INTERACTIVE EFFECT OF WATER TEMPERATURE, NITRATE FERTILIZERS AND SEWAGE EFFLUENT ON DEVELOPMENT, GROWTH AND SURVIVAL OF EMBRYOS AND EARLY LIFE STAGES OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL 1822)

PhD (AQUACULTURE AND FISHERIES SCIENCE) THESIS

SSENFUMA ROBERT

LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

MARCH, 2020

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A THESIS SUBMITTED TO THE FACULTY OF NATURAL RESOURCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN AQUACULTURE AND FISHERIES SCIENCE

LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

MARCH, 2020

DECLARATION

I **Ssenfuma Robert**, declare that this thesis is a result of my own original effort and work, and that to the best of knowledge, the findings have never been previously presented to Lilongwe University of Agriculture and Natural Resources (LUANAR) or elsewhere for the award of any academic qualification. Where assistance was sought, it has been accordingly acknowledged.

Ssenfuma Robert

Signature: 

Date:……11/10/2020……

CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the author's own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within the Lilongwe University of Agriculture and Natural Resources or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on 12.02.2020

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DEDICATION

I dedicate this thesis to my lovely wife, Ssenfuma Dorothy, my mother, Nabaggala Jane, all my sisters; Nansasi Joyce, Nakato Sophie, Babirye Justine, Babirye Patricia, and Naddamba Janet, all my brothers; Kanyike Nelson, and Mulindwa Godfrey and all my beloved children, Nabaggala Purity, Ssenfuma Promise, and Ssenfuma Prosper. All of whom encouraged me to work as a slave and later live like a king.

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ABSTRACT

This study assessed the interactive effect of water temperature, nitrate fertilizers, and sewage effluent on development, growth and survival of embryos and early life stages of African catfish *Clarias gariepinus*. One hundred fertilized eggs were incubated and 30 hatched larvae of *C. gariepinus* were reared in 1000 mL glass flasks for ten days. Three experiments were conducted. Experiments I and II took a 3 x 5, while Experiment III took 3 x 4 x 4 factorial laid out in a completely randomized design. Embryos and larvae were randomly exposed to three temperature levels (25, 28, and 31ºC) in all experiments and five nitrate fertilizer levels (0, 5, 10, 15 and 20 mg/L of NO_3-N) for Experiment I, five sewage effluent levels 0%, 15%, 30%, 45%, and 60% for Experiment II and four nitrate fertilizer levels (0, 5, 10, 15 mg/L of NO_3-N) and four sewage effluent levels (0, 20%, 40% and 60%) for Experiment III in triplicates. Data were subjected to two-way ANOVA for experiments, I and II and three-way ANOVA for experiment III. Orthogonal polynomial contrasts were used to partition significant nitrate and sewage effluent rates into linear, quadratic, cubic and quartic components. The results showed that, the maximum tolerable range of *C. gariepinus* embryos and larvae to nitrate fertilizers reduced from 15 to 20 mg/L of NO_3-N at 25°C to 10 to 15 mg/L of NO_3-N at 28°C and 31°C, while that to sewage effluent reduced from 30% to 45% at 28°C to 15% to 30% at 25°C and 31°C for the embryos and between the control and 15% at 25°C to 31°C for the larvae. The three-way showed significant interactions in the hatching period for the embryos and both yolk absorption period and rate for the larval stage. This study concluded that the effect of both nitrate fertilizers and sewage effluent and their combination on development, growth and survival *C. gariepinus* embryos and larvae depended on temperature. It is therefore recommended that catfish should be bred and raised in water containing less than 10 mg/L of NO_3-N and 15% sewage effluent at 25°C to 31°C

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
FAO	Food and Agricultural Organization
HCG	Human chorionic hormone
LR	Luteinizing hormone
LHRHa	Luteinizing hormone-releasing hormone analogue
SOFIA	State of World Fisheries and Aquaculture
IPCC	Intergovernmental Panel on Climate Change
SRAC	Southern Regional Aquaculture Center
DAWF	Department of Water Affairs and Forestry
NOAA	National Oceanic and Atmospheric Administration
UNAAB	Nigeria. University of Agriculture
PCC	Prairie Climate Centre
WQI	Water Quality Index
USEPA	United States Environmental Protection Agency
EPA	Environmental Protection Agency
USAID	United States Agency for International Development

CHAPTER ONE

INTRODUCTION

1.1 Background

Aquatic ecosystems, including streams, rivers, floodplains and lakes have been turned into final destination of nitrate fertilizers from agriculture runoff and treated sewage effluent from treatment plants (Adebayo *et al.*, 2007; Owa, 2014). The increasing amounts of these effluents have become issues of global concern, especially at this time when aquatic ecosystems are experiencing increasing and varying water temperatures due to climate change. These three factors i.e. nitrate fertilizers, sewage effluent and varying temperatures alter the physico-chemical parameters of water, particularly in the shallow waters which are the breeding grounds of most fishes (Kime, 1995; Owa, 2014; Serdeczny *et al.*, 2017). This is dangerous as it can result into death of the embryonic and early life stages of fish. Destruction of fish at this stage is critical because it may have a faster and disruptive effect on fish stock sizes in natural water body (Kime, 1995).

In sub-Saharan Africa, the major non-point sources of nitrate to aquatic ecosystems are the nitrate fertilizers applied in agricultural fields (Mateo-Sagasta, 2012). The increasing application of nitrate fertilizers has been due to the fact that the soil fertility in this region is deteriorating at a very rapid rate (Mateo-Sagasta, 2012). In order to feed the growing population, some sub-Saharan African countries have adopted and increased the application rate of nitrate fertilizers (OAU, 2006). For example, from 2002 to 2014, on average, the rate of fertilizer application increased from 29.7kg/ha to 36.5kg/ha for Malawi, in Zambia it increased from 26.1 to 46.2kg/ha while that of South Africa and Zimbabwe

was 60.6kg/ha and 29.4kg/ha respectively (FAO, 2017). A considerable amount of these nitrates are carried to the aquatic ecosystems during rain seasons either by leaching or surface runoff (Kremser and Schnug, 2002). For example, in rice fields, more than half of the quantities applied are lost to the surrounding ecosystems by surface runoff (Roy *et al.*, 2006). As a result, its level in surface shallow waters can actually surpass 25 mg/L of NO_3-N (100 mg/L) (Bogardi *et al.,* 1991). These levels are too high, nitrate level above 10mg/L of NO_3-N in drinking water is a potential cause of oxygen deficiency in the blood, a lethal ailment known as methemoglobinemia or "blue baby syndrome" in humans less than six months old (Manassaram *et al.,* 2010). Young mammals such as calves, piglets, lamb and horses, birds, especially chicks are also prone to nitrate poisoning (Jennings and Sneed, 1996). Field studies have also suggested that nitrate fertilizers have contributed towards the reduction of amphibian populations in agricultural fields (Birge *et al.,* 2000) yet not much is known about its effects on embryonic and early stages of fish.

In addition, the content of sewage effluent in natural water bodies has significantly increased in the past few years. This has been attributed to the fact that most of the existing sewage treatment plants in sub-Saharan Africa are either non-functional or overwhelmed by excessive sewage effluents (Naidoo and Olaniran, 2013). As a result, partially or untreated sewage effluents are discharged into the adjacent aquatic ecosystems uncontrollably. The production of sewage has been accelerated by the recently observed population explosion, urbanisation, and improper disposition of domestic wastes in many parts of sub-saharan Africa (Naidoo and Olaniran, 2013). As a result, it has been noted that, during the dry or intermittent or even during the known rainy seasons, inflowing sewage effluent form a large portion of streams, rivers, floodplains and shallow coastal waters of

large water bodies (Kanu and Achi, 2011). Sewage effluent is known to contain pollutants, xenobiotic and stressors (Sowers *et al.*, 2009). The presence of these substances in aquatic ecosystems could be devastating to the inhabitants of the adjacent aquatic ecosystems.

Similarly, the quality and well-being of aquatic ecosystems is temperature dependent because temperature influences the water quality and the physiological processes of the aquatic inhabitants (Fischer *et al.*, 2013). Current studies have reported a dramatic increase and fluctuations of water temperature in most aquatic ecosystems due to climate change. For example, from 1913 to 2000, the water temperature of East Africa Lakes such as Victoria, Edward, Albert, Kivu, Malawi and Tanganyika had warmed by a mean temperature of 0.2°C to 0.7°C on average (Bates, 2008). However, this warming is reportedly more pronounced in the surface and shallow waters than deep water. For example, in Lake Tanganyika, the surface and shallow water has warmed by 1.3°C (Bates *et al*., 2008) which is much higher as compared to the average the increase of the whole lake. Furthermore, the mean global temperature has been predicted to increase by nearly 1-2°C by 2050 (Toulmin, 2009; Thornton *et al.*, 2011) but this will vary from one continent to another and from one place to another. For example, the mean temperature of Africa has been projected to increase by 1.5 - 3°C (IPCC, 2007) while that of the dry interior parts of sub-Saharan Africa has been projected to increase by 5°C by 2050 (Magnusson *et al.*, 2012). Climate predictions also indicate an increase in the frequency, number and intensity of extreme warm temperature events or heat waves across the world which may last for few days with an increase of about 5°C above the normal temperature. These are likely to increase threefold by 2025, with three warming events expected compared to the current rate of one warm event every four years. Moreover, both global warming and warm air

events will maintain higher water temperature for longer periods, mostly in the second half of the century (Barlow *et al*., 2015).

Clarias gariepinus is a shallow-water breeding fish. It spawns its eggs, and its larvae grow up in shallow waters such as streams, rivers, floodplains and shallow coastal waters of large water bodies (Serdeczny *et al.,* 2017). This fish has been reported to be the best candidate for fish farming in Africa (Hossain *et al*., 1998; Hecht and Appelbaum, 1988; Mwanja *et al.*, 2015). However, its greatest challenge is the shortage of fingerlings for both stocking fish ponds and to be used as baits for catching big predatory fish on Lake Victoria (Mwanja *et al.,* 2015). In the past, fingerlings could be collected from shallow waters of natural water bodies, during known breeding seasons. However, at present their numbers have significantly reduced, only a limited amount can be obtained from specific places for example, in Cameroon fingerlings can only be collected from River Nkama where water pollution seems to be less (Walther *et al.*, 2002; Pouomogne, 2008,). The reduction in *C. gariepinus* fingerlings collected from the wild may be accredited to the high sensitivity of its hatchlings to variations in their environment (Rutaisire, 2007), and changes in physico-chemical characteristics of water, which have occurred over the past few decades (Lobell, 2007; WorldFish Center, 2007). In general, the recently observed increasing temperature and warm air events due to climate change, increased inflowing nitrate fertilizers from agricultural fields and large volumes of inflowing sewage effluent from urban areas have been blamed for the fluctuations that have occurred in the physico-chemical characteristics of water bodies (Adebayo *et al.,* 2007; Owa, 2014).

1.2 Problem statement and justification

In Africa, the amounts of nitrate fertilizers from agricultural fields and sewage effluent from urban areas have increased significantly to the extent that, the concentration in surface waters can exceed 25 mg/L of NO_3-N, and inflowing sewage effluent form a large portion of shallow water even during the known rainy seasons (Bogardi *et al.,* 1991; Rim-Rukeh and Agbozu, 2013). The increasing amounts of these substances in natural water bodies may affect aquatic life (Adebayo *et al.,* 2007; Rim-Rukeh and Agbozu., 2013). However, their effect may be temperature dependent because temperature determines the rate of physiological processes that take place in aquatic organisms (Serdeczny *et al.,* 2017). Warm air events, water temperature and its fluctuations have increased during the past few decades due to climate change, to the extent that significant water temperature increments have been detected up to 3000 m deep in the sea (IPCC, 2007). Furthermore, climate predictions also indicate an increase in the frequency, number and intensity of extreme warm temperature events or heat waves across the world which may last for few days with an increase of about 5°C above the normal temperature. The effect of these climate-induced water temperature changes are more pronounced in shallow water aquatic ecosystems such as streams, rivers, floodplains and shallow coastal waters of large water bodies, these are the breeding sites for most aquatic life (Serdeczny *et al.*, 2017; Konan *et al*., 2014). The increasing levels of nitrate fertilizers, sewage effluent and temperature fluctuations may be critical to the survival and the success of the early stages of shallow water breeder (Mbalassa *et al*., 2015). African catfish (*C. gariepinus*) is one of the susceptible fish species because it naturally breeds in shallow waters (Konan *et al*., 2014). Therefore, its embryonic and early life stages are at risk of being exposed to the increasing amounts of

nitrate fertilisers, sewage effluent and water temperature variations in their environment (Rouse *et al*., 1999). Furthermore, *C. gariepinus* is among of the most important fish species in many African countries. For example, in Uganda, it accounts for about 60% of the farmed fish (FAO, 2012, Mwanja *et al.,* 2015). However, its major challenge is the shortage of its fingerling needed for both stocking fish ponds and baits for harvesting wild fish predators such as the Nile perch (Mwanja *et al.,* 2015). The demand for *C. gariepinus* fingerlings has been escalated by the low survival rates of its hatchlings in most African hatcheries. Its high mortality has been attributed to its high sensitivity to fluctuations in its environment (Rutaisire, 2007). In the past, *C. gariepinus* fingerlings for baitfish were entirely collected from the wild, in shallow waters, during known breeding seasons. However, at present their distribution has changed, and its numbers have drastically reduced (Pouomogne, 2007). These changes may have resulted from the observed changes in water quality, which have occurred over the past few decades (Lobell, 2007; WorldFish Center, 2007). The recently observed water temperature variations due to climate change, increased application rates of nitrate fertilizers in agricultural fields and large volumes of inflowing sewage effluent from urban areas may be blamed for these changes in the water quality (Adebayo *et al.,* 2007; Owa, 2014). The demand for *C. gariepinus* fingerling is expected to increase unless measures are put in place to enhance their productivity. Most studies have focused on temperature, nitrate fertizers and sewage effluent as major causes of eutrophication and increased production of aquatic plants (Venugopalan *et al.,* 1998; Tammi *et al.,* 1999; Braga, 2000). Several studies have focused on the effects of temperature (Haylor and Mollah, 1995; Ssenfuma *et al.,* 2011) or sewage effluent on growth and development of *C. gariepinus* (Mdegela *et al.,* 2010; Asem-Hiablie *et al.,*

2013). However, no study has focus on the interaction of these factors. Therefore, the purpose of this study was to assess the interactive effect of water temperature, nitrate fertilisers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*.

1.3 Objectives

1.3.1 General Objective

To determine the interactive effect of water temperature, nitrate fertilizers, and sewage effluents on development, growth and survival of embryos and early life stages of African catfish (*C. gariepinus*)

1.3.2 Specific Objectives

- To determin the interactive effect of water temperature and nitrate fertilizers on development, growth and survival of embryos and early life stages of *C. gariepinus*
- To determine the interactive effect of water temperature and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*
- To determine the interactive effect of water temperature and a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

1.3.3 Hypothesis

- $H_{0:}$ There is no significant interactive effect of water temperature and nitrate fertilizers on development, growth and survival of embryos and early life stages of *C. gariepinus*

- $H_{0:}$ There is no significant interactive effect of water temperature and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

- $H_{0:}$ There is no significant interactive effect of water temperature and a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

CHAPTER TWO

LITERATURE REVIEW

Fish is a high protein food of excellent nutritive quality. It contributes 6.5% of the global protein sources for human consumption and 17% of the global population's intake of animal protein (Shen and Heino, 2014; FAO, 2016). It is especially a valuable protein source in developing nations, where more than 75% of the global fish consumption occurs (Waite, *et al*. 2014). Fish comprises of micronutrients and long chain omega-3 fatty acids that are frequently lacking in the diets of the poor but essential for maternal and child health (Waite, *et al*. 2014). On the African continent, it is an important food source for over 400 million people (Allison, 2011). It employs 12.3 million Africans of which half of these are fishers, 4.9 (7.5%) millions are processors and 0.9 million are fish farmers. Despite these benefits, fish catches from the wild are dwindling yet the demand for fish is rising due to the increasing human population. For example, in Lake Tanganyika, by 2007 fish catches fell by 30% (IPCC, 2007; WorldFish Center, 2007). The only alternative to meeting the high demand of fish is aquaculture. *Clarias gariepinus*, is one of the best candidate for fish farming in Africa (Hecht and Appelbaum, 1988; Hossain *et al*., 1998). It also forms the bulk of baitfish for fishing large predatory fish from wild waters (Mwanja *et al.*, 2015). However, its usage has been limited by the shortage of its fingerlings. This has been attributed to the complicated biology of its early stages. Majority of past studies have focused on temperature, nitrate fertilizer and sewage effluent as major causes of eutrophication and increased production of aquatic plants. This review will focus on direct effects of temperature, nitrate fertilizers and sewage effluent on fish in their environment.

2.1 African Catfish (*Clarias gariepinus*)

African Catfish (*C. gariepinus*) is among the most successful aquatic species on the African continent; its success has been ascribed to the fact that these fishes are efficient opportunists, survivors and equipped to exploit whatever resources are available (Nyamweya *et al*., 2010; Demeke *et al*., 2015). They tolerance a wide range of environmental extremes such as water temperature ranging from 8 to 35ºC as well as breeding and hatching at 17 to 32ºC; salinity ranging from 0 to 12ppt, while the optimal ranges from 0 to 6ppt; oxygen of 0 to 100% saturation. It is an effective and obligate air breather, highly resistant to desiccation, tolerates a wide range of turbidity and pH. It can be reared at a high density and has high annual production. Its production in Zambia indicated a standing crop ranging from 65 to 100 tons/ha (Demeke *et al*., 2015) and a feed conversion ratio (FCR) of up to 1.05 in least cost experimental diets containing 38% crude protein (Uys, 1989).

2.1.1 Production systems

Clarias gariepinus are presently cultured across the world using several production systems varying from very low yielding extensive systems to high yielding intensive systems. The choice of a system suitable for *C. gariepinus* intended for production is perhaps the most significant decision for any prospective aquaculture farmer, and may either result in the failure or success of any aquaculture business. There are two major types of facilities used for catfish farming in Africa; these include ponds and concrete tanks (Akankali *et al*., 2011). Ponds are mainly used in large and small-scale extensive, semi-intensive and intensive farming systems, while tanks are used mainly for the high-density intensive culture of *C. gariepinus* (under either through-flow or recirculation culture). In

making a choice a production system, the following criteria must be taken into consideration: The species to be cultured, the location of production system, aquaculture regulations and financial considerations (Hecht *et al.,* 1996*).* The size of production ponds varies from 36 m^2 to 100 m^2 under subsistence farming conditions to one hectare in size under extensive commercial culture. On semi-intensive commercial farms, the average pond size is 1000 m^2, with an average depth of approximately 1 m (Akankali *et al*., 2011). *Clarias gariepinus* has also been cultured in polyculture with other tilapiine species, predominantly with *Coptodon guinneensis*, *Coptodon rendalli*, *Oreochromis karongae* and *Oreochromis niloticus.* In polyculture systems, *C. gariepinus* is used as predators to control tilapia densities to prevent precocious breeding and stunting (Limbu *et al.,* 2014).

2.1.2 Pre-spawning conditions

After attaining sexual maturity in natural water bodies, *C. gariepinus* are capable of multiple spawning per year. In the wild, sexually mature fish can be found throughout the year but the rainy season is the principal reproductive period. In cultivated fish, sexually mature fish can be found all year-round in ponds only if they are provided with adequate and unrestricted feed (Nwadukwe, 1993). In hatcheries, good quality eggs and sperm of *C. gariepinus* can be acquired continuously when water temperature is steadily maintained at 25°C; but at 30°C the proportion of atretic oocytes escalates and the testis regresses (Legendre *et al.,* 1996).

2.1.3 Spawning in the natural environment

In temperate zones, most teleost species are seasonal spawners. Similarly, in *C. gariepinus*, the reproductive sequences depend on the seasonal environmental cycles. Certain environmental elements, such as water temperature, photoperiod and rainfall, act as cues

for initiating reproduction. Spawning activities begin when minimum water temperatures remain between 18ºC and 30ºC (Demeke *et al*., 2015; Kumakura *et al*., 2003). *C. gariepinus* spawning is restricted to the edges of the lakes, streams and pools where the water current is not strong enough to drift the egg substrates away (Oyelese, 2006). After hatching, the larvae remains under the cover of the submerged vegetation at the water edge which would later serve as a nursing ground for the fry during the yolk sac or larvae stage. The advanced stage subsists on the available plankton within the littoral zone before migrating as juveniles to deeper and larger water bodies where they can prey on fish and other food items (Oyelese, 2006).

2.1.4 Choice of brood fish

Before spawning can be effected, ripe females must first be identified. Suitable females can be identified by their distended tummies and typically red and swollen genital papillae. The readiness of ova can be confirmed by drawing up ova into a tube and inspecting the eggs, which should have a firm, translucent appearance and a diameter of approximately 1 mm. The colour of the female gametes may vary, but if the ova are found to be yellowish and opaque with a "runny" texture, then re-absorption of eggs will have started and it will be too late to induce spawning. It is not practical to judge externally whether male *C. gariepinus* have developed testes, but viable sperm should be exist in males if ripe females are present in the same water body (Britz, 1991). When ripe females are identified, spawning can be induced by either injecting the female with a suitable hormone or manipulated pond environment.

2.1.5 Induced spawning without hormone treatment

In *C. gariepinus,* reproduction in captive environment without any use of hormonal can be successfully achieved when ripe broodfish are placed in newly filled ponds; then the water level is raised while the broodstock is in the pond. However, the numbers of fingerlings obtained are generally very low and poor in quality (Richter, 1976; Ssenfuma *et al.*, 2011), because *C. gariepinus* does not show any parental care, the fry are not easily collected with small scoop nets. Cannibalism among the fry and heavy predation by aquatic insects, frogs and other aquatic animals are accountable for the low fry survival. Owing to these limitations, such a breeding strategies are of little practical importance. In Nigeria, natural spawning of *C. gariepinus* can be achieved by mimicking the flooding situation. Increasing and decreasing water levels and volume intermittently in the ponds may induce natural spawning (Akankali *et al*., 2011).

2.1.6 Hormone-induced breeding

Extensive research has been conducted with regard to the artificial breeding of *C. gariepinus*, and a variety of synthetic and natural hormones have been used to induce spawning. However, the use of homoplastic pituitary glands (i.e. pituitaries taken from the same species being hypophysized), is the practice most widely employed (Akankali *et al*., 2011).

2.1.7 Hypophyzation

Methods and practices used to induce spawning in *C. gariepinus* by hypophyzation are well documented and described by several authors. These practices were predominantly described by Schoonbee and Swanepoel (1988) as well as Britz (1991) as follows; *C. gariepinus* pituitary glands were collected by using a 45 mm diameter hole-saw to make a

hole through the dorsal surface of the fish skull. The hole was carefully made through the frontal and parietal bones, just prior to the posterior fontanel and a cut was made over the pro-otic and exoccipital bones ending just short of the parasphenoid at the bottom of the brain. After the removal of the saw, a circular lump of bones was lifted out carrying with it the brain and pituitary gland, the pituitary gland was clearly visible as a distinct, pea-shaped white organ (± 1 mm diameter in a 1 kg fish). The collected pituitaries could be used straightaway, or it could be maintained whole in 95% alcohol and then kept in a refrigerator (2 – 5°C) for 2 – 3 years. The pituitary dosage administered was determined depending on the weight of the recipient and donor fish and the season of year when the pituitary glands were collected. For a recipient and donor fish of the same weight, a single homogenized pituitary gland which was collected in summer was enough to induce spawning. The pituitary dose must be prepared by collecting the right amount of pituitaries from the alcohol, allow the alcohol to evaporate on a paper towel, then homogenize the pituitary together with a small volume (± 0.5 ml) of sterile water in a grinder. The resultant solution must be diluted further to using sterile water so that each fish receives 1 ml of the resultant solution administered intramuscularly next to the dorsal fin. The latency period between spawning and hypophyzation is temperature dependent. After the estimated period between spawning and hypophyzation had elapsed the female broodfish must were inspected and if ova were freely extruding from the genital papilla, the female was ready for stripping. Oocyte maturation and ovulation of C. *gariepinus* also can be induced by injecting it with pituitary extracts from *C. gariepinus*, common carp, tilapia or *Heterotis* or by injecting it with hCG (Human chorionic gonadotropin) alone or combined with carp pituitary extract (Nwadukwe, 1993).

2.1.8 Synthetic hormones

Traditional approaches of induced breeding in C. *gariepinus* were based on injecting fish with synthetic hormones. A great range of synthetic hormonal treatments have been used to induce breeding in female broodfish. The first synthetic hormone used for artificial reproduction in C. *gariepinus* was 11-deoxycorticosterone-acetate (DOCA), but this steroid hormone induced only oocyte maturation and not ovulation. As a result stripping of eggs could be feasible after treatment with DOCA. It was therefore concluded that ovulation was evoked mechanically (Akankali *et al*., 2011). Further later, two successive injections of 17α-hydroxyprogesterone was used to successfully inducing both oocyte maturation and ovulation (Akankali *et al*., 2011). These synthetic compounds on their own have proved to be less potent sometimes, hence the use of analogues. Hypothalamic hormone analogues (LHRHa) proved efficient in inducing oocyte maturation and ovulation in *C. gariepinus*, but their potency was greatly increased when administered in combination with pimozide, or with other anti-dopamine antagonists (Akankali *et al*., 2011).

2.1.9 Hatchery Procedures

Various hatching procedures have been developed for *C. gariepinus* eggs. These processes differ mainly in the extent of mechanical handling of fertilized eggs resulting in significant differences in fetal survival. Laboratory studies have shown that embryo survival is reduced when procedures involving a high degree of mechanical handling of eggs. For example when funnel breeding technique is used, eggs are separated from their glutinous substance using a solution made from full-cream powdered milk before hatching them in a funnel. When this method was compared with traditional hatching practices in trays, a significant difference in embryo survival was reported (Polling, *et al*., 1987). Egg adhering

substrates ranging from pine tree branches to mesh trays, seem to be effective in hatcheries. However, the most important factors influencing larval survival is water temperature (Hoffman *et al.*, 1991)

2.2 Temperature

The global surface and air temperature of the earth has remained relatively stable at 14°C for the past 11, 000 years (since the ice age) (Solomon *et al*., 2007; IPCC, 2007). However, in the last 100 years the average global surface and air temperature has increased by an average of 0.74°C (range of 0.56°C to 0.92°C), but the rate of this temperature increase has been rapid in the last 50 years (Bates *et al*., 2008; Crowley, 2000). During the 20th century, the average surface and air temperature of the African continent has risen by 0.7°C. Based on IPCC data, the average surface temperature of the African continent is predicted to increase by an average range of 0.3°C to 0.5°C per decade (IPCC, 2007). However, this will vary from one part of the continent to another. For example, the mean temperature of Africa has been projected to increase by 1.5 - 3°C (Lobell, 2007) while that of the dry interior parts of sub-Saharan Africa has been projected to rise by 5°C by 2050 (Magnusson *et al.,* 2012). Furthermore, climate predictions also indicate an increase in the frequency, number and intensity of extreme warm temperature events or heat waves across the world which may last for few days with an increase of about 5°C above the normal temperature. These are likely to increase threefold by 2025, with three warming events expected compared to the current rate of one warm event every four years. Moreover, both global warming and warm air events will maintain higher water temperature for longer periods, particularly in the 2nd half of the century (Barlow *et al*., 2015).

2.2.1 Water temperature and climate change

Globally, air temperature has risen by an average of 0.74°C from 1906 to 2005. Water temperature has also increased with the same trend. However, no specific average increment had been reported. The increment in water temperature has been reflected by an annual sea level increase of 1.8 mm from 1961 to 2005 (IPCC, 2007). In addition, large, deep fresh water lakes of Africa such as Lake Malawi and Lake Tanganyika have warmed up by 0.2 to 0.7°C in the past 100 years (Rosenzweig *et al.*, 2007). This increment in water temperature has been much higher on the surface and shallow water e.g. increment in water temperature has been detected up to 3000 m deep in the sea (IPCC, 2007). This may imply that all water bodies with depth less than 3000 m are experiencing significant increment in water temperature.

2.2.2 Exchange between water and air temperature

Water temperature is the major driver of all physio-chemical and biological characteristics of any given water body (Caissie, 2006). Annual and daily variations in water temperature influence the quality, health and productivity of any given aquatic ecosystems. This is due to the fact that it influences most of the chemical reactions, microbial, physiological and biochemical reactions that take place in aquatic ecosystems (Caissie, 2006; Verones *et al.*, 2010). Heat exchange takes place at the air/water interface. This contributes to about 82% of heat exchanged between air and water hence the most important contributor to the heat of shallow water bodies due to their large surface area to volume ration (Caissie, 2006). The process of air/water surface exchange results in a net gain of long and short wave solar radiations, convection heat influx and evaporation. Friction and precipitation on the surface of water are neglected because they contribute less to the water surface exchange process.

This implies that heat loss or gain is dependent on solar radiation and air temperature (Caissie, 2006).

2.2.3 Daily and Annual temperature variation in shallow waters

In shallow waters, temperature fluctuates both annually and daily. On daily basis, minimum temperature is attained in the morning while the highest temperature is attained between the afternoon and evening (Dallas, 2008; Caissie, 2006). The effects of solar radiation depend on the water volume. Large water volumes have a lower surface area to volume ratio as compared to small water volume, therefore, lower volumes of surface and shallow water warm up quickly as compared to the deeper water (Vannote and Sweeney, 1980; Dallas, 2008).

2.2.4 Effect of water temperature on aquatic organisms

Temperature can be lethal or sub-lethal to aquatic organisms. Lethal temperatures are those that are high enough to cause death while sub-lethal are those that are high enough to cause behavioral changes such as mass movement of fish or migration. Sub-lethal temperatures can also result into changes in the biochemical and physiological processes. For example, high temperature results into high rate of metabolism and respiration, changes in growth and reproductive activities (Langford 1990; Verones *et al.,* 2010). Metabolic activity doubles for every 10°C rise in temperature; this may result into increased demand for food and oxygen, and changes in chemical toxicity (Caissie, 2006; Langford, 1990). This implies that, temperature changes in aquatic ecosystems have the ability to cause multiple impairments in fish behaviour, physiological and biochemical processes.

2.2.5 Response of fish to temperature changes

Temperature influences the distribution of fish in their aquatic environment. This results from the fact that each fish species has a specific range of temperature in which it attains maximum fecundity and body weight without compromising its physiological activities (Vannote and Sweeney, 1980). Fish are highly sensitive to temperature to the extent that they can detect even minor changes of about 0.05^{o}C. In lentic water bodies, fish responds to temperature changes by moving from places where it is unfavourable to where it is close to the optimum for growth (Langford 1990). However, these movements are restricted to the age of fish. For example, eggs and embryos don't move, therefore they are restricted to spawning areas even if temperature changes while larvae respond slowly since their movement is slow (Langford 1990). In lotic water bodies, the environment is divided into water column, streambed interface and substrate habitats depending on temperature. Most aquatic organisms live either on the streambed or at the water surface (Langford 1990). Fitness, reproduction, growth and distribution of these organisms depend on temperature and the speed of water current. Hence each fish species positions itself in a locality where the environment is close to its optimum temperature. For example, the optimum temperate range for *Cyprinus carpio* (carp) is 23 – 29^{o}C, *Salmon trutta* (brown trout) is 7 – 17^{o}C, and micro-invertebrates is 9.1 – 10.6°C (Wallace and Anderson, 1996; Li *et al.,* 2013). However, it should be noted that most aquatic organisms are ectotherms therefore any shift in temperature may directly compromise their body physiological activities (Dallas 2008).

2.2.6 Effect of temperature on early stages of fish

Water temperature has been reported to affect incubation of fish embryos, hatching rates, yolk sac absorption period, larval sizes at hatch, efficiency energy take-up, and larval

survival and growth. Each fish species has a specific temperature range for spawning, embryonic, larval, and juvenile development (Herzig and Winkler, 1986; Ssenfuma, *et al*., 2011). For example, Fish embryos respond to extreme temperatures by hatching at different developmental stages. At the lowest temperatures, some embryos hatch at earlier developmental stages and at highest temperatures embryos hatch at all different stages, and show developmental morphological abnormalities, and many die shortly after hatching (Herzig and Winkler, 1986). Furthermore, juvenile are highly sensitive to temperature fluctuations in their environment and their sensitivity age-dependent i.e. the older the fish, the lessor the sensitivity (Herzig and Winkler, 1986). The survival of 5 day old fish is negatively affected by a fall in temperature from 25°C to 15°C (Hoffman *et al.,* 1991). In contrast, 21 day old fish are not affected by the same range of temperature change (Hoffman *et al.,* 1991).

2.2.7 Effect of temperature on incubation, hatching and yolk absorption period

High water temperature decreases the incubation, hatching and yolk absorption period of fish. Nwosu and Hertzlohner (2000) reported that the latent period from fertilization to hatching of *Heterobranchus longifilis* decreased with increasing temperature. They found out that time from fertilization to hatching was 20-23h at 29°C, 23-32h at 27°C, 26.9-34h at 25°C and 30-38h at 20°C. Findings of their study are also in agreement with those reported by Haylor and Mollor (1995), who reported that the period taken for hatching of *C. gariepinus* eggs at 24°C to 26°C ranged from 27.20-33.47h, at 28°C hatching took 23.03h, and at 30°C to 32°C ranged between 18.27-20.27h. Haylor and Mollor (1995) who incubated *C. gariepinus* embryos in freshwater recorded the yolk absorption time of 74.40-90.24h at lower temperature range of 24-26°C, 63.06h at moderate temperature of 28°C

and 48.96-55.20h at higher temperature range 30-32°C. Increased metabolic activities at higher temperature could have been accountable for the inverse relations between temperature and incubation and hatching period. Low temperature results into low metabolic rates, hence low rate of embryonic development which results into increased incubation and hatching period while high temperature results into high metabolic rates, hence high rate of embryonic development which results into decreased incubation and hatching period (Nwosu and Hertzlohner, 2000). However, it is generally agreed that the optimum hatching temperature for *C. gariepinus* ranged from 25-31^{o}C (Herzig and Winkler, 1986; Haylor and Mollah, 1995; Ajuzic and Appelbaum, 1996)

2.2.8 The effect of water temperature on survival and growth rate yolk sac larvae

The growth rate in length per day increases with at high temperature in freshwater. Britz and Hecht (1987) reported an experiential growth rate of *C. gariepinus* larvae with increasing rearing temperature. Similarly Haylor and Mollah (1995) reported that the number of day-degrees decreased with increasing incubation and rearing temperature until hatching, yolk sac absorption and first feeding in *C. gariepinus.* On the other hand, the survival of *C. gariepinus* yolk sac larvae increased with increasing rearing temperature. Nwosu and Hertzlohner (2000) observed that survival of *H. longifilis* larvae reduced with increasing rearing temperature in fresh water; they recorded the highest survival of 67% at lower temperatures of 25°C, 50% and 33% at moderate temperatures of 27°C and 29°C respectively, and 13% at high temperatures of 32°C.

2.2.9 Effect of temperature on other chemical reactions

Temperature rise, from 10°C to 20°C increased the toxicity of ammonia by a factor of 1.3 to 1.6 in relation to pH, while dissolved oxygen decreases in pure water from 14.2 mg/L at

0°C to 7.5 mg/L at 30°C (Allan and Castillo 2007). Furthermore, as temperature increases, the metabolic activities of the aquatic organisms increase rapidly. This may lead to oxygen depletion from the water and reduced food utilization (Dallas 2008). Thus, changes in water temperature resulting from climate change and global warming may cause changes in the organism's behaviour. Therefore, temperature is an important parameter that can be used to understanding the responses of aquatic organisms to water temperature changes (Dallas 2008).

2.3 Nitrate fertilizers

Globally, the nitrogen cycle has been gradually transformed over the years by human activities such as industrialization of agriculture, increased human population, urbanization, improved animal production systems, increased production of domestic sewage and air pollution (Adebayo *et al.,* 2007; Owa, 2014). These processes have almost doubled the amount of nitrogen added in the ecosystems annually (Vitousek *et al.* 1997). Although all these processes are important in improving food production and the environment for human health on the earth, the increased input of nitrogen in the environment may have serious consequences on the structure and functions of the ecological systems (Bricker *et al.,* 1999). These consequences may be more pronounced in aquatic ecosystems because they are the major destinations of all these wastes (Rabalais 2002). Most studies have focused on nitrates as a chief cause of eutrophication and increased production of aquatic production and not its toxicity to the aquatic organisms. This review will focus on nitrate as a toxic agent to fish in their environment.

2.3.1 Use of inorganic fertilizers

Inorganic fertilizers are chemicals, which are a combination of different essential nutrients that are required by plants to grow. These include: Phosphorus (P), Nitrogen (N) and Potassium (K) (Kremser and Schnug, 2002). They are applied to replenish the soil because during growth, plants absorb a significant amount of nutrients. When harvested, much of these nutrients are exported with them. In order to maintain soil productivity, manures, synthetic fertilizers or municipal wastes must be applied. Africa's soils are devoid of essential nutrients, and it can no longer deliver enough inorganic nutrients to maintain soil health (Wallace and Knausenberger, 1997). Therefore, a mixture of organic and inorganic fertilizers is applied to replenish the soil nutrient reserves. In sub-Saharan Africa, the use of inorganic fertilizers is claimed to be low, but it is the only easy way to provide enough plant nutrients. In fact, many African governments give subsidies on inorganic fertilizer use in cereal production (Smaling, 1993). It is expected that the use of inorganic fertilizers should increase in order to feed the exploding population of Sub-Saharan Africa. Nitrate fertilizers are very soluble in water; as a result they dissolve in water to form nitrates, much of these generated nitrates end up into fresh water ecosystems (Kremser and Schnug, 2002).

2.3.2 Sources of nitrates

The major sources of nitrates released into the environment, surface and drinking water are non-point sources such as excessive use of nitrate-based fertilizers in agricultural fields (Gray, 2008) while point sources include, industrial wastes, human and animal wastes septic/solid disposal, landfills among others (Puckett, I994). In addition to this, organic nitrogenous compounds present in water and soil are broken down to nitrate and nitrite by natural processes such as mineralization, hydrolysis and bacterially activated reactions

(Speiran, 1996). On the other hand, inorganic forms of nitrogen such as ammonium (NH_4^+) are oxidized to nitrite (NO_2-) and then to nitrate (NO_3-). I.e. NH_4^+ —> NO_2- —> NO_3- by aerobic chemo-autotrophic bacteria (Nitrobacter and Nitrosomonas), even if dissolved oxygen decline to a value as low as 1.0 mg O_2/l (Camargo *et al.*, 2005). For example, urea, like a number of other forms of inorganic nitrogen in the soil and natural waters, are transformed to ammonia under aerobic and anaerobic microbial processes. Ammonia is subsequently transformed to nitrate and nitrite (Equation 2.1 to 2.4) (Speiran, 1996).

Urea hydrolysis

$CO(NH_2)_2 + H_2O$ —> $2\ NH_3 + CO_2$ …….…… (Equation 2.1)

Ammonification

$NH_3 + H_2O$ —> $NH_4^+ + OH^-$ ……..……..……...…... (Equation 2.2)

Nitrification

$NH_4^+ + 3/2\ O_2$ —> $2H^+ + NO_2^- + H_2O$ (Nitrosomonas)….. (Equation 2.3)

$NO_2^- + 1/2\ O_2$ —> NO_3^- (Nitrobacter) ……..……. (Equation 2.4)

Net $NH_4^+ + 2\ O_2$ —> $NO_3^- + H_2O + 2\ H^+$ ……..……. (Equation 2.5)

2.3.3 Chemistry of nitrates in water

In aqueous solution, nitrate occurs in an oxidized form (NO_3^-); however, it coexists with nitrite (NO_2^-). The major source of nitrate is the degradation and breakdown of ammonia and organic matter by nitrifying bacteria in the aquatic environment (Lewis and Morris, 1986). In the absence of oxygen, nitrate (NO_3^-) is converted to a more toxic and less stable substance, nitrite (NO_2^-), which exists in very low concentration in water while the reverse is true (Guillette and Edwards 2005). Nitrate present in water is usually absorbed by plants or transformed to nitrogen by denitrifying bacteria (Guillette and Edwards 2005).

2.3.4 Mechanism of nitrate toxicity in fish

Nitrate has been known to be toxic in closed fish culture systems such as fish hatcheries, aquaria and recirculating aquaculture systems (Shimura *et al.* 2002), in animals due to its toxicity in drinking water and in agricultural fields (Bruning-Fann and Kaneene 1993). Nitrate enters the fish body by diffusion via the permeable or gill membranes while nitrite uptake occurs by chloride transport mechanism (Jensen 1996). Within the body, nitrate can either be metabolized to nitrite or accumulate into the body due to the nitrite detoxification process in the liver, which converts the toxic nitrite to the less toxic nitrate (Edwards *et al*. 2004).

2.3.5 Toxic effects of nitrate

Determining nitrate levels as nitrogen (mg/L NO_3-N) and nitrite as nitrogen (mg/L NO_2-N) in surface waters forms the background of water monitoring programs. This is because their levels are general indicators of the nutrient content and the level of pollution of a given water body. It is recommended that nitrate levels in drinking water should be regularly monitored because they are associated with many potential health risks, particularly for babies less than six months old and animals (Fadirani and Mamba, 2005). Nitrate affects synthesis of steroid hormones hence reducing sperm and embryonic viability and motility and fish fecundity (Edwards *et al.,* 2004). The passive diffusion of nitrate (NO_3^-), via the egg and embryo envelop to the developing tissues and the resultant toxic products of its metabolism, i.e. nitrite (NO_2^-) and nitric oxide (NO) (Camargo *et al.,* 2005), may accumulate to toxic levels in the tissues, resulting into death of the young stages of fish (Lundberg *et al.,* 2009; Hickey, 2013). Increased levels of NO may cause carcinogenesis by diffusing into the tissues to form nitrosalting species. Nitrosalting

species have the potential to damage DNA (Lundberg *et al.*, 2009). Nitrite (NO_2^-) and nitric oxide (NO) can also transform Haemoglobin (Hb) into MetHb. This reduces Hb-Oxygen binding and transporting capacity (Kamstra and van der Heul, 1998).

2.3.6 Effects of nitrates to amphibians

Field studies suggest that nitrogenous fertilizers are one of the parameters that have contributed to the reduction of amphibian populations in agricultural fields (Birge *et al.*, 2000). Laboratory experiments have proven that tolerance of amphibians to nitrate toxicity increases with increasing environmental adaptation and body size (Johansson *et al.*, 2001) while toxicity rises with increasing levels and exposure times (Marco *et al*., 1999). Baker and Waights (1993) reported that exposure of *Bufo bufo* tadpoles (common toads) to 9.1 mg/L of NO_3-N for 13 days caused weight loss, reduced feeding activities and survival (15.4%) while Baker and Waights (1993) reported that exposure of *Litoria caerulea* tadpoles (tree frog toads) to 22.7 mg/L of NO_3-N for 16 days caused weight loss, reduced feeding activities and survival (42%). Hecnar (1995) also reported that when he exposed tadpole chorus frog and leopard frog to 10.0 mg/L of NO_3-N using ammonium nitrate for 100 days, he found out that both exhibited lower survival, they showed signs of abnormal behaviour, i.e. they fed and swam less vigorously, they hard transparent and swelled bodies with a lot of digestive-system and head deformities and they suffered from paralysis and oedemas before death.

2.3.7 Toxic effects of nitrates to Fish

Nitrate toxicity varies from one fish species to another and from freshwater to marine fishes. Freshwater fishes are more susceptible to nitrate toxicity than marine fishes. However, the toxicity rises with increasing time of fish exposure and nitrate levels (Scott

and Crunkilton, 2000). Nitrates are harmful to fish even at low concentration. Grabda *et al.* (1974) exposed rainbow trout fry to 5–6 mg/L of NO_3-N for several days, and observed alterations in the hematopoietic centre, peripheral blood and liver damage in addition to the increased blood levels of ferrihemoglobin. Kincheloe *et al.* (1979) exposed some salmonid species to nitrate poisoning for 30 days, and they reported that their embryos and early fry stages had high mortalities at concentrations as low as 2.0 mg of NO_3-N in surface waters of lower total hardness (<40 mg $CaCO_3$/l).

2.4 Sewage effluent

Sewage effluent is defined as a runoff of domestic, commercial or industrial effluent or their mixture carried by water (USEPA, 2004). In African countries, many sewage treatment plants have been constructed to handle the huge amount of sewage effluent from high populated urban areas. However, the efficiency of sewage treatment in these cities has been compromised. This may be due to the fact that most of these treatment plants are either non-functional or overwhelmed by excessive sewage effluent. As a result, partially or untreated sewage effluent are discharged into the adjacent aquatic ecosystems uncontrollably (Adebayo *et al.*, 2007; Rim-Rukeh *et al.*, 2013). In the past, water ecosystems were less polluted, because pollutants were easily managed through natural self-purification processes. However, due to the increased production of domestic, commercial or industrial wastewater known as sewage effluent by the exploding human population, the situation has now changed, and aquatic ecosystems have suddenly become highly polluted (Oyelese, 2006). Much of these pollutants end up into the edges of lakes, rivers, streams, lagoons, flood plans and estuaries, where the water current is not strong enough to drift it (Konan *et al.*, 2014). These shallow water area are the breeding grounds

of *C. gariepinus* (Oyelese, 2006). Furthermore, the availability of sewage effluent in freshwater has become an issue of global concern, as it decreases the water quality yet its demand is rapidly increasing (Byrne *et al*., 2003).

2.4.1 Types and sources of sewage effluent

The volume and type of this effluent is influenced by the population size and a combination of recreation, domestic and industrial activities taking place in the surrounding area (USEPA, 2004). There are four major types of sewage effluent, namely urban, domestic, industrial and agricultural. Urban sewage effluent is a mixture of industrial and domestic effluent, rain water and sewage infiltration, while agricultural effluent is a mixture of wastewater from farm processes, agricultural activities and contaminated ground water (Shatanawi *et al*,. 2007). Domestic sewage effluent is a mixture of human and animal waste originating from washing, bathing, food preparation, personal hygiene, gardening and other domestic activities (DAWF, 1996). Much emphasis has been put on domestic and industrial effluent as of source nutrients and contaminants of the adjacent ecosystems. However, agricultural effluents are gaining much attention due to the increased use of fertilizers and other chemicals (Kremser and Schnug, 2002).

2.4.2 Sewage treatment process

Although, to a certain extent, natural water bodies such as streams and rivers have abilities to purify themselves after effluent have been discharged into them, it is critical that the effluent should be treated in order to remove harmful substances to both human beings and animals before it is released into the adjacent aquatic systems. The process of sewage treatment includes the primary stage which involves the removal of suspended solid substances by settling or screening. This is followed by the secondary stage which involves

the biological removal of dissolved organic substances by the action of living organism such as bacteria. In Africa, most sewage treatment plants stop at this level (EPA, 1997). However, in some plants the process continues to the tertiary level which involves the removal of ammonia and nitrogen. The treated water then goes through a chlorination basin and dechlorination basin for disinfection and removal of chlorine before it is discharged to the adjacent ecosystem (EPA, 1997; Wu, 1999; Naidoo and Olaniran, 2013).

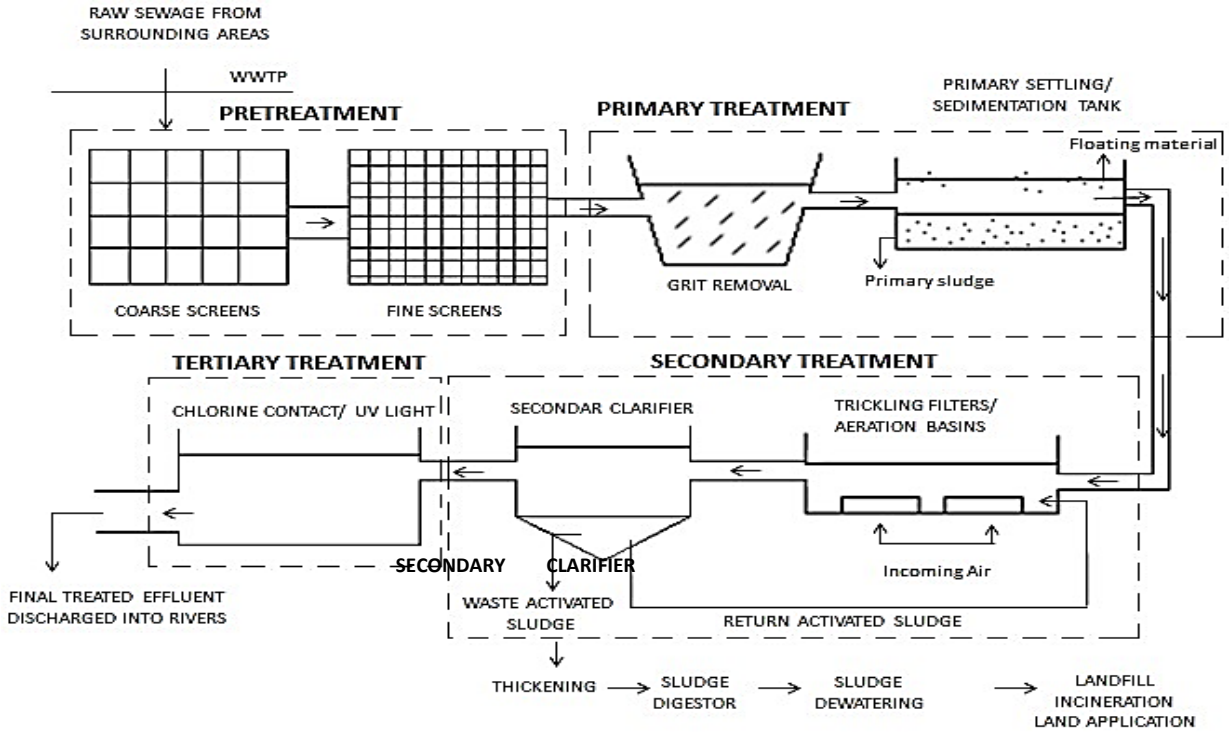

Figure 2.1 Summary of sewage treatment stages

Source: Adapted from EPA (1997).

2.4.3 Sewage treatment quality and standards

In order for the treated water to be discharged into the adjacent water bodies, each treatment plant must get a permit. This specifies standards for the physicochemical parameters of the discharged effluent based on local or international standards (Mara, 2004). The permit sets

minimum standards for dissolved Oxygen (DO), biochemical oxygen demand, pH, total residual chlorine (TRC) for secondary treatment. Permits also describe monitoring methodologies and frequencies in order to certify that the released effluent meets the prescribed standards (Mara, 2004: EPA, 1997).

2.4.4 Effects of sewage effluent to aquatic ecosystems

Sewage effluent are poisonous to aquatic organisms (Bellanca and Bailey, 1977). It is the major source of toxic xenobiotic compounds; reduces DO by increasing algal blooms and bacterial decomposition; increases turbidity from dissolved and suspended organic matter; reduced spawning and feeding habitat through siltation; causes fungal growth; shiftes the species composition and abundance by altering the community quality, as well as adding odor and taste to fish flesh (Wu, 1999; Naidoo and Olaniran, 2013). However, these effects are more critical in summer due to high temperatures and low DO levels. As a result, fish that are highly resistant to pollution are common in summer as compared to those that are less resistant (Naidoo and Olaniran, 2013).

In summary, fitness, reproduction, growth and distribution of fish depend on depends on the optimum condition of their environment. Hence each fish species positions itself in a locality where the environment is close to its optimum temperature. However, these conditions have been altered by the increasing inflowing amounts of nitrate fertilizers from agriculture and sewage effluents treatment plants which have almost doubled in the recent years. These coupled with the increasing and varying water temperature resulting from climate change and extreme warm air events have become issues of global concern. Fish usually responds by moving away from the altered or unsuitable environmental condition. However, eggs, embryos and larvae have limited capacity to move. Hence they end up

being exposed to the increasing amounts of increasing inflowing amounts of nitrate fertilizers, sewage effluent and temperature changes in their environment.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study area and source of broodstock

This study was carried out at Lilongwe University of Agriculture and Natural Resources (LUANAR) Fish Farm, in Lilongwe District of Malawi (latitude 14° 35S' and longitude 33° 50E'). The research period was between October and February 2015/2016 and 2016/2017, which is the natural spawning period of *C. gariepinus.* Fifteen gravid females and ten mature male broodfish of *C. gariepinus* were collected from stocks maintained in ponds at the fish farm. Their individual weights ranged between 401 and 430g and average weight (SD) of 413.7±11.64g for males while that of females ranged between 412 and 486g and average weight (SD) of 448.2±25.55g.

3.2 Acclimatization of broodstock

Broodfish were acclimatized to tank conditions for seven days. Female and male fish were acclimatized separately in 1000L plastic circular tanks filled to 800L with borehole water used for breeding fish at LUANAR fish farm hatchery. Acclimatization tanks were aerated and dark covered using a thick black polyethene. Water temperature was maintained at 28 ± 1^{o}C and pH at 7.1±0.1. The fish were fed at 35% crude protein twice a day at a rate of 5% body weight i.e. at 07:00 and 17:00 hours. During this acclimatization period, a quarter of the water was replaced to remove faecal material and left-over food on a daily basis.

3.3 Broodfish selection

A total of three ready females and two male broodfish were carefully selected from the acclimatization tanks for hormonal injection. Their selection was based on external

morphological characteristics; females that were selected had distended-soft abdomen from which singular-greenish eggs protruded after a gentle application of pressure. Males with pointed, reddish and elongated urino-genital papillae were selected. The selected fish were kept singly in dark covered aerated 60L plastic tanks, with 30 litres of water for 11 h to 12 h.

3.4 Hormone preparation and admission

Broodfish were injected with Aquaspawn® (manufactured by Spawnrite Ltd, Touwsrivier, Southern Cape, South Africa), between 5:30 and 6:30 pm. The hormone was administered intramuscularly in liquid form using a graduated syringe at 0.5 mL/kg body weight for female and 0.25mL/kg for males to induce ovulation and spermatogenesis respectively (Kather Haniffa and Sridhar, 2002). The treated fish were returned and kept singly in dark covered aerated 60L plastic tanks, with 30 litres of water for 11 h to 12 h, and water temperature was maintained at 28 ± 1^{o}C using water heaters with a controlled thermostat.

3.5 Fertilization

Twelve hours after injection, females were seined out of the tanks, dried using a towel and a slight thumb pressure was applied on the belly to push the eggs out of the fish into the plastic bowl. This process was repeated until traces of blood were observed coming out with eggs indicating that the ovaries were empty (Viveiros *et al.*, 2002). A sample of 30 eggs were collected after stripping, and these were used to determine the unfertilized egg sizes. Males were killed, and their abdomens were carefully surgically opened with an incision along the belly (without damaging the gut). The connective tissue of the mesentery was gently cut to remove the tests. Care was taken to minimize bleeding, as this would

obstruct the view of the testes and make their removal difficult. Only the white portions of the testes were removed and gently dried with a towel until all the moisture, blood and mesentery connective tissue had been removed (Dunham and Masser, 2012). The fertilization process involved incising the testes and squeezing the milt over the eggs. The eggs and the sperms were then thoroughly mixed using a clean, soft feather. Saline solution (0.6%) was added, and further agitation was done for a few seconds. Eggs from three females were fertilized by spermatozoa from two males while eggs stripped from different females were kept separately. The time take for the whole practice from stripping to fertilization was about three minutes as recommended by Dunham and Masser, (2012).

3.6 Experimental tanks

Three fibre insulated plastic tanks measuring 2.0 x 1.0 x 1.0 m each were used. These tanks were set indoors in the wet laboratory; each fitted with three thermostatically controlled water heaters, glass mercury thermometer and air blowers. Each tank was filled to 15 cm mark with borehole water and water temperature was raised to 31^{o}C in tank 3 (T_3), 28^{o}C in tank 2 (T_2) and 25^{o}C in tank 1 (T_1).

3.7 Experiments and experimental designs

Three experiments were carried out each with a different design

3.7.1 Experiment one: To determine the interactive effect of water temperature and nitrate fertilizers on development, growth rate and survival of embryos and early life stages of *C. gariepinus*

3.7.1.1 Preparation of test media

Borehole water used in LUANAR fish farm was tested for nitrate concentration before the beginning of the experiment table 4.2. To obtain the required nitrate concentrations, nitrate fertilizer, i.e. Sodium nitrates, prilled $NaNO_3$ (97%) bought from Agricultural Trading Company Limited (ATC) in Malawi was used as the source of nitrates for the experiments. Sodium nitrates, prilled $NaNO_3$ (97%) were also tested to find out whether it had the same concentration as prescribed. A stock solution of 40 mg/L of NO_3-N was prepared from which five dilutions were made i.e. (control) 0, 5, 10, 15 and 20 mg/L of NO_3-N. Each of these dilutions were put into 1000ml glass flasks filled 800ml in triplicates. All nitrates (Nitrate-NO_3) were converted to nitrogen nitrate (of NO_3-N) by (Equation 3.1).

Nitrate-NO_3 (mg/L): NO_3-N = 4.4268:1 (Weschler, 1968)..... (Equation 3.1)

3.7.1.2 Experimental design

This experiment took a 3 x 5 factorial laid out in a completely randomized design. After fertilization, 100 embryos of *C. gariepinus* were incubated in 1000ml glass flasks filled to 800ml mark with borehole water containing one of the five concentrations of inorganic nitrate (0 (control), 5, 10, 15 and 20 mg/L of NO_3-N) levels and each flask was put in a water bath set at one of the three temperatures (25, 28, and 31ºC) levels in triplicates. Air stones were placed in each flask and in each water bath to ensure oxygen supply and temperature homogeneity respectively. Thermometers were also placed in each water bath

to monitor temperature. The test solutions for the embryo stages were renewed at the end of the hatching period, while that of the larval stage was renewed after every two days.

3.7.2 Experiment two: To determine the interactive effect of water temperature and sewage effluent development, growth and survival of embryos and early life stages of *C. gariepinus*

3.7.2.1 Sourcing experimental samples

Experimental samples were collected from Likangala River, located in Zomba district of Malawi. The river was selected because; it originates from Zomba Plateau, flows through highly deforested Zomba Plateau, Zomba city, rural and irrigation agriculture areas and terminates into Lake Chilwa. Lake Chilwa is the second most productive lake in terms of *C. gariepinus* fisheries in Malawi (Phiri *et al.*, 2010). Owing to this, the river receives nitrates from agricultural fields; untreated poorly deposited domestic wastes from the city and rural households and treated sewage effluent from Zomba wastewater treatment plant. Sewage originates from the University (Chancellor College), Zomba Central Prison, hospitals and a fraction of residential houses from the city (Pullanikkatil *et al*., 2015). The whole treatment process; starts with screening, settlement and removal of large solid particles; followed by tank settlement and removal of suspended particulate organic matter; then biological degradation of dissolved substances by sprinkling the effluent on bacterial ladened rocks and uptake of nutrients by lattice (*Pistia stratiotes*) in a series of two maturation ponds before its final release into Likangala River. However, the treatment plant has been blamed of poor or partial treatment of wastewater (Pullanikkatil *et al*., 2015). Zomba district sewage treatment plant was chosen for the study because its outlet discharges the final effluent into Likangala River.

3.7.2.2 Experimental design

This experiment took a 3 x 5 factorial laid out in a completely randomized design. One hundred embryos of *C. gariepinus* were incubated in 1000ml glass flasks filled to 800ml and each flask were subjected to one of 25, 28, and 31ºC temperatures levels and one of these four dilutions of sewage effluent (0%, 15%, 30%, 45% and 60%) levels, replicated three times. Aeration, temperature homogeneity embryos and larvae rearing were handled as in experiment I (section 3.7.1.2).

3.7.3 Experiment three: To determine the interactive effect of water temperature and a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

3.7.3.1 Test media preparation

Groundwater and inorganic nitrates were sourced and handled as in experiment I. The test solution was collected from the sewage effluent outlet before it mixes with the river water (S3). Samples were collected from five other sites: 100m (S1) and 50m (S2) upstream before the sewage outlet, the sewage effluent outlet after mixing with the river water (S4), 50m (S5) and 100m (S6) downstream from the sewage outlet. Samples for water quality analysis were collected in 300 ml bottles. Nitric acid (1 mL) was used to acidify samples for metal analysis. Acidification was done to avoid metal ions adsorption to the container wall. Samples for metal analysis were kept at room temperature while the other samples were chilled and transferred to a refrigerator set at 4ºC. All these samples were taken to the laboratory for analysis. Some water quality parameters samples were measured on site. These include; dissolved oxygen, temperature, turbidity, total suspended solids, pH and

electric conductivity were measured using a hydrolab water quality checker (DS5X multiprobe sonde (Hach, USA*)*. Sewage effluent samples for experiment II and III were collected at the same time.

3.7.3.2 Experimental design

This experiment took a 3x4x4 factorial laid out in a split-plot design. After fertilization, 100 embryos of *C. gariepinus* were incubated in 1000ml glass flasks filled to 800ml mark with a mixture made by one of the four levels of sewage effluent (0%, 20%, 40% and 60%) levels and one of the four concentrations of inorganic nitrate (0 (control), 5, 10, and 15 mg/L of NO_3-N) levels and each flask was placed in a water bath set at one of the three temperatures (25, 28 and 31ºC) levels in triplicates. Air stones were put in each beaker and in each water bath to ensure oxygen supply and temperature homogeneity, respectively. Mercury in glass thermometers was also placed in each water bath to monitor temperature. The test solutions for the egg and embryo stages were renewed at the end of the hatching period. The larvae were raised for ten days in the nitrate concentrations and temperatures of incubation. The test for solution was renewed every two days.

3.8 Survival of embryos, incubation period, hatching period and hatching rate

A separate sample of embryos was incubated alongside with the experimental flasks at all temperatures. A subsample of 40 to 60 embryos (average) was taken and observed under stereo-loupe microscope connected to a video screen. Fertilized eggs (embryos) were those which cleaved or differentiated 30 to 80 minutes after fertilization. Another sample of 30 embryos, 10 at a time were collected from each replicate after every 4 hours. Embryos were replaced after counting was completed. A pipette was used to remove all unfertilized eggs

which turned white 14 h post-fertilization. Dead and unfertilized eggs (showed no signs of development after 4 hours and turned cloudy 8 hours post-fertilization) were counted twice, using a stereo-loupe microscope connected to a video-screen. Survival was determined using (Equation 3.2) (Okunsebor *et al*., 2015)

$$\text{Survival rate of embryos} = \frac{\text{Number of fertilized eggs before hatching}}{\text{Number of fertilized eggs}} * 100 \ldots.(\text{Equation 3.2})$$

The incubation period began from the time of egg fertilization to the first hatch while the hatching period was defined as the period from egg fertilization to the time when 50% of *C. gariepinus* embryos had hatched. At the end of hatching period all unhatched embryos, hatched larvae and dead larvae were counted, and the hatching rate (HR) were determined by equation (3.3) (Radonic *et al.*, 2007)

$$\text{HR} = \frac{\text{hatched larvae}}{\text{Hatched larvae + dead larvae + non hatched eggs}} * 100 \quad \ldots\ldots\ldots \text{(Equation 3.3)}$$

3.9 Yolk absorption rate

Yolk absorption rate was determined by the rate of reduction in the yolk volume. Immediately after fertilization, 20 embryos were randomly selected, and their yolk sac length (L) and yolk height (H) were measured using a stereo-loupe microscope connected to a video screen with a graduated slide. The yolk sac volume was determined using the equation (3.4) (Borode and Oyintoke, 2005).

$$V = \pi/6 \cdot LH^2 \quad \text{(Equation 3.4)}$$

Where

V is the yolk sac volume (mm^2)

L is yolk length (mm)

H is yolk height (mm)

This was repeated in each replicate after the first larvae had hatched, then at 50% hatching and every two day for the larval stages. Linear regression was also made to predict the yolk absorption rate at 50%, 80% and 100% (Kamler *et al.,* 1994) but this was verified by the actual measurements. The average rate of yolk absorption was determined daily using the equation (3.5) (Borode and Oyintoke, 2005):

$$\text{Yolk absorption rate} = (nI - nF)/t \quad \text{(Equation 3.5)}$$

Where:

- nI = initial average yolk volume,
- nF = final average yolk volume,
- t = rearing period

3.10 Yolk absorption period and larval survival rate

The yolk-sac period started from the end of the hatching period to when 50% of the larvae fully absorbed their yolk-sac. This was determined by visual observation. Mortality was determined by collecting, counting and recording daily the number of dead larvae for ten days. Percentage survival was determined by the equation (3.6) below (Radonic *et al*., 2007).

(%) Survival = $\frac{\text{Number of live larvae at the end 10 days x 100}}{\text{Number of live larvae after hatching}}$ ……..… (Equation 3.6)

3.11 Growth rate

The growth rate (GR) was determined daily by randomly measuring the length of 9 larvae from each treatment, three from each replicate using an ocular micrometer mounted on a light microscope for ten days. The average rate of growth in length was calculated using the equation (3.7) below by Borode and Oyintoke (2005):

GR = (Lf – Li)/t ……..…….……..… (Equation 3.7)

Where:

Lf = final average length of larvae,

Li = initial average length of larvae

t = rearing period.

3.12 Feeding

After 50% of the larvae had absorbed two-thirds of their yolk-sac, the larvae were fed on naturally occurring zooplankton. These zooplanktons were collected from well-fertilized fish ponds from Bunda Fish Farm using a plankton net (64 micro net). The fry were fed 3 times a day *ad libitum* for each treatment (Oyelese, 2006; Adewumi, 2015).

3.13 Water quality parameters

Some water quality parameters such as, conductivity, temperature, DO, pH and salinity were measured directly using a *Hydrolab (*DS5X *multiprobe* sonde (Hach, *USA).* PO_4^{3-} and NO_3^-, was measured using UV/Visible spectrophotometer (model T90, Wagtech Projects, China). COD and BOD were measured using standard methods as by Ademoroti (1996).

Samples were incubated in BOD bottle at 20°C for 5 days. DO maintained using a RESUN low noise air pump (LP-100, RESUN aquarium, Guangdong, China).

3.14 Data analysis

After homoschedasticity and normality tests, data were subjected to two-way analysis of variance ANOVA for the interactions between temperature and nitrate fertilizers, and water temperature and sewage effluent (Equation 3.8 and 3.9). The interactions among water temperature, nitrate fertilizers, and sewage effluent were subjected to three-way analysis of variance ANOVA (Equation 3.10). Means that were found significantly interacting were compared using Turkey's test in a one-way ANOVA. Orthogonal polynomial contrasts were used to partition significant nitrate and sewage effluent rates into linear, quadratic, cubic and quartic components (Equation 3.11). Data were analysed using R statistical analysis software (version R 3.4.3 and R-studio 0.9.8).

3.15 Models for two way and three way analysis

$Y_{ijk} = \mu + T_i + N_j + (TN)_{ij} + \varepsilon_{ijk}$ ..... (Equation 3.8)

$Y_{ijkl} = \mu + T_i + S_k + (TS)_{ik} + \varepsilon_{ikl}$ ..... (Equation 3.9)

$Y_{ijkl} = \mu + T_i + N_j + S_k + (TN)_{ij} + (TS)_{ik} + (NS)_{jk} + (TNS)_{ijk} + \varepsilon_{ijkl}$ ..... (Equation 3.10)

Where,

Y_{ijkl} = l^{th} observed response value when level *i* of Water temperature, level *j* of Nitrate fertilizer and level *k* of Sewage effluent is used.

μ = general effect

α_I = effect due to the fact level *i* of Water temperature was used.

β_j = effect due to the fact level *j* of Nitrate fertilizer was used.

γ_k = effect due to the fact level *j* of Nitrate fertilizer was used

$(\alpha\beta)_{ij}$ = effect due to the interaction of i^{th} level of Water temperature and the j^{th} level of Nitrate fertilizer

$(\alpha\gamma)_{ik}$ = effect due to the interaction of i^{th} level of Water temperature and the k^{th} level of Sewage effluent

$(\beta\gamma)_{jk}$ = effect due to the interaction of j^{th} level of Nitrate fertilizer and the k^{th} level of Sewage effluent

$(\alpha\beta\gamma)_{ijk}$ = effect due to the interaction of i^{th} level of Water temperature, j^{th} level of Nitrate fertilizer and the k^{th} level of Sewage effluent

ε_{ijkl} = the random error, represents the variation in the response values when the i^{th} level of Water temperature, j^{th} level of Nitrate fertilizer and the k^{th} level of Sewage effluent

3.16 Polynomial equations

$$Y = \beta_0' + \beta_1 ' X + \beta_2 ' X^2 + \beta_3 ' X^3 + \beta_4 ' X^4 + \beta_5 ' X^5 \quad \text{(Equation 3.11)}$$

If only β_1 significant, the relationship is linear, if β_1 and β_2 are significant, then the relationship is quadratic, etc.

CHAPTER FOUR

RESULTS

4.1 Physico-chemical parameters and heavy metals, of borehole water and sewage effluent used in the currents experiments

At the beginning of the experiment, borehole water used in LUANAR fish farm, sewage effluent, water from Likangala river were tested for nitrate concentration, heavy metals and other physical chemical parameters (Table 4.1).

Table 4.1 Summary of physico-chemical parameters and heavy metal, of borehole water and sewage effluent before and after discharge in Likangala River

Parameters	**Sampling points**						
	Borehole water	**Upstream (100m)**	**Upstream (50m)**	**Sewage effluent**	**After mixing with stream water**	**Downstream (50m)**	**Downstream (50m)**
pH	6.82 ± 0.3^{abc}	7.0 ± 0.4^{a}	7.1 ± 0.2^{a}	6.4 ± 0.2^{c}	6.6 ± 0.2^{bc}	6.8 ± 0.1^{abc}	6.9 ± 0.1^{ab}
Temp (^{o}C)	23.5 ± 0.7^{a}	21.0 ± 1.0^{d}	20.9 ± 1.3^{d}	23.1 ± 1^{b}	22.8 ± 0.6^{b}	22.6 ± 0.6^{b}	21.8 ± 1.1^{d}
EC (µS/cm)	94.78 ± 3.4^{g}	108 ± 15.2^{f}	128 ± 10.3^{e}	546 ± 26.0^{a}	380 ± 25.4^{b}	337.5 ± 6.3^{c}	238 ± 14.6^{d}
TDS (ppm)	15.93 ± 7.9^{g}	54 ± 8.2^{f}	64 ± 4.3^{e}	273 ± 14.5^{a}	204 ± 6.5^{b}	168.5 ± 10.5^{c}	119 ± 20.0^{d}
Turbidity(FNU)	14.07 ± 1.1^{c}	19.6 ± 1.0^{c}	21.1 ± 2.3^{c}	69.2 ± 8.5^{a}	48.8 ± 4.0^{b}	49.1 ± 3.0^{b}	42.2 ± 2.1^{b}
SO_4^{2-} (mg/L)	1.44 ± 0.31^{c}	1.16 ± 0.2^{e}	1.18 ± 0.4^{e}	3.4 ± 0.4^{a}	2.0 ± 0.1^{b}	2.0 ± 0.3^{b}	1.3 ± 0.2^{d}
PO_4^{3-} (mg/L)	0.62 ± 0.1^{d}	0.64 ± 0.2^{d}	1.14 ± 0.5^{d}	20.1 ± 1.2^{a}	10.1 ± 1.4^{b}	10.6 ± 1.6^{c}	8.5 ± 0.5^{c}
NO_3^{-} (mg/L)	1.64 ± 0.40^{d}	5.79 ± 1.3^{c}	5.4 ± 0.4^{c}	36.1 ± 12.8^{a}	34.3 ± 1.7^{ab}	35.6 ± 1.7^{a}	32.8 ± 1.1^{b}
Ca (mg/L)	6.7 ± 0.5^{f}	8.6 ± 1.4^{e}	11.3 ± 2.1^{d}	15.8 ± 3.2^{a}	13.2 ± 1.4^{c}	13.3 ± 2.0^{c}	14.1 ± 2.1^{b}

(Continued)

Parameters	Sampling points						
	Borehole water	**Upstream (100m)**	**Upstream (50m)**	**Sewage effluent**	**After mixing with stream water**	**Downstream (50m)**	**Downstream (50m)**
Na (mg/L)	8.4±1.2^{f}	7.8±0.4^{e}	9.3±1.2^{d}	33.4±2.5^{a}	22.4±2.3^{b}	21.2±1.6^{c}	15.2±2.4^{d}
K (mg/L)	1.6±0.12^{d}	0.9±0.2^{e}	1.0±0.3^{e}	13.5±3.2^{a}	7.3±1.2^{b}	3.5±1.1^{c}	1.9±0.2^{d}
Mg (mg/L)	1.6±.06^{e}	3.0±0.6^{d}	3.9±1.2^{c}	5.5±1.1^{a}	4.7±0.9^{b}	4.8±0.7^{b}	4.9±1.4^{b}
Fe (mg/L)	1.9±0.7^{c}	0.6±0.1^{g}	0.8±0.1^{f}	3.6±0.2^{a}	3.0±0.3^{b}	1.2±0.1^{d}	1.0±0.2^{e}
Al (mg/L)	0.33±0.03^{d}	0.30±0.1^{d}	0.39±0.06^{c}	0.80±0.2^{a}	0.63±0.1^{b}	0.64±0.2^{b}	0.18±0.1^{e}
Mn (mg/L)	bdl	0.11±0.02^{e}	0.24±0.1^{d}	0.69±0.3^{a}	0.41±0.1^{b}	0.47±0.1^{b}	0.69±0.2^{b}
Cu (mg/L)	bdl	bdl	bdl	0.05±0.02^{a}	0.04±0.01^{a}	0.04±0.02^{a}	0.03±0.01^{a}
Cr (mg/L)	bdl	bdl	bdl	0.05±0.01^{a}	0.05±0.01^{a}	Bdl	bdl
Pb (mg/L)	bdl	bdl	bdl	0.05±0.02^{a}	0.05±0.01^{a}	0.04±0.01^{a}	bdl
Zn (mg/L)	bdl	bdl	bdl	0.05±0.01^{a}	Bdl	Bdl	bdl

bdl: below detection limit, Means (mean ± SE) with the same letters do not differ significantly

Table 4.1.shows that the Physico-chemical parameters of sewage effluent were almost all above the required range relevant for the survival, growth and development of *C. gariepinus* eggs, embryos and larvae (Table 4.1). In the preliminary experiment, few embryos hatched and no larvae survived at 80% sewage effluent level and above. However, since all samples used in the current study were diluted to at least 60%, all the parameters were within the required range in each experimental unit.

4.2 Interactive effect of temperature and nitrate fertilizers on development, growth and survival of embryos and early life stages of *C. gariepinus*

4.2.1 Interactive effect of temperature and nitrate fertilizers on development, growth and survival of *C. gariepinus* embryos

There were two-way significant interaction ($p < 0.05$) between water temperature and nitrate fertilizers on incubation period, hatching period and hatching rate of *C. gariepinus* embryos and their linear, quadratic and cubic orthogonal polynomial trends of nitrate in temperature and nitrate fertilizers interaction Table 4.2. This implied that the increasing levels of nitrate fertilizers from the control to 20 mg/L of NO_3-N had different effects on development, growth and survival of *C. gariepinus* embryos at 25°C, 28°C and 31°C. However, no significant two-way interactions ($P > 0.05$) were noted between water temperature and nitrate fertilizers on survival of embryos (Table 4.3 and 4.4).

Table 4.2 Two-way interactive effect of temperature and nitrate fertilizers and their orthogonal polynomial contrasts on incubation period, hatching period and hatching rate of *C. gariepinus* embryos

T (ºC)	N (mg/L of NO_3-N)	Incubation period (h)	Hatching period (h)	Hatching rate (%)
25	0	24.3 ± 0.6^{c}	30.3 ± 0.9^{d}	68.3 ± 1.7^{def}
	5	26.7 ± 0.6^{b}	34.3 ± 0.8^{c}	67.9 ± 1.6^{def}
	10	26.6 ± 0.7^{b}	37.0 ± 1.2^{bc}	65.7 ± 3.9^{def}
	15	28.7 ± 0.6^{b}	40.0 ± 1.2^{ab}	65.8 ± 2.4^{def}
	20	31.3 ± 0.7^{a}	41.0 ± 0.6^{ab}	62.6 ± 4.7^{f}
28	0	20.3 ± 0.6^{efg}	21.7 ± 0.3^{hi}	88.2 ± 2.9^{ab}
	5	20.0 ± 1.1^{fg}	26.3 ± 0.7^{ef}	85.2 ± 2.6^{ab}
	10	21.7 ± 0.6^{def}	27.6 ± 0.6^{de}	82.6 ± 2.2^{bc}
	15	22.6 ± 0.5^{cd}	28.7 ± 0.6^{de}	72.7 ± 3.9^{de}
	20	23.3 ± 0.6^{cd}	30.3 ± 0.3^{d}	64.5 ± 3.7^{ef}
31	0	18.3 ± 0.6^{g}	19.3 ± 0.3^{i}	92.4 ± 2.9^{a}
	5	19.3 ± 1.3^{g}	21.7 ± 0.3^{hi}	88.4 ± 2.6^{ab}
	10	19.7 ± 0.6^{fg}	22.3 ± 0.3^{ghi}	83.8 ± 2.2^{abc}
	15	20.3 ± 1.3^{efg}	24.0 ± 0.6^{fgh}	75.2 ± 3.9^{cd}
	20	22.3 ± 0.6^{cde}	25.3 ± 0.3^{efg}	67.7 ± 3.7^{def}
	p-value	$p = 0.002$	$p < 0.001$	$p = 0.027$
	Linear	S	S	S
	Quadratic	S	S	NS
	Cubic	S	NS	NS
	Quartic	NS	NS	NS

T = temperature, N = nitrate, Means (mean ± SE) with the same letters do not differ significantly, NS = non-significant, S = significant

4.2.1.1 Effect of temperature and nitrate fertilizers on survival of *C. gariepinus* embryos

There were no significant two-way interaction effect ($p > 0.05$) of temperature and nitrate fertilizers on the survival of *C. gariepinus* embryos (Tables 4.3 and 4.4). This implied that the effect of increasing nitrate fertilizers on survival of *C. gariepinus* embryos was independent from that of water temperature. The main effect of water temperature and nitrate fertilizers and their orthogonal polynomial linear and quadratic effects respectively on the survival of *C. gariepinus* embryos were significant ($p < 0.05$) (Fig 4.1 and 4.2).

Table 4.3 Main of effects nitrate on survival rate of *C. gariepinus* embryos

Nitrates (mg/L of NO_3-N)	**Survival rate of embryos (%)**
0	91.3±0.78[a]
5	91.9±1.01[a]
10	89.2±1.6[a]
15	83.3±1.2[b]
20	77.2±1.3c
p-value	$p < 0.007$
Linear	S
Quadratic	S
Cubic	NS
Quartic	NS

NS = non-significant, S = significant, Means (mean ± SE) with the same letters do not differ significantly

Table 4.4 Main effects of temperature on survival rate of *C. gariepinus* embryos

Temp (^{O}C)	Survival rate of embryos (%)
25	84.9±1.2^{c}
28	86.6±1.0^{ab}
31	88.3±0.9^{a}
p-value	$p = 0.023$
Linear	S
Quadratic	S

NS = non-significant, S = significant, Means (mean ± SE) with the same letters do not differ significantly

4.2.1.1.1 The main effect of nitrate on survival of C. gariepinus embryos

The significant cubic trend of the main effect of nitrate showed a decrease in survival of *C. gariepinus* embryos with increasing levels of nitrate (Figure 4.2). The highest survival of *C. gariepinus* embryos was observed at 5mg/L of NO_3-N; this was not significantly different ($p > 0.05$) from those obtained at 0 mg/L of NO_3-N and 10 mg/L of NO_3-N, but they were significantly different ($p < 0.05$) from those observed at 15 mg/L of NO_3-N, and 20 mg/L of NO_3-N. Significant differences ($p < 0.05$) were also recorded between the number of *C. gariepinus* embryos obtained at 15 mg/L of NO_3-N and 20 mg/L of NO_3-N (Figure 4.2, Table 4.3). These results suggest that *C. gariepinus* embryos can survive in nitrate water up to a range of 10 to 15 mg/L of NO_3-N, beyond which their survival reduced.

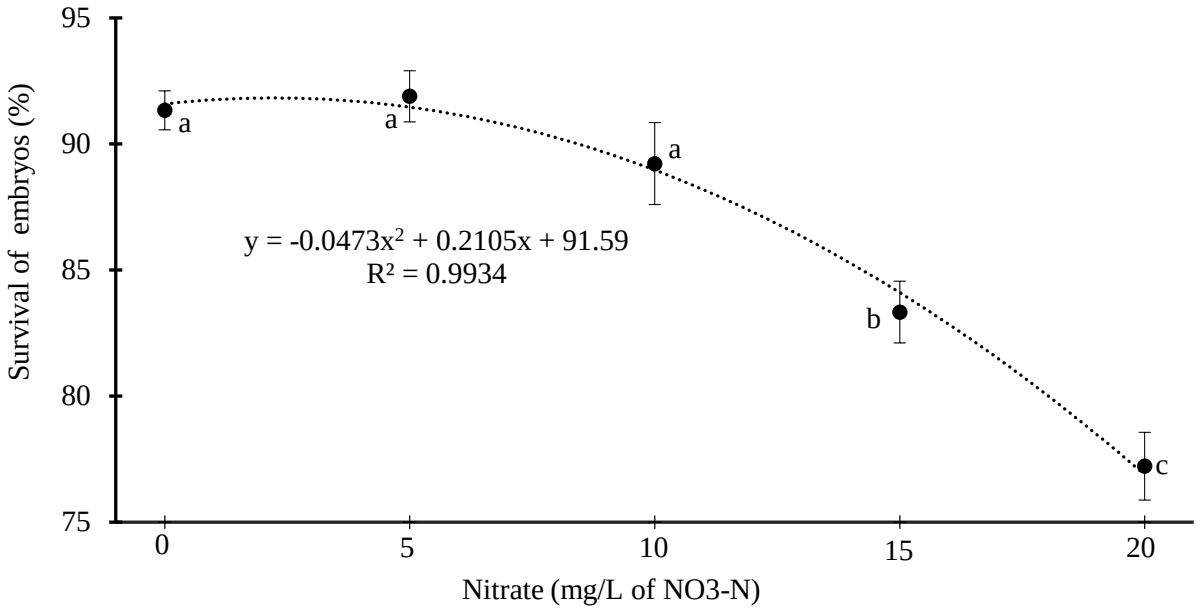

Figure 4.1 Quadratic main effect of nitrate on survival *C. gariepinus* embryos (Means with the same letter are not significantly different ($p > 0.05$)).

4.2.1.1.2 The main effect of temperature on survival of C. gariepinus embryos

The significant linear trend of the main effect of temperature showed an increase in the survival of *C. gariepinus* embryos with increasing incubation temperature (25°C-31°C) (Figure 4.1, Table 4.4). However, no significant differences ($p > 0.05$) were observed between the survival of *C. gariepinus* embryos obtained at 28°C and 31°C but these were significantly higher ($p < 0.05$) than those observed at 25°C. These results suggest a direct relationship between water temperature and survival of *C. gariepinus* embryos with a suitable temperature range of 28°C to 31°C.

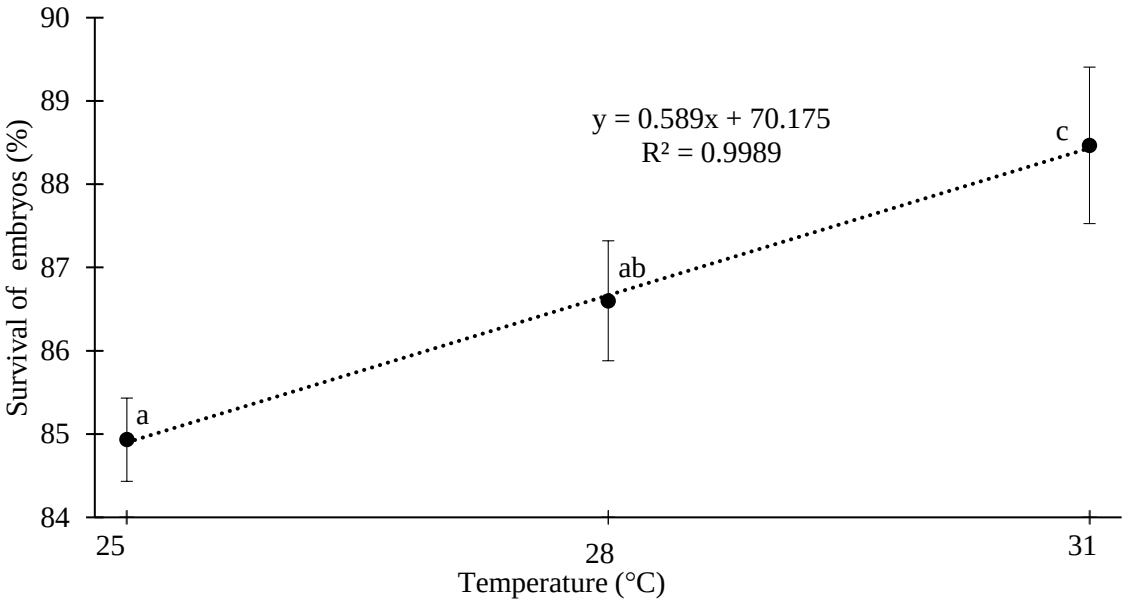

Figure 4.2 Linear main effect of temperature on survival of *C. gariepinus* embryos (Means with the same letter are not significantly different ($p > 0.05$)).

4.2.1.2 Effect of temperature and nitrate fertilizers on incubation period of *C. gariepinus* embryos

4.2.1.2.1 Interactive effect of temperature and nitrate fertilizers on incubation period of *C. gariepinus* embryos

There was a two-way significant interaction ($p < 0.05$) between temperature and nitrate fertilizers and its orthogonal polynomial quadratic effect of nitrate on the incubation period of *C. gariepinus* embryos (Table 4.2, Figure 4.3). The cubic trend indicated significant differences ($P < 0.05$) between the control (0 mg/L of NO_3-N) and 20 mg/L of NO_3-N at all incubation temperatures (25°C to 31°C). However, no significant differences ($P > 0.05$) were noted in the incubation period when *C. gariepinus* embryos were incubated in water containing 5 to 15 mg/L of NO_3-N at all incubation temperatures (25 to 31°C) (Figure 4.3). This suggests that the presence of nitrate in incubation water delayed the development and

growth of *C. gariepinus* embryos. However, at 25°C the development rate was significantly ($p<0.05$) less in the control as compared to nitrate polluted water while at 28°C and 31°C the embryos tolerated a maximum of 10 to 15mg/L of NO_3-N and 15 to 20 mg/L of NO_3-N respectively.

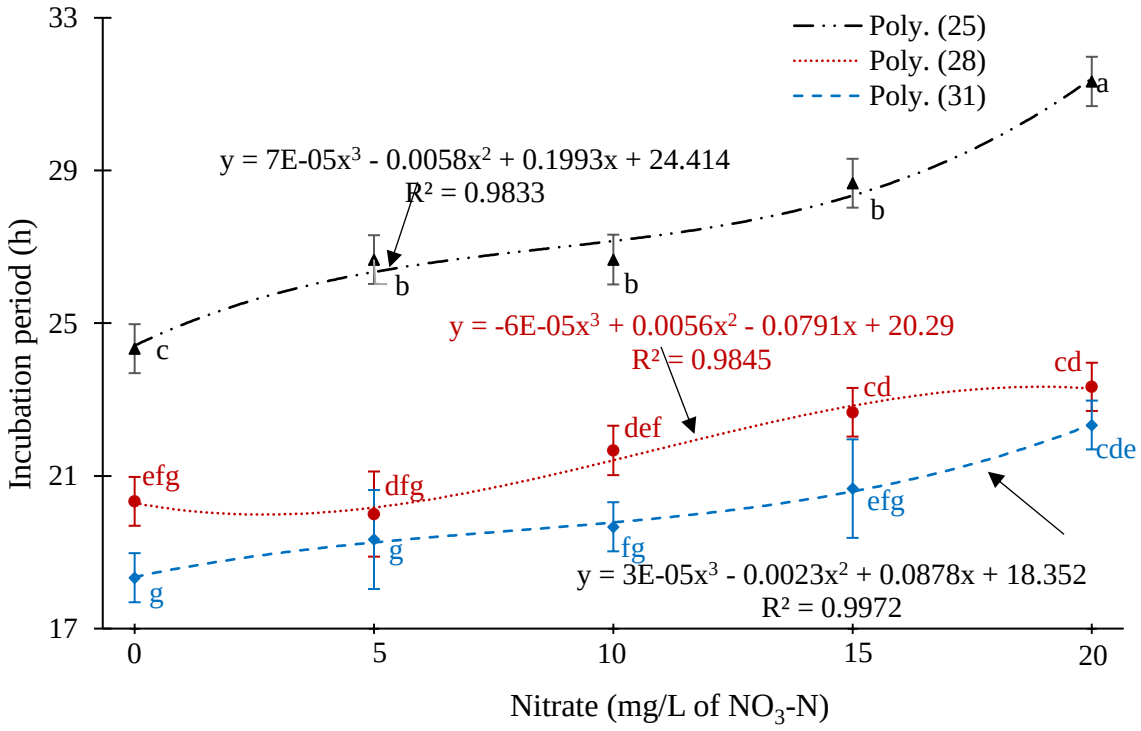

Figure 4.3 Cubic effect of nitrate in temperature and nitrate fertilizers interaction on the incubation period of *C. gariepinus* embryos

(Means (mean ± SE, n=45) with the same letters do not differ significantly)

4.2.1.3 Effect of temperature and nitrate fertilizers on hatching period of *C. gariepinus* embryos

4.2.1.3.1 Interactive effect of temperature and nitrate fertilizers on hatching period of *C. gariepinus* embryos

There was a two-way significant interaction ($p < 0.05$) between temperature and nitrate fertilizers and its orthogonal polynomial quadratic effect of nitrate on the hatching period

of *C. gariepinus* embryos (Table 4.2, Figure 4.4). The quadratic trend indicated significant differences ($p < 0.05$) between the control and all nitrate polluted water (5 to 20 mg/L of NO_3-N) at all incubation temperatures (25 to 31°C). However, no significant differences ($p > 0.05$) were noted in hatching period when *C. gariepinus* embryos were incubated in water containing 10 to 20 mg/L of NO_3-N at all incubation temperatures (25 to 31°C). Significant differences ($p < 0.05$) were noted in hatching period when *C. gariepinus* embryos were incubated in the control and water containing 20 mg/L of NO_3-N at all incubation temperatures (25 to 31°C) (Figure 4.4). These results suggest that the presence of nitrate fertilizers in hatching water delayed the development, growth and hatching process of *C. gariepinus* embryos. However, at 28 to 31°C no significant differences ($p > 0.05$) were noted in hatching period between 5 and 15 mg/L of NO_3-N while at 25°C significant differences ($p < 0.05$) were noted in the same nitrate concentration.

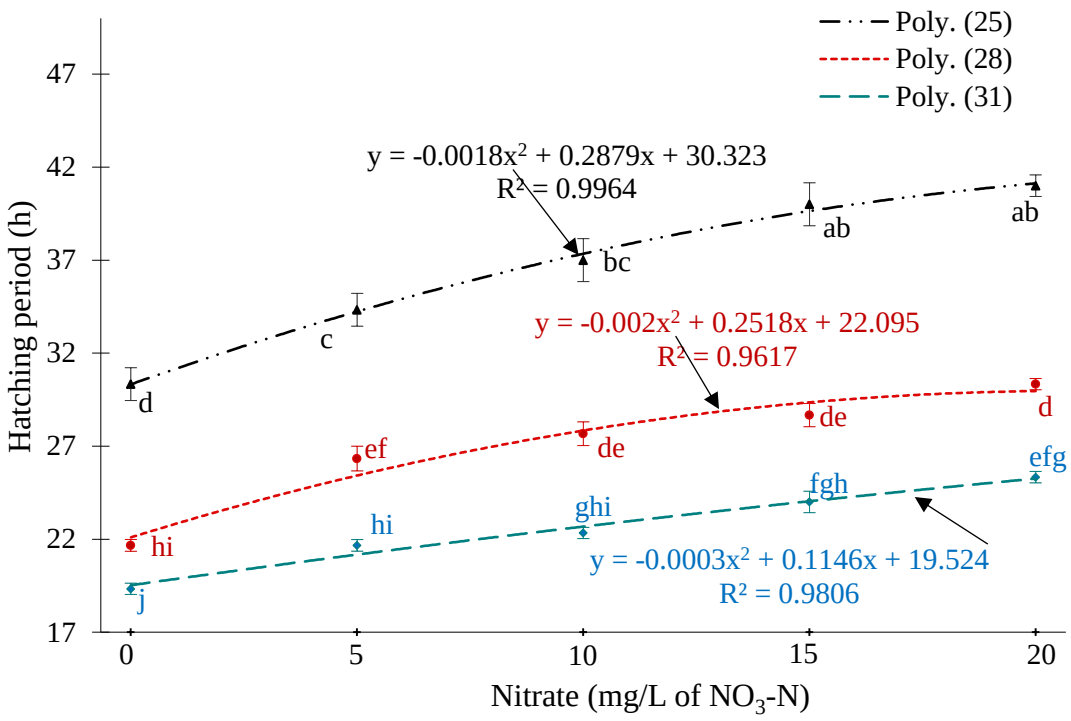

Figure 4.4 Quadratic effects of nitrate in temperature and nitrate fertilizers interaction on hatching period of *C. gariepinus* embryos

(Means (mean ± SE, n=45) with the same letters do not differ significantly)

4.2.1.4. Effect of temperature and nitrate fertilizers on hatching rate of *C. gariepinus* embryos

4.2.1.4.1 Interactive effect of temperature and nitrate fertilizers on hatching rate of *C. gariepinus* embryos.

There was a two-way significant interaction (p<0.05) between temperature and nitrate fertilizers and its orthogonal polynomial linear effect of nitrate on the hatching rate of *C. gariepinus* embryos (Table 4.2). The significant linear trend showed that, at incubation temperatures of 28 and 31°C there were no significant differences (p > 0.05) in the hatching rates of *C. gariepinus* embryos between the control and water containing nitrate up to range of 10 to 15 mg/L of NO_3-N. Significant differences (p < 0.05) were noted

between 10 and 20 mg/L of NO_3-N at 28 and 31°C, but no significant differences ($p > 0.05$) were noted in the incubation periods between the control and all nitrate levels (0-20 mg/L of NO_3-N) at 25°C (Figure 4.5). The results suggest that *C. gariepinus* embryos could be successfully hatched in water containing a maximum range of 10 to 15 mg/L of NO_3-N at 28°C and 31°C and 20 mg/L of NO_3-N at 25°C.

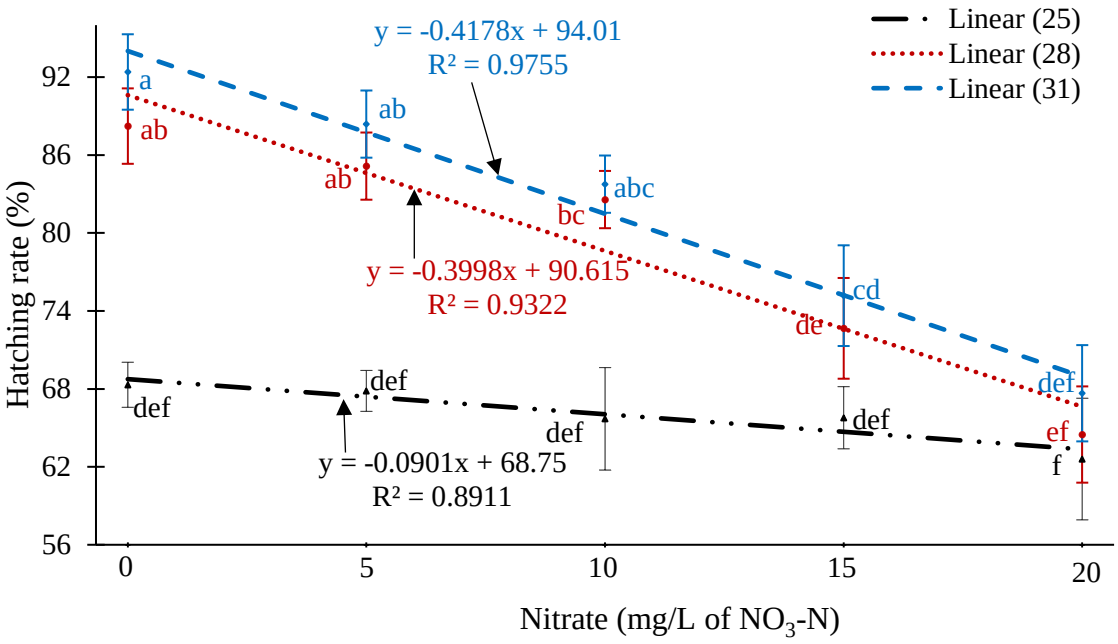

Figure 4.5 Linear effects of nitrate in temperature and nitrate fertilizers interaction on hatching rate of *C. gariepinus* embryos

(Means (mean ± SE, n=45) with the same letter are don't differ significantly ($p > 0.05$)).

4.2.2 Interactive effect of temperature and nitrate fertilizers on development, growth and survival of *C. gariepinus* larvae

There were significant interaction ($p < 0.05$) between water temperature and nitrate fertilizers on growth rate and survival of *C. gariepinus* larvae and their quadratic orthogonal polynomial trends of nitrate in temperature and nitrate fertilizers interaction Table 4.5. This implied that the increasing levels of nitrate fertilizers from the control to

20 mg/L of NO_3-N had different effects on development, growth and survival of *C. gariepinus* larvae at 25°C, 28°C and 31°C. However, no significant two-way interactions ($P > 0.05$) were noted between water temperature and nitrate fertilizers on yolk absorption period and yolk absorption rate (Table 4.6 and 4.7).

Table 4.5 Two-way interactive effect of temperature and nitrate fertilizers and their orthogonal polynomial contrasts on growth rate and survival of *C. gariepinus* larval

T (ºC)	N (mg/L of NO_3-N)	Growth rate (mm/day)	Survival rate (%)
25	0	0.86 ± 0.02^{cd}	48.9 ± 1.1^{ed}
	5	0.85 ± 0.03^{cd}	47.8 ± 2.2^{def}
	10	0.80 ± 0.03^{cd}	43.3 ± 1.9^{efg}
	15	0.72 ± 0.03^{de}	40.0 ± 1.9^{g}
	20	0.67 ± 0.02^{e}	30.0 ± 1.9^{h}
28	0	1.09 ± 0.02^{a}	70.0 ± 1.9^{abc}
	5	1.05 ± 0.02^{ab}	64.4 ± 2.2^{bc}
	10	1.05 ± 0.03^{ab}	63.3 ± 3.3^{c}
	15	0.92 ± 0.04^{cd}	45.6 ± 2.2^{efg}
	20	0.69 ± 0.02^{ed}	38.9 ± 1.1^{g}
31	0	1.12 ± 0.03^{a}	73.3 ± 1.9^{a}
	5	1.06 ± 0.03^{a}	71.1 ± 2.2^{ab}
	10	1.07 ± 0.03^{a}	70.0 ± 3.3^{abc}
	15	0.95 ± 0.03^{cb}	53.3 ± 1.9^{d}
	20	0.74 ± 0.01^{d}	41.1 ± 2.9^{fg}
	p-value	$p = 0.002$	$p = 0.007$
	Linear	S	S
	Quadratic	S	S
	Cubic	NS	NS
	Quartic	NS	NS

T = temperature, N = nitrate, Means (mean ± SE) with the same letters do not differ significantly, NS = non-significant, S = significant

4.2.2.1 Effect of temperature and nitrate fertilizers on growth rate of *C. gariepinus* larvae

4.2.2.1.1 Interactive effect of temperature and nitrate fertilizers on the growth rate of *C. gariepinus* larvae

There was a two-way significant interaction (p <0.05) between temperature and nitrate fertilizers and its orthogonal polynomial quadratic effect of nitrate on the growth rate of *C. gariepinus* larvae (Table 4.5, Figure 4.6). The quadratic trend indicated that there were no significant differences (p> 0.05) in the growth rate of *C. gariepinus* larvae between the control and up to 10 mg/L of NO_3-N at 28°C and 31°C and 15 mg/L of NO_3-N at 25°C. Significant differences (p< 0.05) were noted when *C. gariepinus* larvae were nursed in water containing 10 and 20 mg/L of NO_3-N at all incubation temperatures (25 to 31°C). Furthermore, significant differences (p< 0.05) were also noted when *C. gariepinus* larvae were reared in the control and water containing 15 mg/L of NO_3-N at 28 and 31°C while at 25°C, significant differences (p< 0.05) were noted when *C. gariepinus* larvae were reared in the control and water containing 20 mg/L of NO_3-N (Figure 4.6). The results suggest that *C. gariepinu*s larvae could be successfully grown in water containing a maximum range of 10 to 15 mg/L of NO_3-N at 28°C and 31°C and 15 to 20 mg/L of NO_3-N at 25°C.

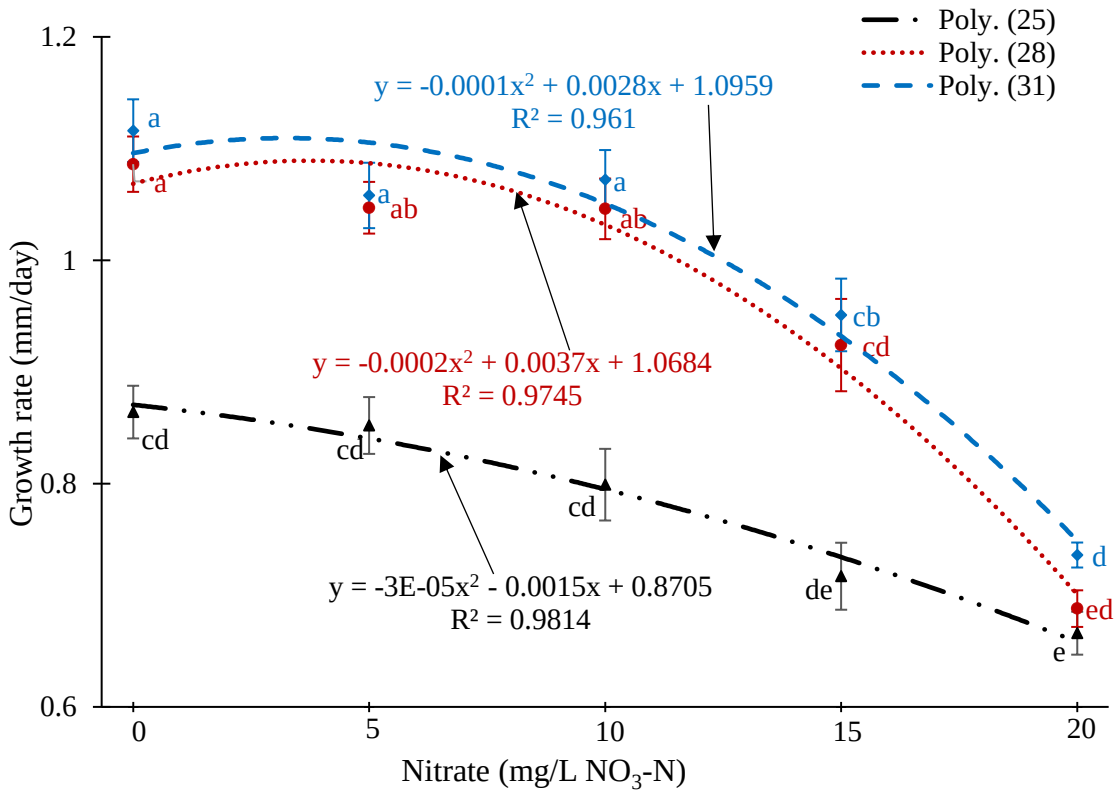

Figure 4.6 Quadratic interactive effect of nitrate in temperature and nitrate fertilizers interaction on growth rate of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letters don't differ significantly ($p > 0.05$))

4.2.2.2 Effect of temperature and nitrate fertilizers on the yolk absorption period of *C. gariepinus* larvae

There was no two-way significant interaction ($p > 0.05$) between temperature and nitrate fertilizers on the yolk absorption period of *C. gariepinus* larvae (Tables 4.6 and 4.7). These results suggest that water temperature had an independent effect from nitrate fertilizers. The main effect of temperature and nitratefertilizers and their orthogonal polynomial linear effects of both temperature and nitrate fertilizers on *C. gariepinus* larvae were significant ($p > 0.05$) (Figure 4.7 and 4.8).

Table 4.6 Main of effects nitrate on yolk absorption period and yolk absorption period of *C. gariepinus* larvae

Nitrates (mg/L of NO_3-N)	**Yolk absorption period/h**	**Yolk absorption rate/Day**
0	67.3 ± 4.8^{c}	0.83 ± 0.08^{a}
5	71.3 ± 6.2^{c}	0.77 ± 0.08^{ab}
10	79.3 ± 7.1^{bc}	0.69 ± 0.061^{abc}
15	91.3 ± 6.8^{b}	0.62 ± 0.051^{bc}
20	104.0 ± 8.5^{a}	0.52 ± 0.063^{c}
p-value	$p < 0.001$	$P < 0.001$
Linear	S	S
Quadratic	NS	NS
Cubic	NS	NS
Quartic	NS	NS

NS = non-significant, S = significant, Means (mean ± SE) with the same letters do not differ significantly

Table 4.7 Main effects of temperature on yolk absorption period and yolk absorption rate of *C. gariepinus* larvae

Temp (^{O}C)	Yolk absorption period/h	Yolk absorption rate/Day
25	104.8±5.5[a]	0.52±0.04[c]
28	80.0±3.0[b]	0.67±0.04[b]
31	63.2±4.0[c]	0.87±0.05[a]
p-value	$p < 0.001$	$P < 0.001$
Linear	S	S
Quadratic	NS	S
Cubic	NS	NS

NS = non-significant, S = significant, Means (mean ± SE) with the same letters do not differ significantly

4.2.2.2.1 Effect of nitrate on the yolk absorption period of *C. gariepinus* larvae

The linear trend of nitrate main effect indicates that the yolk absorption period of *C. gariepinus* larvae increased with increasing levels of nitrate (Figure 4.7). The least yolk absorption period was obtained at 0 mg/L of NO_3-N, this was not significantly different ($p > 0.05$) from that, obtained at 5 mg/L of NO_3-N and 10 mg/L of NO_3-N. The highest yolk absorption period was obtained at 20 mg/L of NO_3-N this was significantly higher ($p < 0.05$) than that obtained at 15 mg/L of NO_3-N. There were significant differences ($p < 0.05$) between incubation periods obtained at 10 mg/L of NO_3-N and 15 mg/L of NO_3-N. These results suggest that *C. gariepinus* larvae can effectively digest and utilize their yolk sac in water with a maximum of 10 to 15 mg/L of NO_3-N, beyond which the yolk sac digestion and utilization is reduced.

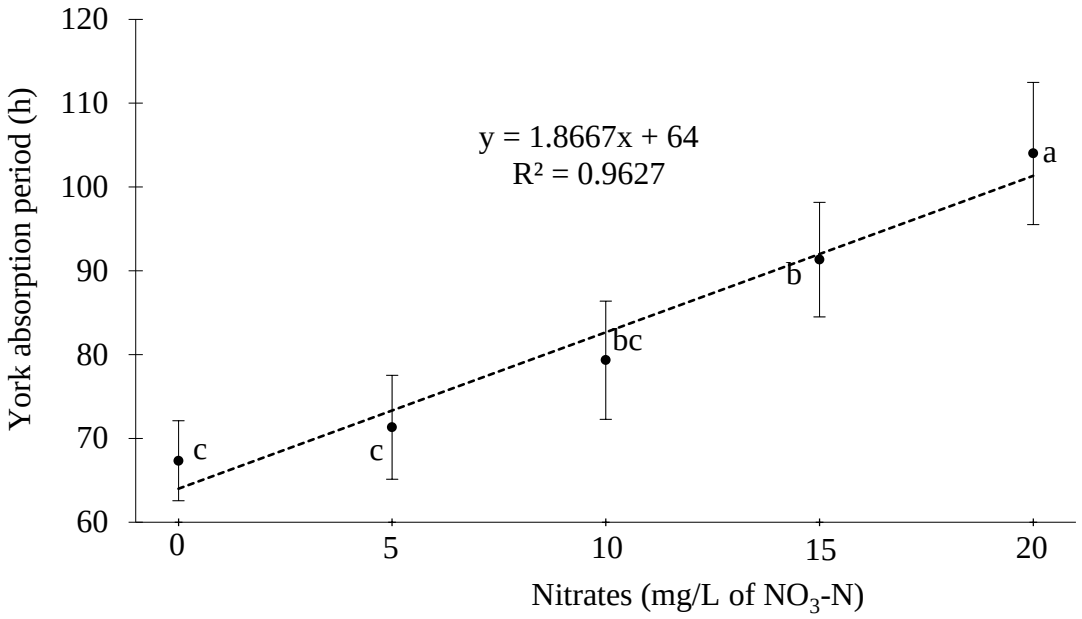

Figure 4.7 Linear main effect of nitrate on yolk absorption period of *C. gariepinus* larvae. (Means with the same letter don't differ significantly ($p > 0.05$)).

4.2.2.2.2 Main effect of temperature on yolk absorption period of *C. gariepinus* fertilized larvae

The linear trend of temperature indicated a decrease in the yolk absorption period of *C. gariepinus* larvae with increasing larval rearing temperature (25°C-31°C) (Figure 4.8). The highest yolk absorption period was observed at 25°C, this was significantly higher ($p < 0.05$) than that observed at 28°C. The least yolk absorption period was obtained at 31°C, this was not significantly lower ($p > 0.05$) than that obtained at 28°C. This implies that yolk digestion and utilization in *C. gariepinus* larvae increases with increasing temperature between 25°C and 28°C.

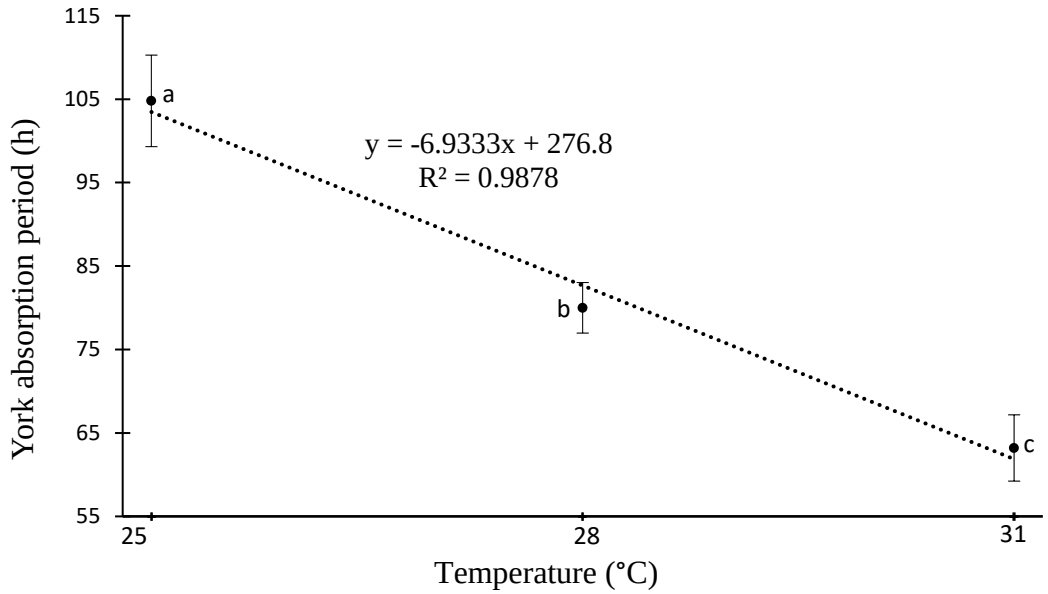

Figure 4.8 The linear main effect of temperature on yolk absorption period of *C. gariepinus* larvae

(Means with the same letter don't differ significantly ($p > 0.05$)).

4.2.2.8.3 Effect of temperature and nitrate fertilizers on yolk absorption rate of *C. gariepinus* larvae

There was no two-way significant interaction ($p > 0.05$) between temperature and nitrate fertilizers on the yolk absorption period of *C. gariepinus* larvae (Tables 4.6 and 4.7). The main effects of temperature and nitrate fertilizers and their orthogonal polynomial linear effects of both temperature and nitrate fertilizers on fertilized *C. gariepinus* larvae were significant ($p<0.05$) (Figure 4.9 and 4.10).

4.2.2.3.1 Main effect of nitrate on yolk absorption rate of *C. gariepinus* larvae

The linear trend of nitrate main effect indicates that the yolk absorption rate of *C. gariepinus* larvae decreased with increasing levels of nitrate (Figure 4.9). The highest yolk

absorption rate was observed at 0 mg/L of NO_3-N; this was not significantly higher ($p > 0.05$) than that obtained at 5 mg/L of NO_3-N and 10 mg/L of NO_3-N. The least yolk absorption rate was observed at 20 mg/L of NO_3-N; this was not significantly different ($p > 0.05$) from that observed at 15 mg/L of NO_3-N and 10 mg/L of NO_3-N. These results suggest that the highest yolk sac digestion and utilization in *C. gariepinus* larvae takes place between 0 and 10 mg/L of NO_3-N.

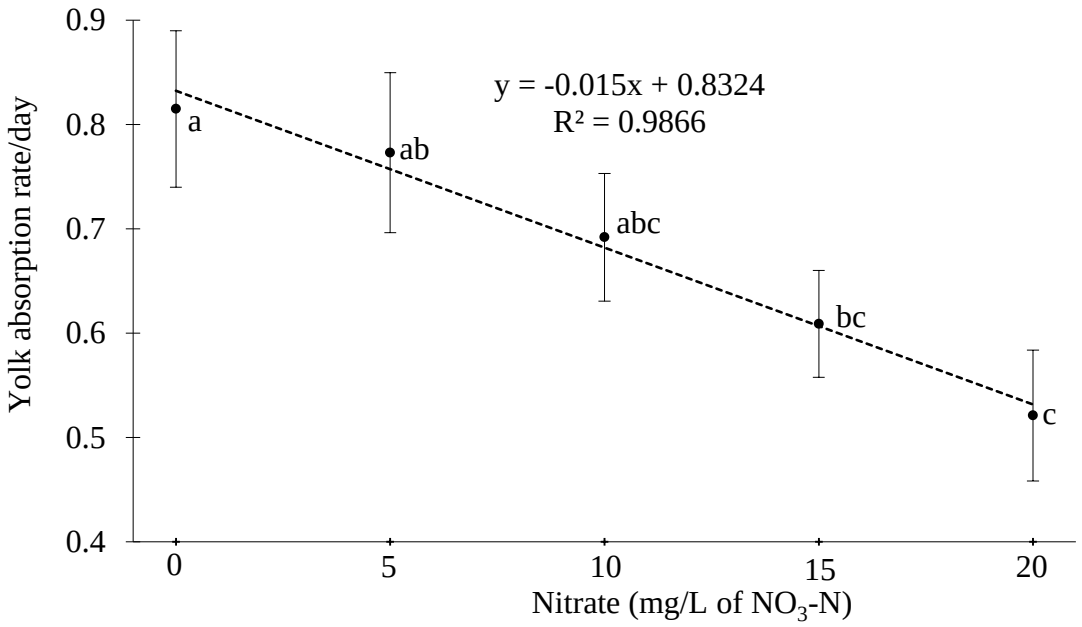

Figure 4.9 The linear main effect of nitrate on yolk absorption rate of *C. gariepinus* (Means with the same letter don't differ significantly ($p > 0.05$)).

4.2.2.3.2 Main effect of temperature on yolk absorption rate of *C. gariepinus* larvae

The linear trend of temperature indicated an increase in the yolk absorption rate per day of *C. gariepinus* larvae with increasing larval rearing temperature (25°C-31°C) (Figure 4.10). The highest yolk absorption rate was observed at 28°C, this was significantly higher ($p <$

0.05) than that observed at 28°C. The least yolk absorption period was obtained at 31°C, which was not significantly lower ($p > 0.05$) than that observed at 28°C (Figure 4.10).

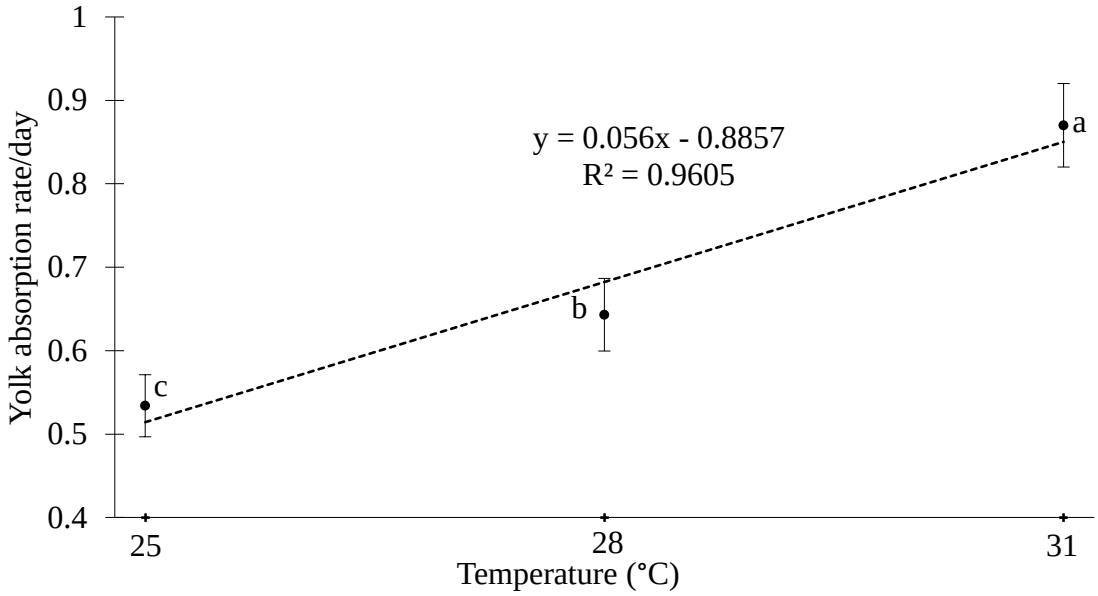

Figure 4.10 Linear main effect of temperature on yolk absorption rate of *C. gariepinus* larvae

(Means with the same letter don't differ significantly ($p > 0.05$)).

4.2.2.4 Effect of temperature and nitrate fertilizers on survival of *C. gariepinus* larvae

4.2.2.4.1 Interactive effect of temperature and nitrate fertilizers on survival of *C. gariepinus* larvae

There was a two-way significant interaction ($p < 0.05$) between temperature and nitrate fertilizers and its orthogonal polynomial quadratic effect of nitrate on the survival of *C. gariepinus* larvae (Table 4.5, Figure 4.11). The quadratic trend indicated that there were no significant differences ($p > 0.05$) in the survival of *C. gariepinus* larvae between the control and water containing up to 10 to 15 mg/L of NO_3-N at 28°C and 31°C and 15 to 20 mg/L of NO_3-N at 25°C. Significant differences ($p < 0.05$) were noted when *C. gariepinus*

larvae were nursed in the control and water containing 15 mg/L of NO_3-N at all nursing temperature (28 to 31°C). Furthermore, significant differences ($p < 0.05$) were also noted when *C. gariepinus* larvae were nursed in water containing 10 and 20 mg/L of NO_3-N at all incubation temperatures (25 to 31°C) (Figure 4.11). The results suggest that *C. gariepinu*s larvae could be successfully survive in water containing a maximum range of 10 to 15 mg/L of NO_3-N at 28°C and 31°C and 15 to20 mg/L of NO_3-N at 25°C.

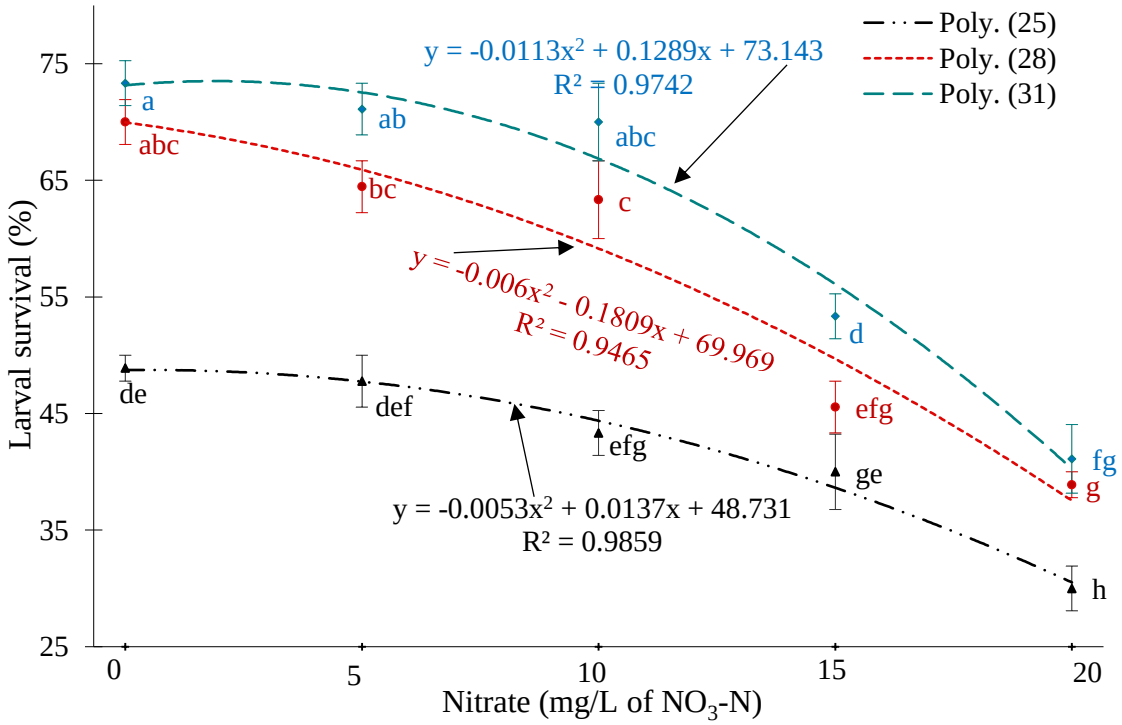

Figure 4.11 The quadratic effects of nitrate in temperature and nitrate fertilizers interaction on the survival of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letters don't differ significantly ($p > 0.05$)).

In summary the results suggest that higher temperatures of 28°C to 31°C promoted development, growth and survival of *C. gariepinus* embryos and larvae up to a maximum range of 10 to 15 mg/L of NO_3-N, while lower temperature of 25°C promoted it up to a maximum range of 15 to 20 mg/L of NO_3-N. This implied that the effect of nitrate on development, growth and survival of *C. gariepinus* embryos and larvae depended on temperature i.e. it was high at higher temperature of 28°C to 31°C than at lower temperatures of 25°C.

4.3 Interactive effect of temperature and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

4.3.1 Interactive effect of temperature and sewage effluent on development, growth and survival of *C. gariepinus* embryos

The results showed that there were significant interactions ($p < 0.05$) between the temperature and sewage effluent on survival of embryos and hatching period of *C. gariepinus* embryos (Table 4.4). There was no significant interactions ($p > 0.05$) noted between the temperature and sewage effluent on incubation period and hatching rate *C. gariepinus* embryos (Tables 4.5 and 4.6).

Table 4.8 Two-way interactive effect of temperature and sewage effluent and their orthogonal polynomial contrasts on survival rate of embryos and hatching period of *C. gariepinus*.

T (ºC)	Sewage effluent (%)	Survival rate of embryos (%)	Hatching period (h)
25	0	82.7±1.2^{cde}	30.7±0.3^{de}
	15	81.3±0.7^{de}	31.3±0.9^{cd}
	30	79.6±2.0^{e}	32.3±0.7^{bc}
	45	75.7±1.9^{f}	33.0±0.6^{ab}
	60	65.3±0.9^{g}	34.3±0.3^{a}
28	0	85.7±1.3^{abc}	22.3±0.3^{g}
	15	85.6±1.5^{abc}	22.0±0.6^{gh}
	30	84.7±1.2^{bcd}	23.0±0.6^{g}
	45	81.7±1.5^{cde}	29.3±0.3^{ef}
	60	75.0±1.2^{f}	31.3±0.3^{cd}
31	0	89.3±2.7^{a}	20.3±0.3^{i}
	15	88.7±1.9^{ab}	20.7±0.7^{hi}
	30	87.6±1.7^{ab}	22.3±0.3^{g}
	45	81.0±1.5^{de}	28.0±0.6^{f}
	60	80.3±0.9^{e}	30.3±0.3d^{e}
	p-value	$p = 0.0033$	$p < 0.001$
	Linear	S	S
	Quadratic	S	S
	Cubic	NS	S
	Quartic	NS	NS

NS = non-significant, S = non-significant, T = temperature, N = nitrate, Means (mean ± SE) with the same letters do not differ significantly

4.3.1.1 Effect of temperature and sewage effluent on survival of embryos of *C. gariepinus*

4.2.1.1.1 Interactive effect of temperature and sewage effluent on survival of *C. gariepinus* embryos

There was a two-way significant interaction ($p< 0.05$) between temperature and sewage effluent and its orthogonal polynomial quadratic effect of sewage effluent on embryos of *C. gariepinus* (Table 4.8, Figure 4.12)**.** The trend quadratic indicated that there were no significant differences ($p> 0.05$) in the survival of *C. gariepinus* embryos (Figure 4.12), between the control and water containing up to 45% sewage effluent at 28°C and 30% at 25°C and 31°C. Significant differences ($p < 0.05$) were noted in the survival of *C. gariepinus* embryos between 30% and 60% sewage effluent levels at all the three incubation temperature (25°C to 31°C). These results suggest that the maximum tolerable range for the survival of *C. gariepinus* embryos to sewage effluent was 45% to 60% at 28°C and 30% to 45% at 25°C and 31°C (Figure 4.12).

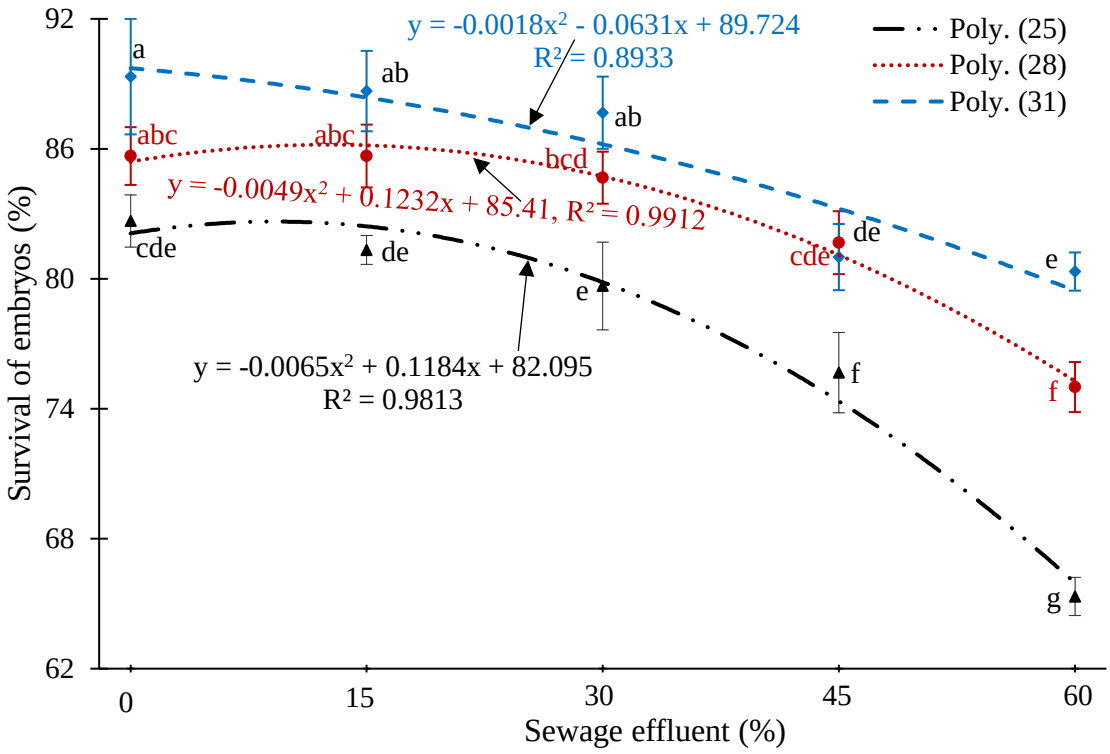

Figure 4.12 Quadratic effect of sewage effluent in temperature and sewage effluent interaction on the survival of *C. gariepinus* embryos

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.3.1.2 Effect of temperature and sewage effluent on incubation period of *C. gariepinus* embryos

4.3.1.2.1 Interactive effect of temperature and sewage effluent on incubation period of *C. gariepinus* embryos

There was no two-way significant interaction ($p > 0.05$) between temperature and sewage effluent on incubation period of *C. gariepinus* embryos (Tables 4.9 and 4.10). However the main effects of temperature and sewage effluent and their linear effects on incubation period of *C. gariepinus* embryos were significant ($p<0.05$) (Figure 4.13 and 4.14).

Table 4.9 Main of effects sewage effluent on hatching rate and incubation period of *C. gariepinus* embryos

Sewage effluent (%)	**Incubation period(h)**	**Hatching rate (%)**
0	21.2 ± 0.8^{c}	77.7 ± 2.7^{a}
15	21.4 ± 0.7^{c}	74.8 ± 2.7^{ab}
30	22.3 ± 0.7^{bc}	74.4 ± 2.8^{ab}
45	24.0 ± 0.6^{ab}	69.0 ± 3.4^{b}
60	24.8 ± 0.6^{a}	61.2 ± 2.8^{c}
p-value	$p < 0.001$	$p < 0.001$
Linear	S	S
Quadratic	NS	S
Cubic	NS	NS
Quartic	NS	NS

NS = non-significant, S = non-significant**,** (Means (mean ± SE, n=45) with the same letter are not significantly different ($p > 0.05$)).

Table 4.10 Main effects of temperature on hatching rate and Incubation period of *C. gariepinus* embryos

Temperature (^{O}C)	Incubation period (h)	Hatching rate (%)
25	24.6±0.5[a]	62.5±2.1[a]
28	22.3±0.5[b]	71.7±1.7[b]
31	21.3±0.5[b]	80.0±1.8[b]
p-value	p <0.001	p <0.001
Linear	S	S
Quadratic	NS	S

NS = non-significant, S = non-significant, (Means (mean ± SE, n=45) with the same letter are not significantly different (p > 0.05)).

4.3.1.2.2 Main effect of sewage effluent on incubation period of *C. gariepinus* embryos

Figure 4.13 showed that sewage effluent had no significant effect (p > 0.05) on the incubation period of *C. gariepinus* embryos up to range of 30% to 45%, beyond which the incubation period started increasing significantly. Significant differences (p < 0.05) were noted in the incubation period of *C. gariepinus* embryos between 45% and 60% but no significant differences (p > 0.05) in the incubation period of *C. gariepinus* embryos were noted between 30% and 45% sewage effluent levels (Figure 4.13). This trend suggests that *C. gariepinus* embryos withstood sewage effluent up to a maximum range of 30% to 45%, beyond which they become susceptible. This implied that the embryos of *C. gariepinus* develop faster at lower than at high sewage effluent level.

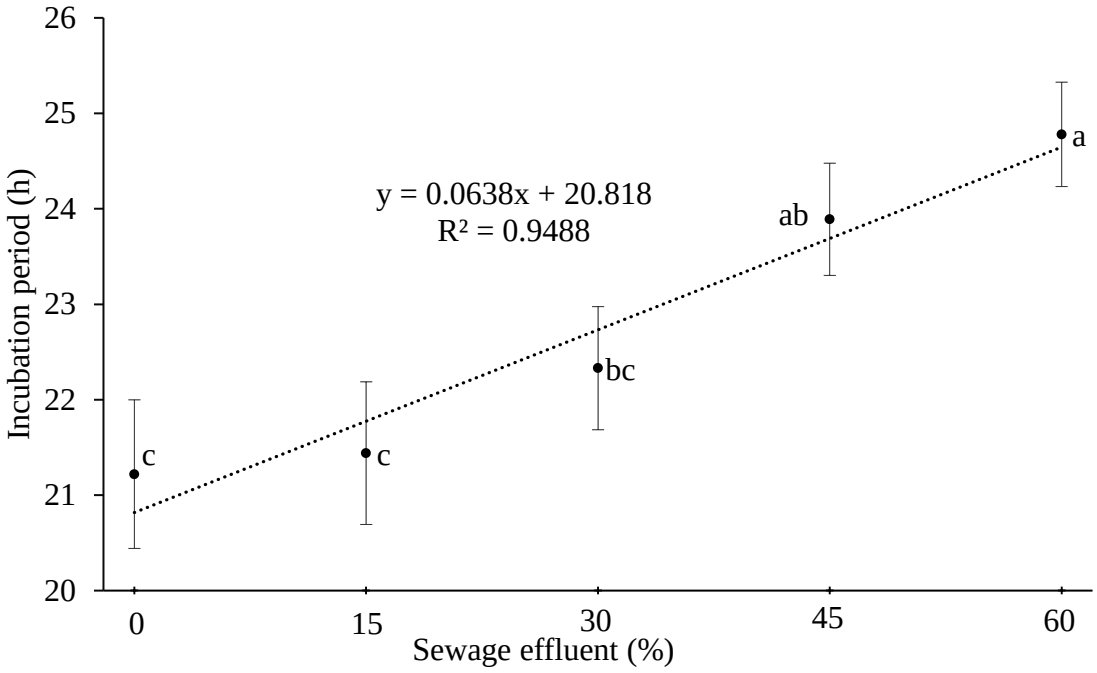

Figure 4.13 The linear main effect of sewage effluent on incubation period of *C. gariepinus* embryos

(Means with the same letter don't differ significantly ($p > 0.05$)).

4.3.1.2.3 Main effect of temperature on incubation period of *C. gariepinus* embryos

Figure 4.14 indicated that the incubation period of *C. gariepinus* embryos decreased with increasing temperature. The linear trend further showed that the longest incubation period of *C. gariepinus* embryos was observed at 25°C, this was significantly different ($p < 0.05$) from that which was obtained at 28°C. No significant differences ($p > 0.05$) were noted between the incubation period of *C. gariepinus* embryos at 28°C and 31°C. These results suggest that the incubation period of *C. gariepinus* embryos was shorter at high temperatures and longer at low temperatures. This implied that the embryos of *C. gariepinus* develop faster at higher than at low temperatures.

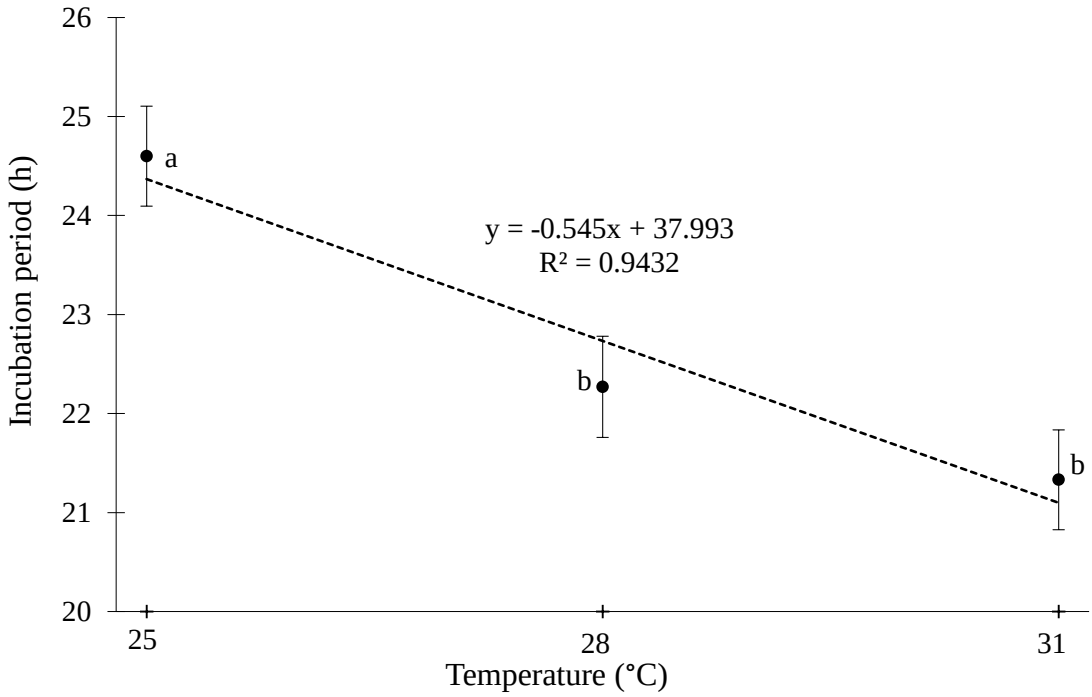

Figure 4.14 The linear effect of temperature on incubation period of *C. gariepinus* embryos.

(Means with the same letter don't differ significantly ($p > 0.05$)).

4.3.1.3 Effect of temperature and sewage effluent on hatching period of *C. gariepinus* embryos

4.3.1.3.1 Interactive effect of temperature and sewage effluent on hatching period of *C. gariepinus* embryos

There was a significant two-way interaction ($p < 0.05$) between temperature and sewage effluent and its orthogonal polynomial cubic effect of sewage effluent on hatching period of *C. gariepinus* embryos (Table 4.8, Figure 4.15.). The cubic trend indicated that there were no significant differences ($p > 0.05$) between the control and water containing up to 30% sewage effluent at 28°C and 15% at 25°C and 31°C. Significant differences ($p < 0.05$) were noted in the hatching period of *C. gariepinus* embryos between 15% and 45% and

45% and 60% sewage effluent levels at all the three incubation temperatures (25 to 31°C). These results suggest that maximum tolerable range for hatching *C. gariepinus* embryos to sewage effluent was 30% to 40% at 28°C and 15% at 25°C and 31°C.

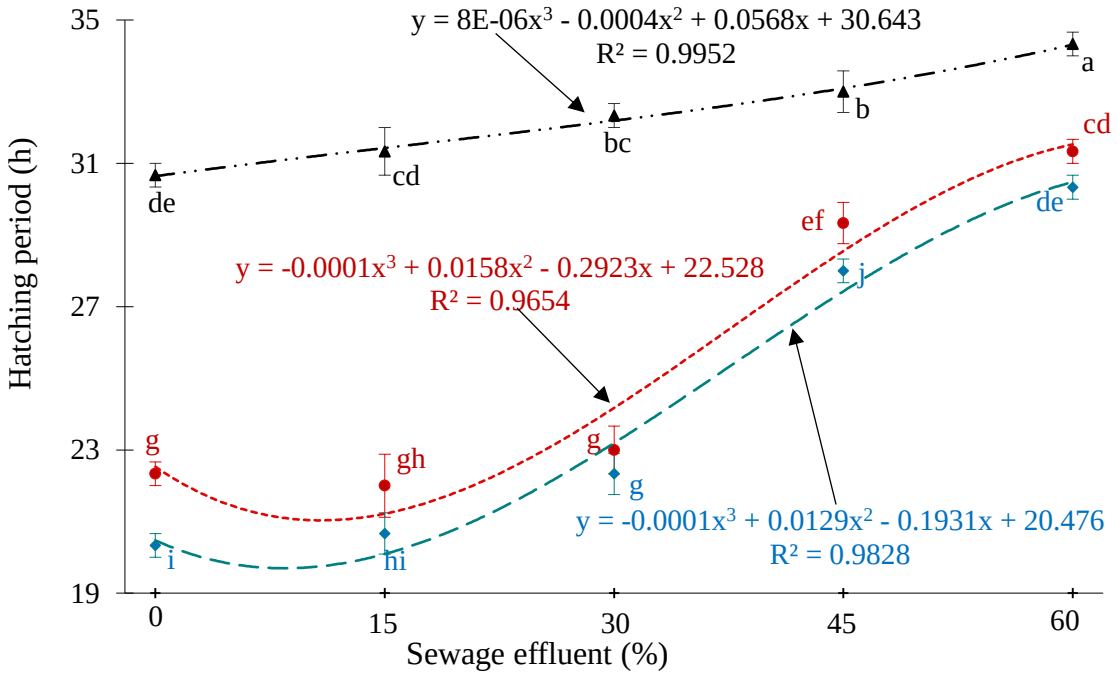

Figure 4.15 The cubic effect of sewage effluent in temperature and sewage effluent interaction on the hatching period of *C. gariepinus* embryos

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.3.1.4 Interactive effect of temperature and sewage effluent on the hatching rate of *C. gariepinus* embryos

There was no two-way significant interaction ($p > 0.05$) between temperature and sewage effluent of sewage effluent on the hatching rate of *C. gariepinus* embryos (Tables 4.9 and 4.10). However, the main effect of temperature and sewage effluent and the orthogonal polynomial quadratic effects respectively on the hatching rate of *C. gariepinus* embryos were significant ($p<0.05$) (Figure 4.16 and 4.17).

4.3.1.4.1 Main effect of sewage effluent on the hatching rate of *C. gariepinus* embryos

The quadratic trend of sewage effluent on the hatching rate of *C. gariepinus* embryos (Figure 4.16) indicated that there were no significant difference ($p>0.05$) in the hatching rate of *C. gariepinus* embryos between the control and 30%. Significant differences ($p < 0.05$) were however, noted in the hatching rate of *C. gariepinus* embryos between 30% and 60% sewage effluent levels. These results suggest that *C. gariepinus* embryos withstood a maximum range of 30% to 45% sewage effluent level, above which the embryos start dying.

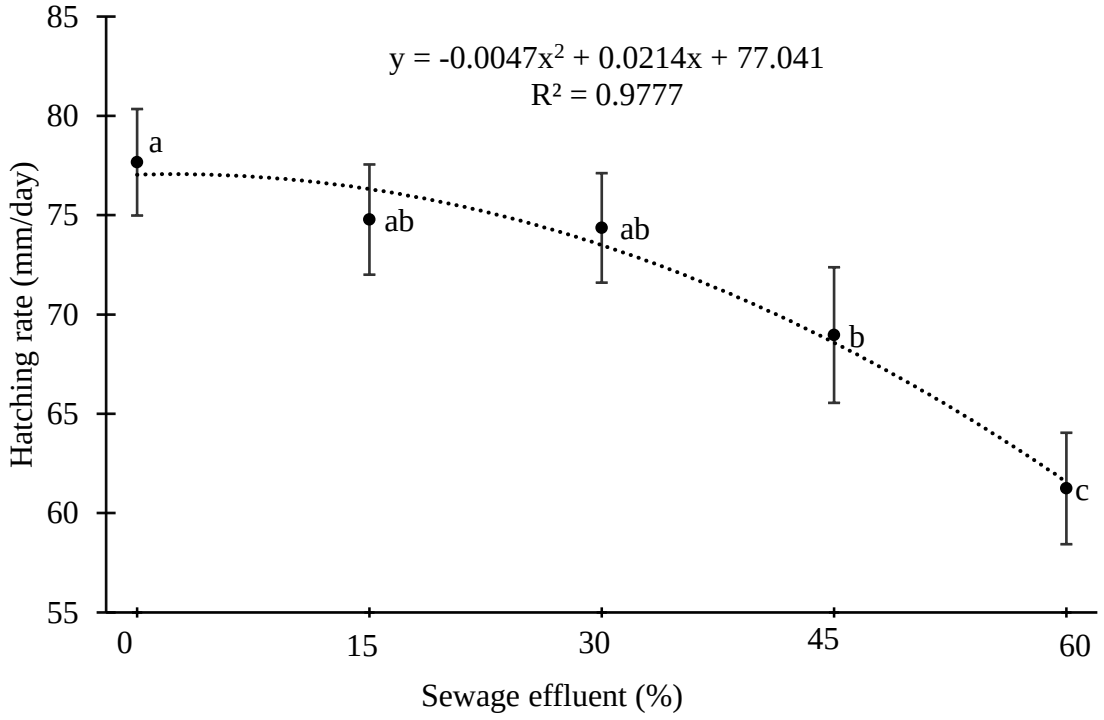

Figure 4.16 The quadratic main effect of sewage effluent on the hatching rate of *C. gariepinus* embryos

(Means with the same letter are not significantly different ($p > 0.05$)).

4.3.1.4.2 Main effect of temperature on the hatching rate of *C. gariepinus* embryos

Figure. 4.17 indicated that the hatching rate of *C. gariepinus* embryos increased with increasing temperature. The results further noted that the highest hatching rate of *C. gariepinus* embryos was obtained at 31°C; this was significantly different ($p < 0.05$) from that which was obtained at 28°C and 25°C. Significant differences ($p < 0.05$) were also n observed between the hatching rate at 28°C and 30°C.

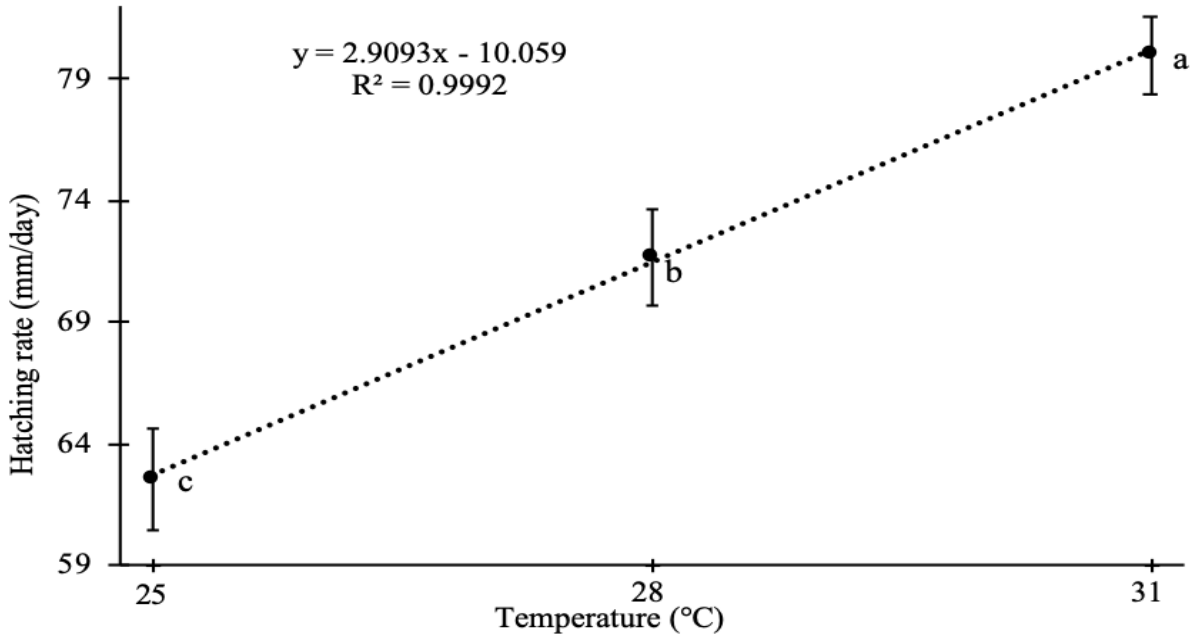

Figure 4.17 The linear effect of temperature on the hatching rate of *C. gariepinus* embryos (Means with the same letter don't differ significantly ($p > 0.05$)).

4.3.2 Interactive effect of temperature and sewage effluent on development, growth and survival of *C. gariepinus* larvae

The results indcated that there were significant interactions ($p < 0.05$) between the temperature and sewage effluent on growth rate, yolk absorption period, yolk absorption rate and larval survival (Table 4.11 and 4.12).

Table 4.11 Two-way interactive effect of temperature and sewage effluent and their orthogonal polynomial contrasts on survival of growth rate, yolk absorption period, yolk absorption and survival rate of *C. gariepinus* larvae

T (ºC)	Sewage effluent (%)	Yolk absorption period (h)	Yolk absorption Rate (mm/Day)
25	0	74 ± 1.0^{e}	0.58 ± 0.04^{d}
	15	90 ± 1.7^{c}	0.51 ± 0.02^{de}
	30	100 ± 2.6^{b}	0.41 ± 0.03^{fg}
	45	102 ± 1.7^{b}	0.38 ± 0.01^{g}
	60	110 ± 1.0^{a}	0.33 ± 0.02^{g}
28	0	58 ± 1.0^{g}	0.91 ± 0.04^{b}
	15	74 ± 1.0^{e}	0.68 ± 0.03^{c}
	30	84 ± 1.7^{d}	$0.55\pm0.04d^{e}$
	45	90 ± 1.7^{c}	$0.52\pm0.03d^{e}$
	60	94 ± 1.0^{c}	$0.48\pm0.02e^{f}$
31	0	48 ± 1.7^{h}	1.09 ± 0.05^{a}
	15	50 ± 1.0^{h}	0.88 ± 0.04^{b}
	30	56 ± 2.0^{g}	0.82 ± 0.03^{b}
	45	64 ± 2.0^{f}	0.68 ± 0.04^{c}
	60	78 ± 1.7^{e}	0.60 ± 0.02^{cd}
	p-value	$p < 0.001$	$p < 0.001$
	Linear	S	S
	Quadratic	S	S
	Cubic	NS	NS
	Quartic	NS	NS

NS = non-significant, S = non-significant, T = temperature, N = nitrate, Means (mean ± SE) with the same letters do not differ significantly

4.3.2.1 Effect of temperature and sewage effluent on yolk absorption period of *C. gariepinus* larvae

4.3.2.1.1 Interactive effect of temperature and sewage effluent on yolk absorption period of *C. gariepinus* larvae

There was a two-way effluent significant interaction ($p < 0.05$) between temperature and sewage and its orthogonal polynomial quadratic interactive effect of sewage effluent on yolk absorption period of *C. gariepinus* larvae (Table 4.11, Figure 4.18). The quadratic trend indicated that at rearing temperature of 31°C and 28°C, the yolk absorption period of *C. gariepinus* larvae was significantly higher ($p < 0.05$) in sewage effluent polluted water than in the control while at 25°C. No significant differences ($p > 0.05$) were observed between the yolk absorption period of *C. gariepinus* larvae in the control and 15% sewage effluent levels. Significant differences ($p < 0.05$) were observed in yolk absorption period of *C. gariepinus* larvae between 15% and 30% sewage effluent levels while at 25°C significant differences ($p < 0.05$) were observed between yolk absorption period of *C. gariepinus* larvae at both 15%, 30% and 60% sewage effluent levels at all the three incubation and rearing temperatures (25°C, 28°C and 31°C). These results suggests that the rate of yolk sac absorption was high between the control and 15% at 28°C and 31°C and 15% to 30% at 25°C than at high at levels.

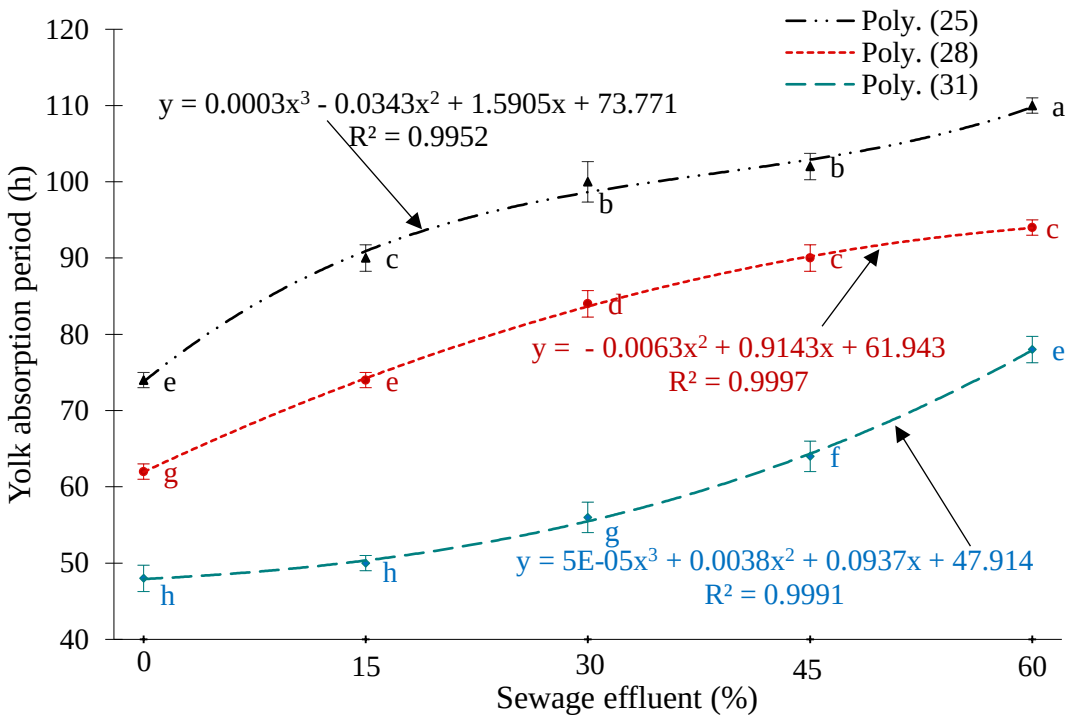

Figure 4.8 The cubic effects of temperature and sewage effluent interaction on yolk absorption period (h) of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.3.2.2. Effect of temperature and sewage effluent on yolk absorption rate of *C. gariepinus* larvae

4.3.2.2.1 Interactive effect of temperature and sewage effluent on yolk absorption rate of *C. gariepinus* larvae

There was a two-way significant interaction ($P < 0.05$) between temperature and sewage effluent and its orthogonal polynomial quadratic effect of sewage effluent on yolk absorption rate of *C. gariepinus* larvae (Table 4.11, Figure 4.19). The quadratic trend showed that at rearing temperature of 31°C and 28°C, the yolk absorption rate of *C. gariepinus* larvae was significantly higher ($p < 0.05$) in the control than in sewage effluent polluted water while at 25°C no significant differences ($p > 0.05$) were observed between

the yolk absorption rate of *C. gariepinus* larvae in the control and 15% sewage effluent levels. Significant differences ($p < 0.05$) were also noted in the yolk absorption rate of *C. gariepinus* larvae between 30% and 60% sewage effluent levels at all rearing temperatures (25-31°C). These results suggests that the yolk sac absorption rate was high between the control and 15% sewage effluent at 28°C and 31°C and at 15% to 30% at 25°C.

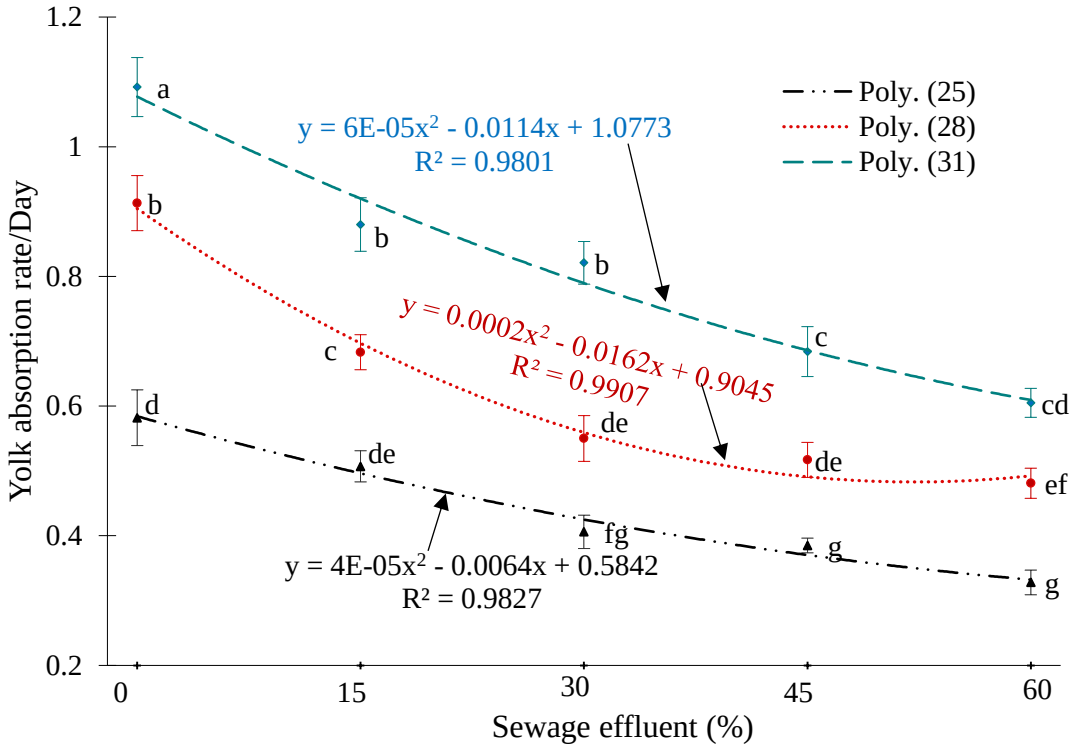

Figure 4.19 Quadratic effect of sewage effluent in temperature and sewage effluent interaction on yolk absorption rate of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

Table 4.12 Two-way interactive effect of temperature and sewage effluent and their orthogonal polynomial contrasts on survival of growth rate and survival rate of *C. gariepinus* larvae

T (ºC)	Sewage effluent (%)	Growth rate (mm/day)	% Larval rate survival
25	0	0.88 ± 0.03^{eg}	44.4 ± 2.2^{de}
	15	0.79 ± 0.03^{hi}	36.7 ± 1.9^{efg}
	30	0.73 ± 0.01^{ij}	32.2 ± 2.2^{g}
	45	0.66 ± 0.04^{jk}	24.4 ± 1.1^{h}
	60	0.56 ± 0.03^{l}	23.3 ± 1.9^{h}
28	0	1.09 ± 0.02^{ab}	71.1 ± 2.2^{a}
	15	0.96 ± 0.03^{cd}	58.9 ± 2.9^{b}
	30	0.86 ± 0.02^{gh}	44.4 ± 2.9^{de}
	45	0.71 ± 0.01^{jk}	41.1 ± 2.2^{def}
	60	0.63 ± 0.02^{kl}	35.6 ± 2.9^{fg}
31	0	1.11 ± 0.02^{a}	75.6 ± 2.2^{a}
	15	1.02 ± 0.02^{bc}	70.0 ± 3.3^{a}
	30	1.03 ± 0.02^{bc}	53.3 ± 3.3^{bc}
	45	0.94 ± 0.02^{de}	48.9 ± 4.0^{cd}
	60	0.80 ± 0.04^{hi}	38.9 ± 2.9^{efg}
	p-value	$p = 0.011$	$p = 0.005$
	Linear	S	S
	Quadratic	NS	NS
	Cubic	NS	NS
	Quartic	NS	NS

NS = non-significant, S = non-significant, T = temperature, N = nitrate, Means (mean ± SE) with the same letters do not differ significantly

4.3.2.3 Effect of temperature and sewage effluent on growth rate of *C. gariepinus* larvae

4.3.2.3.1 Interactive effect of temperature and sewage effluent on growth rate of *C. gariepinus* larvae

There was a two-way significant interaction ($P < 0.05$) between temperature and sewage effluent and its orthogonal polynomial linear effect of sewage effluent on growth rate of *C. gariepinus* larvae (Table 4.12, Figure 4.20.). The linear trend showed an inverse relationship between the growth rate of *C. gariepinus* larvae and sewage polluted water at all incubation and rearing temperatures (25-31°C). Significant differences ($p < 0.05$) were noted in the growth rate of *C. gariepinus* larvae between the control and 15% and 15% and 60% sewage effluent levels at all rearing temperatures (25-31°C). These results suggest that the growth rate was high between the control and 15% sewage effluent. This implied that the presence sewage effluent in rearing water retarded growth.

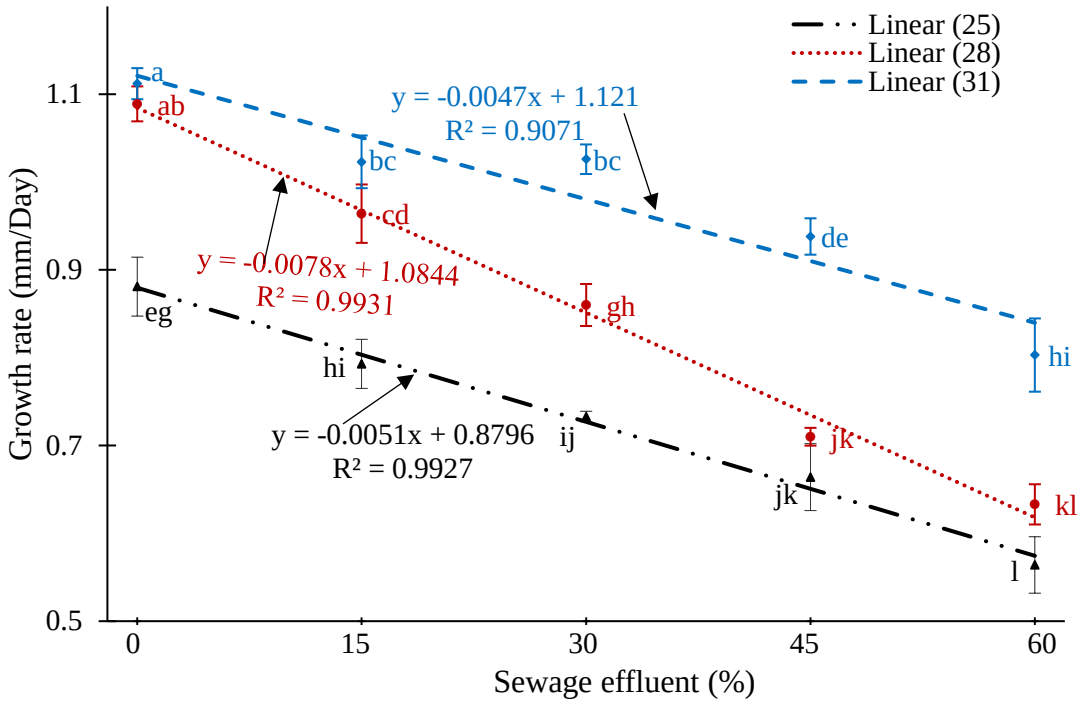

Figure 4.20 Linear effect of sewage effluent in temperature and sewage effluent interaction on the growth rate of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.3.2.3 Effect of temperature and sewage effluent on the survival of *C. gariepinus* larvae

4.3.2.3.1 Interactive effect of temperature and sewage effluent on survival of *C. gariepinus* larvae

There was a two-way significant interaction ($p < 0.05$) between temperature and sewage effluent and its orthogonal polynomial quadratic interactive effect of sewage effluent on the survival of *C. gariepinus* larvae (Table 4.12, Figure 4.21). The linear trend showed that at all rearing temperatures (25°C to 31°C), there was an inverse relationship between the survival of *C. gariepinus* larvae and sewage effluent polluted water. The results further showed that at incubation and rearing temperature of 25°C and 28°C, the survival rate of

C. gariepinus larvae was significantly lower ($p < 0.05$) in sewage effluent polluted water than in the control, while at 31°C significant differences ($p < 0.05$) were observed in the survival of *C. gariepinus* larvae between the control and 30%. Significant differences ($p < 0.05$) were also observed in the survival of *C. gariepinus* larvae between the 30% and 60% sewage effluent levels at all rearing temperatures (25-31°C). These results suggest tolerance limit for the survival of *C. gariepinus* larvae to sewage effluent was 15% at 31°C and control at 25°C and 28°C.

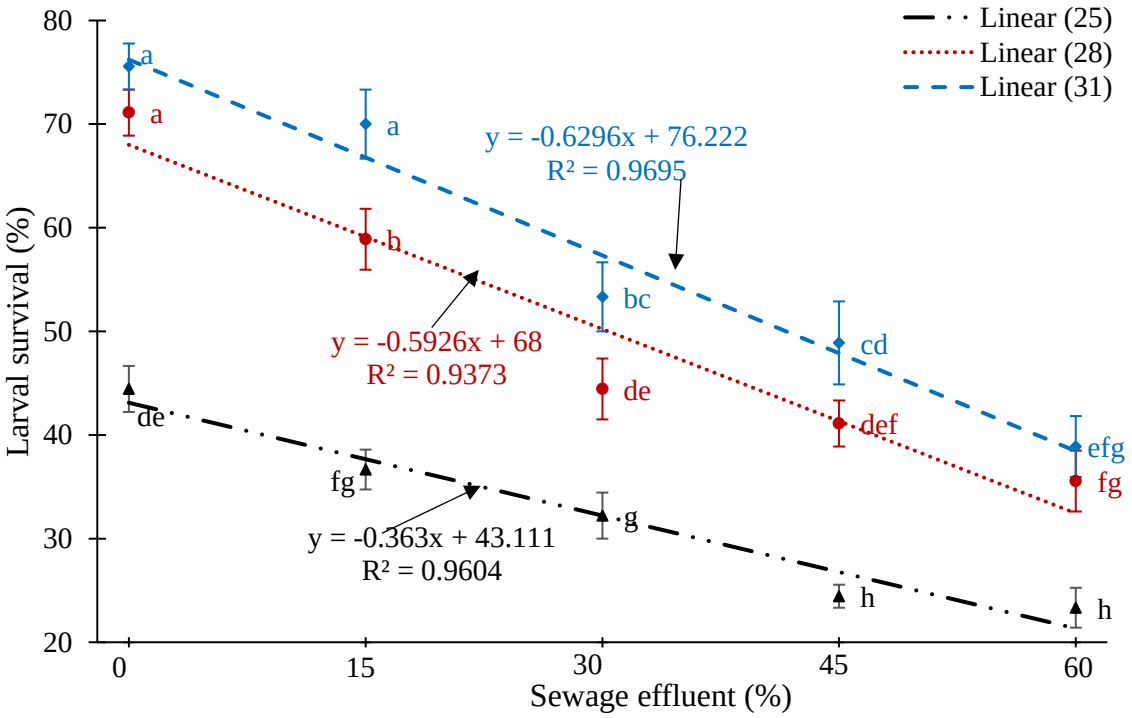

Figure 4.21 Linear effect of sewage effluent in temperature and sewage effluent interaction on the survival of *C. gariepinus* larvae

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

In summary the interaction results suggest that moderate temperature of 28°C promoted development, growth and survival of *C. gariepinus* embryos at sewage effluent level between 30% to 45% while water between the control and 15% sewage effluent promoted growth and survival of *C. gariepinus* larvae at all rearing temperature of 25°C to 31°C.

4.4. Interactive effect of water temperature and a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

The results of the two-way interactions between temperature, nitrate and temperature and sewage effluent showed significant interactions ($p<0.05$). *Clarias gariepinus* embryos and larvae could tolerate a maximum range of 10 to 15 mg/L of NO_3-N at higher temperatures of 28°C and 31°C, while at lower temperatures of 25°C the tolerance limit ranged from 15 to 20 mg/L of NO_3-N for the hatching rate, growth rate and larval survival. Temperature of 28°C showed the highest maximum range for the survival of *C. gariepinus* embryos of 45% to 60% sewage effluent as compared to 30% to 45% at 25°C and 31°C. However, at all rearing temperatures (25°C and 31°C) the tolerance limit of *C. gariepinus* larvae to sewage effluent ranged from the control to 15%. Nitrate level in sewage effluent was as low as 36.1mg/L NO_3- (8.87 mg/L of NO_3-N) (Table 4.1), as compared to the 10 mg/L of NO_3-N which is the observed tolerable limit of *C. gariepinus* embryos and larvae to nitrate in the present study. Therefore, it is not clear from the two-way interactions, whether the increasing levels of nitrate in the sewage effluent could cause any further significant effect. Owing to this, the three-way interaction test was conducted between sewage effluent, and nitrate fertilizers at different temperature levels.

Table 4.13 Three-way ANOVA for temperature a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

	T	N	S	TN	TS	NS	TNS
Survival of embryos (%)	***	***	***	ns	*	Ns	Ns
Incubation period (h)	***	***	***	*	ns	Ns	Ns
Hatching period (h)	***	***	***	**	***	***	***
Hatching rate (%)	***	***	***	**	ns	Ns	Ns
Yolk absorption period/h	***	***	***	ns	**	***	***
Yolk absorption rate/Day	**	***	***	ns	***	***	**
Growth Rate (mm/day)	***	***	***	**	***	Ns	Ns
Larval Survival (%)	***	***	***	**	*	Ns	Ns

***= p value < 0.001, **= p value < 0.01, *= p value < 0.05, T = temperature, N = nitrate, S =sewage effluent, ns = not significantly, ns = non-significant, s = non-significant

Table 4.14 Three-way interactive effect of temperature a mixture of nitrate fertilizers and sewage effluent the hatching period, growth rate, yolk absorption period and yolk absorption rate of *C. gariepinus*

		Hatching period (h)			Yolk absorption period (h)			Yolk absorption rate/day		
NO_3-N	**SE**	**25°C**	**28°C**	**31°C**	**25°C**	**28°C**	**31°C**	**25°C**	**28°C**	**31°C**
0	0	30.7 ± 0.3^{k}	22.3 ± 0.3^{j}	20.3 ± 0.3^{f}	78 ± 2^{j}	64 ± 2^{f}	48 ± 1^{e}	0.68 ± 0.02^{a}	0.71 ± 0.01^{ab}	1.08 ± 0.03^{a}
5	0	33.6 ± 0.3^{ij}	27.0 ± 0.6^{h}	21.7 ± 0.3^{f}	84 ± 3^{i}	68 ± 2^{e}	48 ± 2^{e}	0.68 ± 0.03^{a}	0.75 ± 0.03^{a}	0.99 ± 0.02^{b}
10	0	36.7 ± 0.3^{fg}	27.0 ± 0.6^{h}	22.0 ± 0.6^{f}	102 ± 3^{f}	80 ± 2^{d}	56 ± 2^{d}	0.54 ± 0.02^{cd}	0.65 ± 0.02^{bc}	0.84 ± 0.03^{cd}
15	0	41.3 ± 0.7^{c}	29.3 ± 0.3^{h}	24.7 ± 0.3^{ef}	114 ± 4^{cd}	92 ± 2^{c}	72 ± 3^{b}	0.46 ± 0.02^{efg}	0.57 ± 0.01^{d}	0.66 ± 0.03^{ef}
0	20	32.3 ± 0.6^{j}	23.0 ± 0.6^{j}	22.3 ± 0.3^{f}	82 ± 2^{ij}	72 ± 1^{e}	48 ± 1^{e}	0.62 ± 0.02^{b}	0.70 ± 0.02^{ab}	0.91 ± 0.04^{b}
5	20	35.3 ± 0.7^{gh}	34.0 ± 0.6^{f}	31.0 ± 0.6^{cd}	96 ± 3^{g}	72 ± 4^{e}	48 ± 1^{e}	$0.58\pm0.02b^{c}$	0.75 ± 0.03^{a}	0.96 ± 0.02^{b}
10	20	37.3 ± 0.7^{ef}	34.0 ± 0.6^{f}	33.0 ± 0.6^{bcd}	108 ± 3^{e}	84 ± 2^{d}	60 ± 4^{cd}	0.49 ± 0.02^{def}	0.58 ± 0.02^{cd}	0.70 ± 0.03^{e}
15	20	41.7 ± 0.3^{bc}	37.7 ± 0.3^{bc}	35.7 ± 0.3^{abc}	118 ± 2^{bc}	92 ± 2^{c}	80 ± 2^{a}	0.46 ± 0.01^{efg}	0.56 ± 0.03^{d}	0.59 ± 0.04^{fg}
0	40	33.0 ± 0.6^{ij}	29.3 ± 0.3^{h}	28.0 ± 0.6^{de}	90 ± 3^{h}	72 ± 2^{e}	56 ± 2^{d}	0.51 ± 0.02^{de}	0.70 ± 0.03^{ab}	0.82 ± 0.03^{c}
5	40	38.0 ± 0.6^{ef}	36.7 ± 0.3^{ce}	33.0 ± 0.6^{bcd}	98 ± 3^{fg}	84 ± 1^{d}	60 ± 3^{cd}	0.52 ± 0.01^{cde}	0.57 ± 0.02^{d}	0.71 ± 0.03^{e}

(Continued)

		Hatching period (h)			Yolk absorption period (h)			Yolk absorption rate/day		
NO_3-N	**SE**	**25°C**	**28°C**	**31°C**	**25°C**	**28°C**	**31°C**	**25°C**	**28°C**	**31°C**
10	40	38.0 ± 0.5^{ef}	35.7 ± 0.3^{e}	34.0 ± 0.6^{abc}	114 ± 2^{cd}	96 ± 3^{bc}	72 ± 2^{b}	0.49 ± 0.01^{def}	0.53 ± 0.03^{de}	0.69 ± 0.03^{e}
15	40	43.0 ± 0.6^{ab}	39.0 ± 0.6^{b}	37.7 ± 0.3^{ab}	120 ± 3^{b}	100 ± 2^{b}	84 ± 3^{a}	0.43 ± 0.01^{fg}	0.46 ± 0.02^{e}	0.52 ± 0.03^{gh}
0	60	34.3 ± 0.3^{hi}	31.3 ± 0.3^{g}	30.3 ± 0.3^{cd}	102 ± 3^{f}	84 ± 4^{d}	64 ± 2^{c}	0.37 ± 0.02^{h}	0.55 ± 0.03^{d}	0.68 ± 0.04^{e}
5	60	39.7 ± 0.3^{d}	37.7 ± 0.3^{bc}	32.3 ± 3.7^{bcd}	110 ± 2^{cde}	92 ± 2^{c}	68 ± 2^{b}	0.49 ± 0.02^{ef}	0.55 ± 0.03^{d}	0.71 ± 0.03^{e}
10	60	38.7 ± 0.3^{de}	37.0 ± 0.6^{ce}	35.0 ± 0.6^{abc}	120 ± 4^{b}	100 ± 2^{b}	84 ± 3^{a}	0.43 ± 0.02^{g}	0.46 ± 0.02^{e}	0.49 ± 0.02^{h}
15	60	43.3 ± 0.7^{a}	40.3 ± 0.3^{a}	39.3 ± 0.3^{a}	132 ± 2^{a}	108 ± 1^{a}	84 ± 3^{a}	0.42 ± 0.01^{g}	0.45 ± 0.02^{e}	0.51 ± 0.03^{gh}
p-value		$p < 0.001$			$p < 0.001$			$p < 0.001$		
Linear		S			S			S		
Quadratic		S			S			S		
Cubic		NS			S			S		
Quartic		NS			NS			NS		

SE = sewage effluent (%), Means (mean ± SE) with the same letters do not differ significantly, NS = non-significant, S = significant

Table 4.8 indicated that there was a three-way significant interaction ($p< 0.05$) among temperature and a mixture of nitrate fertilizers and sewage effluent and its orthogonal polynomial effect of nitrate fertilizer and sewage effluent on hatching period, yolk absorption period and yolk absorption rate of *C. gariepinus* embryos and larvae. However, no significant three-way interaction ($p< 0.05$) among temperature and a mixture of nitrate fertilizers and sewage effluent were observed on incubation period, hatching rate, growth rate, survival rate of *C. gariepinus* embryos and larvae. Only variables that showed three-way interaction where included in this table.

4.4.1 Effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the hatching period of *C. gariepinus* embryos

4.4.1.1 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the hatching period of *C. gariepinus* embryos

There was a three-way significant interaction ($p< 0.05$) among temperature and a mixture of nitrate fertilizers and sewage effluent and its orthogonal polynomial quadratic effect of nitrate fertilizer and sewage effluent on the hatching period of *C. gariepinus* embryos (Table 4.13, Figure 4.22). This implied that the interactive effect of sewage effluent and nitrate fertilizer on the hatching period was different at the three incubation temperatures (25°C, 28°C and 31°C).

Within each temperature level, at 25°C, the shortest hatching period was obtained when *C. gariepinus* embryos were hatched in the control (0 mg/L of NO_3-N). Significant differences ($p<0.05$) were noted in the hatching period between each level of nitrate, i.e. 0 and 5 mg/L of NO_3-N, 5 and 10 mg/L of NO_3-N, and 10 and 15 mg/L of NO_3-N at all levels of sewage

effluent. At 28°C, the shortest hatching period was obtained when *C. gariepinus* embryos were hatched in the control (0 mg/L of NO_3-N). This was significantly shorter ($p<0.05$) than that obtained at 5 mg/L of NO_3-N at all levels of nitrate. Significant differences ($p<0.05$) were noted in the hatching period between 5 and 15 mg/L of NO_3-N at 20%, 40% and 60% sewage effluent levels while there were no significant differences ($p> 0.05$) in the hatching period between 5 and 15 mg/L of NO_3-N at 0% sewage effluent level. At 31°C, the shortest hatching period was obtained when *C. gariepinus* embryos were hatched in the control (0 mg/L of NO_3-N). This was significantly shorter ($p<0.05$) than those obtained at 10 mg/L of NO_3-N at 20%, 40% and 60% sewage effluent levels, while there were no significant differences ($p> 0.05$) in the hatching period between 10 and 15 mg/L of NO_3-N at the same sewage effluent levels. No significant differences ($p> 0.05$) in the hatching period were noted when *C. gariepinus* embryos were hatched between 0 and 15 mg/L of NO_3-N at 0% sewage effluent. In general, these results showed that within each temperature level, 25°C, 28°C and 31°C, the hatching period was shortest in the control at all levels of nitrate fertilizer and sewage effluent, suggesting that the rate of egg development was high in the control than in polluted water.

These results further suggest that the hatching period increased with increasing levels of both pollutants, i.e. nitrate and sewage effluent within each temperature level. This implies that the growth and development decreased with increasing levels of pollutants in hatching water.

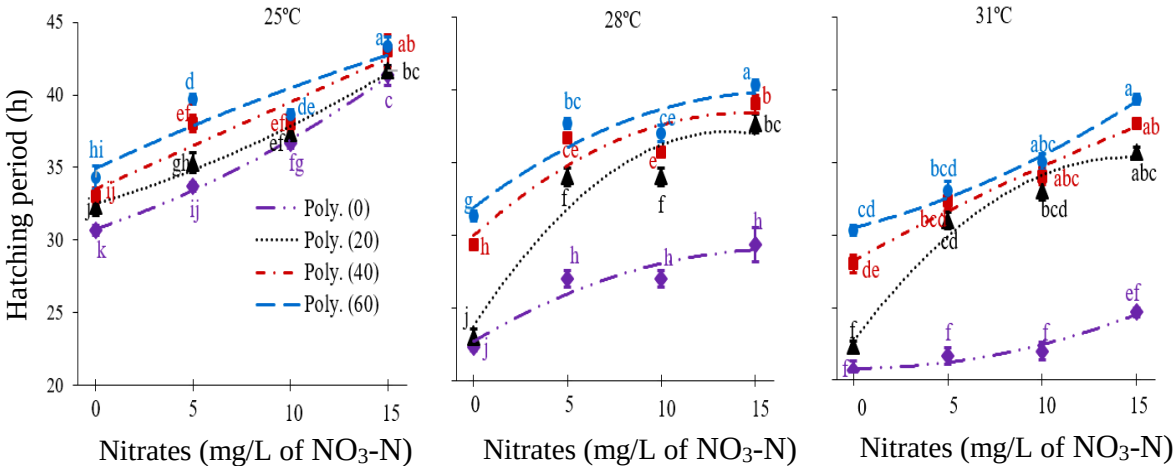

Figure 4.22 Quadratic effects of nitrate in temperature and a mixture of nitrate fertilizers and sewage effluent interaction on hatching period of *C. gariepinus* embryos, incubated at 25°C, 28°C and 31°C

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.4.2 Effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the yolk absorption period of *C. gariepinus* larvae

4.4.2.1 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the yolk absorption period of *C. gariepinus* larvae

There was a three-way effluent significant interaction ($p< 0.05$) among temperature and a mixture of nitrate fertilizers and sewage and its orthogonal polynomial cubic effect of nitrate fertilizer and sewage effluent on the yolk absorption period of *C. gariepinus* larvae (Table 4.13, Figure 4.23). This implied that the interactive effect of sewage effluent and nitrate fertilizer on the yolk absorption period was different at the three rearing temperatures (25°C, 28°C and 31°C).

Within each temperature level, at 25°C, the shortest yolk absorption period was obtained when *C. gariepinus* larvae were reared in the control (0 mg/L of NO_3-N). Significant differences ($p<0.05$) were noted in the yolk absorption period between each level of nitrate i.e. 0 and 5 mg/L of NO_3-N, 5 and 10 mg/L of NO_3-N, and 10 and 15 mg/L of NO_3-N at all levels of sewage effluent (0 to 60%). At 28°C, significant differences ($p<0.05$) in the yolk absorption period were noted between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N at all levels of sewage effluent (0 to 60%). However, there were no significant differences ($p> 0.05$) in the yolk absorption period when *C. gariepinus* larvae were reared between 0 and 5 mg/L of NO_3-N and 5 and 10 mg/L of NO_3-N at 20% and 0% sewage effluent levels respectively. Significant differences ($p<0.05$) were noted in the yolk absorption period when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 0%, 20% and 60% sewage effluent levels, but no significant differences ($p> 0.05$) in the yolk absorption period were noted when *C. gariepinus* larvae were nursed between 10 and 15 mg/L of NO_3-N at 40% sewage effluent level. At 31°C, significant differences ($p<0.05$) in the yolk absorption period were noted between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N at all levels of sewage effluent (0 to 60%). However, there were no significant differences ($p> 0.05$) in the yolk absorption period when *C. gariepinus* larvae were reared between 0 and 5 mg/L of NO_3-N at 0%, 20% and 40% sewage effluent levels, but no significant differences ($p> 0.05$) in the yolk absorption period were noted when *C. gariepinus* larvae were reared between 0 and 5 mg/L of NO_3-N at 60% sewage effluent level. Significant differences ($p<0.05$) were noted in the yolk absorption period when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 0%, 20% and 40% sewage effluent levels, but no significant differences ($p> 0.05$) in the yolk absorption

period were noted when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 60% sewage effluent level. These results suggest that the yolk sac consumption decreased with increasing levels of both pollutants in the rearing water.

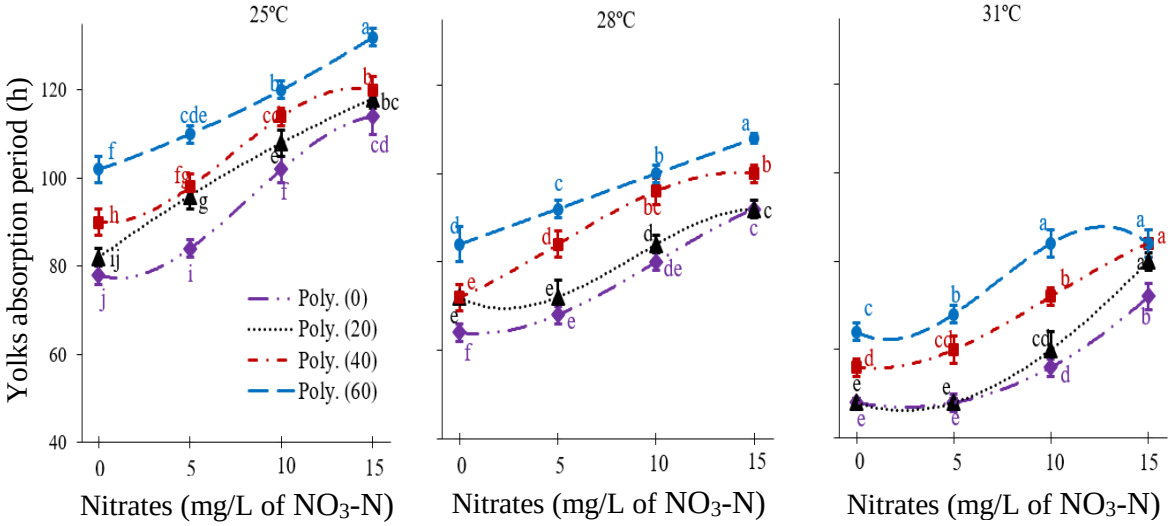

Figure 4.23 Cubic effects of nitrate in temperature and a mixture of nitrate fertilizers and sewage effluent interaction on yolk absorption period of *C. gariepinus* larvae incubated at 25°C, 28°C and 31°C

(Means (mean ± SE, n=45) with the same letter don't differ significantly ($p > 0.05$)).

4.4.3 Effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the yolk absorption rate of *C. gariepinus* larvae

4.4.3.1 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the yolk absorption rate of *C. gariepinus* larvae

There was a three-way significant interaction ($p< 0.05$) among temperature and a mixture of nitrate fertilizers and sewage effluent and a crossover orthogonal polynomial cubic effect of nitrate fertilizer and sewage effluent on the yolk absorption rate of *C. gariepinus* larvae (Table 4.13, Figure 4.24). This implied that the interactive effect of sewage effluent

and nitrate fertilizer on the yolk absorption rate was different at the three rearing temperatures (25°C, 28°C and 31°C).

At 25°C, significant differences ($p<0.05$) were noted between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N when *C. gariepinus* larvae were reared at 0%, 20% and 40% sewage effluent levels, but at 60% sewage effluent, significant differences ($p<0.05$) were noted between the control (0 mg/L of NO_3-N) and 5 mg/L of NO_3-N. No significant differences ($p> 0.05$) in the yolk absorption rate were noted when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at all levels of sewage effluent (0 to 60%).

At 28°C, significant differences ($p<0.05$) in the yolk absorption rate were noted between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N at all levels of sewage effluent (0 to 60%). At 40% significant differences ($p<0.05$) were noted in the yolk absorption rate between the control (0 mg/L of NO_3-N) and 5 mg/L of NO_3-N but that this was not significant differences ($p> 0.05$) from that obtained between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N. No significant differences ($P> 0.05$) in the yolk absorption rate were noted when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 20%, 40% and 60% sewage effluent levels. However, significant differences ($P<0.05$) were noted in the yolk absorption rate when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 0% sewage effluent.

At 31°C, significant differences ($p<0.05$) in the yolk absorption rate were noted between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N at 20%, 40% and 60% sewage effluent levels. Although at 40%, significant differences ($p<0.05$) were noted between the control (0 mg/L of NO_3-N) and 5 mg/L of NO_3-N but that this was not significant different

(p> 0.05) from that obtained between the control (0 mg/L of NO_3-N) and 10 mg/L of NO_3-N. At 0% sewage effluent, significant differences (p<0.05) in the yolk absorption rate were noted between the control (0 mg/L of NO_3-N) and 15 mg/L of NO_3-N. No significant differences (p> 0.05) in the yolk absorption rate were noted when *C. gariepinus* larvae were reared between 10 and 15 mg/L of NO_3-N at 0%, 20% and 40% sewage effluent levels. However, significant differences (p<0.05) were noted in the yolk absorption rate when *C. gariepinus* larvae were nursed between 10 and 15 mg/L of NO_3-N at 60% sewage effluent.

These results suggest that the yolk absorption rate decreased with increasing levels of both pollutants, i.e. nitrate fertilizers and sewage effluent within each temperature level. This implies that the yolk sac consumption decreased with increasing levels of both pollutants in nursing water.

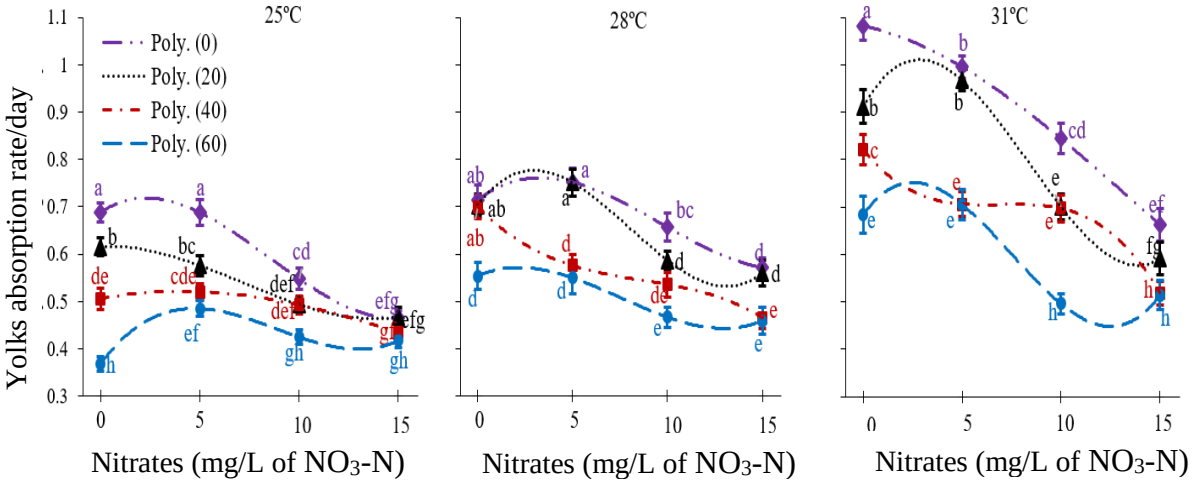

Figure 4.24 Cubic effect of temperature and a mixture of nitrate fertilizers and sewage effluent interaction on yolk absorption rate of *C. gariepinus* larvae incubated at 25°C, 28°C and 31°C.

(Means with the same letter don't differ significantly (p > 0.05)).

Table 4.9. Three-way interaction of temperature and a mixture of nitrate fertilizers and temperature and their orthogonal polynomial effects on hatching rate, incubation period, hatching period, growth rate and larval Survival (mean±SE, n = 45) of *C. gariepinus*.

	T	N	S	TN	TS	NS	TNS
Survival of embryos (%)	Q***	L***	C*	NS	Q*	NS	NS
Incubation period (h)	Q***	C***	C***	C*	ns	ns	ns
Hatching period (h)	Q**	C***	Q***	L*	Q***	Q**	Q*
Hatching rate (%)	L***	Q***	Q*	Q*	ns	ns	ns
Yolk absorption period/h	Q***	C***	Q***	ns	Q*	L***	C***
Yolk absorption rate/Day	Q***	C***	L***	ns	Q*	L***	C*
Growth Rate (mm/day)	Q***	C***	C***	L***	Q**	ns	ns
Larval Survival (%)	Q***	L***	Q**	Q**	L**	ns	ns

***= p value < 0.001, **= p value < 0.01, *= p value < 0.05, T = temperature, N = nitrate, S= sewage effluent ns = not significantly

In summary, the three-way interactions showed that the tolerable limit was highest at 31°C followed by 28°C and the least was at 25°C but within each level it decreased with increasing levels of nitrate fertilizers x sewage effluent.

CHAPTER FIVE

DISCUSSION

5.1 Interactive effect of water temperature and nitrate fertilizers on development, growth and survival of embryos and early life stages of *C. gariepinus*

5.1.1 Water quality parameter

During this study, all water quality factors relevant for the survival, growth and development of *C. gariepinus* embryos, embryos and larvae except nitrate and temperature were within the accepted range in all experimental treatments, i.e. pH, DO, BOD, COD, Nitrite, Phosphates, TDS, ammonia and salinity (Onada and Ogunola, 2017; Demeke *et al*., 2015). pH increased with increasing nitrate levels. However, it didn't differ significantly ($p > 0.05$) in all treatments. Nitrate levels were attained by adding $NaNO_3$ (97%). The results of the present experiment could have been affected by the ionic imbalance between K^+ and Na^+ (in the internal and external environment of the experimental organisms) if the concentration of Na^+ increased sustainably. However, this was explained by Romano and Zeng (2009) who carried out a similar experiment on highly susceptible shrimp, and they added KCl in a mole ratio of 46:1, Na: K in order to balance K^+ and Na^+. They discovered that both ions had the same effect. Therefore the findings of this experiment were as a result of the two factors that varied; temperature (25-31°C) and nitrate (0 – 20 mg/L of NO_3-N). *C. gariepinus* larvae were also fed on natural zooplankton after 50% had absorbed two-thirds of their yolk sacs, in order to avoid starvation and cannibalism (Folkvord, 1991). The effect of feeding was not tested in the present experiment. However, all larvae were fed uniformly at three times *ad libitum* in all treatments in order to avoid bias in experimental results (Britz, 1988).

5.1.2 Main effect of temperature on the survival of fertilized *C. gariepinus* embryos

The direct relationship between temperature and the survival of fertilized *C. gariepinus* embryos can be linked to the high metabolic rates at high temperatures. High metabolic rate increases the rate of egg and embryonic development (da Silva Longo and de Oliveira Nuñer, 2010; Okunsebor, *et al.,* 2015). In addition, high temperature increases the activities of digestive enzymes, this may have increased the rate and efficiency at which the yolk sac food reserves are metabolized and utilized to satisfy the metabolic requirements for the elevated development rate (Laurel *et al.*, 2008; Baxter, 1969). High metabolism may have resulted into energetic and active embryos with high survival rate as observed in the current study. Similar outcomes were reported by Okunsebor *et al.*, (2015), who used water at 26ºC, 28ºC, 30ºC and 32ºC to incubate embryos of *Heterobranchus bidorsalis*, another catfish and they concluded that the health of fertilized *H. bidorsalis* embryos increased with increasing temperature from 26-30°C.

5.1.3 Main effect of nitrate on survival of *C. gariepinus* embryos

The present work showed a decrease in the survival of embryos of *C. gariepinus* with increasing levels of nitrate. The cubic trend further revealed that *C. gariepinus* embryos were resilient to nitrate up to 10 to 15 mg/L of NO_3-N. The high survival of *C. gariepinus* embryos below 10 mg/L of NO_3-N could be linked to what was reported by Caspers (1978). He reported that each species is tolerant to some degree of environment variation or range and beyond or near its tolerable limit; the species evokes a biological response which may cause limited survival or death. In the present study, 10 to 15 mg/L of NO_3-N and below may be regarded as the tolerable range of *C. gariepinus* embryos to nitrate fertilizers. The death of *C. gariepinus* embryos beyond 10 to 15 mg/L of NO_3-N can be linked to the toxic

effects of nitrate on development of aquatic organisms as suggested by Rouse *et al.*, (1999). Nitrate is a toxic substance; it passively diffuses through the embryonic envelop into the inner developing cells (Camargo *et al.*, 2005). In the cells, the tissues respond by metabolizing it into nitrite (NO_2^-), nitric oxide (NO) and other bioactive nitrogen oxides in the presence of oxygen. In the absence of oxygen, the process of nitrate metabolism into nitric oxide (NO) becomes dysfunctional but instead, the process of metabolizing it into nitrite (NO_2^-) and ammonia is enhanced (Lundberg *et al.*, 2009). Adult fish protect themselves from nitrite (NO_2^-) and nitric oxide (NO) toxicity by reversing these processes when conditions are favourable. However, embryos have insignificant or negligible protection, simply because at this stage, they have not yet developed specialized organs for detoxification such as the liver (Hickey, 2013). In the present study, the onset of cleavage is a fragile stage (Yokota *et al.*, 2000), the toxicity of nitrate at levels higher than 10 to 15 mg/L of NO_3-N may have caused death of *C. gariepinus* embryos. In contrast to these results, are those reported by Kincheloe *et al.*, (1979) who reported that nitrate levels of 1-10 mg/L (2 mg/L of NO_3-N) was mildly toxic to embryos and embryos of the rainbow, Lahontan, Chinook salmon or steelhead. However, these outcomes are in line with those reported by Beiniarz *et al.*, (1996) who carried out an experiment to examine the effect of eutrophication and pollution on carp production. He exposed carp embryos to 15 mg/L NO_3^- (3.4 mg/L of NO_3-N),150 NO_3^-/L (33.9 mg/L of NO_3-N) and 500mg NO_3^-/L (113.1 mg/L of NO_3-N), and he found significant differences ($p < 0.05$) at all levels of nitrate. Rouse *et al.* (1999) reported that nitrate toxicity can cause developmental abnormalities such as tail, body, head and digestive deformities.

5.1.4 Interactive effect of nitrate and temperature on incubation and hatching period of *C. gariepinus* embryos

The result of the present work showed that the presence of nitrate in incubation water reduced the development and growth rate of *C. gariepinus* embryos at all incubation temperatures. The explanation for this may be attributed to what was suggested by Lundberg *et al*. (2009) and Hickey (2013), who reported that the diffused and metabolism of nitrate in the developing embryos results into high concentrations of nitrate (NO_3^-), and its toxic products of metabolism, which include nitrite (NO_2^-) and nitric oxide (NO) in their tissues. In an effort to eliminate these substances, the developing embryos respond by increasing metabolic rate through increasing the energy requirements for cellular metabolism defence, homeostatic mechanism and growth restoration potential. As a result, the maintenance energy of the developing embryos increases at the expense of growth energy. High maintenance means less energy for growth. This may have reduced the rate of development resulting into the decreased incubation and hatching period with increasing level of nitrate in the present study. Temperature may have supplied much of the energy requirement for maintenance (Laurel *et al.*, 2008). This may be the explanation for the high rate of embryonic development observed at high temperatures of 28ºC and 31ºC as compared to 25ºC observed in both the incubation and hatching period at all levels of nitrate. Temperature increased not only the demand for metabolites to satisfy the requirement of the elevated metabolism but also the bioavailability of nitrate and its toxic products of metabolism by increasing nitrate uptake and the rate at which it is metabolized in the cells. In response, the developing cells increased their maintenance energy by elevating the energy required for detoxification (von Westernhagen, 1988; Lundberg *et al.*,

2009, Laurel *et al.,* 2008). This could have reduced the growth energy, hence reducing the rate of development. These outcomes are in agreement with those reported by Baker and Waight (1993) who reported that exposure of common toads (*Bufo bufo*) to nitrate levels ranging between 9 and 22.6 mg NO_3-/L (2 to 5.1 mg/L of NO_3-N) resulted into reduced growth. They further reported that nitrate has the same effect at both low and high nitrate levels. The similarity of their study with the present work is that nitrate retarded growth rate of *C. gariepinus* embryos at nitrate levels as low as 5 mg/L of NO_3-N (22.14mg NO_3^- /L) but it differed in that in the current work; growth retardation increased with increasing levels of nitrate. The present results are also in line with those reported by Hecnar (1995) who reported that the severity of nitrate on toads increased with increasing nitrate concentration.

5.1.5 Interactive effect of nitrate and water temperature on hatching rate of *C. gariepinus* embryos

The results revealed that at 28 and 31°C, *C. gariepinus* embryos were tolerant to nitrate levels up to 10 to 15 mg/L of NO_3-N beyond which they are susceptible. However, at 25°C the embryos are tolerant up to 20 mg/L of NO_3-N. The explanation for this may be linked to the passive diffusion of nitrate (NO_3^-) via the egg and embryo envelop to the developing tissues and the toxic products of its metabolism, which include nitrite (NO_2^-) and nitric oxide (NO) (Camargo *et al.,* 2005). The accumulation of these substances in the tissues (Lundberg *et al.,* 2009; Hickey, 2013) may have resulted into death of the developing embryos in the current study. Increased levels of NO may cause carcinogenesis by diffusing into the tissues to form nitrosalting species, which have the potential to damage DNA (Lundberg *et al.,* 2009). Nitrite (NO_2^-) and nitric oxide (NO) can also transform

haemoglobin (Hb) into MetHb. This reduces Hb-oxygen binding and transporting capacity (Kamstra and van der Heul, 1998). At this stage, the embryos and their circulation system are in their developmental stage making them unable to withstand the low oxygen at nitrate levels higher than 10 to 15 mg/L of NO_3-N at 28°C and 31°C. Furthermore, when temperature interacts with nitrate, temperature increased the general metabolic rate of the developing embryos (Laurel *et al.,* 2008), therefore the demand for oxygen to satisfy the high metabolic rate may have exceeded its supply resulting into death of the embryos at high temperature of 28°C and 31°C as compared to low temperature of 25°C. These results seem to be contradicting with the recommended nitrate levels i.e. 90mg NO_3-/L (20 mg/L of NO_3-N) for warm water fishes (USEPA, 2002; Kross, 2002). However, in the current work, these recommendations seems to be true when embryos were incubated at 25°C, but at higher temperature of 28°C and 31°C nitrate posed a significant effect at 10 to 15 mg/L of NO_3-N. The reason for this could be as suggested by Nwosu and Hertzlohner (2000), who reported that at lower temperatures the rate of metabolism is low. Therefore, the demand and supply of metabolites such as oxygen are more stable at 25°C. This may have made the embryos at 25°C stronger and able to break the chorion during hatching as compared to those at 28°C and 31°C.

5.1.6 Interactive effect of nitrate and temperature on growth rate of *C. gariepinus* larvae

The results indicated that the tolerable limit for the growth rate of *C. gariepinus* larvae was 10 to 15 mg/L of NO_3-N at 28°C and 31°C while that at 25°C was 15 mg/L of NO_3-N beyond which their growth was reduced. The reduction in growth rate beyond the tolerable limit can be linked to what was reported by Kamstra and van der Heul (1998). Kamstra and

van der Heul explained that growth retardation was as a result of the formation and reduction of methemoglobin (MetHb). In most aquatic animals, nitrate transforms haemoglobin (Hb) into MetHb, this reduces Hb-oxygen binding and transporting capacity. The reversal of this process by MetHb reductase in fish is an energy consuming process (Camargo *et al.*, 2005; Van Bussel *et al*., 2012). This implied that the presence of nitrate in breeding water increased maintenance energy for the growing larvae. As a result, most of the energy which would have been used for growth is used for nitrate detoxification. Further explanation was given by van Bussel *et al.* (2012), who reported that the presence of nitrate in water decreases feed conversion efficiency (FCE). This suggests that nitrate is a toxic substance to fish, and it requires a substantial amount of energy to be detoxified. The current study, further showed that levels of 10 mg/L of NO_3-N were obtained at high temperature of 28°C and 31°C and 15 mg/L of NO_3-N at low temperature of 25°C. These findings are in line with those of Westin (1974) whose recommendation was a maximum concentration 5.7 mg/L of NO_3-N for optimal growth and health of trout. Related results were published by Schram *et al*., (2014) who cultured juvenile African catfish (154.3±7.5g) at 25.4-25.6°C and recommended a maximum of 140mg/L NO_3- for proper growth. However, the differences between the results of these two experiments could have arisen due to differences in the age of fish cultured and temperature. In the same experiment, Schram cultured juvenile African catfish at increasing levels of nitrates. They found out that the concentration of nitrates in juvenile African catfish blood plasma increased nearly linearly with increasing levels of nitrates in their environment, though the ratio of nitrate concentration in the culture environment to the blood plasma was considerably low, i.e. 0.15 to 0.25. Scott (1993), Schram *et al*., (2014) and Davidson *et al.*, (2014) further

explained that the difference between the ratios was due to the fact that nitrate uptake into the fish's body through the gills, and surface body cells is a passive process and that nitrate permeability into the bronchial of the freshwater fish gills is low. In the present study, the effect of nitrate on the fish larvae increased with increasing temperature; this could have resulted from the fact that temperature increased the rate of diffusion into the animal cell (Scott, 1993).

5.1.7 Main effect of nitrate on the yolk absorption period and yolk absorption rate of *C. gariepinus* larvae

The results showed that, beyond 10 to 15 mg/L of NO_3-N the yolk absorption period increased while the yolk absorption rate of *C. gariepinus* larvae decreased with increasing levels of nitrate. This implied that the tolerable limit for the consumption of *C. gariepinus* larvae yolk sac was 10 to 15 mg/L of NO_3-N beyond which it reduced (Caspers 1978, Kincheloe *et al.,* 1979). Nitrate is a toxic substance which reduces the oxygen carrying capacity of the red blood cells within the fish body by transforming haemoglobin (Hb) into MetHb (Camargo *et al.,* 2005; Van Bussel *et al*., 2012). In humans, this condition results into a blue baby syndrome when children under six months of age are given water with nitrate levels above 10 to 15 mg/L of NO_3-N. Other young animals such as calves, lamb, piglets, chicks and colts are also affected (Jennings and Sneed, 1996; Manassaram *et al.,* 2010). In the present study, nitrate levels above 10 to 15 mg/L of NO_3-N may have reduced the yolk utilization efficiency by reducing oxygen supply, resulting into reduced yolk consumption hence increased yolk absorption period and reduced rate yolk absorption at higher levels of nitrate.

5.1.8 Main effect of temperature on the yolk absorption period and yolk absorption rate of *C. gariepinus* larvae

The results showed that, the yolk absorption period decreased linearly while the yolk absorption rate of *C. gariepinus* larvae increased quadratically with increasing temperature. High metabolism at higher temperatures could have been accountable for the inverse relationship between temperature and incubation period and the direct relationship between temperature and yolk absorption rate (Haylor and Mollah, 1995). The explanation for this could be linked to that given by Nwosu and Hertzlohner, (2000), who reported that temperature is the major driver of the physiological activities that take place in the fish's body. High temperature not only increased the rate of larval growth and development but also the metabolic demands for substances such as yolk sac metabolites (Laurel *et al.*, 2008). This may have resulted into the high rate of yolk sac absorption at high temperatures in the present study. These outcomes are in line with those resented by Haylor and Mollor (1995) who reared *C. gariepinus* embryos in freshwater and reported the yolk absorption period of 74.40-90.24h at lower temperature range of 24-26°C, 63.06h at moderate temperature range of 28°C and 48.96-55.20h at high temperature range 30-32°C. In the present study, the increasing temperature from 25°C to 31°C could have increased physiological activities that consumed the yolk sac at a faster rate.

5.1.9 Interactive effect of nitrate and temperature on larvae survival of *C. gariepinus*

The results indicated that the tolerable limit for the survival of *C. gariepinus* larvae ranged between 10 to 15 mg/L of NO_3-N at 28°C and 31°C while that at 25°C was 45 mg/L of NO_3-N beyond which their survival decreased. This could have been due to the fact that temperature increased the rate of nitrate diffusion and metabolism resulting into the

accumulation of nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO) and other toxic oxides into the cells of the developing *C. gariepinus* larvae (Lundberg *et al*., 2009; Scott, 1993). At this stage, specialized organs for detoxification such as the liver are still in their developing stage (Hickey, 2013). The accumulation of nitrates and its products of metabolism may have caused nitrate poisoning, resulting into death of the larvae. These results contradict those of Davidson *et al.* (2011a, 2011b) and Pedersen *et al.* (2012). Davidson, cultured rainbow trout at low level nitrate of 13 mg/L of NO_3-N and high level nitrate 99 ± 7 mg/L of NO_3-N at 12.9-14.0°C while Pederson, cultured rainbow trout at low-level nitrate of 50 mg/L of NO_3-N and high-level nitrate 200 mg/L of NO_3-N at 15.5°C and both reported no difference in survival. The contradiction in the results could have arisen from the differences in susceptibility of fish to nitrate at different ages. Embryos, larvae, and juveniles have been reported to be the most susceptible life stages to toxicants than adult fishes (Sprague, 1985). Related to the present study are results published by Davidson *et al.* (2014) who cultured rainbow trout at low-level nitrate of 30 mg/L of NO_3-N and high-level nitrate 91 mg/L of NO_3-N suggest cumulative reduction of survival at 91 mg/L of NO_3-N. The cause of this was not clear to them.

In summary, increasing temperature not only increased the rate of nitrate diffusion and metabolism within the developing embryos and larvae but also metabolic rates. High metabolic rates increased the demand for substrates such as oxygen and food metabolites. Nitrate, on the other hand, reduced the supply of oxygen by converting blood haemoglobin to MetHb. As a result, the inefficient oxygen supply reduced the rate at which food and food reserves are metabolized. Inadequate supply of both oxygen and food metabolites to

satisfy the requirements for the elevated metabolic rate may break down the normal physiological processes resulting into either death or reduced development, growth and survival of embryos and larvae of *C. gariepinus*.

5.2 Interactive effect of sewage effluent and water temperature on development, growth and survival of embryos and early life stages of *C. gariepinus*

5.2.1 Physicochemical parameters

Physicochemical parameters of sewage effluent were all above the required range by Malawi Bureau of Standards (MBS). This could have been the reason why in the preliminary experiment, few embryos hatched and no larvae survived at 80% and above sewage effluent. Since all samples used in the current study were diluted to at least 60%, all the parameters were within the required range in each experimental unit. RESUN low noise air pump (LP-100, RESUN aquarium, Guangdong, China) was also used to provide oxygen in order to balance the low DO, high BOD and COD during the experiment. Therefore, the differences in the results of the current findings could have risen from the temperature differences and the presence or absence of the sewage effluent but within the acceptable range.

5.2.2 Interactive effect of temperature and sewage effluent on survival of *C. gariepinus* embryos

The results indicated that the survival rates of *C. gariepinus* embryos increased with rising temperature from 25°C and 31°C while their tolerance limit to sewage effluent went up to 45% at 28°C as compared to 30% at 25°C and 31°C. This can be linked to what was reported by Cao *et al*. (2009). Cao *et al* explained that during egg and embryonic stage, the yolk sac absorbs sewage effluent and during yolk utilization, the effluent is transferred with the yolk sac metabolites to the developing tissues. In the tissues, the effluent may accumulate to toxic levels, especially at this early stage when most of the defensive mechanisms are not yet fully developed (Fent and Hunn., 1993; Sidell and Hazel., 1987).

This may have caused sewage effluent toxicity resulting into death of embryos in the current study. Temperature, on the other hand, may have supplied much of the energy requirement for metabolism processes such as cellular metabolic defence, homeostatic mechanism and growth restoration (Okunsebor *et al*., 2015; Heming and Buddington, 1988), hence supplying some of the energy required for detoxification of some toxins found in sewage effluent. This could explain why egg survival was highest at 31ºC and 28ºC as compared to 25ºC at all levels of sewage effluent. At 28ºC the tolerable limit for the survival of *C. gariepinus* embryos to sewage effluent went up to 45%. This may have been due to the fact that 28ºC is the suitable temperature for hatching *C. gariepinus* embryos i.e., the temperature at which all the physiological and biochemical activities are at their best (Ssenfuma *et al.,* 2011; Okunsebor, *et al.,* 2015). Similar outcomes were reported by Okunsebor, *et al.,* (2015) who used water at 26ºC, 28ºC, 30ºC and 32ºC to incubate embryos of *Heterobranchus bidorsalis* another catfish and they concluded that the health of fertilized *H. bidorsalis* embryos increased with increasing temperature from 28-30°C.

5.2.3 The main effect of sewage effluent on incubation period of *C. gariepinus* embryos

The direct relationship between the incubation period of *C. gariepinus* embryos and increasing sewage effluent levels may be linked to the explanation given by Cao *et al*., (2009), Von Westernhagen, 1988 and Sowers *et al*. (2009). These reported that xenobiotics in sewage effluent passively diffuse through the egg and embryonic envelop. These accumulate in the developing tissues, and in an effort to eliminate and metabolize these substances, tissues respond by increasing the energy requirements for metabolic activities such as cellular metabolism defense, homeostatic mechanism and growth restoration potential. In the present study therefore, the increased maintenance energy may have

reduced the energy required for development and growth of the embryos. However, at sewage effluent levels below 30% the developing embryos were less sensitive because they have a protective layer called the chorion that prevented the entry of such substances (Osman *et al.*, 2007; Dubińska-Magiera *et al.,* 2016).

5.2.4 Interactive effect of temperature and sewage effluent on hatching period of *C. gariepinus* embryos

The results indicated that *C. gariepinus* embryos could tolerate sewage effluent up to 30% at 25°C and 28°C while at 31°C the tolerance could only go up to 15%, beyond which the hatching period of *C. gariepinus* embryos is compromised. The explanation for this could have been given by Cao *et al*., (2009). They reported that when fish embryos are exposed to sewage effluent, they absorb the effluent into both the developing tissues and yolk sac food reserve. At levels below the tolerable range, the developing embryos were less sensitive because they have a protective layer called the chorion which acted as an effective barrier to prevent the entry of such substances (Osman *et al.,* 2007; Dubińska-Magiera *et al.*, 2016). At high levels of sewage effluent, the xenobiotic contents of sewage such as PPCPs, cosmetics and oils have the ability to alter the yolk sac structure. These chemicals alter the yolk sac structure by combining all oil globulins that are dispersed in the yolk sac into one large oil drop. This not only reduces the surface area over which these oils are metabolised but also the metabolic activities of the yolk sac (Heming and Buddington, 1988). In the present study, since the effluent used in the experiments originated from the university, prison, hospitals and a fraction of residential houses of Zomba city (Pullanikkatil *et al*., 2015), it is most likely that it had xenobiotic contents. Such contents in the effluent could have reduced rate of development as well as weakening the embryos,

hence making them to stay for a longer period before hatching. On the other hand, high temperature increased the rate of metabolism, this may explain why the hatching period was low at high temperatures at 28ºC and 31ºC as compared to lower temperatures of 25ºC at all levels of sewage effluent with a non-crossover trend (Laurel *et al.,* 2008, Von Westernhage, 1988). Furthermore, the results of the present study showed that at 28°C, the embryos were more resilient to sewage polluted water than at other incubation temperatures, i.e. 25°C and 31°C. The explanation for this could have been given by Ssenfuma *et al*., (2011), who reported that 28°C is the best temperature for hatching *C. gariepinus* embryos. It is the temperature at which the physiological activities are at best. The effect due to sewage effluent could have been overcome by the effect due to temperature to up to 30%.

5.2.5 The main effect of sewage effluent on the hatching rate of *C. gariepinus* embryos

The survival of *C. gariepinus* embryos below 30% could be linked to what was reported by Caspers (1978) and Kincheloe *et al.,* (1979). They reported that each species is tolerant to some degree of environment variation or range and beyond or near its tolerable limit; the species evokes a biological response which may cause limited survival or death. In the present study, 30% may be the tolerable limit for the survival *C. gariepinus* embryos. The death of *C. gariepinus* embryos at sewage effluent levels beyond 30% in the present study can be attributed to what was explained by Heming and Buddington (1988) and Von Westernhage (1988). These reported that during this stage, sewage effluent are absorbed into both the developing tissues and in the yolk sac food reserves by diffusion, these are either metabolized or biotransformed into less toxic substances in the developing tissues. At this stage, the embryos have not yet developed specialized organs such as the liver.

Therefore, the rate at which these xenobiotics are processed is low. As a result, at levels above 30%, xenobiotics may accumulate to toxic levels and result into death of the embryos before hatching. These outcomes are in line with those reported by Cao *et al.* (2009), who incubated Japanese medaka embryos in 100% secondary sewage effluent collected from Beijing sewage treatment plant and obtained 36% hatching success in the effluent as compared to 89.5% in the control, however, he did not report the reason for this.

5.2.6 Interactive effect of temperature and sewage effluent on yolk absorption period and rate of *C. gariepinus* larvae

The results indicated that the highest rate of yolk sac consumption was obtained in the control at 28ºC and 31ºC and below 15% sewage effluent levels at 25ºC. This may be attributed to the fact that some PPCPs found in sewage effluent in little amounts such as clofibric acid, benzofibric acid and other lipid regulators caused digestive disorders in yolk sac larvae (Raldúa *et al.* 2008). As a result the larvae may have failed to absorb some macro and micro nutrients, a condition known as malabsorption syndrome. Furthermore, the presence of PPCPs in sewage effluent could have resulted into reduced yolk sac consumption hence increasing yolk absorption period and reduced yolk absorption rate at high sewage effluent levels. In addition, the yolk sac consumption rate is a function of the animal's physiological activities. The higher the metabolic rate, the higher the rate of yolk consumption and the shorter the yolk absorption period (Sowers *et al.*, 2009). High temperature increases metabolic rates. This may explains why the yolk sac consumption was high at high temperatures at 28ºC and 31ºC as compared to 25ºC at all sewage effluent levels. Furthermore, high temperature increased the demand for food metabolites to satisfy the metabolic requirements for the elevated metabolic rate (Laurel *et al.*, 2008, Von

Westernhage, 1988), this may explain why the sewage effluent tolerance limit was high at the highest temperature of 31ºC.

5.2.7 Interactive effect of temperature and sewage effluent on growth rate of *C. gariepinus* larvae

The lower growth rate in sewage polluted water as compared to the control at all rearing temperatures (25ºC to 31ºC) can be attributed to increased enzyme production and enzyme mediated activities required to metabolise xenobiotics found in sewage effluent (Sowers *et al.*, 2009). In the current study, the energy required for growth could have been diverted to eliminate and metabolise xenobiotics hence retarding growth at high sewage effluent level. Temperature off-sets some maintenance energy as well as maintaining metabolic rates high enough to enable metabolism and elimination of toxic xenobiotics at high rates (Laurel *et al.*, 2008; Okunsebor *et al.*, 2015). This may explain why the growth rate was high at high temperatures at 28ºC and 31ºC as compared to 25ºC at all sewage effluent levels with a non-crossover trend.

5.2.8 Interactive effect of temperature and sewage effluent on survival of *C. gariepinus* larvae

The results indicated that the tolerable limit for the survival rate of *C. gariepinus* larvae to sewage effluent was 15% at 28°C and 31°C while that at 25°C was in the control. This could have been due to the fact that, when larvae of fish are exposed to sewage effluent in their environment, they absorb the effluent into their tissues (Cao *et al.*, 2009; Fent and Hunn, 1993). At lower concentrations the developing larvae respond by either eliminating them or metabolizing them into less toxic substances (Lundberg *et al*., 2009; Sowers *et al.* 2009; Hickey, 2013). At the larval stage, these processes take place at a slow pace, simply

because the specific organs to perform those activities such as the liver, kidneys and others are still in their early stages of development (Heming and Buddington, 1988). In addition, the absorbed xenobiotics may accumulate along the development stages from eggs to embryos to toxic levels in the larvae (Weis and Weis, 1989). This may also explain the death of larvae at this stage of development. Furthermore, it should be noted that the lethal concentration of toxins in sewage effluent is a function of cellular metabolic defence, homeostatic mechanism and growth restoration. In the current study, temperature may have supplied much of the energy requirement for metabolic processes such as cellular metabolic defence, homeostatic mechanism and growth restoration (Heming and Buddington, 1988; Von Westernhage, 1988). This could explain why the survival and tolerance limit to sewage effluent was highest at 28ºC and 31ºC as compared to 25ºC. High temperature increased metabolic rates. This increases the demand for food metabolites to satisfy the metabolic requirements for the elevated metabolic rate (Laurel *et al.*, 2008). However, metabolites come along with toxic xenobiotics that have been accumulating in the food reserves. This could have increased the bioavailability of toxins to the cells of the developing larvae which may result to death (Von Westernhage, 1988; Laurel *et al.*, 2008).

5.3 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on development, growth and survival of embryos and early life stages of *C. gariepinus*

5.3.1 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on the hatching period of *C. gariepinus* embryos

The long hatching period at high levels of sewage effluent and nitrate fertilizers as compared to the lower levels can be linked to the fact that the yolk sac and developing tissues of the embryos absorb these substances. In order to eliminate them, the tissues responded by converting them to less toxic substances. Nitrate (NO_3^-) is converted to a less toxic nitric oxide (NO) in the presence of oxygen or to ammonia (HNO_4-) in the absence of oxygen. Nitric oxide (NO) and ammonia (HNO_4-) are then eliminated from the cells (Lundberg *et al.*, 2009; Hickey, 2013). Other toxic xenobiotic content of sewage effluent are also eliminated or metabolized to less toxic substances and then eliminated (Sowers *et al.*, 2009). In the current study, the long incubation period may have been due to the fact that the process of elimination and metabolism of sewage effluent, nitrate fertilizers and their products of metabolism which required the developing embryos to increase their maintenance energy at the expense of growth energy (von Westernhagen, 1988; Lundberg *et al*., 2009). At high concentration of these pollutants, xenobiotic contents of sewage effluent such as PPCPs, cosmetics and oils and nitrate fertilizers may be absorbed into the yolk sac which is the energy reserved for the developing embryo. The presence and accumulation of these toxic xenobiotics may have reduced the rate at which the yolk sac was absorbed while increasing maintenance energy, hence reducing the supply of metabolites to the developing embryos. This may have resulted into long hatching periods at high levels of sewage effluent and nitrate fertilizers. The results further showed that the

shortest hatching period was obtained at high temperatures of 28ºC and 31ºC while the longest were obtained at lower temperature of 25ºC. High temperature may have supplied some of the energy required for maintenance, as well as increasing the rate at which the yolk sac was metabolized (eming and Buddington, 1988).

5.3.2 Interactive effect of temperature and a mixture of nitrate fertilizers and sewage effluent on yolk absorption period and yolk absorption rate of *C. gariepinus* larvae

The decreasing rate of yolk sac consumption with increasing levels of nitrate and sewage effluent pollutants in rearing water, may have been due to the fact that, when these substances are absorbed into the larvae, they are either taken up by the living cells or the stored in the yolk sac. The living cells eliminate these substances through their physiological activities while the contents of the yolk sac have no mechanism of eliminating them. The accumulation of nitrate and sewage effluent pollutants in the yolk sac, may limit its physiological activities by reducing its size, altering its structure as well as deforming it (Lundberg *et al*., 2009; Sowers *et al.*, 2009; Hickey, 2013). Xenobiotic contents of these pollutants may also cause incomplete yolk incomplete yolk circulation, alteration of both yolk sac osmoregulation process and volume (von Westernhagen, 1988). This results into reduced yolk utilization, which is specifically dangerous to fish at larval stage, because energy requirements from the yolk reserve approximately increases ten times shortly after hatching. High temperature may have increased the rate at which these pollutants, and their contents are eliminated from the larvae body cells before they reach the yolk sac (Heming and Buddington, 1988). In the current study, the increasing levels of nitrate and sewage effluent may have altered the yolk absorption rate, causing increased

yolk absorption period and reduced yolk absorption rate. Furthermore, high temperature increased the activities of digestive enzymes while low temperatures reduced the activities of digestive enzymes (Baxter, (1969). In the present study, lower temperature may have reduced the rate at which the yolk was digested, resulting into higher yolk absorption period and low yolk absorption rate at lower temperature of 25°C as compared to those obtained at high of temperature of 28°C and 31°C.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study concluded that:

- The effect of nitrate fertilizers on *C. gariepinus* embryos and larvae was dependent on temperature i.e. both embryos and larvae tolerated a maximum range of 10 to 15 mg/L of NO_3-N at 28°C to 31°C and 15 to 20 mg/L of NO_3-N at 25°C.

- The effect of sewage effluent on *C. gariepinus* embryos and larvae was dependent on temperature i.e. embryos tolerated a maximum range of 30 to 45% sewage effluent at 28°C and 15 to 30% at 25°C and 31°C while larvae tolerated a maximum range between the control and 15% at all temperatures (25°C to 31°C).

- The effect of a mixture of both nitrate fertilizers and sewage effluent was dependent on temperature i.e. significant differences were observed in the hatching rate for the embryo stage and yolk absorption period and the yolk absorption rate for the larval stage.

6.2 Recommendations

It is therefore recommended that:

- *C. gariepinus* embryos and larvae farmed in water containing less than 10 to 15 mg/L of NO_3-N should be incubated and reared at high temperatures of 28°C to 31°C while those cultured in water containing between 15 and 20 mg/L of NO_3-N should be incubated and reared at low temperature of 25°C.

- Embryos and larvae of *C. gariepinus* should be incubated at moderate temperature of 28°C in water polluted with sewage effluent of less than 15%.

- Environmental scientists and managers should monitor and put in place strict measures and buffers to avoid nitrate levels from raising above 10 to 15 mg/L of NO_3-Nand sewage effluent above 15% if they are to conserve the breeding places of *C. gariepinus*

- A study should be carried out to determine whether the increased rate of yolk absorption at high levels of both sewage effluent and or nitrate fertilizer affects the mouth and eye opening of *C. gariepinus* larvae

- A study should be carried out to determine the exact level of nitrate which can be tolerated by the embryos and larvae of *C. gariepinus* other than the range that have been provided in this study

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APPENDICES

Appendix 1. The main effects and two-way (temperature and nitrate) interactions ANOVA for fertilized eggs, Survival of embryos, yolk absorption period and yolk absorption rate of *C. gariepinus* embryos in an experiment with three temperatures and five levels of nitrate

Source	**DF**	**SS**	**MS**	**F-value**	**Pr(>F)**	**contrasts**			
						L	Q	C	Qa
Fertilized eggs (%)									
Temp (^{O}C)	2	7.244	3.622	0.472	0.628	ns	ns	-	-
Nitrates (mg/l)	4	63.689	15.922	2.077	0.109	ns	ns	ns	ns
Nitrates (mg/l) X Temp (^{O}C)	8	20.978	2.622	0.342	0.942	ns	ns	ns	ns
Error	30	230	7.667						
Survival of embryos (%)									
Temp (^{O}C)	2	83.333	41.667	4.271	0.023	**	ns	-	-
N (mg/L NO_3-N)	4	1402.8	350.7	35.949	0.007	***	***	ns	ns
Nitrates (mg/l) X Temp (^{O}C)	8	58.000	7.25	0.743	0.653	ns	ns	ns	ns
Error	30	292.70	9.8						

(Continued)

Source	DF	SS	MS	F-value	Pr(>F)	contrasts			
						L	Q	C	Qa
Yolk absorption period/h									
Temp (^{O}C)	2	13139	6570	80.51	<0.001	***	-	-	-
Nitrates (mg/l)	4	8144	2036	24.951	<0.001	***	ns	ns	ns
T x N	8	957	120	1.466	0.211	ns	ns	ns	ns
Error	30	2448	82						
Yolk absorption rate/Day									
Temp (^{O}C)	2	0.909	0.455	21.353	<0.001	***	***	-	-
Nitrates (mg/l)	4	0.550	0.138	6.453	<0.001	***	ns	ns	ns
T x N	8	0.034	0.004	0.204	0.987	ns	ns	ns	ns
Error	30	0.639	0.021						

***= p value < 0.001, ns= non-significant, T = temperature, N = nitrate. Orthogonal polynomial contrasts were used to partition significant Nitrate levels into linear (L), quadratic (Q), cubic (C) and quartic (Qa) components.

Appendix 2. The main effects and two-way interactions ANOVA for yolk absorption period, yolk absorption rate, growth rate and hatching rate of *C. gariepinus* larvae in an experiment with three levels of temperature and five levels of nitrate

Source	DF	SS	MS	F value	Pr(>F)	contrasts			
						L	Q	C	Qa
Yolk absorption period/h									
Temp (^{O}C)	2	13139	6570	80.51	<0.001	***	ns	-	-
Nitrates (mg/l)	4	8144	2036	24.951	<0.001	***	ns	ns	ns
T x N	8	957	120	1.466	0.211	ns	ns	ns	ns
Error	30	2448	82						
Yolk absorption rate/Day									
Temp (^{O}C)	2	0.909	0.455	21.353	<0.001	***	***	-	-
Nitrates (mg/l)	4	0.550	0.138	6.453	<0.001	***	ns	ns	ns
T x N	8	0.034	0.004	0.204	0.987	ns	ns	ns	ns
Error	30	0.639	0.021						

(Continued)

Source	DF	SS	MS	F value	Pr(>F)	contrasts			
						L	Q	C	Qa
Growth rate (mm/Day)									
Temp (^{O}C)	2	1.132	0.566	86.491	<0.001	-	-	-	-
Nitrates (mg/l)	4	1.886	0.471	72.060	<0.001	***	***	ns	ns
T x N	8	0.176	0.022	3.356	0.002	***	***	ns	ns
Error	30	0.785	0.007						
Larval Survival (%)									
Temp (^{O}C)	2	3141	1570.6	104.279	<0.001	-	-	-	-
Nitrates (mg/l)	4	4805	1201.4	79.77	<0.001	***	***	ns	ns
Nitrates (mg/l) X Temp (^{O}C)	8	411	51.481	3.418	0.007	***	**	ns	ns
Error	30	451.85	15.062						

***= p value < 0.001, ns= non-significant, T = temperature, N = nitrate. Orthogonal polynomial contrasts were used to partition significant Nitrate levels into linear (L), quadratic (Q), cubic (C) and quartic (Qa) components.

Appendix 3. The main effects and two-way interactions ANOVA for fertilized eggs, survival of embryos, incubation and hatching period and hatching rate of *C. gariepinus* egg in an experiment with three levels of temperature and five levels of sewage effluent

Source	**DF**	**SS**	**MS**	**F value**	**Pr(>F)**	**contrasts**		
						L	Q	C
Fertilized eggs (%)								
Temp (^{O}C)	2	2.72	1.361	0.306	0.739	ns	ns	ns
Sewage (%)	3	1.56	0.519	0.117	0.949	ns	ns	ns
Sewage (%) X Temp (^{O}C)	6	15.94	2.657	0.598	0.729	ns	ns	ns
Error	24	106.67	4.444					
Survival of embryos (%)								
Temp (^{O}C)	2	478.4	239.2	41.399	<0.001	***	**	ns
Sewage (%)	3	813.9	271.3	46.955	<0.001	***	**	ns
Sewage (%) X Temp (^{O}C)	6	98.3	16.4	2.835	0.0033	**	*	ns
Error	24	138.7	5.8					
Incubation period (h)								
Temp (^{O}C)	2	71.17	35.58	80.063	<0.001	***	ns	-
Sewage (%)	3	64.31	21.44	48.229	<0.001	***	ns	ns
Sewage (%) X Temp (^{O}C)	6	2.61	0.44	0.979	0.461	ns	ns	ns
Error	24	10.67	0.44					

(Continued)

Source	DF	SS	MS	F value	Pr(>F)	contrasts		
						L	Q	C
Hatching period (h)								
Temp (^{O}C)	2	369.4	184.7	316.61	<0.001	***	*	ns
Sewage (%)	3	337.6	112.5	192.88	<0.001	***	***	***
Sewage (%) X Temp (^{O}C)	6	64.6	10.8	18.46	<0.001	***	***	***
Error	24	14	0.6					
Hatching rate (%)								
Temp (^{O}C)	2	3419	1709.3	97.306	<0.001	***	ns	-
Sewage (%)	3	1455	485.1	27.615	<0.001	***	**	ns
Sewage (%) X Temp (^{O}C)	6	9	1.4	0.081	0.998	ns	ns	ns
Error	24	422	17.6					

***= p value < 0.001, ns= non-significant, T = temperature, N = nitrate. Orthogonal polynomial contrasts were used to partition significant Nitrate levels into linear (L), quadratic (Q), cubic (C) and quartic (Qa) components.

Appendix 4. The main effects and two-way interactions ANOVA for yolk absorption period, yolk absorption rate, growth rate and hatching rate of *C. gariepinus* larvae in an experiment with three levels of temperature and five levels of sewage effluent

Source	**DF**	**SS**	**MS**	**F value**	**Pr(>F)**	**contrasts**		
						L	Q	C
Yolk absorption period (h)								
Temp (^{O}C)	2	20322	10161	376.33	<0.001	***	ns	ns
Sewage (%)	3	5976	1992	73.778	<0.001	***	**	ns
Sewage (%) X Temp (^{O}C)	6	270	45	1.002	0.033	**	**	ns
Error	24	1152	30					
Yolk absorption rate/day								
Temp (^{O}C)	2	1.9295	0.9648	106.96	<0.001	***	**	ns
Sewage (%)	3	1.2575	0.4192	46.473	<0.001	***	**	ns
Sewage (%) X Temp (^{O}C)	6	0.2021	0.0337	3.735	<0.001	***	*	ns
Error	96	0.8659	0.009					
Growth rate/day								
Temp (^{O}C)	2	1.343	0.6715	122.75	<0.001	***	ns	ns
Sewage (%)	3	0.5582	0.1861	34.017	<0.001	***	*	ns
Sewage (%) X Temp (^{O}C)	6	0.0192	0.0032	0.005	<0.011	*	ns	ns
Error	96	0.5251	0.0055					

(Continued)

Source	DF	SS	MS	F value	Pr(>F)	contrasts		
Larval Survival (%)								
Temp (^{O}C)	2	3919	1960	111.40	<0.001	***	ns	ns
Sewage (%)	3	5155	1718	97.704	<0.001	***	*	ns
Sewage (%) X Temp (^{O}C)	6	357	60	3.385	0.005	**	ns	ns
Error	24	422	18					

***= p value < 0.001, ns= non-significant, T = temperature, N = nitrate. Orthogonal polynomial contrasts were used to partition significant Nitrate levels into linear (L), quadratic (Q), cubic (C) and quartic (Qa) components.