for each treatment from Day 49 to Day 104.

The total body length of the fish from T0.25 (162.78 ± 2.01mm) differed ($p \leq 0.05$) to that of T0 (173.24 ± 2.42mm) and T1.5 (174.89 ± 2.21mm) on Day 77, all treatments on Day 91, and T0.5 (220.08 ± 2.56mm) on Day 104, where it was always seen to be smaller.

The only growth rate, in terms of total body length of the fish, that differed ($p \leq 0.05$) was relative growth rate during Period 91-104, which saw T0.25 (19.66 ± 2.23%) and T0.5 (17.61± 2.77%) to show a greater growth rate compared to T0 (11.56 ± 0.74%).

**Table 5.13** Total body length (mean mm ± SE) recorded from Day 49 to Day 104 for African catfish that received Aquahatch during early development.

| Day | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | **0** | **0.25** | **0.5** | **1.5** | **p-value** |
| **49** | 89.00±1.23 | 86.68±1.18 | 88.30±1.35 | 91.37±1.20 | 0.068 |
| **64** | 132.56±1.73 | 133.34±1.76 | 134.82±1.78 | 133.91±1.49 | 0.809 |
| **77** | $173.24^{a}$±2.42 | $162.78^{b}$±2.01 | $167.93^{ab}$±1.93 | $174.89^{a}$±2.21 | 0.0004 |
| **91** | $199.21^{a}$±2.71 | $184.16^{b}$±2.61 | $196.54^{a}$±3.03 | $195.48^{a}$±2.37 | 0.001 |
| **104** | $223.66^{ab}$±2.64 | $220.08^{b}$±2.51 | $230.48^{a}$±2.93 | $225.75^{ab}$±2.42 | 0.049 |

Different superscripts (e.g. $^{a, b}$) in the same row indicate significant differences ($p \leq 0.05$).

**Table 5.14** Growth rates (absolute (mm), relative (%) and specific (%)) in terms of total body length (mean ± SE) recorded from Day 49 to Day 104 for African catfish that received Aquahatch during early development.

| | | Period | 49-64 | 64-77 | 77-91 | 91-104 |
|---|---|---|---|---|---|---|
| **Absolute Growth Rate for total body length (mm)** | **Treatment inclusion level (mL/L)** | **0** | 43.56±1.75 | 37.52±2.37 | 25.97±4.05 | 24.46±1.88 |
| | | **0.25** | 46.65±3.35 | 29.44±2.98 | 21.38±2.94 | 35.92±3.71 |
| | | **0.5** | 46.52±2.17 | 34.06±3.50 | 27.66±4.15 | 33.94±5.09 |
| | | **1.5** | 42.54±1.81 | 40.98±2.62 | 20.59±3.25 | 30.27±3.49 |
| | | **p-value** | 0.489 | 0.056 | 0.459 | 0.165 |
| **Relative Growth Rate for total body length (%)** | **Treatment inclusion level (mL/L)** | **0** | 49.19±2.36 | 30.77±2.85 | 15.44±2.57 | $11.56^{b}$±0.74 |
| | | **0.25** | 53.97±4.12 | 22.48±2.56 | 13.18±1.85 | $19.66^{a}$±2.23 |
| | | **0.5** | 52.87±2.80 | 25.56±2.93 | 16.55±2.53 | $17.61^{a}$±2.77 |
| | | **1.5** | 46.81±2.53 | 32.13±1.63 | 12.02±2.05 | $15.50^{ab}$±1.80 |
| | | **p-value** | 0.314 | 0.0502 | 0.492 | 0.066 |
| **Specific Growth rate for total body length (%/day)** | **Treatment inclusion level (mL/L)** | **0** | 2.45±0.17 | 2.08±0.09 | 1.17±0.11 | 0.693±0.072 |
| | | **0.25** | 2.60±0.12 | 2.18±0.09 | 1.09±0.11 | 0.737±0.044 |
| | | **0.5** | 2.63±0.09 | 1.95±0.07 | 1.33±0.09 | 0.612±0.071 |
| | | **1.5** | 2.27±0.09 | 2.04±0.09 | 1.32±0.10 | 0.732±0.076 |
| | | **p-value** | 0.154 | 0.298 | 0.274 | 0.581 |

Different superscripts (e.g. $^{a, b}$) in the same column indicate significant differences ($p \leq 0.05$).

Table 5.15 presents the exponent b for the body condion equation calculated for each treatment during Period 49-104. The exponet b was derived from the slope of the relationship between the natural logarithm of the individual weight and the natural log of total body length of the African catfish from each treatment. It was analysed by means of simple linear regression with a 95% prediction interval. There was no difference ($p \geq 0.05$) between the treatments for b, with a mean of 2.97 ± 0.017. The b value for each treatment concentration also did not differ ($p \geq 0.05$) to that of b=3 given by Fultons body condition for isometric growth.

**Table 5.15** Exponent b for body condition equation (mean ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Period | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 49-104 | 3.00±0.05 | 3.00±0.03 | 2.93±0.03 | 2.96±0.03 | 0.426 |

Table 5.16 presents the body condition of the African catfish from Day 49 to Day 104 between treatments. There were no differences ($p \geq 0.05$) between treatments for body condition on any of the days. There was however, a tendency on Day 64 ($p = 0.091$) and Day 104 ($p = 0.086$) for higher concentration treatments to result in a greater condition factor, with T0.5 having the greatest condition factor on all days. The mean body condition for the whole trial was 0.846 ± 0.030.

**Table 5.16** Body condition (mean ± SE) recorded from Day 21 to Day 104 for African catfish that received Aquahatch during early development.

| Day | Treatment inclusion level (mL/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 1.5 | p-value |
| 49 | 0.670±0.121 | 0.726±0.083 | 1.002±0.114 | 0.897±0.115 | 0.146 |
| 64 | 0.595±0.154 | 0.672±0.113 | 1.070±0.162 | 0.929±0.136 | 0.091 |
| 77 | 0.613±0.114 | 0.686±0.08 | 0.948±0.128 | 0.839±0.109 | 0.157 |
| 91 | 0.657±0.13 | 0.725±0.084 | 1.081±0.133 | 0.921±0.135 | 0.086 |
| 104 | 0.752±0.166 | 0.777±0.099 | 1.151±0.147 | 1.027±0.144 | 0.156 |

### 5.1.6 Body proximate composition

A summary of the body proximate composition (moisture, crude protein, ash, and crude lipid) recorded on Day 21 and Day 104, for African catfish that received Aquahatch during early development, is presented in Table 5.17. Differences ($p \leq 0.05$) in ash and crude lipid content between treatments were found, but no differences ($p \geq 0.05$) were found for the moisture and crude protein content between treatments. The difference ($p \leq 0.05$) in ash was found on Day 21, where T0 (2.13 ± 0.04%) had a higher ash content then that of T1.5 (1.96 ± 0.03%). The ash content did not differ ($p \geq 0.05$) between treatments on Day 104, with a mean of 1.95 ±

0.08%. Crude lipid content did not differ ($p \geq 0.05$) between treatments on Day 21, with a mean of 4.28 ± 0.08%. On Day 104, the crude lipid content of T0 (10.47 ± 0.15%) and T1.5 (10.59 ± 0.31%) was greater than that of T0.25 (9.81 ± 0.15%). The mean moisture content on Day 21 and 104 was 83.15 ± 0.14% and 73.01 ± 0.18%, respectively. The mean crude protein content was 10.17 ± 0.12% on Day 21, and 14.99 ± 0.17% on Day 104.

**Table 5.16** A summary of the body proximate composition for moisture, crude protein, ash, and crude lipid (mean % ± SE) recorded on Day 21 and Day 104 for African catfish that received Aquahatch during early development.

| | | Day | 21 | 104 |
|---|---|---|---|---|
| **Moisture (%)** | **Treatment inclusion level (mL/L)** | **0** | 83.06±0.22 | 73.00±0.28 |
| | | **0.25** | 83.16±0.36 | 72.90±0.38 |
| | | **0.5** | 82.81±0.25 | 72.90±0.42 |
| | | **1.5** | 83.58±0.26 | 73.25±0.40 |
| | | **p-value** | 0.246 | 0.898 |
| **Crude protein (%)** | **Treatment inclusion level (mL/L)** | **0** | 10.40±0.19 | 14.92±0.41 |
| | | **0.25** | 10.17±0.30 | 15.29±0.33 |
| | | **0.5** | 10.32±0.25 | 15.09±0.32 |
| | | **1.5** | 9.79±0.23 | 14.66±0.32 |
| | | **p-value** | 0.280 | 0.635 |
| **Ash (%)** | **Treatment inclusion level (mL/L)** | **0** | $2.13^{a}$±0.04 | 2.20±0.26 |
| | | **0.25** | $2.06^{ab}$±0.05 | 1.96±0.10 |
| | | **0.5** | $2.06^{ab}$±0.03 | 1.82±0.11 |
| | | **1.5** | $1.96^{b}$±0.03 | 1.81±0.10 |
| | | **p-value** | 0.011 | 0.259 |
| **Crude lipid (%)** | **Treatment inclusion level (mL/L)** | **0** | 4.16±0.14 | $10.47^{a}$±0.15 |
| | | **0.25** | 4.41±0.23 | $9.81^{b}$±0.15 |
| | | **0.5** | 4.31±0.09 | $10.23^{ab}$±0.22 |
| | | **1.5** | 4.25±0.16 | $10.59^{a}$±0.31 |
| | | **p-value** | 0.751 | 0.018 |

Different superscripts (e.g. $^{a, b}$) in the same column indicate significant differences ($p \leq 0.05$).

### 5.1.7 Cephalosomatic index and viscerosomatic index

Cephalosomatic and viscerosomatic index gives the head and visceral organ weight, respectively, as a percentage of the total body weight of the African catfish fish (%). These were recorded on Day 104 and are illustrated in Figure 5.6. The mean cephalosomatic and viscerosomatic index was 31.02 ± 0.15% and 9.55 ± 0.11%, respectively. Differences were recorded for both the cephalosomatic ($p = 0.017$) and viscerosomatic index ($p = 0.010$). These differences occurred between T0 (31.65 ± 0.28%) which had a larger head weight to total body weight proportion than that of T1.5 (30.49 ± 0.28%), and between T0.25 (9.09 ± 0.26%) which had a smaller visceral organ weight to total body weight proportion than that of T0.5 (9.96 ± 0.22%).

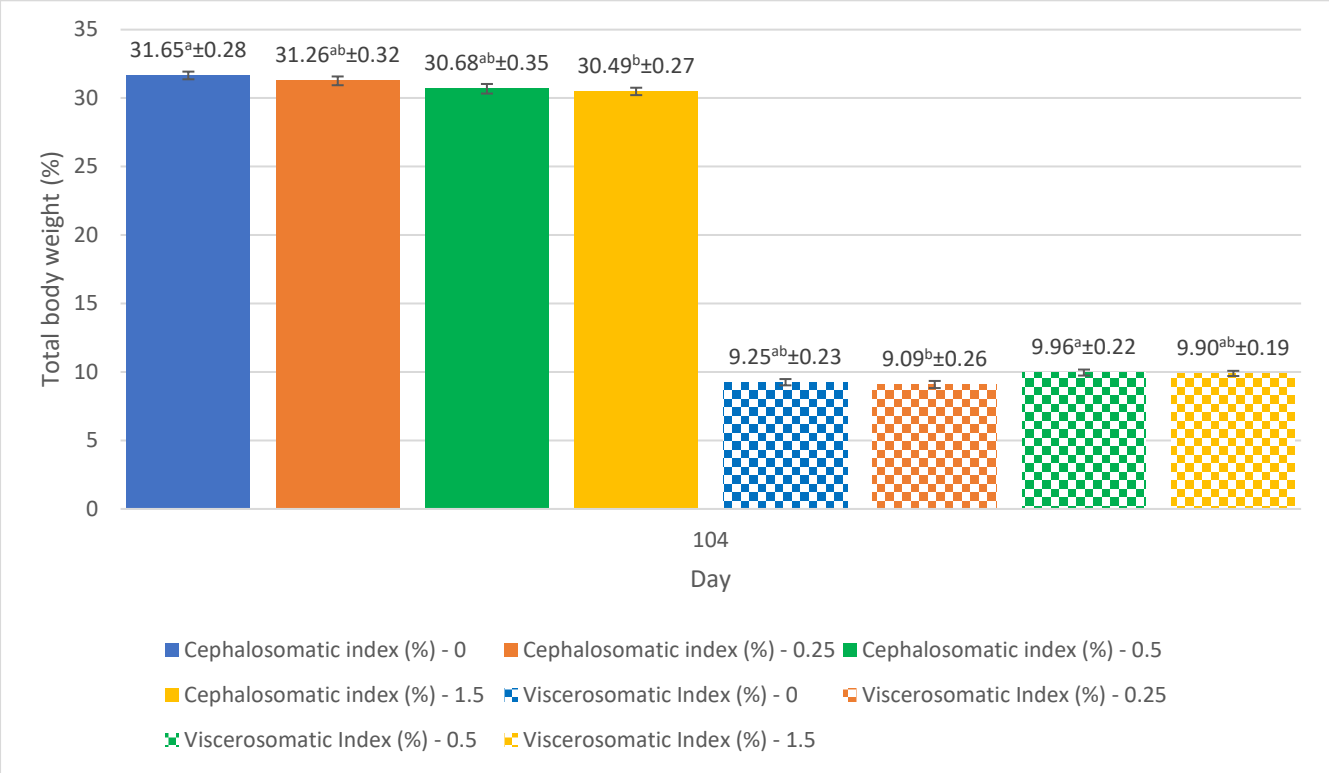


**Figure 5.6** Cephalosomatic index and viscerosomatic Index of the African Catfish that received Aquahatch during early development recorded on Day 104.

## 5.2 Discussion

Throughout the current trial the R-squared value for the data was seen to be very low, which can be attributed to a high variation within the groups. The high variation within the groups can be accredited to the high inherent variation within an African catfish population (Martins *et al.*, 2005). This variation is especially prominent during the juvenile stages of African catfish that is characterized by high growth and development rates, which result a large degree of variation within a population. Despite the low R-squared value, significant differences in the data recorded were present, these differences will be discussed below.

### 5.2.1 Haematocrit

Haematological parameters, such as haematocrit, provide an indication of the health status and stress condition of the fish and are often used to detect problems that are not visually apparent. Poor haematocrit values have been associated with stress, diseases, and poor nutrition (Hattingh & Van Pletzen, 1974; Martins *et al.*, 2008; Hansen *et al.*, 2015). The B vitamin and nucleotide components of Aquahatch are seen in other studies to have the most pronounced effect on haematocrit values (Li & Gatlin, 2006; Ringo *et al.*, 2012; Hansen *et al.,* 2015; Huu, 2016). In the current trial, the method used to supplement Aquahatch did not have an effect on the haematocrit values for the African catfish, and the results obtained were comparable to other African catfish and finfish studies (Robinson & Lovell, 1978; Andrews & Murai, 1979; Limsuwan & Lovell, 1982; Ng *et al.*, 1997; Morris *et al.*, 1998; Mohamed & Ibrahim, 2001; Welker *et al.*, 2011).

### 5.2.2 Mortality

On Day 104 of the current trial, there is an indication in the total mortality that corresponds to higher treatment concentrations negatively affecting the survival of the African catfish. This would contradict the majority of studies done on the nutrients that are included in the Aquahatch formulation, as these nutrients are seen to rather help improve survival, especially during periods of stress. L-carnitine has been indicated in the improvement of the survival of fish in several studies, such as in African catfish under exercise stress (Ozorio *et al.*, 2005), for chinook salmon subjected to acute ammonia poisoning (Tremblay & Bradley, 1992), in guppies exposed to xenobiotics (Schreiber *et al.*, 1997) and in ornamental cichlid exposed to cold shock (Harpaz *et al*, 1999). Nucleotide supplements have been see to improve the survival of disease infected fish (Burrells *et al.*, 2001b; Li *et al.*, 2004). Vitamins may be oversupplied and accumulate in the body at toxic levels, called hypervitaminosis, which negatively effects survival. However, these conditions are not likely to occur under practical farming conditions and do not occur for water-soluble vitamins, such as the B vitamins found in Aquahatch (Tacon, 1992).

The mortality levels in the current trial was considerably high, the cause of which is not thought to be accredited to Aquahatch as the same high mortality levels were also experienced by the control. High mortality rates in African catfish production systems are not uncommon. There are a number of factors which can contribute to the incidence of mortalities, and most commonly include poor water quality, disease, stress, and cannibalism.

In terms of water quality, the DO levels throughout trial were in the optimal range for African catfish. There was a gradual decline in the DO levels over the course of the experiment as the fish used more oxygen as they increased in size. African catfish have the ability to breathe atmospheric oxygen, therefore the DO is not a considerable limiting factor. The pH was low throughout the experiment but still fell within the tolerable pH range of 5-9 (Ndubuisi *et al.*, 2015).

The main concerns were higher than optimal temperatures in the early stages of the trial and the elevating total ammonia levels throughout the trial.

The mean temperature for the current trial was within the optimal range for African catfish production however, there were periods in the early stages in which the temperature increased to the upper limits, i.e. higher than 35°C (Britz & Hecht, 1987; Prokesova *et al.*, 2015; Santi *et al.*, 2017). This was due to the trial being conducted in summer with the air conditioning unit frequently experiencing problems. These temperatures, despite not being in the ideal range, are still tolerated by African catfish. The temperatures were also not maintained throughout the day, with a decline experienced during the night-time. Due to the temperature still maintaining tolerable levels for African catfish, temperature was not suspected to be the main cause of the high mortality rates but possibly led to some stress.

High total ammonia levels were another water quality concern. Steps were taken to try and manage the increasing rate for example, partial and complete water exchanges, cleaning of the filtration unit, and maintaining water pH below 7. Total ammonia can be separated into two components, $NH_3$ and $NH_4^+$, of which $NH_3$ is the more toxic form which can only being tolerated at low levels, whilst $NH_4^+$ can be tolerated at much higher levels by African catfish. The proportion of these components in the water is dependent mainly on pH and temperature (Durborow *et al.*, 1997). An increase in pH and temperature leads to a higher proportion of $NH_3$, whereas a decrease leads to a high proportion of $NH_4^+$. Schram *et al.* (2010) studied the effect of elevated ammonia levels on African catfish and it was advised that the $NH_3$ water levels should never exceed 0.34mg/L. The estimated levels of $NH_3$ in the current trial, was judged to never exceed this limit, due to a pH below 7 being maintained (Durborow *et al.*, 1997). Schram *et al.* (2010) did not experience any mortalities even at the two highest total ammonia levels of 72.2mg/l and 275.1mg/L with a pH above 7 for a prolonged period of 34

days, demonstrating the ability of African catfish to tolerate extreme levels of ammonia stress. In the current trial, conditions potentially contributing to ammonia stress to such a high extent were not reported, thus indicating that the ammonia levels were not the main cause of the high mortality levels experienced in the current trial. Although not contributing to mortalities, the high ammonia levels resulted in an increase in FCR and a decline in SGR, with the latter two parameters as potentially indicative of increased stress levels in the African catfish.

By looking at the nature of the mortalities, the main cause becomes more apparent. Period 21-36 experience an elevated daily mortality rate for all treatments, with a mean of 2.44 ± 0.13 fish/day as opposed to a mean of 0.564 ± 0.025 fish/day for remainder of the trial. This period was also associated with a high feeding rate, 40.7 ± 1.41%. Despite high feeding rates being seen to reduce mortalities in African catfish, as a result of cannibalism (Al-hafedh & Ali, 2004), excessive feeding rates also show adverse effects on African catfish.

Ruptured intestine syndrome (RIS) is a common non-infectious disease related to the over-feeding of juvenile African catfish, and commonly occurs at 2-8 weeks after hatch. The syndrome is characterised by five stages that include the following: (1) local inflammation in the hind gut resulting in (2) rupturing of the gut, (3) peritonitis producing a haemorrhagic smelling fluid in the abdominal cavity. This is followed by (4) an internal ulcer of the ventral abdominal wall, and finally (5) the disappearance of a great part of the gut and ventral abdominal wall (Boon *et al.*, 1987; Schippers *et al.*, 1992; Hariati *et al.*, 1994).

Boon *et al.* (1987) found a feeding rate of 27% to cause RIS and result in a 23.9% mortality rate. Period 21-36 had a mean feeding rate of 40.7 ± 1.41%, which is well above the feeding rates associated with RIS, as found in the above-mentioned study. During Period 21-36 deceased African catfish were found with swollen or missing abdomens. This together with the high feeding rate and the age of the fish leads us to believe RIS was the main cause of the mortalities during Period 21-36. Since no differences between treatments in the rate of mortalities during and soon after Period 21-36 were found, initial indications suggest Aquahatch offered no support to the African catfish in helping them manage elevated feeding rates and RIS.

After Period 21-36 the feeding rates were reduced, with no further signs of RIS or other illnesses and diseases. An improvement in the daily mortality rates were seen for all treatments however, these rates were still considerably high. Mortalities at this time were noticed during sampling periods where mainly skeletons or parts of fish were found. Therefore, cannibalism is determined to be the main cause of the mortalities past Day 36. Cannibalism is the act of consuming whole, or a major part, of an individual of the same species. There are many genetic, behavioural and environmental factors affecting the rate of cannibalism in

African catfish such as stocking density, size variation, food availability, lighting (Solomon & Udoji, 2011). In the current trial, a low stocking density and a high degree of variation in individual weight were determined to be the main cause of cannibalism.

The use of incorrect stocking densities, both low and high, can have adverse effects on African catfish. In the current trial, a stocking density on Day 21 of 2 fish/L with mean individual fish weight of 0.159 ± 0.007g was used. Haylor (1991) and Van de Nieuwegiessen *et al.* (2008) found signs of chronic stress and increased incidents of aggressive behaviour at low stocking densities for African catfish. Even though no differences in mortalities were experienced in both these studies, these adverse effects have the potential to lead to higher mortalities. The lowest stocking density used in the above-mentioned studies were 4 fish/L with a mean individual fish weight of 10g in Van De Nieuwegiessen *et al.* (2008)'s study and 50 fish/L with mean individual fish weight of 0.05g in Haylor (1991)'s study. The stocking density in the current trial was considerably lower and is likely to have emphasised negative effects associated with low stocking density of African catfish resulting in the high mortality rates.

A high degree of growth variation is often the main cause of cannibalism in African catfish. Younger fish have a higher growth potential than older fish therefore, growth variation is at its highest during the juvenile stages. "Shooters" is a common term to describe juvenile catfish which grow at a much faster rate than others of the same age, resulting in obvious differences in sizes (Biu *et al.*, 2015). The larger size of the shooter causes a higher degree of dominance and aggressive behaviour therefore, these shooters exhibit a greater degree of cannibalism towards other fish. A large variation in the individual size of the fish was noticed throughout the current trial. The high mortality rates experienced were also found to be comparable to that of other studies that noted cannibalism and size variation to be the main cause of mortalities. Yang *et al.* (2015) was one such study, where catfish (*S. asotus*), fed to saturation, only had a 36.3% survival, which was even lower when starved. Biu *et al.* (2015) found a 59.75% survival in unsorted African catfish (*C. gariepinus*) with the highest survival being 85.63% which is owed to weekly sorting. Survival as poor as 1.02% have been found in African catfish when no form of grading has been applied (Mollah *et al.*, 1999). Reports of high mortalities due to cannibalism, brought about by growth variation, have not only been seen in catfish but also in other fish species, such as sea bass (Katavic *et al.*, 1989), Cod (Folkvord, 1991), yellowtail (Sakakura & Tsukamoto, 1996), koi-carp (Altaff & Janakiraman, 2013), and barrumandi (Ribeiro *et al.*, 2015). In an effort to reduce the rate of cannibalism in the current trial, a small number of fish that were visually considered to be shooters were removed during sampling periods. However, the variation in the individual size of the fish became too large over the course of the trial resulting in the procedure being discontinued. This may have helped reduce the rate of cannibalism but was unsuccessful at stopping it all together.

Due to factors such as cannibalism and RIS being the suspected greatest cause of mortalities, one cannot say for certain that the differences in mortalities experienced was as a result of Aquahatch.

### 5.2.3 Feeding

The feeding rate indicates the amount of feed provided to the fish. This is important to know as growth of the fish is directly linked to the amount of feed provided and how well the fish are able to utilise the feed (Li & Robinson, 2008). The most common way to describe how well the fish utilise the feed for growth is through the use of the FCR calculation. The higher the FCR, the more feed is needed to grow the fish to a given mass and *vice versa*. Many factors affect FCR, such as fish health, genetics, environment, fish size, body composition, feed type and feeding management (Robinson & Li, 2015).

During feeding, the African catfish readily accepted the feed and consumed it immediately. However, during Period 98-104 there was a difference between the highest (T1.5 = 3.16 ± 0.05%) and lowest (T0.25 = 2.92 ± 0.06%) feeding rates which amounted to 0.24%. Although statically it was significantly different, this small difference was judged not to have caused any biological effect between African catfish from different treatments, as the feeding rate between treatments from Day 21 to Day 104 shows no differences.

The feeding rate may have been too restictive at times, possibly contributing towards the agrressive and cannibalistic nature noticed in the African catfish. The feeding rate was decreased as the fish grew which is normal practise since the metabolic requirements decrease as the fish grow. The lowering of the feeding rate was also aimed at slowing down the rate of deterioration of the water quality with regards to total ammonia. However, this reduction found the feeding rate to fall below the suggested rate of 6% (Al-hafedh & Ali, 2004) which would help to reduce cannibalism in African catfish of a similar size. Al-hafedh & Ali (2004) however also found that high feeding rates would also contribute to a deterioration in water quality.

The FCR from Day 21 to 104 showed no difference between treatments with a mean of 0.868 ± 0.005. An FCR below that of 1 indicates that the African catfish utilised the feed very efficiently. FCR is also a good indication of stress in fish, with stress causing a higher FCR. Periods in the trial where the intermediate FCR was greater than 1 includes Period 21-36 (which has previously been discussed to be associated with RIS), Period 77-84 and Period 91-98. During Period 77-84 a difference was noted between treatments seen with T0.25 having a higher FCR than that of T0 and T1.5. At the start of this period a full system water replacement was done in order to lower the total ammonia levels. Even though this was aimed at improving the water quality, it is possible that the procedure itself caused stress to the fish.

The differences in FCR between treatments could indicate that the fish adapted differently to this stressor, and therefore requires further research. The FCR was also shown to generally increase over the course of the trial, which is expected as the fish grow (Robinson & Li, 2010). The FCR's experienced in the current trial are comparable to that of other African catfish and finfish studies (Torreele *et al.*, 1993; Serrini *et al.*, 1996; Ozorio *et al.*, 2001a; Welker *et al.*, 2011; Zhao *et al.*, 2017).

### 5.2.4 Growth parameters

The most consistent difference between treatments was seen in individual body weight and length. For the majority of the days, T1.5 was seen to have a greater individual weight and length when compared to that of T0.25. With a greater individual weight, it is expected that total body length would be greater as well. The greater individual weight is thought to be less as a result of treatment effect, and more due to a difference in stocking density caused by mortalities. Stocking density can either be expressed as the number of fish or the total biomass of fish (Ellis *et al.*, 2001). Although significant only on Day 104, T1.5 consistently had a higher cumulative mortality than T0.25. This resulted in regular days that saw T1.5 having a lower biomass, although not significantly different, than that of T0.25. These differences throughout the trial are thought to have caused a biological effect which resulted in significantly different individual weights and therefore, lengths. In the current trial, there was a positive correlation between the mortalities and individual weight. This correlation was seen to generally increase over time, with a correlation coefficient (r) of 0.408 on Day 36 to 0.722 on Day 104. The correlation between individual weight and biomass was initially positive (r = 0.339 on Day 36), but then decreased to a negative correlation by the end of the trial (r = -0.230 on Day 104). This shows that both stocking density, in terms of biomass and number of fish, had an influence on the individual weight of the fish, with lower stocking densities resulting in larger individual weights. Lower stocking densities which cause higher individual weights have been reported in numerous aquaculture studies (Hengsawat *et al.*, 1997; Hossain *et al.*, 1998; M'balaka *et al.*, 2013). This is due to the fish having more space in which they can grow. Further evidence in stocking density effecting individual weight is also provided by the absolute growth rate for individual weight, which was shown in regular periods in the trial where it was greater in T1.5 compared to T0.25. This is supported by Kareem & Olanrewaju (2015) who studied the effect of stocking density on African catfish and also found lower stocking densities to increase absolute growth rate. With no other consistent difference between treatments for the various growth parameters calculated for biomass, length, and body weight, the treatment method used for Aquahatch is judged not to have had an effect on post-treatment growth of African catfish. The growth rates experienced throughout the current trial were comparable to

other African catfish and finfish studies, indicating good growth (Ozorio, 2001; Desai *et al.*, 2010; Hien & Doolgindachbaporn, 2011a, b; Zhao *et al.*, 2017).

### 5.2.5 Body condition

The body condition factor ($CF=W/L^b$) represents the relationship between the weight and length of a fish, and is also an indicator of its well-being, health and nutritional status (Luckhoff *et al.*, 2005). The length-weight relationship is given by $W=aL^b$, where W is the fish weight, L is the fish length, and a and b are derived from the regression equation logW=log a + b log L (a being the intercept and b being the slope of the regression). The value for b gives an indication of the type of growth experienced. When b = 3 isometric growth is experienced.This is when when the weight and length of the fish grow in proportion to each other. This is an assumption made by Fulton's condition factor, a popular condition factor used in aquacaulture (Jones *et al.*, 1999). However, this assumption is not always correct, with b values that vary from 3 resulting in allometric growth (Froese, 2006). When b > 3, the fish grows faster in weight than in length, become more round as length increases. When b < 3 the opposite is true, the fish grows faster in length than in weight becoming more slender as the length increases. The b value for African catfish growth is generaly found to lie within the range of 2.699-3.254 (Fishbase, 2019). Many factors can effect the b-value such as age, sex, season, maturation, fullness of the gut, feed, fat reserves and musculature (Le Cren, 1951; Barnham & Baxter, 1998; Khallaf *et al.*, 2003; Luckhoff *et al.*, 2005; Froese, 2006; Hossain *et al.*, 2006; Tarkan *et al.*, 2006; Muchlisin *et al.*, 2010).

In the current trial, the b values for each treatment were not seen to differ from one another aswell as that of 3, given by Fultons conditon factor.Therefore, isometric growth was experienced in the trial. However, the determined b-value for each treatment was still used to calculate the condition factor rather than using Fulton's condition factor, as it results in a more accurate representation (Froese, 2006). The condition factor of the fish were found to be comparable to that of other African catfish and catfish studies (Serrini *et al.*, 1996; Luckhoff *et al.*, 2005; Ozorio *et al,*. 2009; Yaghobi *et al.*, 2014). There was an tendency (Day 64 $p = 0.091$ and Day 91 $p = 0.086$) that the condition factor of the fish increased with increasing Aquahatch concentration. This is suspected to have resulted from a greater b-value, although not significantly different ($p \geq 0.05$) for the control and T0.25 compared to T0.5 and T1.5. Luckhoff *et al.* (2005) recommended that one should aim for a condition factor of 1.04 for African catfish, anything below 0.856 would be an indication of negatively influencial circumstances affecting growth. The conditon factor, for the higher treatment levels, were closer to 1.04 compared to the lower treatment levels which fell below 0.856.

### 5.2.6 Body composition

Fish body proximate composition is an important parameter to measure in terms of the nutrition of the fish, as it gives an indication of how the nutrients provided to the fish are being utilised (Breck, 2014). The L-carnitine component of Aquahatch is seen to have the greatest effect on the body composition in other studies. L-carnitine has been shown to affect the utilization of fats and proteins as an energy source by increasing the utilization of fats, and therefore, decreasing the utilization of proteins as an energy source. Soltan *et al.* (2016) demonstrated this effect in common carp, where the inclusion of L-carnitine in the diet reduced the body fat percentage and increased the protein percentage. This effect was not noticed in the current trial. The body composition parameters that were affected in the current trial on Day 21, saw a decrease in ash with increasing concentrations of Aquahatch. This is in accordance with El-Sayed *et al.* (2010) who found a negative correlation between L-carnitine supplementation levels in the diet and ash content in the body composition of Nile tilapia. However, this pattern in El-Sayed *et al.* (2010) study is expected to have resulted from differences seen in other body composition parameters, such as fat and protein, for which L-carnitine is known to potentially have a greater effect on. No differences were observed in the other body composition parameters on Day 21 of the current trial which would have had an effect on ash content. On day 104, the difference in the crude lipid composition can be accredited to the difference in the individual weight of the fish, the cause of which has been previously explained. Size is known to effect body proximate composition, and it is commonly seen that larger fish have a greater crude lipid content compared to smaller fish (Breck, 2014).The results obtained in the current trial for body proximate composition are comparable to that of other African catfish and finfish studies (Mohamed & Ibrahim, 2001; Ozorio *et al.*, 2001a; Desai *et al.*, 2010; Zhao *et al.*, 2017). This gives an indication that the nutrients received by the fish in the current study were adequate.

### 5.2.7 Cephalosomatic index and viscerosomatic index

Dressing yield represents the proportion to which various body components contribute to the total weight of the fish. Two components of the dressing yield include the cephalosomatic and viscerosomatic index which identify the contribution of the head and visceral organ weight to the total body weight of a fish. The skeletal muscles of the African catfish that also forms part of the dressing yield represent the economically most important part of the fish, whereas the head and organs are seen as waste or by-products. Therefore, one would aim for the treatment to improve dressing yield by a greater percentage of the weight being contributed by skeletal muscles rather than the head and organs.

In the current trial, the cephalosomatic index of the fish is shown to decrease with increasing concentrations of Aquahatch, with a difference of 1.16% between the control and T1.5. This

may indicate that Aquahatch supplementation is in favour of increased soft tissue growth (i.e. fat and protein) which makes up the majority of the body weight, rather than hard tissue growth (i.e. bone) which makes up the majority of the head weight. The cephalosomatic index is not a commonly reported growth parameter for the nutrients found in Aquahatch. Tekle *et al.* (2004) was one of the few studies that found L-carnitine supplementation to affect the cephalosomatic index of Mozambique tilapia. These differences were not seen between the control and treatments, but rather between L-carnitine supplementation levels of 250mg/kg and 750mg/kg feed, which had the lowest and highest cephalosomatic index, respectively. This in addition to the current trial may indicate that L-carnitine has the potential to reduce the cephalosomatic index within certain limits.

In the current trial, the viscerosomatic index was seen to be greater at higher Aquahatch concentrations. Some of the major organs that make up the visceral organs and contribute the most to the weight include the liver, stomach and intestines, with large fat deposits also contributing a significant amount. These organs, referred to as offal, are often discarded during the processing of the fish. Despite an increased viscerosomatic index resulting in increased offal being discarded, an increase in the weight of these organs may indicate altered functionality and fat metabolism around these organs. Increased stomach and intestine weight may indicate possible altered digestive function, whilst increased liver weight may indicate possible altered health status and immune function.

A study conducted by Desai *et al.* (2010), which had a comparable percentage body weight to the current trial, found no difference in the visceral organ index in Asian catfish supplemented with L-carnitine. However, when the liver weight was taken into account separately, Desai *et al.* (2010) found that increasing concentrations of L-carnitine resulted in a smaller contribution of the liver to the total body weight, possibly due to less fat being deposited in the liver. In the current trial, despite not differentiating the liver from the rest of the visceral organs, the visceral organ index follows the opposite trend seen by Desai *et al.* (2010) with regards to the liver weight. The difference in the visceral organ weight experienced in the current trial points to possible further investigation into how Aquahatch impacts the separate organs that make up the visceral organs. This may provide some direction to where a possible benefit of the product lies.

### 5.2.8 A comparison of the three sampling methods

Three sampling methods were used in the current trial to obtain data for the fish. These methods as well as the image analysis technique employed were characterized by advantages and disadvantages, which are discussed below.

A variety of forms of image analysis have been used in aquaculture research (Ruff *et al.*, 1995; Blonk *et al.*, 2010; Verdal *et al.*, 2014; Naslund, 2014; Petrtyl *et al.*, 2014; Man, 2016). However, it is often the case that such image analysis techniques require expensive equipment and computer programs that are difficult to operate and are not readily accessible to the everyday farmer. The image analysis procedure used in the current trial made use of an open platform image analysis program, ImageJ. To analyse images on ImageJ a computer with access to the internet, a smart phone with a decent quality camera, a ruler and a container to hold the fish are required. This equipment was deemed to be easily accessible and affordable to most farmers.

African catfish are a hardy species however, excessive handing, which is a common occurrence during sampling, may be a cause of stress. This potentially leads to diseases, impaired growth and poor survival (Adeyemo *et al.*, 2009). This is where one of the advantages of using image analysis, to obtain data during fish sampling, lies. Using image analysis to collect data on the fish during sampling helped to greatly increase the efficiency of sampling and therefore, the African catfish spent less time being handled. With regards to SM1 used image analysis to capture the total length of the fish which would usually be done by individually sedating and manually measuring the fish. Sedation of the fish dramatically increases the time required for sampling as it involves an induction period and a recovery period, which can both take in excess of 5-10 minutes (Ogretmen & Gokcek, 2013; Adeshina *et al.*, 2016). Since measuring is done individually and requires some time for accuracy, sedation would also have to be done individually to avoid complications, such as over-sedation. By measuring and sedating individually, a considerable amount of time is required to sample even a few fish. With image analysis, a picture can quickly be captured of all the fish at once and later be analysed to obtain desired morphological measurements, in this study's case, the total length of the fish. This allows for the fish to be sedated as a group since the manual, individual length measurement is not required. Even though not implemented, it was also discovered that sedation was not entirely necessary in order to take length measurements of the fish when using image analysis. Sedation is mainly used when manually taking length measurements to reduce stress and make it easier to handle and position the fish. Since the type of handling and positioning required in manual measurements is not required in image analysis, African catfish have the potential to be measured with image analysis, without the use of sedation.

Even if done correctly, the sedation process still has the potential to weaken the fish, making it vulnerable to aggressive behaviour and cannibalistic tendencies. Therefore, the exclusion of sedation during sampling might be beneficial to the survival of the fish.

Only total length of the fish was measured in the current trial, however by using image analysis there was the potential to capture more morphological measurements, provided they were in the same plane, as a 2D image was used.

In SM3, image analysis was used to count the total number of fish in each tank at a later stage. Counting the fish manually would involve counting them one by one and placing them into their original tank. This process requires a large amount of handling and a prolonged period in which the fish are crowded, all causing additional stress. By using image analysis, the procedure to count the fish had a considerably shorter handing and crowding time than that required for manual counting, potentially reducing stress. Image analysis was also found to be more accurate at capturing data when sampling. The increased accuracy of image analysis is mainly due to increased potential for human error when manually capturing data. Mistakes such as, inconsistent uniformity of measurements, miscounting and recording inaccurate measurements of the fish are common when sampling a large number of fish. This either results in having to re-record data if found to be incorrect, resulting in additional stress being placed on the fish, or if realized too late, the incorrect data will have to be excluded. Image analysis helps to reduce the amount of human error and allows, in some cases, for images rather than the fish to be revised to obtain the correct data if mistakes are made. Capturing images of the fish also allows for a better visual analysis of health and abnormalities. Petrtyl *et al.* (2014) who compared manual measurements with image analysis measurements of fish come to the same conclusion. In the study, it was found that a high degree of error and variation occurred when manual measurements were taken and compared to measurements from image analysis. It was also found that the accuracy of manual measurements was more dependent on the experience of the researcher. Therefore, image analysis provides, even an inexperienced researcher, a way of measuring morphological characteristics of fish with a high degree of accuracy and uniformity.

There were, however, some disadvantages in the image analysis method used. The main disadvantage found was that the analysis of the image required a considerable amount of time. Therefore, one would save time on fish handling, but analysis time would be increased.

In SM1, a factor that increased the analysis time was recalibration between images in order to take measurements. The longer analysis time is due to a set-point not being used, if it was used it would avoid the procedure of recalibration.

In SM3, a problem that was encountered during analysis of the images was due to the growth of the fish which led to them being crowded in the holding container. This made differentiating individual fish and obtaining a fish count difficult.

Since the problem was only discovered when analysing the images at a later stage, days that were difficult to differentiate between fish were excluded from the results. This was later avoided by using a larger container to hold the fish while capturing the image, which allowed for clearer individual identification.

The main advantage of SM1 is its potential to capture a greater variety of morphological information. However, increasing the amount of information gathered also increases the sampling time and the handling time. Therefore, only a few of the fish could be sampled in a given time frame. In the current trial, the number of fish sampled during SM1 was limited to 10 fish (5% of Day 21 stocking density). When performing statistical analysis, outliers are removed. Since multiple individual fish per tank are analysed in SM1, it provides numerous data points. If classified as an outlier, these individual data points can be removed rather than the whole tank, avoiding the deletion of treatment replicates.

The main advantage of SM2 is that it can be carried out in a relatively short period without the use of sedation. This method was potentially the least stressful sampling method out of the three used. SM2 allowed a greater proportion of the fish in the tank to be sampled, within a given time period, than that of SM1. This was limited to limited to 20 fish (10% of Day 21 stocking density). SM2 however, provides the least amount of information on the fish compared to the other sampling methods. It also results in whole tanks being discarded when classified as outliers during statistical analysis of the data.

The main advantage of SM3 is that it samples all the fish in the tank, providing the most accurate representation of the fish and changes over time, when compared to the other sampling methods.SM3 does not require sedation which reduces the stress placed on the fish. The disadvantage of SM3 includes potential sampling related stress affecting the entire tank rather than a proportion of it and the removal of outliers during data analysis resulting in treatment replicates being removed. In SM3, although avoided using image analysis, miscounting the number of fish is a common human error, especially with an increased number of fish. If not detected this can result in over or under estimation of data recorded.

Certain situations may not allow each sampling method to be used at each sampling period. A combination of the three sampling methods may be used. The advantages and disadvantages for each sampling method and the image analysis procedure will allow one to have a better understanding of which method is best suited to their needs.

## 5.3 Conclusions

The current trial explored a novel method of supplementing a commercially available mixture of L-carnitine, nucleotides and B vitamins in the form of Aquahatch into the culture water of

African catfish during their larval and early fry developmental period, in an effort to improve post-treatment growth performance. Information on the potential use of three sampling methods and image analysis as sampling tool, was also presented.

Acceptable growth for African catfish was experienced in the Trial. The greater individual fish size, in term of live weight and body length, that was seen for T1.5 when compared to T0.25 was not ascribed to the Aquahatch supplementation but rather to a difference in stocking density, in terms of number of fish and biomass, that resulted from a difference in mortalities. The main causes of mortalities were suspected to be as a resulted of ruptured intestinal syndrome (RIS) and cannibalism. The size difference influenced parameters such as the crude lipid content and absolute growth rate of the fish.

The results obtained in the trial leads us to believe that the supplementation of Aquahatch in the water, during the early developmental stages of African catfish, does not have an effect on the potential post-treatment growth of the fish.

This trial provides a benchmark study for Aquahatch as a nutrient supplement and its potential to improve growth and wellbeing of freshwater fish. Future studies need to further refine the Aquahatch supplementation method and level. Inconclusive results that require further investigation include increasing Aquahatch inclusion levels decreasing in the cephalosomatic index and improving the body condition for the African catfish.

# Chapter 6

# General conclusion and recommendations

The global development of the African catfish (*C. gariepinus*) industry is bottlenecked by the limited supply of quality seed stock. The juvenile phase of African catfish is characterised by poor survival which can be attributed to early developing fish being highly prone and sensitive to nutrient deficiencies. Such nutrient deficiencies can affect development and ultimately growth potential. Research on effective methods to improve the nutrient accessibility and supply during early development may help to address development and growth concerns of African catfish.

Aquahatch is a recently released liquid nutrient supplement for aquaculture species, which consists of L-carnitine, nucleotides and B vitamins. The potential synergistic influence of these components in African catfish has not yet been investigated. The nutrients in Aquahatch are all water-soluble and therefore presents an opportunity to administer the product in the culture water of African catfish.

This study presents novel findings on the use of Aquahatch as a supplement to improve early development in African catfish in terms of growth and yolk sac utilization, and the subsequent effect on growth performance post-supplementation. Due to the novel nature of the study, findings were related to studies that report on the respective nutrients that are included in the Aquahatch formulation. Studies often reported conflicting results for the growth-enhancing effects of L-carnitine and nucleotides, while B vitamins showed no added beneficial results above certain inclusion levels.

An Aquahatch inclusion level of 0.25mL/L resulted in a consistently shorter body length compared to the control during the larval stage. The smaller size is considered unfavourable as the transition to exogenous feeding is facilitated by larvae having a larger body size.

Aquahatch supplementation did not yield an improved survival, as a decrease in survival was reported in this study. The decline in survival with higher Aquahatch inclusion levels can potentially be attributed to an oversupply of nutrients, which caused a deterioration in water quality, as evident in the reported higher total ammonia levels. The highest total ammonia level was linked to the highest Aquahatch supplementation concentration, however, their relationship was not supported by high mortalities. It is thus hypothesized that Aquahatch exerted a potential protective effect by minimising the degree of ammonia stress experienced by the fish. Inconclusive results that require further investigation include differences from the control that were observed around the time associated with the development of oral ingestion

capabilities. This includes larvae having a shorter body length with Aquahatch supplementation and the highest Aquahatch inclusion level resulting in a larger larvae yolk sac volume.

African catfish treated with Aquahatch at an inclusion level of 1.5mL/L were found to have a greater individual size, in terms of live weight and total body length, then that of the fish subjected to the 0.25mL/L Aquahatch inclusion level. This size difference, however, cannot be ascribed to the Aquahatch supplementation but rather to a difference in stocking density that resulted from a difference in mortalities. The main causes of mortalities were found to include ruptured intestinal syndrome (RIS) and cannibalism. As a result, the size difference influenced parameters such as the crude lipid content and absolute growth rate of the fish. Inconclusive results that require further investigation include increasing Aquahatch inclusion levels decreasing in the cephalosomatic index and improving the body condition for the African catfish.

In conclusion, the findings of this study do not provide evidence that the supplementation of Aquahatch in the culture water, during the early developmental stages of African catfish, provide any beneficial effect for the parameters measured during and after the treatment period. Therefore, the additional expense that will be incurred with the supplementation of Aquahatch would not be justified. However, this study provides a benchmark for Aquahatch as a nutrient supplement and its potential to improve yolk sac utilization, growth and the wellbeing of freshwater fish.

## 6.3 Recommendations

From this study a few recommendations can be made for similar studies conducted in the future.

The inclusion level of Aquahatch and the method of culture water supplementation still needs to be refined. A wider range of Aquahatch inclusion levels need to be tested. However, the economics of supplementation, especially within the culture water, must also be justified. Water supplementation in larger systems may possibly cause it to become uneconomical at high inclusion levels.

For further research into water supplementation, it is recommended to test the individual nutrient stability in the water over time. The spectrophotometric analysis approach used to determine Aquahatch concentration proved to not be reliable. Therefore, this analysis method needs to be refined and compared to a chemical analysis approach of treatment concentration in the water.

In the current study, a longer treatment period was not possible due to rapid deterioration of water quality. To extend the treatment period for water supplementation, individual systems can be used with their own filtration units to prevent deterioration of water quality. The effect of common water quality management practises, such as water replacements, may alter its effectiveness and must be accounted for when testing in larger systems for a prolonged period.

Water, as a means of nutrient supplementation, may be more beneficial to fish species that have a longer yolk-sac period or to saltwater species (as they drink more water than freshwater species).

It is also recommended that alternative methods of providing Aquahatch to the yolk-sac larvae be investigated in conjunction with the method used in the current study. Alternative methods include supplementation on the broodstock diet, possibly allowing for the transfer of nutrients from broodstock to offspring, and inclusion during egg incubation, for possible absorption through the egg membrane. Since Aquahatch is a newly formulated nutrient supplement, feed supplementation studies must be conducted. Aquahatch can possibly be used to enrich live feed or decapsulated artemia cysts (which constitutes the main diet for early fry), or diets deficient in the nutrients contained in the Aquahatch formulation.

The concentrations of individual nutrients, contained in the Aquahatch formulation, must also be tested in the fish before and after supplementation. This will help to determine if the nutrients are taken up effectively and when further supplementation is required. Results of the current study indicated that Aquahatch was possibly utilised effectively only after the development of oral ingestion capabilities. The nutrients that make up the Aquahatch formulation should be tested individually and in various combinations in order to determine which combination is most beneficial to the fish.

Since indications of altered ammonia stress tolerance with Aquahatch supplementation was seen in the study, further investigation is required to determine the validity of this observation. It is recommended that Aquahatch supplementation be done in environments with varying levels of ammonia in the culture water. However, since results link Aquahatch with elevated ammonia levels, the cause of this needs to first be determined.

The low number of replicates in Trial 2 is a likely reason why no statistical difference on survival during the treatment period was detected. Increased replication of treatments is required when further investigation into the effect of Aquahatch on survival is done.

An in-depth investigation into Aquahatch's potential to alter dress-out percentage, and organ development and health may demonstrate an alternative effect of Aquahatch. One area of fish production performance that still requires testing with Aquahatch is immune stimulation.

If further research is conducted on Aquahatch, more regular sampling needs to be conducted during the larvae and fry stages. This study deducted that the combination of the three sampling methods used in Trial 3 and the image analysis technique, can be useful in improving the fish sampling procedures. However, the capturing of the image for image analysis needs to be standardized in order to reduce image analysis time.

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