

MAKERERE



UNIVERSITY

**BIO-PHYSICAL SUITABILITY OF SALINE CRATER LAKES OF WESTERN UGANDA
FOR *ARTEMIA* PRODUCTION**

BY

NKAMBO MUJIBU

**BSc. FISHERIES & AQUACULTURE (MAKERERE UNIVERSITY), MSc. WATER AND
COASTAL MANAGEMENT (UNIVERSITY OF BERGEN), MSc. AQUACULTURE
(GHENT UNIVERSITY)
REG. NO: 2012/HD13/18426U**

SUPERVISORS

**PROF. FREDRICK.W. BUGENYI
COLLEGE OF NATURAL SCIENCES, DEPARTMENT BIOLOGICAL
SCIENCES, MAKERERE UNIVERSITY**

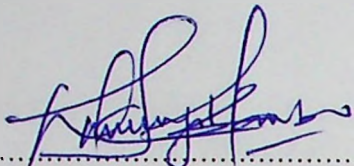
**Dr. JOHN S. BALIRWA
NATIONAL FISHERIES RESOURCES RESEARCH INSTITUTE,
NATIONAL AGRICULTURAL RESEARCH ORGANISATION**

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DECLARATION

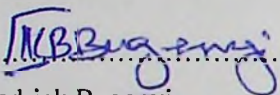
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Nkambo Mujibu

APPROVAL

This thesis/dissertation has been submitted for examination with the approval of the following supervisors:

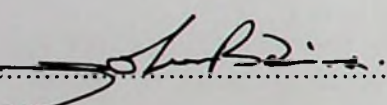
Signature..... 

Prof. Dr. Fredrick Bugenyi

Department of Biological Sciences,

College of Natural Sciences (CONAS), Makerere University

Date..... 13-12-2019

Signature..... 

Dr. John S. Balirwa

National Agricultural Research Organisation (NARO)

Date..... 13/12/2019

DEDICATION

I can only thank the Almighty for this achievement because without the gift of life and abilities He has bestowed on to me this work would not have been possible. Daddy from my early childhood you always talked to me and showed that with hard work the sky is the limit. You have always inspired and motivated me even when things appeared so oblique and impossible. For your love, commitment and investment in my education even when you had so little. I dedicate this work to you and my

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GENERAL ABSTRACT

Despite *Artemia* being the most widely used live feed for successful larval weaning and rearing, unreliable quality, not being readily available and expensive especially to small farmers hinders its use. This study explored production of *Artemia* using waters from saline crater lakes of western Uganda. Local *Artemia* production will contribute towards reducing the low survivals in hatcheries more especially for African catfish (*Clarias gariepinus*) by providing affordable *Artemia* of reliable quality and quantity to the hatchery operators. Limno- biophysical chemistry was used to identify the best suited lakes for bio-manipulation to produce *Artemia*. Limno-bio-physical chemical findings indicated the studied lakes ranged between $0.2\pm0.0\text{m}$ and $2.3\pm0.3\text{m}$ (depth), $0.0\pm0.0\text{mg l}^{-1}$ and $205.0\pm15.3\text{mg l}^{-1}$ for salinity, $27.9\pm0.3^{\circ}\text{C}$ and $34.4\pm2.4^{\circ}\text{C}$ for temperature, $18.6\pm0.1\text{ }\mu\text{scm}^{-1}$ and $106.3\pm3.5\text{ }\mu\text{scm}^{-1}$ for conductivity, $1.7\pm0.4\text{ mg l}^{-1}$ and $6.0\pm1.0\text{mg l}^{-1}$ for Dissolved Oxygen and 9.6 ± 0.1 and 11.5 ± 1.0 for pH, with no occurrence of local *Artemia* resources. Other than Lake Bagusa where *Anabaena circinalis* was found to dominate the algal biomass, *Spirulina platensis* was the dominant algal biomass in majority of these lakes. Lake Kikorongo had the highest zooplankton diversity, having *Brachionus calyciflorus* as the most abundant (50 to 100 individuals /litre). Hatchability experiments using five commercial *Artemia* strains: *Artemia franciscana* from Great Salt Lake (GSL), *A. franciscana* from salt ponds in Vinh Chau (VC), Chinese strain (Chinese), TUZ Parthenogenetic *Artemia* from Kazakhstan (TUZ) and Parthenogenetic *Artemia* strain from Siberia (PAS) using waters from crater lakes indicated *Artemia franciscana* (VC) to have significantly higher hatching percentage of 93.9 ± 29.4 and 79.8 ± 15.2 for lake Bagusa, 89.3 ± 6.9 and 92.7 ± 3.6 for lake Katwe, 83.4 ± 10.7 and 83.5 ± 9.1 for lake Maseche in the dry and wet season respectively ($P < 0.05$). Bucket culture experiments of the best performing commercial *Artemia* strains from the hatchability experiments indicated survival and mean total length of GSL *Artemia* nauplii cultured at salinities of 40 and 60g/l to be significantly higher than that cultured at salinities of 80 and 100g/l. VC *Artemia* nauplii cultured at salinity of 40g/l was found to have significantly higher growth rate (length increment) than those at salinities of 60g/l, 80g/l and 100g/l (Asymptotic sig (2-sided tested) ≤ 0.000). Pond culture experiments showed that the highest mean total lengths of GSL and VC *Artemia* nauplii were $921.0\mu\text{m}$ and $923.0\pm91.9\mu\text{m}$ respectively when cultured at salinities of 60g/l for 10days. No *Artemia* nauplii were found to survival beyond day 10 of culture at both salinities of 60 and 80g/l. The mean survival of both GSL and VC *Artemia* nauplii did not significantly vary across the treatments (60 and 80g/l) after the same culture period in the salt ponds (Asymptotic sig (2-sided tested) ≥ 0.200). Although more research is needed to establish the cause for the high mortalities after 8 and 10days of culturing in buckets and ponds respectively, this results show that it possible to culture *Artemia* using waters from alkaline saline crater once these waters are bio-manipulated to favorable conditions for *Artemia* production.

CHAPTER ONE

1.0 General introduction

1.1 Background

Approximately 18% of Uganda's total surface is covered by water. Lakes Victoria, Albert, Kyoga, Edward and George are Uganda's major water bodies that contribute to the largest proportion of Uganda's fisheries production. Lake Victoria contributes about half of Uganda's total annual fish catches. The fish catches in these lakes are dominated in composition by the Nile perch (*Lates niloticus*), Nile tilapia (*Oreochromis niloticus*) and Mukene (*Rastrineobola argenticia*). The Nile perch and Nile tilapia dominate regional and international export market while Mukene has been predominant on the local market (UNCTAD, 2014). Despite Uganda's huge fisheries potential, the fisheries industry only began to grow with the rest of the economy in the late 1980s (UNCTAD, 2014). Fish exports are reported to have grown from less than 1 million USD to more than 45 million USD in 1996 before peaking off in 2005 with an approximate 39,000 metric tonnes of fish exported mainly to the EU, generating about 143 million USD (Mbowa, Odokonyero, & Taabu, 2017). Since then, Uganda's fish exports have been on a decline with fish exports of 17,000 metric tones reported in 2014. The decline has been attributed to the decline in capture fisheries production. The decline in capture fisheries production was attributed to over fishing (Kasozi, Rutaisire, Nandi and Sundaray, 2017), capture of immature fish using indiscriminate fishing gears, and pollution more especially of Lake Victoria. Despite the decline, Uganda's fisheries sector remains second to coffee in contributing to the countries' foreign exchange earnings. This persistently high contribution of forex earning from fish could be attributed to the reported increase in unit value of fish from 3,200 to 7,700

USD between 2005 and 2014, respectively (Mbowa, Odokonyero, & Taabu, 2017). Meanwhile, coffee is reported to be currently contributing USD 312 million (<https://tradingeconomics.com/uganda/exports>).

The decline in fish production from capture fisheries is considered a big threat to Uganda's foreign exchange earnings, domestic income and food security (Mbowa, Odokonyero, & Taabu, 2017). Therefore, increasing aquaculture production has huge potential to bridge the gap between the declining production from capture fishers and the increasing demand (Namatovu, 2018). As a means of bridging the gap between the declining stocks and the increasing fish demand, the Government of Uganda (GoU) has encouraged commercialisation of aquaculture through various initiatives like the aquaculture promotion strategy throughout the country using a USD 30 million fund in 2007, Improvement of the Aquaculture Research and Development Center (ARDC) facility at Kajjansi to demonstrate commercial earthen pond aquaculture and training local fish farmers in commercial aquaculture production with funds from GoU and People's Republic of China in 2009, development of the draft Aquaculture Policy in 2012 (UNCTAD, 2014). It is difficult to increase fish production much above its current level on a sustainable basis solely on the improvement in management of capture fisheries in the country. Despite GoU regulating the capture fisheries sector by introducing vessel identification number plates as a means of managing the number of fishers on the various water bodies, the problem of over fish has persisted (<https://thefishsite.com/articles/uganda-to-increase-aquaculture-production>). There is need to learn from experiences of countries where insufficient fish production has been addressed through adoption of aquaculture. The strategy is not solely as a livelihood measure as was the case throughout Africa in the 1950s when aquaculture was introduced (FAO, 2007), but

as an enterprise based on practicing commercial fish farming. The ever declining catches from capture fisheries have triggered a deliberate shift in effort from capture fisheries towards aquaculture (Lyudmila, Aleksandr, Elena, & Kirill, 2014).

In order to maintain her export fish position as well as ensuring increased animal protein in-take for her population, GoU is promoting modern aquaculture to boost the fisheries production amidst declining production from capture fisheries (<https://thefishsite.com/articles/uganda-to-increase-aquaculture-production>). Namatovu et al (2018) and NaFIRRI (2012) point to a number of factors rendering aquaculture to be such a viable option for increasing fisheries production in Uganda. These factors include; the conducive policy environment, increased donor and private sector interest in investing in Uganda's Aquaculture industry, local industries getting engaged in fish feed production, availability of raw materials like mukene (*Rastrineobola argenticia*), soya bean, maize among others for fish feed formulation, research and farmer training facilities at ARDC in Kajjansi, the conducive climate with high temperatures of 25-35°C, suitable for fish growth, suitable aquaculture species including the Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and high market demand for fish at local, regional and international level.

Despite the conducive environment and supportive factors, Uganda's aquaculture production is yet to match its potential (Jagger & Pender, 2001). Limited supply of good quality feed and seed are among the key bottlenecks for aquaculture development in Uganda. Other factors impeding aquaculture development in Uganda include limited technical support to farmers, weak research extension linkages, and lack of funding support from financial institutions, among others (Kasozi,

et al., 2017). A reliable source of feed and seed are key requirements for sustainable aquaculture industry (Garcia-Ortega & Huisman, 2001; Soundarapandian & Saravanakumar, 2009; Achionye-nzeh, Olumuji, & Bello, 2012; Nabulime, Rutaisire, Ssekaayi, Smith, & Kabasa, 2015; Egwenomhe, Aliu, & Lawrence, 2017). Fish seed requires readily available source of live food, especially at the larval stage and *Artemia* has proved to be the most suitable organism for this purpose for more than 85% of the cultured fish species (Gopalakrishnan, Raju, & Thaker, 1989; Mahmood, Haider, & Saikat, 1993; Soundarapandian & Saravanakumar, 2009). Despite *Artemia* having proved to be the most suitable starter feed for fish (Olurin, & Oladapo, 2012), its supply from the natural sources has been reported as inadequate to satisfy its ever increasing demand in the aquaculture industry (Kulasekarapandian & Ravichandran, 2003). It is because of this shortage that attempts have been made to inoculate *Artemia* in suitable saline habitats (Kulasekarapandian & Ravichandran, 2003).

Artemia also commonly referred to as brine shrimps (Kaiser, Gordon, & Paulet, 2006; Mahmood et al., 1993), are small zooplankton (adult size: about 1 cm) occurring worldwide in natural hypersaline lakes (Mohebbi, 2010; Ben Naceur, Jenhani, & Romdhane, 2012; Riddle, Baxter, & Avery, 2013), ponds and lagoons, as well as in man-operated solar saline works (Abatzopoulos et al., 2006). *Artemia* is a crustacean belonging to the phylum Arthropoda, class Brachiopoda, sub-order Anostraca, family Artemiidae and genus *Artemia*. It has unique osmoregulatory capacity which makes it survive in harsh environmental conditions (Soltanian, 2007; Dai et al., 2011). Its osmo-regulation capacity allows it to survive in diverse environments with salinities ranging between approximately 10 and 340 g/l (Soltanian, 2007; Agh et al., 2008), diverse ionic composition and temperature regimes (Ben Naceur, et al., 2012). Under extreme conditions,

Artemia is able to produce diapausing cysts having a dormant embryo enveloped in a rigid shell which enables it to survive unfavorable environmental conditions (Van Stappen, Lavens, & Sorgeloos, 1998; Kaiser et al., 2006; Dai et al., 2011). Cysts are produced in large quantities and often found floating on the water surface (Kulasekarapandian & Ravichandran, 2003). It is the specific structure of the cyst shell that enables the *Artemia* cysts to float on the water surface.

Artemia cysts stay viable for several years if the processing and packing is well done and, during storage the dried embryos (diapause) stay in a state of arrested metabolism until favorable conditions are available (Kaiser, Gordon, & Paulet, 2006; Podrabsky & Hand, 2015; Robbins et al., 2010). Under the influence of environmental cues the diapause can be terminated, allowing the cyst to hatch under appropriate hatching conditions. It is the emerging larva ("nauplius") that is used worldwide as an alternative to natural plankton diet for fish and shrimp larvae. Following specific enrichment procedures, the nutritional properties of the hatched nauplii can be enhanced to provide the different nutritional requirements for fish and shrimp larvae. It is these unique properties together with *Artemia*'s ease and versatility for use which have led to its worldwide use in hatcheries (Malunood et al., 1993).

1.2 Global Distribution and production of *Artemia*

World-wide there are more than 50 geographical strains of *Artemia* (Graviel, 2000), many of which have been commercially harvested, processed and distributed for use as live feed in aquaculture. The majority of these strains come from the Great Salt Lakes of Utah in USA, where more than 80% of the world's commercial *Artemia* cysts are harvested (Sorgeloos, Lavens, Leger, & Tackaert, 1991). Since its first description, about 600 *Artemia* sites have been found

and described throughout the world and their populations have been characterized. The distribution of these sites in Africa is very uneven and much of it unknown. This might be attributed to *Artemia* exploration activities in Africa being very few and as such does not give a precise picture of the actual occurrence of *Artemia* in Africa.

Ever since the discovery of *Artemia* as a suitable starter feed for fish and shell fish, its demand has been on the increase. Over 1,500 metric tons of dry *Artemia* cysts are marketed world-wide for use in fish and shellfish hatcheries (Soltanian, 2007). It is because of the ever increasing demand for *Artemia* cysts that Abatzopoulos et al., (2006) pointed to the need for exploring other *Artemia* natural sources. While the last decades have seen a relative expansion in the exploration of *Artemia* sites (and corresponding increase in knowledge of their population) in most continents, especially Africa, the knowledge of *Artemia* sites has remained rather scanty. The species *A. salina* is known from the Mediterranean basin, but also from a number of salt pans in South Africa. Parthenogenetic strains have been described for a few places scattered over the continent, especially in its southern part. A systematic survey in sub-Saharan Africa, however, is non-existent, except for South Africa, where intensive research has been conducted over the last decade. A review by Kaiser *et al.*, (2006) listed about 130 African *Artemia* sites: this is a very low figure, taking into account the dimensions of the continent, and the vast part of it where suitable climatological conditions exist for the occurrence of various types of hypersaline ecosystems; for comparison: in Spain alone about 50 sites have been described. Apart from North and South Africa, however, the list compiled by Kaiser et al., 2006 is very limited; for Central and Eastern Africa, it only lists a few sites in Kenya.

1.3 Problem statement

Sufficient good quality seed to stock in grow-out production systems is one of the important contributors to commercial aquaculture production (Sorgeloos, Dehasque, Dhert, and Lavens, 1995). Despite aquaculture production increasing from 285 metric tons in 1999 to over 95,000 metric tons in 2010/11 (MAAIF-DFR, 2012), seed production for all the farmed fish in the country remains insufficient (Mwanja, Rutaisire, Ondhoro, Ddungu and Aruho, 2015). Scarcity of affordable good quality larval weaning and advanced nursing feeds is one of bottle necks for mass fish seed production (Mwanja et al., 2015). Although freshwater zooplanktons are a cheaper option for larval weaning and exogenous feeding, sustenance of their culture has proved to be difficult (Mwanja et al., 2015). Despite *Artemia* being an option most widely used for weaning and larval rearing with high survivals realised (Sorgeloos et al., 1993; Soltanian, 2007), unreliable quality, not being readily available and expensive especially to small farmers hinder its use (Mwanja et al., 2015). So far, no artificial feed formulation has been effective in completely replacing *Artemia* (Soltanian, 2007). This study therefore sought to produce novel *Artemia* production methods using waters from saline crater lakes of western Uganda. Novel *Artemia* production methods will contribute towards its local production, thereby resulting in increased availability of affordable, reliable good quality supplies for use in local hatcheries for increased survival of fish larvae in hatcheries, especially for African catfish (*Clarias gariepinus*). This study also provided an opportunity to investigate the limno-chemistry of these saline crater lakes and its contribution towards *Artemia* production.

1.3 General Objectives

To develop novel methods for *Artemia* production using waters from the saline crater lakes of Western Uganda, as a mean of supporting improved survivals in Ugandan hatcheries.

1.4 Specific Objectives

- i) To determine biophysical and chemical parameters of five (5) selected saline crater lakes of Western Uganda.
- ii) To identify local *Artemia* strains in five (5) selected saline crater lakes of Western Uganda.
- iii) To hatch both local and commercial *Artemia* strains using water samples collected from five (5) selected saline lakes of Western Uganda.
- iv) To culture local and selected commercial *Artemia* strains *ex-situ* (bucket cultures) using water samples collected from selected saline crater lakes.
- v) To culture local and selected commercial *Artemia* strains *in-situ* (pond cultures) using waters from selected saline crater lakes.

1.5 Conceptual Framework

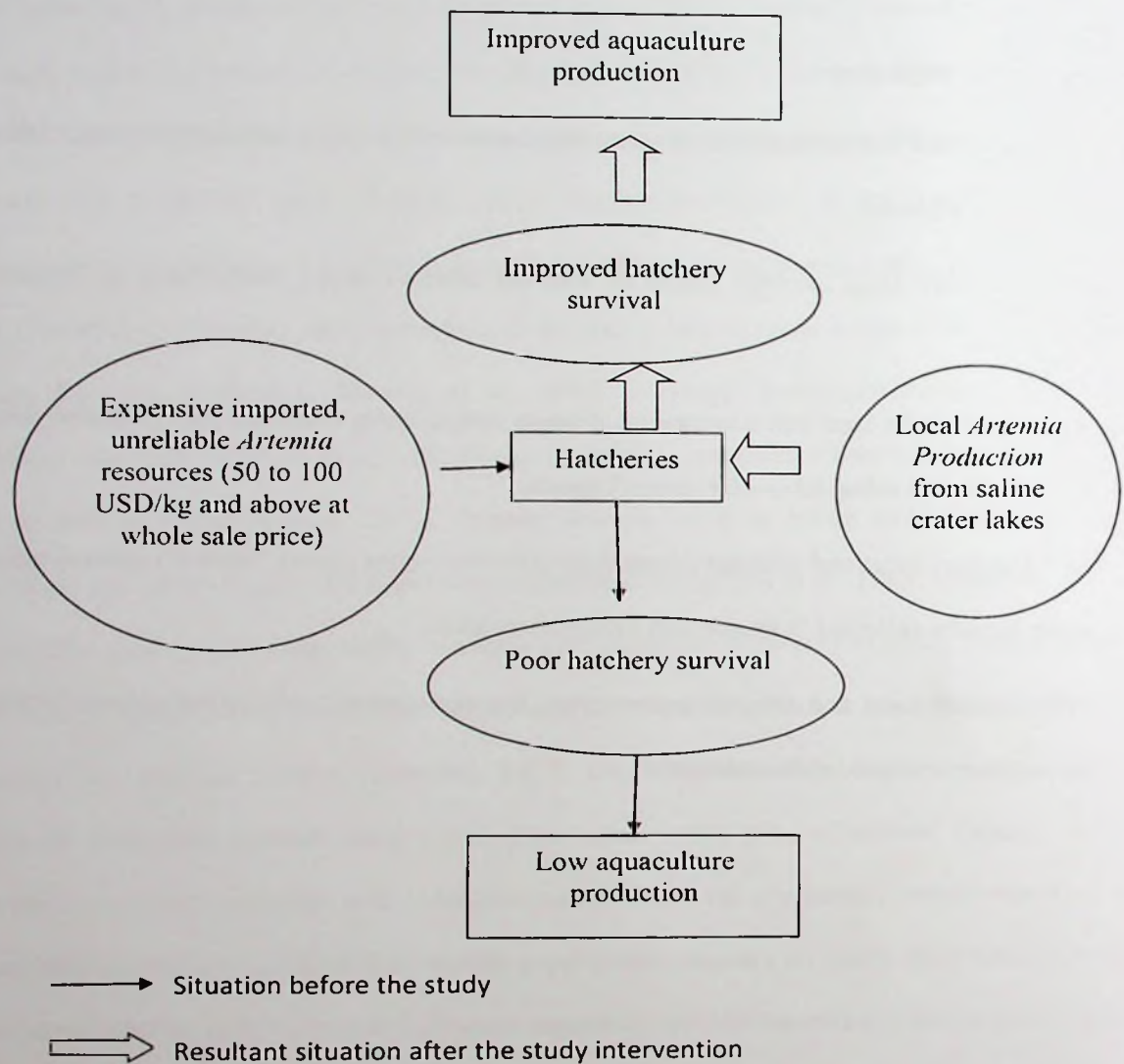


Figure 1. 1. The conceptual framework, showing the situation before the study and envisaged/resultant situation after the study intervention

1.6 *Applicability of the research findings*

Findings of this study provide useful information to guide production of *Artemia* biomass in closed systems like tanks and ponds adjacent to these hypersaline systems with preferred salinity ranges being between 60 and 80g/l.

This study shows that there are no local occurring *Artemia* strain contrary to earlier suggestion by Ogello et al (2014). However, commercial *Artemia* strains can be cultured using the saline lake waters, by bio-manipulation to remove potential *Artemia* predators through filtration, reducing salinity to preferred ranges (60 - 80g/l), by diluting with freshwater and regular mixing of the waters in *Artemia* culture ponds to improve aeration.

Artemia also plays a complex ecological role of controlling algal blooms in man-operated salt ponds. Culturing of *Artemia* in these saline craters will not only play a role in providing relatively cheap *Artemia* resources but also plays a role in cleaner salt production. *Artemia* being filter feeding organisms (Soundarapandian *et al.*, 2009), its production in the salt pans contribute to cleaner salt production. As a mechanism of acquiring food, *Artemia* filters large volumes of water, thereby trapping and extracting algae, phytoplankton plus other suspended solids from the water. The algae, phytoplanktons and suspended solids are impurities which affect the salt crystallization process and thereby affecting the quality of salt produced from the salt pans. As a result, *Artemia* production in the salt pans will be beneficial in increasing the rate of salt crystallization and better quality production. Proper management of *Artemia* population will also provide opportunity for production of valuable products, in the form of *Artemia* cysts and biomass.

1.7 *Structure of the Thesis*

This thesis comprises of eight chapters. Each chapter (except chapters 1 and 8) allows an independent publishable journal Article format i.e Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion and References.

Chapter 1 ('General Introduction'), Provides a detailed review of the background information on *Artemia* use and distribution. This chapter gives insights into Uganda's fish production from both capture and culture fisheries, the fisheries contribution to the country's foreign exchange earnings, dominant capture and culture fish species, aquaculture potential to supplement the dwindling stocks, impediments to aquaculture development in Uganda and how a reliable starter feed in form of locally produced *Artemia* can boost aquaculture production. This chapter provides a general overview of the research problem, study objectives, significance, thesis structure and organization.

Chapter 2 ('Use, Production and Assessment of Local *Artemia* resources in Uganda and Africa: A review'), contributes to the state of knowledge on how much *Artemia* is produced and used in Uganda and Africa. It also explores the existing knowledge on availability of *Artemia* resources in Uganda and Africa. This chapter points to the high prices of imported *Artemia* in Uganda and Africa as whole and how it has led to limited use of *Artemia* by hatchery operators. Since no artificial diet has been developed to replace *Artemia* as a starter feed, many farmers in Uganda and Africa experience very poor survival in hatcheries. No local *Artemia* resources were found in Uganda despite speculation of occurrence of *Artemia* resources around salt lakes in Uganda by Ogello et al (2014). The few reported *Artemia* resources in Africa are mainly found in Southern

and Northern Africa (Kaiser et al., 2006), with some *Artemia* production sites in “infancy” reported in Kenya (Ogello et al., 2014). Since no artificial diet so far has been able to completely replace *Artemia* as a starter feed, and imported *Artemia* being costly to many farmers especially smallholders, there is need to explore local *Artemia* production, so as to improve survival among hatcheries in Uganda and Africa.

Chapter 3 (‘Limnological evaluation of the alkaline, saline crater lakes of western Uganda’) addressed Objective one of this study, bringing out the physico-chemical characteristics of selected alkaline saline crater lakes of western Uganda. Other than previous study by Mungoma (1990) where the physical and chemical parameters of some of the alkaline saline crater lakes were studied, there is scanty information. Understanding of the physico-chemical characteristics of these lakes plays a central role in understanding their potential for *Artemia* production, as well as occurrence of local *Artemia* resources in these lakes. It is also useful to have a proper understanding of the physico-chemical characteristics of considered lakes, so as to guide the right bio-manipulation of the waters of these lakes for *Artemia* production. Eight selected saline crater lakes from western Uganda were surveyed with physico-chemical data of temperature, pH, conductivity, salinity, dissolved oxygen, transparency, total depth and seasonality taken *in-situ*. Water samples were collected at geo-referenced points for detailed chemical analysis in the laboratory. All the study lakes were confirmed to be alkaline and saline in nature as per previous studies, except Lake Kikorongo which was found to be totally fresh, contrary to findings by Mungoma (1990). Other than Lake Murumuli, which was found to be a carbonate-chloride lake, the rest of the selected studied lakes were found to be carbonate-sulphate lakes. Lakes Munyanyange and Nyamunuka were found to be seasonal lakes, with no major water inflows while lakes Katwe, Bagusa, Murumuli, Maseche and Bunyampaka were found to be permanent

with fresh water inflow within their catchments. Salinity of these lakes were significantly higher in the dry season. These study findings meant that lakes Kikorongo, Munyanyange and Nyamunuka were dropped from consideration for *Artemia* production.

Chapter 4 ('Plankton and Fish biodiversity of alkaline saline crater lakes of Western Uganda') investigated the zoo and phytoplankton, as well as fish occurring in these lakes. This contributes in addressing the biological component of objective one in this study. The information of the plankton and fish biodiversity of the alkaline saline lakes of western Uganda is required in the selection of the most suited lakes for *Artemia* production. Zooplankton and fish are mainly predators to *Artemia*, while phytoplanktons are either food for *Artemia*, potential sources of toxins or competitors. Prior to this study no other investigations had been done in these lakes, focusing on the plankton and fish biodiversity, yet they are important for understanding their ecosystems functioning. Rotifera zooplanktons were the only zooplankton found in Lake Katwe. Lakes Maseche and Kikorongo had both Rotifera and copepod zooplanktons. None of the studied lakes were found to have Cladocerans both in the dry and wet season samplings throughout the study period. Blue-Green algae (Cynobacteria) were found to be the dominant phytoplankton in these studied saline crater lakes, followed by green algae (Chlorophyceae). Other than Lake Kikorongo which was reported to have African catfish (*Clarias gariepinus*) during the rainy season, none of the study lakes was found to have fish during the entire study period. These findings further support the elimination of Lake Kikorongo from being considered for *Artemia* production since it has so many *Artemia* predators.

Chapter 5 ('*Artemia* Occurrence, salinity and ionic rates of saline crater lakes of Western Uganda') investigated the occurrence of both *Artemia* biomass and cysts in the five selected saline crater lakes of Western Uganda. The salinity and ionic rates of these waters in relation to suitability for *Artemia* occurrence was also examined. Findings of this chapter responded to objective two of this study. Ogello et al., (2014) alluded to the existence of *Artemia* resources on the shores of the saline crater lakes in Western Uganda. It was therefore a target to investigate if this assertion was true. If these lakes had locally occurring *Artemia* strains, then it would have been these that would be given priority for bucket and pond cultures. The salinities and ionic rates of these lakes were compared with those of sea water with an aim of further establishing which of these lakes had salinity and ionic rates close to that of sea. Lakes with salinities and ionic rates comparable to that of sea would be more preferred for *Artemia* production, since many of these *Artemia* resources have been reported to inhabit the sea shores. Prior to this study, no comprehensive study had ever been conducted to investigate occurrence of local *Artemia* resources in Uganda. Although salinities of all the study lakes were higher than that of sea both in the dry and wet season, their $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ ionic rates were found to be significantly lower than those of the sea. There was no significant difference in the $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ ionic rates in the different studied lakes. No *Artemia* cyst or biomass was found in the studied lakes during the three year of sampling period. It was based on these findings that commercially available *Artemia* strains were used in the hatchability, *ex-situ* (bucket) culture and *in-situ* (pond) culture experiments.

Chapter 6 ('Hatchability of selected commercial *Artemia* strains using waters from the five (5) selected saline crater lakes of Western Uganda') examined the ability of waters from alkaline,

saline crater lakes of western Uganda to break *Artemia* diapause to cause hatching. The findings of this chapter addressed objective three of this study. The ability of these bio-manipulated waters to break *Artemia* diapauses and cause hatching is an indication that these waters can offer conducive conditions for *Artemia* survival. *Artemia* hatchability experiments were set using different selected commercial strains. The strains which showed the highest hatchability was considered the more suited for production, using waters from the saline crater lakes. It is the best performing *Artemia* strain in the hatchability experiments which were used in the bucket (*ex-situ*) and Pond (*in-situ*) culture experiments. Waters from lakes Katwe, Bunyampaka, Maseche, and Bagusa were able to break diapauses in *Artemia franciscana* from Great Salt Lake (GSL), *A. franciscana* from salt ponds in Vinh Chau (VC), Chinese strain (Chinese), TUZ Parthenogenetic *Artemia* from Kazakhstan (TUZ) and Parthenogenetic *Artemia* strain from Siberia (PAS) (commercial strain). *Artemia franciscana* (VC) had significantly higher hatching percentage than all tested strains ($p < 0.05$). This was followed by *A. franciscana* (GSL) from the Great Salt Lake. Lakes Katwe, Bunyampaka and Maseche waters presented the highest hatching percentages, therefore were best suited for *Artemia* production. It is based on these finding that VC and GSL strains were selected for bucket and pond cultures, using Lake Katwe waters.

Chapter 7 ('Performance of Selected *Artemia* strains under Bucket (*ex-situ*) and Pond (*in-situ*) culture using saline waters from Lake Katwe') explores the production of *Artemia* in buckets (*ex-situ*) and ponds (*in-situ*) using bio-manipulated waters from alkaline, saline crater lakes in Western Uganda. This chapter investigated the performance of selected commercial *Artemia* strains at different salinities in buckets and pond cultures using waters from alkaline, saline crater lakes. It is of paramount importance that before inoculating *Artemia* in natural systems, the

optimal salinity range which is a key limiting physical parameter for *Artemia* production under controlled conditions (Kaiser, et al., 2006) is determined. The bucket cultures at different salinity ranges (40, 60, 80 and 100g/l) were used to examine the optimal salinity ranges for culturing the selected commercial *Artemia* strains using waters from saline crater lakes of western Uganda. The observed optimal salinity ranges where the selected commercial *Artemia* strains had the highest survival and growth performance were the one adopted for the pond (in-situ) cultures. The findings from the bucket culture experiments responded to objective 4 while pond cultures findings responded to objective five of the study. This chapter therefore seeks to establish possibility of culturing selected commercial *Artemia* strains by bio-manipulation of the waters from alkaline, saline crater lakes of Western Uganda. Bio-manipulation was done to maintain the preferred salinities, as well as removing potential predators while maintaining potential *Artemia* food organisms. Under bucket cultures (ex-situ) both GSL and VC strains were able to survive for an eight-day culture period at salinities of 40g/l and 60g/l. Although it had very low survivals at salinities of 80g/l and 100g/l in day 6 and 8 of culturing, GSL had survivals in all treatments throughout the entire 8 day culture period. Whereas *A. franciscana*, Vinh chau (VC) strain showed significantly high growth performance at salinity of 40g/l, with growth performance decreasing with increasing salinity, GSL strain had no significant difference in growth performance across the different treatments. Under pond cultures, both GSL and VC strains were able to survive upto day 10 at salinity treatments of 60 and 80g/l. Although the GSL nauplii was significantly longer than the VC nauplii at the start in the treatment of 60g/l, no significant difference was observed in the nauplii length at day 2, 4 and 6. In the treatment of 80g/l, there was no significant difference in *Artemia* length of both strains until day 8 and 10, where GSL nauplii were found to be significantly longer. Survival for both *Artemia* strains decreased with

increasing culture period in all treatments with none of the two strains being able to survive longer than 10 days after inoculation in culture ponds thereby being unable to attain reproductive maturity. These findings showed that it was possible to culture both GSI and VC *A. franciscana* strains in salt ponds adjacent to Lake Katwe using waters from Lake Katwe and Kyabamba fresh water spring. The best *Artemia* performance was realized at salinity of 60g/l. More research trials are recommended to establish the hindrances to *Artemia* strains attaining reproductive maturity and encysting if commercial production is to be done successfully using waters from these alkaline saline crater lakes.

Chapter 8 synthesizes the results in all the chapters and discusses their implications for *Artemia* production in the alkaline saline crater lakes of Western Uganda. It also includes conclusions and future recommendations on the development of *Artemia* production.

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CHAPTER TWO

Use, Production and Assessment of Local *Artemia* resources in Uganda and Africa: A review

2.0 Abstract

Literature on existing *Artemia* habitats, their use and production in Uganda and Africa as a whole was reviewed. In view of *Artemia*'s role as a starter feed in the aquaculture industry, its scarcity and high cost (US\$ 50-100 per kg), there is need for commercial development and exploitation of local *Artemia* resources. In this study, a desk review of literature was conducted, and in addition several potential *Artemia* sites visited and surveyed in Uganda. This study revealed neither local occurring *Artemia* resources nor its commercial production exists in Uganda and no documentation of use. The limited or lack of use of *Artemia* resources is possibly due to it being expensive with its use in Africa generally restricted to the few commercial fish hatcheries. Literature pointed to North African countries bordering the Mediterranean Sea to be leading in the number of *Artemia* sites and production in Africa. This is attributed to the high effort in the identification of *Artemia* resources in this region.

Key words; *Artemia* use; live feeds; *Artemia* production sites in Uganda and Africa

2.1 Introduction

Artemia (Crustacea; Anostraca) typically inhabit hypersaline environments (Gajardo, Parraguéz, Beardmore & Sorgeloos, 2001; Kaiser, Gordon, & Paulet, 2006; Ogello, Kembenya, Githukia, Nyenje, & Munguti, 2014). Although Schlosser is reported to have the first description of *Artemia* in salt pans of Lymington, Hampshire (England) in 1755 (Kuenen and Becking, 1938;

Asem. 2008), records in Lake Urmia date back to 982 by unknown Iranian author (Asem, 2008; Ben Naceur, Jenhani, & Romdhane, 2012; Kara & Amarouayache 2012). Mankind has known *Artemia* for centuries with native Amerindian and Lybians reported to have been using it as food ages ago (Asem, 2008). It is a primitive arthropod having a segmented body, with leaf-like appendages known as thoracopods. The adult varies in length between 8-10mm and 10-12mm for males and females respectively. Both males and females have an approximate width of about 4mm (FAO 2015). *Artemia* is reported to have bisexual and parthenogenetic populations that are similar morphologically and biometrically (Triantaphyllidis, Abatzopoulos & Sorgeloos, 1998; Ben Naceur, Jenhani, & Romdhane, 2009; Munoz and Pacios, 2010; Castro-Mejía et al., 2011). Bisexual *Artemia* populations include *Artemia franciscana* and *Artemia persimilis* which have been classified as the new world *Artemia* populations and are usually diploid while their parthenogenetic relatives (*A. salina*, *A. sinica*, *A. urmiana* and *A. tibetiana*) which are polyploids are referred to as the Old World populations (Triantaphyllidis, Criel, Abatzopoulos & Sorgeloos, 1997; Baxevanis, Kappas, & Abatzopoulos, 2006; Maccari, Amat, & Go'mez, 2013). Bisexual populations are found on all continents, except the Antarctica while parthenogenetic populations are endemic to Europe, Asia and Australia (Triantaphyllidis et al., 1997). Reproduction in *Artemia* is by either ovoviviparity, where eggs hatch into live young ones within the body before being released or oviparity where metabolism and embryonic development in the embryo are arrested to form diapausing cysts (Ben Naceur et al., 2009; Sugumar 2010). The latter may be a crucial strategy for surviving unfavorable environment conditions, where the cysts remain in this stage of arrested metabolism but remain viable for many years (Papeschi, Alba, Paula, Amat, & Rosa, 2008). Different conditions have been reported to be responsible for the switch between ovoviviparity and oviparity.

Artemia has been reported to have a wide geographical distribution (Kaiser et al., 2006; Ben Naceur et al., 2009), occurring in all continents with the exception of the Antarctica (Papeschi et al., 2008; Ben Naceur et al., 2009; Ben Naceur et al., 2012). Over 600 habitats of *Artemia* populations have been reported world-wide in natural salt water bodies and man-made salterns (Van Stappen 2002; Ogello et al., 2014). The distribution of these populations are reportedly reflected in the paths of some migratory birds, as well as intentional inoculations by man for commercial purposes (Ben Naceur et al., 2009; Munoz, Green, Figuerola, & G'omez, 2013). It is adapted to survive and live under hypersaline conditions in salt lakes, ponds, coastal salt lagoons, pans and solar saltworks (Kaiser et al., 2006; Ben Naceur et al., 2009; Ogello et al., 2014). They have been reportedly found in athalassohaline and thalassohaline environments, with salinities as high as 340g/l (Ben Naceur et al., 2009; Ogello et al., 2014). *Artemia* is reported to withstand stressful conditions imposed by hypersaline environments such as fluctuating temperatures, salinity, and ionic composition (Gajardo and Beardmore 2012; Ogello et al., 2014). Being highly osmotolerant (Ogello et al., 2014) and the ability to have an interchangeable life cycle which can switch between diapausing cysts and live nauplii depending on environmental conditions are some of the key adaptations that enable it to withstand stressful conditions (Kaiser et al., 2006; Ben Naceur et al., 2009; Gajardo & Beardmore 2012). Salinity has been reported as the main environmental factor affecting its distribution (Vanhaecke, Tackaert, & Sorgeloos, 1987; Kaiser et al., 2006), with other factors such as temperature, light intensity, food availability seeming to be more responsible its numbers and densities (Vanhaecke et al., 1987).

Artemia is among the most widely used live feed for larviculture of fish and shell fish (Sorgeloos, Dhert & Candreva, 1991; Philips 2010), with over 2000 metric tons of dry *Artemia* cysts produced and marketed world-wide annually (FAO 1996; Triantaphyllidis, Abatzopoulos, Miasa & Sorgeloos, 1996; Ogello et al., 2014). Despite man having known *Artemia* for centuries, its use as food for larvae in the aquaculture industry began only in the 1930s when it was discovered to be nutritionally rich food for new hatched larvae (Bengtson, Leger, & Sorgeloos, 1991; FAO 1996; Ogello et al., 2014). Since then, *Artemia* has been collected from natural saline lakes and coastal salt works. As a result of the rapid expansion of the aquaculture production more especially in 1970's, the demand for *Artemia* exponentially increased, exceeding production (FAO 1996; Sorgeloos et al., 1991). Since then, limited supply of *Artemia* has become a big impediment to the expansion of the aquaculture (Sorgeloos et., 1991; FAO 1996; Coutteau & Sorgeloos 1997; Maung, Bu, & Htun, 2008), with many countries importing *Artemia* at prices ranging between US\$ 50-100 per kg (Jaccarini & Martens 1990; Bengtson et al., 1991). Annually, its production has been reported to fluctuate, hence affecting market prices, as well as raising hatchery production costs (Maung et al., 2008; Schwartz 2008).

Rotifers and *Artemia* are among the extensively used zooplanktons for larval rearing both in marine and freshwater aquaculture (Bengtson et al., 1991; Akbary, Hosseini. & Imanpoor, 2011; Rathod et al., 2014). Availability as an off-the-shelf feed in form of dormant cysts is one of the key advantages of *Artemia* over other live feeds (Leger, Bengtson, Sorgeloos, Simpson, & Beck, 1987; Akbary et al., 2011). *Artemia* is easy to hatch (Tarekegn 2015), tolerant to unfavorable environmental changes, with a wide size range and different physical forms (Leger et al., 1987). If well packaged, it can stay viable for so many years as cysts (Bengtson et., 1991; Ogello et., 2014). All these render it more versatile and preferable in comparison to other live feeds. Other

competitive advantages of *Artemia* use are the high nutritional value for fish larvae and the ease in improvement of its nutritional value through enrichment techniques (Akbari et al., 2011). Through bioencapsulation *Artemia* is used as a route for transferring essential nutrients including ascorbic acid (Immanuel, Citarasu, Sivaram, Shankar, & Palavesam, 2007; Akbari et al., 2011), probiotics (Arndt and Wagner 2007; Seenivasan, Saravana. Bhavan, Radhakrishnan & Shanthi, 2012), as well as spawning hormone (Ogello et al., 2014), medication like anti-biotics (Nkambo, Bugenyi, & Nayiga, 2015) and other feed supplements like Eicosapentaenoic acid (EPA) and decosahexanoic acid (DHA) to the fish (Han, Geurden, & Sorgeloos, 2000). Ascorbic acids are necessary in the synthesis of collagen required in formation of connective tissue and bone matrix (Dabrowski & Blom 1994). *Artemia* production in salt ponds has also been documented to play a vital role in controlling algal blooms and thereby improving the salt crystallization process as well as leading to better salt quality (Ogello et al., 2014). *Artemia* is currently one of the model organisms which have been put to use in a number of environmental eco-toxicology, evolutionary and genetic studies (Baxevanis, Kappas, & Abatzopoulos, 2006; Ben Naceur, Jenhani, El Cafsi, & Romdhane, 2008; Munoz & Pacios 2010; Ogello et al., 2014).

Very little is known about *Artemia* use, production and occurrence in Uganda and the African continent. In addition to review of literature, exploratory visits were made to potential *Artemia* sites in the alkaline saline crater lakes of Western Uganda with an aim of determining its occurrence, production potential, and use.

2.2 Potential *Artemia* sites in Uganda

Other than the reported discovery of a few *Artemia* samples on the shore of a salt lake in Western Uganda in June, 2008 by Prof. Bill Daniels and Dr. Ron Phelps of the Department of Fisheries

and Allied Aquacultures, Auburn University, Alabama-USA, and claims of the occurrence of *Artemia* in some saline crater lakes of Uganda by Ogello et al., 2014, there is no literature available on its occurrence in Uganda. A feasibility study funded by the Ministry of Finance, Planning and Economic Development (MoFPED) in 2010/11 reported lesser flamingo which are reported *Artemia* dispersal agents (Childress, Nagy and Hughes, 2007) to be frequent visitors of these saline crater lakes in Western Uganda. Also *Spirulina*, potential food for *Artemia* was reported to be present in all the considered lakes in the feasibility study of 2010/11. This gave an indication that *Artemia* resources might be available in these lakes in some seasons much as it was not found under the feasibility study (Unpublished data). It was on the basis of this information that saline lakes of western Uganda were considered potential *Artemia* sites. The study feasibility financed by MoFPED in 2010/11 provided preliminary information to this study. The surveyed lakes included lakes Katwe, Munyanyange, Maseche, Kikorongo, Nyamunuka, Nshweera, Murumuri, Bunyampaka and Bagusa (Fig 2.2.1). The visited and surveyed lakes were chosen because of their high salinities, since *Artemia* is known to inhabit hypersaline environments.



Figure 2.2. 1; Map showing the location of saline crater Lakes Katwe, Munyanyange, Nyamunuka, Banyampaka, Kikorongo, Bagusa and Maseche visited for potential *Artemia* sites.

2.3 Status and Implications

The limited literature highlighting the use of *Artemia* in Ugandan and in African hatcheries could be attributed to the small number (three) of commercial hatcheries in Uganda with a majority of them still dependant on un-standardised technologies and practices (Mwanja, Rutaisire, Ondhoro, Ddungu & Aruho, 2015). Only 31% of the Ugandan hatchery operators have been reported to use imported *Artemia* alone in weaning fish larvae (Mwanja et al., 2015). The small percentage of farmers using *Artemia* is due to its high cost (US\$ 200 per kg), FAO (2011). The lack of literature on the use of *Artemia* in the other East African countries may be due to aquaculture being small scale and rural with no commercial hatcheries as was reported in Tanzania (Chenyambuga, Mwandya, Lamtane, & Madalla. 2014), and hence no need for *Artemia* resources. In Tanzania, aquaculture is mainly a subsistence activity for the rural poor households

in some coastal and inland areas (Mallya 2007). These small scale farmers commonly depend on green water cultures for plankton production and rely on simple hatcheries that do not require *Artemia* resources. Fry production demands for such small scale hatcheries are not as high, and therefore, even with poor hatchery survival they can still have enough fingerlings for the subsistence demand.

This study showed that Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) are the leading aquaculture species in Africa with Egypt and Nigeria having the highest number of private commercial hatcheries. However, the majority of hatcheries lack accreditation and certification systems and therefore unregulated (Brummett 2007). Uganda and other African countries, like Cameroon, Ghana and Zimbabwe were reported to be mainly dependent on semi-commercial systems for seed production (Brummett 2007). This is in agreement with finding from Mwanja et al. (2015) where the majority of Ugandan hatcheries were reported to be weaning fish larvae on natural feeds or a combination of natural feeds with dry diets, hence the low use of *Artemia* in Ugandan hatcheries. Many small hatcheries in Nigeria have been reported to be weaning and culture catfish larvae to fingerling stage using green water cultures (Adewumi & Olaleye, 2011). Micro-algae and rotifers have been reported to be the main targeted feed from green water cultures. The choice of rotifers for weaning fish larvae was reported to have an advantage of low cost, albeit with the difficulty to maintain pure cultures as the main disadvantage (Francis, 2007). Commercial hatcheries being generally few, coupled with lack of regulation, accreditation, and certification systems in Africa; this makes information about hatchery practices to be very scanty.

Unlike in other continents where its populations have been well studied, reports documenting local *Artemia* resources in Africa are scarce, with little work done on characterisation of the known local *Artemia* resources (Triantaphyllidis et al., 1996; Triantaphyllidis et al., 1998). Although *Artemia* population have been reported to be found in natural salt lakes and man-made salterns in the tropical, sub-tropical and temperate climatic zones (FAO 1996), they were not found in the studied lakes. Previous unpublished findings from a short study funded by the Belgian Development Cooperation (BTC) in 2010 where lakes Katwe, Bunyampaka, Kikorongo, Murumuri and Bagusa were surveyed, indicated that these lakes had no local *Artemia* resources but with huge potential for its production (Nkambo et al., un-published). Other than the speculations made about the existence of *Artemia* cysts on the shores of salt lakes in Western Uganda (Ogello et al., 2014), there is no literature confirming its existence in Uganda. This could be attributed to the fact that with the exception of a 2 day exploratory visits done in 2008 by Prof. Patrick Sorgeloos of *Artemia* Reference Center (ARC), Ghent University in Belgium and the short feasibility study which was funded by BTC in 2010, there have been no efforts directed towards identifying *Artemia* resources in Uganda.

Due to favorable environment like the hypersaline lakes and salt lagoons, most African countries have been reported to have potential habitats for *Artemia* (Ogello et al., 2014) and therefore potential for its production. The majority of *Artemia* populations in Africa have been collected in countries with a coastline including Algeria, Egypt, Libya, Morocco (Kara and Amarouayache 2012) and Tunisia (Triantaphyllidis et al., 1998; Ben Naceur et al., 2008). Other African countries where *Artemia* resources have been identified include Kenya (Munoz and Pacios 2010), Madagascar, Mozambique, Namibia, Nigeria, Niger, Senegal, and South Africa

(Triantaphyllidis et al., 2006). Most African *Artemia* habitats have been reported in countries bordering the Mediterranean region, with Tunisia reported to have 23 sites (Ben Naceur et al., 2012), and this has been attributed to the high effort in the identification of *Artemia* resources in this region (Kaiser et al., 2006). In 2010 Africa was reported to have a total of 92 *Artemia* localities, 57 of which were undescribed, with unknown reproductive mode, 8 and 5 of which were undescribed parthenogenetic and sexual *Artemia* populations respectively (Munoz & Pacios, 2010). The 92 localities in Africa compared to the 172 in Europe is a small number, bearing in mind that Africa is several times bigger than Europe and with a more favorable climate. This difference points to the gap that needs to be filled by increasing research efforts toward identifying more *Artemia* resources in Africa.

Although very few studies have been directed towards reproductive characterization of *Artemia* strains in Africa, both parthenogenetic and sexually reproducing populations have been reported (Triantaphyllidis et al., 1998; Kaiser et al., 2006; Munoz & Pacios, 2010). It should be noted that a good number of *Artemia* resources so far identified in these African countries are not local but were rather intentionally or accidentally introduced (Munoz & Pacios, 2010), with some others being introduced by agents of dispersal like the migratory birds (Triantaphyllidis et al., 1998; Munoz et al., 2013). One of the well documented example is the Kenyan *Artemia franciscana* which was introduced between 1984 and 1986 in a collaborative project between Kenya Marine & Fisheries Research Institute (KMFRI) and the Belgian Agency for Development Cooperation (BADC) with an aim of assessing the potential for *Artemia* production in the Kenyan coastal salt works (Radull et al., 1992; Mremi 2011; Ogello et al., 2014). *Artemia franciscana* is now reported to have spread along the Kenyan coast probably by agents of dispersal and has

permanently established (Munguti, Kim, & Ogello, 2014; Ogello et al., 2014). Eight salt companies are reported to have *Artemia* inoculation policies with production in commercial and artisanal salt ponds already on-going (Ogello et al., 2014). *Artemia* cysts genetically similar to Kenyan *A. franciscana* populations have been reported at Tanga on the Tanzanian coast (Ogello et al., 2014). These might have been dispersed from the Kenyan coast but no literature pointing to *Artemia* production in Tanzania was found in this present study. Another example of intentional introductions are those of sexual *A. franciscana* used as live feed in aquaculture, which was introduced in the Mediterranean basin and have been reported to be quickly replacing the native species (Amat, Hontoria, Navarro, Viera, & Mura, 2007; Kara & Amarouayache 2012). Since success of invasion and impact depends on competitiveness and trophic interaction in the environment (Amat et al., 2007), from biodiversity conservation perspective it is important that local *Artemia* resources are developed. It is important to note that in all the reviewed literature very little is hinted about commercial *Artemia* production in Africa.

2.4 Conclusions

There is generally very little information on *Artemia* in Uganda and Africa. Much as the distribution of *Artemia* is well studied in many countries, especially Europe, America and Australia, much less is known about *Artemia*, its use, production and distribution in Africa. Even among the identified local African *Artemia* resources, the majority is not yet developed to allow for commercial exploitation. Despite the urgent need for affordable *Artemia* resources for use in Ugandan and African aquaculture, no commercial *Artemia* production sites were found in Africa. This situation justifies the pressing need for documentation of *Artemia* use, exploitation and development in Africa.

2.5 Recommendations

For sustainable Aquaculture development in Uganda and Africa as a whole, more effort in form of research and development need to be directed towards identifying, establishing and development of local *Artemia* resources. This will allow for improved performance of Uganda and African hatcheries, hence addressing the current shortages in quality seed supply caused by lack of a suitable live starter feed.

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CHAPTER THREE

Limnological evaluation of the alkaline saline crater lakes of western Uganda

3.0 Abstract

Eight alkaline, saline crater lakes in western Uganda were investigated between 26th February and 3rd March, 2014, with an aim of determining their physico-chemical conditions for comparison with similar environments in other parts of the world having *Artemia* production. The lakes studied were: Katwe, Kikorongo, Murumuli, Nyamunuka, Munyange, Bunyampaka, Bagusa and Maseche. Surface and Bottom measurements of physico-chemical parameters were taken *in-situ* from at least three geo-referenced points in each lake. Water samples for alkalinity, nutrients and detailed ionic composition analysis were taken from each of the sampled points. In the laboratory, the collected water samples were analysed following APHA standard operating procedures (APHA, 1990). Apart from Lake Kikokongoro with salinity of 0.00mg/l, all the studied lakes were alkaline (pH 9.58 ± 0.1 to 11.5 ± 1.0 , alkalinity ranged from 12534.4 ± 1.6 mg/l in Lake Kikorongo to 110205.0 ± 7.1 mg/l in Lake Katwe) and saline (92.3 ± 7.6 g/l in lake Maseche to 205.0 ± 15.3 g/l in lake Nyamunuka) in nature. With the exception of Lake Murumuli which was found to be a Carbonate-chloride lake, the rest of the sampled lakes were carbonate-sulphate, contrary to earlier studies by Mungoma (1990) which reported lakes like Katwe and Kikorongo to be Carbonate-chloride. The very high salinity and carbonate-sulphate dominance in these lakes makes them potential *Artemia* habitats.

Key words: Alkaline, saline, carbonate-chloride, carbonate-sulphate, lakes

3.1 Introduction

The western part of Uganda is richly endowed with crater lakes, many of which are alkaline saline. They are small (surface area 1 – 3km²) unique water bodies occurring in the basin associated with the western Rift valley. These lakes have unique physical and chemical characteristics in comparison to the big fresh water systems like Lakes Victoria, Edward, Albert, Kyoga and George, Lakes Katwe, Munyanyange, Nyamunuka, Bagusa, Murumuri, Maseche, Bunyampaka and Kikorongo, all of which belong to the Katwe-Kikorongo volcanic system in western Uganda were the only lakes considered in this study. Lake Katwe with an average area of 2.5km² is the largest among these lakes (Kirabira, Kasedde, & Semukuuttu, 2013; Nixon; Nixon, Morton, & von Knorring, 1971). This area is characterized by volcanic soils, hot and dry climate and a bimodal rainfall pattern. March to May and September to November are the rain seasons in this area. The area receives a mean annual rainfall range of 60 – 70cm with mean monthly temperatures ranging between 19.0±2.8°C and 28.0±3.2°C. Savannah grassland and dry woodland mosaic are the dominant vegetation of Katwe-Kikorongo Volcanic system (Mungoma, 1990). These lakes exhibit considerable temporal variations in volume and surface area. Their total depth range between less than 1 to 6m (Kirabira et al., 2013). Stable alkaline conditions in previously studied alkaline systems have been attributed to a unique combination of climate, geological and topographical conditions (Grant, 2006). These lakes have been categorized by earlier studies into three groups; carbonate-chloride, sulphate-chloride or chloride, depending on the dominant anion(s) (Mungoma, 1990; Camargo et al., 2005). Matagi (2004) used soda lakes to refer to highly alkaline hypersaline environments and these have been described as the earth's most stable high pH environments (Grant, 2006). These lakes usually have pH above 9.6 (Grant, 2006; Matagi, 2004) and are associated with low Mg²⁺ and Ca²⁺ content with evaporation rates

as high as 2,160mm/year (Kasedde, 2016). In some cases, the evaporation rates often exceed water inflow rates (Grant, 2006), therefore they are susceptible to drying in extreme dry seasons. Other areas other than the East African Rift Valley where such environments occur include arid and semi-arid areas of tropical and/or subtropical rain-shadow deserts like in North America and continental interiors of Asia (Grant, 2006). The ionic composition and concentration in these lakes plays an integral role in determining their limnochemical characteristics and resource exploitation by the surrounding communities. Lakes Katwe and Bunyampaka are the only lakes among these alkaline saline lakes where commercial common salt (NaCl) extraction (Matagi, 2004) occurs. The majority of the other alkaline, saline lakes have not been commercial exploited.

In comparison to fresh water systems and other inland water bodies, the limnology and hydrobiology of saline systems have not been given as much attention (Stenger-Kovács et al., 2014). Despite the vast research done on these lakes which dates back from Beadle (1932, 1974), Holmes & Harwood (1932), von Knorring & DuBois (1961), Arad & Morton (1969), Kilham (1971a, b), Melack & Kilham (1972), Hecky and Kilham (1973), Melack (1976, 1978), Melack et al., (1982) and so on, none of these research focused on the limnology of these lakes. It was therefore the aim of this study to conduct a limnological examination of selected alkaline, saline crater lakes in western Uganda to provide information on the physico-chemical features of these less known unique habitats and provide a basis for comparison with similar environments in other parts of the world.

3.2 Materials and Methods

3.2.1 Study Area



Figure 3.2. 1; Map showing the selected alkaline saline crater lakes of Western Uganda considered in this study, and inset, location map of Uganda.

Table 3.2. 1: GPS location and hydrological characteristics of the studied lakes

Lakes	Coordinates		Altitude (H a.s.l)(m)	lake Surface Area (km ²)	Average Depth (mean±SD) (m)
	Long. E	Lat. N			
Katwe	029.87033°E	00.13217°S	897	2.45	2.1±0.7
Munyanyange	029.88591°E	00.13513°S	915		0.2±0.0
Nyamunuka	029.98743°E	00.09344°S	929		0.2±0.0
Murumuri	029.99186°E	00.07323°S	887		0.2±0.0
Bunyampaka	030.12819°E	00.03765°S	893	0.43	0.20±0.1
Bagusa	030.17958°E	00.09793°S	884		1.9±0.5
Maseche	030.19019°E	00.09355°S	918		1.3±0.2
Kikorongo	030.01228°E	00.01190°S	939	1.03	2.3±0.3

3.2.2 General assessment of the lake environments

A Global Positioning System (GPS) unit (GARMIN 12XL) was used to determine coordinates and the altitude / elevation (m) above sea level (H. a.s.l) of the different points of sample collection in each of the sampled lake. Visual observations of water color, number of fresh water inflows, and percent vegetation cover were made at each of the studied lake. Fringing vegetation in the littoral zone around each of the studied lake was characterized and the major plant species identified, where possible. All these parameters were examined at the beginning and end of both the wet and dry season for two years.

3.2.3 Determination of physico-chemical parameters

At each lake, selected physical and chemical parameters (depth (m), temperature (°C), dissolved oxygen concentration (DO, mg/l), pH, Conductivity (in $\mu\text{S.cm}^{-1}$) and salinity (mg/l) were measured *in situ* from geo-referenced sampling points from the surface (0.5m), 2m and 5m where possible. Six (6) to eight (8) sampling points were set on each lake depending on the surface area. These were measured twice in the wet and dry season for a two year sampling regime with *in-situ* measurement and water samples taken between 09:00am and 03:00pm on each day of sampling. Water temperature (°C), dissolved oxygen concentration and conductivity were measured using a YSI oxygen/temperature/conductivity meter (Model YSI 550A), pH was determined using an OAKTON pH Tester 30, while salinity was measured with a refractometer. From each of the selected sampling points, depth (m) was determined using a portable depth finder (Hondex PS-7). The depth finder was lowered just below the water surface, while pointing to the lake bottom, the button was pushed and the depth reading was taken from the backlit screen.

Surface water samples for chemical and nutrient characteristics of each of the studied lakes were collected using a Van Dorn water sampler from geo-referenced sampling points. These were stored in pre-washed and pre-rinsed (distilled water) Nalgene bottles for analysis in the laboratory. In the field all water samples in pre-rinsed Nalgene bottles were kept in a cooler box containing dry ice and later transferred to the National Fisheries Resource Research Institute (NaFIRRI) laboratory in Jinja where the water samples were analysed for the following parameters: alkalinity, bicarbonate (HCO_3^-), Carbonate (CO_3^{2-}), Sulphate (SO_4^{2-}), Silicates (SiO_2), Chloride (Cl^-), Fluoride (F), Sodium (Na), Magnesium (Mg), Calcium (Ca), Potassium (K), and Hydrogen Sulphide (H_2S), Sodium, Magnesium, Calcium, Potassium, Chloride (Cl), Fluoride (F), and silicon following Standard Operating Procedures APHA, 1990.

3.2.4 Determination of Nutrients concentrations

Water samples for dissolved nutrients; soluble reactive phosphorus (SRP), ammonia-nitrogen ($\text{NH}_3\text{-N}$) and nitrite-nitrogen ($\text{NO}_2\text{-N}$) were filtered through 47mm pore Whatman GF/C filter papers and analysed by spectrophotometric methods following procedures by Stantoin *et al.*, 1977. Water samples were also analysed for total suspended solids (TSS).

3.3 Results and Discussion

3.3.1 General environmental characteristics of selected alkaline, saline lakes

Lakes Munyanyange, Murumuri and Nyamunuka were reported by the locals to be seasonal lakes and sometimes evaporating to dryness during the dry seasons. Lakes Katwe, Bunyampaka, Bagusa and Maseche are permanent lakes but vary in water volumes between the dry and wet seasons reported by the locals (Table 3.3.1).

Table 3.3. 1: Selected environmental characteristics of the selected alkaline, saline Lakes of Western Uganda

Lake	General environment characteristics			
	Seasonality	Waters	% Vegetation cover	Fringing vegetation
Katwe	Permanent but experience variations in water volume	Thick, slimy & Peanut soup green in color	35	Acacia woodland, <i>Euphorbia candelabra</i> , savanna grassland and <i>Cyperus lavigatus</i> on the shoreline
Munyanyange	Seasonal, sometimes evaporates to dryness in dry season	Slimy & green in color	45	Savannah grassland, scattered thorny shrubs, <i>Euphorbia candelabra</i> , <i>Cyperus lavigatus</i>
Nyamunuka	Seasonal, sometimes evaporates to dryness in dry season	Thick slimy & Dark green in color	80	Savannah grassland Woodland and scattered shrubs
Murumuli	Seasonal, sometimes evaporates to dryness in dry season	Thick slimy & Dark green in color	90	Acacia woodland
Bunyampaka	Permanent but experience variations in water volume	Thick slimy & Peanut soup green	60	Woodland dominated by <i>Euphorbia candelabra</i> . Other plant species include: <i>Acacia siberiana</i> , wild palms, <i>Caparis tomentosa</i> . Shoreline has wetland dominated with <i>Cyperus lavigatus</i> and wild palms.
Bagusa	Permanent but experience variations in water volume	Thick, slimy, Pinkish on shore and Reddish-brown offshore	80	Grassland, woodland, wild palms. Shoreline dominated by <i>Cyperus lavigatus</i>
Maseche	Permanent but experience variations in water volume	Thick slimy & Peanut soup green in color	75	Grassland, woodland, wild palms. Shoreline dominated by <i>Cyperus lavigatus</i>
Kikorongo	Permanent but experience variations in water volume	greenish in color	80	Grassland, shrubs and woodland.

Lakes Katwe, Bunyampaka, and Maseche waters were found to be pea-nut soup green in color (Figure 3.3.1) while Lakes Nyamunuka and Murumuri waters were dark green in color. Lakes

Kikorongo and Munyanyange waters were greenish in color. Lake Bagusa waters were pink along the shore and reddish brown offshore (Figure 3.3.2).



Figure 3.3. 1: *Pea-nut soup green waters from Lake Katwe*



Figure 3.3. 2; *Cyperus lavigatus* with the Pinkish waters near the shores of Lake Bagusa which turns reddish brown towards the middle of the lake.

Lakes Katwe and Munyanyange had the lowest vegetation cover, estimated to be 35% and 45% respectively. This was followed by Lake Bunyampaka, with an estimated vegetation cover of 60%. The rest of the other studied lakes had vegetation cover estimates of 75% and above (Table 3.3.1). Lakes Katwe, Bagusa and Maseche were found to have freshwater wetlands surrounding them in some parts. *Cyperus lavigatus*, savannah woodland dominated by *Acacia* woodland and *Euphorbia candelabra* were the dominant vegetation surrounding these lakes.

3.3.2 Physical characteristics of selected alkaline, saline lakes

Table 3.3. 2: Mean±SD of in-situ measured of selected physical characteristics of the study alkaline, saline crater lakes of Western Uganda

Lake	Mean ±SD					
	Depth (m)	D.O (mg/l)	pH(-)	Temp. (°C)	Salinity(g/l)	Cond (µs/cm)
Katwe	2.1±0.7	2.6±0.2	9.9±0.1	27.9±0.3	180±67.8	104.5±6.4
Munyanyange	0.2±0.1	1.7±0.4	10.8±0.4	34.4±2.4	101.0±7.1	59.7±8.2
Nyamunuka	0.2±0.2	2.6±0.3	11.5±1.0	30.5±3.1	205.0±15.3	100.5±0.6
Murumuri	0.2±0.0	1.7±0.5	11.1±1.3	32.0±0.8	162.8±34.2	106.3±3.5
Bunyampaka	0.20±0.1	2.20±0.6	9.58±0.1	30.33±1.5	199.50±16.4	103.90±4.3
Bagusa	1.9±0.5	3.2±0.8	10.5±0.4	32.1±2.0	199.5±16.4	103.9±4.3
Maseche	1.3±0.2	2.9±0.4	10.9±0.4	30.0±0.7	92.3±7.6	71.2±1.3
Kikorongo	2.3±0.3	6.0±1.0	10.4±0.0	28.9±0.4	0.0±0.0	18.6±0.1

With the exception of Kikorongo and Katwe whose average depth was above 2m, the rest of the sampled lakes were found to have an average depth less than 2m. The highest measured dissolved oxygen (DO) was 6.0±1.0mg/l in Lake Kikorongo, followed by lakes Bagusa and Maseche which had D.O levels of 3.2±0.8 and 2.9±0.4mg/l respectively. In all the sampled lakes the pH was high and way above neutral, ranging between 9.58±0.1 and 11.5±1.0, measured in lakes Bunyampaka and Nyamunuka respectively. The highest measured temperatures were 34.4±2.4°C, followed by 32.1±2.0°C and 32.0±0.8°C in lakes Munyanyange, Bagusa and Murumuri respectively. The lowest measured temperatures were 27.9±0.3°C and 28.9±0.4°C in lakes Katwe and Kikorongo respectively. Of all the sampled lakes, it was only Lake Kikorongo which had fresh waters. The rest of the sampled lakes were found to be hypersaline with salinities ranging between 92.3±7.6g/l and 205.0±15.3g/l in lakes Maseche and Nyamunuka respectively. The conductivity in all the studied lakes ranged between 10.5±0.6µS/cm and 106.3±3.5µS/cm which were the lowest and highest conductivities recorded in lakes Nyamunuka and Murumuri respectively (Table 3.3.2).

3.3.3 Chemical composition of selected alkaline, saline crater lakes

Lakes Katwe ($110205.0 \pm 7.1 \text{ mg/l}$), Nyamunuka ($101254.5 \pm 6.4 \text{ mg/l}$), and Maseke ($76909.1 \pm 7.0 \text{ mg/l}$) had the highest alkalinities while lakes Kikorongo ($12534.4 \pm 1.6 \text{ mg/l}$) and Munyanyange ($12767.2 \pm 329.3 \text{ mg/l}$) had the lowest alkalinities. All the studied lakes were found to have carbonate and bicarbonates as the dominant anions with lakes Nyamunuka and Kikorongo having the highest and lowest carbonate concentrations, respectively. Calcium was found to be the dominant cation in all the studied lakes. Lakes Bunyampaka, Katwe and Maseke had the highest sodium concentrations while lakes Murumuli, Bunyampaka and Katwe respectively had the highest Chloride concentration (Table 3.3.3).

Table 3.3. 3: *Mean ±SD of selected chemical parameters of the different Alkaline, saline lakes considered in this study*

Parameter	Mean±SD of the concentration of the different chemical parameters in each lake							
	Bagusa	Bunyampaka	Katwe	Murumuli	Nyamunuka	Munyanyang	Kikorongo	Maseche
Alkalinity (mg/l)	66161.7±3.5	65391.7±200.3	110205.0±7.1	66050.5±71.4	101254.5±6.4	12767.2±329.3	12534.4±1.6	76909.1±7.0
Calcium (mg/l)	147.7±17.2	674.5±77.1	1275.7±1.4	368.5±0.7	677.5±0.7	124.0±5.7	111.0±1.4	631.3±6.9
Carbonates (mg/l)	46398.3±142.6	59151.0±8.5	31905.0±7.1	37250.5±0.7	67804.0±5.7	8315.2±21.4	8333.5±0.5	43628.0±7.1
Chloride (mg/l)	164.3±8.5	203.1±35.9	202.3±0.0	1140.0±0.7	102.0±2.1	134.0±8.5	8.4±0.5	183.4±4.6
Bicarbonates (mg/l)	19518.3±319.4	6090.8±20.0	78302.0±2.8	28753.5±4.9	34451.0±1.4	4650.0±70.7	4205.5±7.8	33241.2±56.6
Sulphates (mg/l)	293.0±7.3	243.9±20.6	244.9±0.6	143.0±4.2	181.7±0.3	194.0±14.1	154.0±28.3	233.9±3.2
Sodium (mg/l)	84.5±4.7	135.9±1.4	134.6±2.1	67.8±0.2	92.6±0.7	89.9±9.4	62.2±1.4	106.6±1.9
Magnesium (mg/l)	33.8±0.1	176.4±45.8	557.3±2.8	114.5±7.1	246.5±0.7	32.1±4.1	27.4±0.4	226.4±2.0
Potassium (mg/l)	43.1±1.6	78.6±3.7	65.1±3.5	31.0±1.4	48.6±0.1	45.4±7.0	30.2±0.3	56.7±1.0
Hydrogen sulphide (mg/l)	15.0±0.2	1.2±0.1	13.8±1.4	4.3±0.7	7.4±0.2	1.6±0.1	0.4±0.1	7.7±0.6
Total silica (mg/l)	21.8±1.1	10.6±3.5	28.8±1.5	16.2±0.7	19.3±0.2	10.0±0.3	11.3±0.1	19.9±1.0
Fluoride (mg/l)	0.4±0.0	0.7±0.0	0.8±0.1	0.3±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.9±0.4

3.3.3b Nutrient status of selected alkaline, saline lakes

Table 3.3. 4; Mean±sd of selected nutrients concentrations in the selected Alkaline, saline lakes considered in this study

Lake	Mean ± SD for Nutrients and TSS				
	NH ₃ -N mg/l	NO ₂ -N mg/l	NO ₃ -N mg/l	SRP mg/l	TSS mg/l
Katwe	8.03±0.28	1.48±0.05	0.19±0.04	64.93±2.91	281.00±30.29
Bunyampaka	7.78±2.55	1.68±0.10	0.29±0.11	32.90±13.68	3103.00±2122.09
Maseche	7.20±1.44	1.30±0.10	0.17±0.03	32.47±0.61	8462.85±5782.53
Munyanyange	6.53±0.68	1.83±0.15	0.24±0.01	15.53±1.76	1984.32±176.88
Bagusa	13.45±1.89	1.98±0.13	0.47±0.04	12.23±1.34	5241.86±1305.56
Murumuri	6.13±0.03	1.31±0.27	0.23±0.04	14.15±7.99	35175.23±93.96
Kikorongo	0.04±0.01	0.15±0.07	0.21±0.01	9.40±0.71	90.03±10.20
Nyamunuka	7.7±1.21	1.7±0.11	0.23±0.04	82.01±0.91	91833.33±186.23

Lake Kikorongo had the lowest concentrations of NH₃-N, NO₂-N, NO₃-N, SRP and TSS. Among the sampled Lakes, Bagusa had the highest NH₃-N, NO₂-N, and NO₃-N concentrations while Lake Nyamunuka had the highest SRP and TSS (Table 3.3.4 above).

3.4 Discussion

The riparian community confirmed that Lake Munyanyange, Murumuri and Nyamunuka evaporate to dryness during the extreme dry seasons. These lakes have no major inflows and the intense solar heating probably experienced are the main reason why they evaporate to dryness in the extreme dry seasons. Although soda lakes are characterized by high evaporation rates (Grant, 2006), the presence of many fresh water inflows around Lakes Katwe, Bunyampaka, Bagusa and Maseche ensures that these lakes do not evaporate to dryness even in the dry season hence making them permanent lakes (Musinguzi & Katikiro, 2000). The high variations in the volume of these lakes can be attributed to the very high evaporation rates experienced in saline lakes (Jones & Grant, 1999; Grant, 2006; Kasedde, 2016). Melack (1978) reported Lake Nyamunuka to have evaporated to dryness for a period of two weeks in May 1971. This could have been mainly because it has few small fresh water inflows which are not enough to maintain the water level during the extreme dry seasons.

The peanut soup green, dark green and greenish color in lakes Katwe, Bunyampaka and Maseche, Nyamunuka and Murumuri, Kikorongo and Munyanyange respectively observed in this study might be attributed to the cyanobacteria and algal blooms (Mungoma, 1990; Jones & Grant, 1999; Matagi, 2004). Lakes Kikorongo and Maseche waters were peanut soup green in color, while Lake Bagusa waters were brown in earlier limnologicals studies (Mungoma, 1990). East African Saline lakes have unique cyanobacteria community (Hadjembes, 2006). *Arthrospira fusiformis* which is among the dominant cyanobacteria community in these lakes is the world's fastest growing phytoplankton (Fazi et al., 2016). With the exception of Lake Kikorongo whose

waters were not thick and slimy, the waters in the rest of the other sampled lakes were found to be thick and slimy. The thickness and slimy nature of the waters in alkaline and saline lake waters might be attributed to the high algal abundance and high evaporation rates in hypersaline conditions which leave behind lots of organic matter (Jones & Grant, 1999; Matagi, 2001; Grant, 2006). Lakes Katwe, Munyanyange and Bunyampaka experience severe impacts of overgrazing and deforestation. Deforestation and overgrazing are among the major drivers of reduced vegetation in the hypersaline rift valley lakes in Tanzania and Ethiopia (Matagi, 2004). A lot of vegetation has been cleared around lakes Katwe and Bunyampaka by salt miners who use it in construction and re-enforcing of salt crystallization pans. The Vegetation is also used to cover the extracted salt, as a way of protecting it from rain during storage while waiting for buyers. The high vegetation cover in the other studied lakes (Murumuri, Nyamunuka, Bagusa, Maseche and Kikorongo) could be because the lakes are found in Queen Elizabeth National park (QENP) where there are protected from encroachment by Uganda Wildlife Authority (UWA). The freshwater wetlands around Lakes Katwe, Bagusa and Maseche are maintained by ground water and seasonal streams flowing from highlands surrounding these lakes (Grant, 2006). Musinguzi et al. (2000) reported lakes Katwe, Munyanyange and Nyamunuka to be surrounded by pure strands of *Cyperus lavigatus*. Salinity plays a key role in determining the emergent vegetation in the littoral zones of saline lakes (William & Fawn, 2009). This might explain why *Cyperus lavigatus*, a species that is adapted to such saline environments was found to be the dominant surrounding vegetation in many of these alkaline saline lakes. In a biodiversity assessment of the flamingo lakes of East African, savannah woodland, bushland and shrubland were reported to be the dominant vegetation in the alkaline rift valley lakes of East Africa. *Euphorbia candelabra* were reported in most highland forests surrounding these lakes, while *Cyperus lavigatus*

dominated the surrounding wetland vegetation (Matagi, 2004). *Cyperus lavigatus* and *Cyperus rotundus* are among plants reported to be tolerant to difficult conditions in arid and semi-arid areas (Alsherif, Ayesh, Allogmani, & Rawi, 2012). This region has hot and dry climate with high evaporation rates (Mungoma, 1990), and it is mainly the plants like *Cyperus lavigatus* and *Euphorbia candelabra* with ability to survive in such harsh conditions that are abundant to be the dominant vegetation.

Previous limnological studies of alkaline, saline lakes in Uganda reported these lakes to be shallow, with only lakes Kikorongo and Mahega being the only ones reported to have depth exceeding 5m (Mungoma, 1990). The studied lakes being shallow can also probably be attributed to their mode of formation. The lakes were formed by water filling a series of shallow depressions which were formed by tectonic activities during the formation of the great East African rift valley (Grant, 2006; Matagi, 2004). Although some crater lakes of western Uganda like Bunyonyi (average depth 39m) are very deep (www.nemaug.org/atlas/atlas_wetlands.pdf), endorheic, saline lakes have been described to be typically small (<50ha) and shallow (<1m) (Stenger-Kovács et al., 2014). With the exception of Lake Kikorongo, the rest of the sampled lakes had very low dissolved oxygen (DO) levels (<3.5mg/l). The low DO could be explained by the high decomposition rates of dead algae since these lakes are reported to be amongst the most productive aquatic systems in the world (Grant, 2006). Earlier studies have reported dissolved oxygen levels as low, less than 1mg/l in these lakes (Mungoma, 1990). Alkaline, saline lakes have generally high pH above 9.6 (Grant, 2006; Matagi, 2004). This might be reason to explain the high observed pH in the investigated lakes. With the very high evaporation rates experienced in these lakes, the dissolved minerals are concentrated into alkalis, with pH ranging from 8.5 in dilute lakes to around 12 in highly concentrated lakes (Grant, 2006). Other East African alkaline,

saline lakes like Nakuru (10.5), Elmenteita (9.4), Manyara (10.2), Reshitani (9.2) and Baringo (9.2). Lakes Naivasha and Lake Naibungo (9.2) and Lake Momela (10.4) have similarly high pH ranges (Melack & Kilham, 1974).

The highest and lowest measured temperatures of $34.4 \pm 2.4^\circ\text{C}$ and $27.9 \pm 0.3^\circ\text{C}$ measured in lakes Naivasha and Lake Naibungo respectively were higher than the reported mean monthly temperatures of this region of $19.0 \pm 2.8^\circ\text{C}$ and $28.0 \pm 3.2^\circ\text{C}$ from previous studies by Mungoma 1990. This could be attributed to global warming, climate change and weather variability. This area experiences a hot and dry climate compared to the surrounding highlands (Mungoma, 1990). Such climates are characterized by high atmospheric temperature. These alkaline saline lakes being small and shallow with no major out flow, they are very sensitive to weather and climate changes. Small changes have been reported to cause large and sometimes irreversible changes in the nature of saline lakes (Hammer, 1990). Shallow closed-basin saline lakes of Northern Great Plains were reported to be sensitive to changes in precipitation and evaporation (Evans & Prepas, 1996). Like the shallow closed-basin saline lakes of the Northern Great Plains, the alkaline saline lakes of Uganda were found to be closed with no major outflow. This implies that they are also very sensitive to weather and climate variations.

Lake Kikorongo was found to be fresh with a salinity of $0.0 \pm 0.0\text{g/l}$. This is contrary to previous literature which reported lake Kikorongo to be saline (Hadjembes, 2006; Mungoma, 1990). The rest of studied lakes were hypersaline. With the exception of Lake Maseche, whose salinity was slightly below 100g/l , the rest of the sampled lakes had salinities above 100g/l (Table 3.3.2). Such hypersaline lakes with high pH above 10 have been referred to as soda lakes (Matagi, 2004). These lakes experience temporal variations in salinities as high as 50g/l (Mungoma, 1990). Lake Kikorongo has been reported to receive fresh water from floods coming from the neighboring Lake George during the rain seasons (Hecky & Kilham, 1973; Mungoma, 1990).

This together with precipitation affects salinity in closed lakes (Saros & Fritz, 2000) and could be responsible for the reduction in salinity observed in Lake Kikorongo. From studies of potential effect of climate change on ion chemistry and phytoplankton communities in prairie saline lakes, precipitation and evaporation were reported among the factors affecting salinity in these lakes (Evans & Prepas, 1996). Evans et al., (1996) also reported local difference in surface and ground water to have an effect on brine concentration and composition in closed saline lakes.

Conductivity has been reported as a useful indicator for the levels of available inorganic constituents in water and plays an important role in determining the availability of aquatic organisms in certain environments (Gupta & Mrinal, 2013). The observed high conductivity in these lakes could be attributed to the high concentration of inorganic ions in these lakes.

The low alkalinities, salinities and conductivities in Lake Munyanyange can be attributed to it being a seasonal lake. Also the samples and measures were taken a few days after rains in the area. It usually requires some residence time for the lake waters to infiltrate down to the salt rich parent rock which leads to diffusion of salts from the parent rock to the lake waters (Yan, Marschner, Cao, Zuo, & Qin, 2015). As the rain waters spend more time in the lake, the salinity, alkalinity and conductivity increase. The low alkalinity, salinity and conductivity in Lake Kikorongo could probably be explained by dilution effect from fresh water incoming water Lake George during the rainy season floods (Mungoma, 1990).

In this study, all lakes were found to have carbonates as the dominant anions, followed by sulphates. This is in agreement with earlier studies where carbonates were reported to be the major dissolved anions in soda lakes of East Africa (Jones & Grant, 1999). Modern and

Holocene carbonate sedimentology of two saline volcanic maar lakes in Southern Australia reported carbonates to be among the common components in sediments of modern salt lakes (Last & De Deckker, 1990). The high carbonate concentrations in these lakes waters might be caused by incoming waters dissolving the carbonates from the sediments. Jones and Grant (1999) reported the high concentration of carbonates as major dissolved anions to be caused by evaporative concentration. Contrary to the current study findings, previous studies by Mungoma (1990) reported lakes Katwe and Kikorongo to have more chlorides than carbonates. Chloride concentrations in Lake Katwe might have reduced due to the excessive salt extraction while in Kikorongo it might be the dilution caused by rains and the reported floods from lakes George (Hecky & Kilham, 1973; Mungoma, 1990). With the exception of SiO_2 and F which are lower than all the other chemical compounds considered in this study are higher than those reported in earlier studies by Mungoma (1990). The high concentration of nitrogenous compounds including $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$ in these lakes (table 3.3.4) can be attributed to the high algal biomass found in these lakes where upon decay and subsequent nitrification lots of nitrogenous compound like nitrates are released in these highly productive lakes. Lake Kikorongo having the lowest concentrations of $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, SRP and TSS can be attributed to it being the least productive among the studied lakes. Total phosphorous concentrations were reported to be frequently high in saline lakes in the Canadian prairies. The high phosphorous concentration was associated with the conservation of phosphorous in these lakes (Evans, Arts, & Roberts, 1996). In the same way, phosphorous might be conserved in these saline crater lakes of western Uganda like it is in the phosphorous-rich saline lakes of the Canadian prairies since these lakes are also found in areas experiencing high evaporation rates exceeding precipitation rates and in closed drainage basins (Jones & Grant, 1999).

3.5 Conclusions

All the studied lakes were alkaline and saline in nature as reported in the previous studies except, Lake Kikorongo which was found to be fresh with a salinity of zero. With the exception of Lake Murumuli which was found to be a Carbonate-chloride lake, the rest of the sampled lakes are carbonate-sulphate lakes which is contrary to earlier studies which reported lakes like Katwe and Kikorongo to be Carbonate-chloride lakes.

Climate change and human activities such as rock salt extraction, water diversion to salt pans, deforestation, and livestock grazing are threatening the ecological status of these lakes more especially those outside the protected areas like Katwe and Munyanyange. Could the observed increment in water temperatures in the study lakes and a reduction in salinity more especially in Lake Kikorongo be a sign of climate change? For this to be confirmed, it requires routine and continuous monitoring of these lakes. Although the majority of these lakes are found in Queen Elizabeth National Park (QENP) which is a protected area, there is need to offer additional protection to these unique systems to safeguard them from human induced climate change. Alkaline saline lakes in Carpathian Basin which are under Protection by Law (Stenger-Kovács et al., 2014) can be useful examples.

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CHAPTER FOUR

Planktonic and fisheries biodiversity of alkaline saline crater lakes of western Uganda

4.0 Abstract

Artemia being a potential prey to a broad range of aquatic organisms, it was important to understand the plankton and fish biodiversity of these lakes before bio-physical manipulation of their waters for its production. Saline crater lakes in Western Uganda were investigated to establish plankton and fish biodiversity. The average depth (m), salinity (g/l), temperature (°C), conductivity ($\mu\text{S}/\text{cm}$), dissolved Oxygen (mg/l) and pH were measured *in-situ* at each of the sample collection point. The Mean $\pm$ SD of the different physico-chemical parameters in the sampled lakes ranged between $0.2\pm0.0\text{m}$ and $2.3\pm0.3\text{m}$ for depth, $0.0\pm0.0\text{g}/\text{l}$ and $205.0\pm15.3\text{g}/\text{l}$ for salinity, $27.9\pm0.3^\circ\text{C}$ and $34.4\pm2.4^\circ\text{C}$ for temperature, $18.6\pm0.1\ \mu\text{scm}^{-1}$ and $106.3\pm3.5\ \mu\text{Scm}^{-1}$ for conductivity, $1.7\pm0.4\ \text{mg}/\text{l}$ and $6.0\pm1.0\text{mg}/\text{l}$ dissolved Oxygen and 9.6 ± 0.1 and 11.5 ± 1.0 for pH. With the exception of Lake Bagusa, where *Anabaena circinalis* was found to dominate the algal biomass, Bunyampaka and Nyamunuka where no *Spirulina platensis* was found, the rest of the studied lakes had *Spirulina platensis* dominating their algal biomass. All the studied lakes had relatively low zooplankton abundance (< 10 individuals per litre) and biodiversity, with Lake Kikorongo having the highest zooplankton biodiversity and *Brachionus calyciflorus* as the most abundant at 50 to 100 individuals /litre. None of the study lakes had fish. This could be explained by the high salinities in these lakes.

Key words: Zooplankton, Phytoplankton, saline, alkaline, lakes

4.1 Introduction

Planktons are the main food for juvenile fish (Lind, 1965) and major players in terms of both primary and secondary production in aquatic ecosystems (Boero et al., 2008). Plant-like minute organisms that continuously drift in the water are referred to as phytoplankton, while minute animal-like organisms, unable to synthesis food are referred to as zooplankton (www.biology-online.org/dictionary/Phytoplankton). Whereas fish constitute a key component in the aquatic systems as primary, secondary or tertiary consumers, phytoplankton and zooplankton play vital roles as primary producers and primary consumers respectively. Plankton are not only food organisms for fish fry, fingerlings and adult fish but also influence key abiotic features, such as transparency in aquatic systems (Joshi, 2009). Saline systems have been reported to have a generally low biodiversity (Hammer, 1986), with diatoms being typical and ecologically dominant among algal biomass in alkaline saline systems (Stenger-Kovács et al., 2014). Rotifera, Cladocera, Copepoda and Anostraca species occur in saline systems but with their abundance and biodiversity decreasing with increasing salinity (Hammer, 1993). East African saline lakes generally have more rotifers in their zooplankton assemblages than either copepods or cladocerans, with the dominant species of Rotifera, Copepoda and Cladocera reported to change with the salinity gradient (Green, 1993). Alkaline saline crater lakes are very productive environments (Grant, 2006; Harper et al., 2003), with prokaryotic photosynthetic primary production the driving force behind nutrient recycling in these systems (Jones & Grant, 1999). Community evenness decrease with increasing nutrient concentration, with few species favored being dominant (Harper et al., 2003). Certain species like *Dunaliella* sp dominate the saline waters of Utah Lake in the USA in abundance (Larson & Belovsky, 2013), while 'Spirulina'

Arthrospira fusiformis is dominant in hypersaline Lake Bogoria (Harper et al., 2004). Lake Bogoria has no macro-zooplankton with lesser flamingo (*Phoeniconaias minor*) being the only grazers in it (Harper et al., 2003).

Several fish species more especially amphihaline ones have been described to have physiological mechanisms which enable them migrate between freshwater and sea water. Such species can tolerate, adapt or even acclimate to salinity, alkalinity and ionic compositions levels outside the conventional freshwater and seawater conditions (Brauner, Gonzalez, & Wilson, 2012). While both native and exotic species were found in waters with salinities less than 30mg/l⁻¹ in a study of fish distribution in inland saline waters in Victoria, Australia, no inland fish species were found at salinities above 30mg/l (Chessman & Williams, 1974). Saline lakes with salinity levels below 20mg/l⁻¹ can have fish species of commercial value (Hadjembes, 2006). Several species can tolerate salinities as high as 15,000mg/l with only the nine-spined stickle back reported to exist at salinities of 20,000mg/l (Rawson & Moore, 1944). *Oreochromis alcalicus graham*, *O. amphimelas*, and *O. alcalicus* are endemic in lakes Magadi and Natron which are among the East African saline lakes (Matagi, 2004).

Environmental factors including salinity and nutrient in hypersaline systems are key factors influencing biodiversity in saline environments (Larson & Belovsky, 2013). Saline lakes have limited micro-organisms species biodiversity (Harper et al., 2003). Salinity and nutrient concentration in hypersaline lakes are strong determinants of phytoplankton diversity, with species richness decreasing with increasing salinity and increasing with increasing nutrient concentration (Larson & Belovsky, 2013). Despite the inverse proportionality between salinity

and aquatic biodiversity, the relationship between salts and biodiversity is still not well understood (Derry, Prepas, & Herbert, 2003; Ríos-Escalante, 2013). Despite the numerous fish and plankton biodiversity studies in fresh water systems, very little of such studies have been conducted in saline systems (Jones & Grant, 1999; Larson & Belovsky, 2013). The aim of this study is therefore to investigate fish and plankton biodiversity in selected saline crater lakes in western Uganda so as to determine the relationship between fish and plankton biodiversity.

4.2 Materials and Methods

4.2.1 Study area

Lakes considered in this study are small unique water bodies found in Katwe-Kikorongo volcanic system in western Uganda. Lake Katwe is the largest among them, an average area of 2.5km² (Nixon, Morton, & von Knorring, 1971) and located at (029.87033°E, 00.13217°S). Others are Munyanyange (029.88591°E, 00.13513°S), Nyamunuka (029.98743°E, 00.09344°S), Bagusa (030.17958°E, 00.09793°S), Murumuri (029.99186°E, 00.07323°S), Masech (030.19019°E, 00.09355°S), Bunyampaka (030.12819°E, 00.03765°S) and Kikorongo (030.01228°E, 00.01190°S). Among these lakes Bagusa and Kikorongo were at lowest and highest altitude of 884m and 939m above sea level (a. s. l) respectively. These lakes are mostly alkaline and saline in nature with dominant anions being carbonates and sulphates (Nkambo, Bugenyi, Naluwairo, Nayiga, & Waswa, 2015). They exhibit considerable temporal variations in volume and surface area. The total depth of these lakes range between less than one meter to six meters (Kirabira, Kasedde, & Semukuutu, 2013).

4.2.2 Zooplankton and phytoplankton diversity

Data collection in this study was done between the 26th of February and 3rd of March, 2014, a period towards the end of the dry season in the region. A Global Positioning System (GPS) unit (GARMIN 12XL) was used to take GPS coordinates and the altitude/elevation above sea level (H. a.s.l) of the different set sampling points on each of the sampled lake.

Geo-referenced sampling points were set on each of the sampled lakes to ensure consistence in the sampling. Zooplankton samples were obtained by filtering four liters of water collected from every set geo-referenced sampling point through a 50 μ m mesh zooplankton net. The samples obtained after filtration were preserved in 95% ethanol and carried to the National Fisheries Resources Research Institute (NaFIRRI) laboratory, Jinja, for identification to the lowest possible taxonomic level and counted under an inverted microscope. Using a Van Dorn water sampler, water samples for phytoplankton analysis were collected at a depth of 0.5m in lakes whose average depth was more than 1m. For the very shallow lakes (depth < 0.5m), surface water samples were collected for plankton analysis. 500mls of the collected water samples were preserved using Lugol's solution in pre-rinsed Nalgene bottles which were kept in a cooler box containing dry ice and later transferred to the NaFIRRI. In the laboratory, phytoplankton in the collected water samples were identified to the lowest possible taxonomic level and the wet biomass of each of the identified group determined.

At every sampling point where water samples for plankton analysis were taken, selected physical and chemical parameters (depth (m), temperature ($^{\circ}$ C), dissolved oxygen concentration (DO (mg/l), pH, Conductivity (μ S/cm) and salinity (g/l) were also measured *in-situ* at the lake Surface

and bottom. Where lakes were too shallow, physico-chemical measurement were taken at surface. Water temperature, dissolved oxygen concentration and conductivity were measured using a YSI oxygen/temperature/conductivity meter (Model YSI 550A), pH was determined using an OAKTON pH Tester 30, while salinity was measured with a refractometer. The depth was determined using a portable depth finder (Hondex PS-7).

4.2.3 Fish Biodiversity

All the study lakes deeper than 1m were sampled for fish by setting gill nets of 1 and 2 inch mesh sizes, of 2m by 50m in these lakes in the evening at 5pm and removing them to sample for the catches the following morning at 7am. This was followed by seining using seine nets of 2inch mesh size, with dimensions of 2X75m a day after retrieving the gillnets. In addition to setting gill and seine nets, consultations were made among communities around each of the studied lakes whether they have ever seen or got any fish from these lakes.

4.3 Results

4.3.1 Physico-chemical parameters

Table 4.3. 1; Mean±SD of the selected measured physico-chemical in the selected studied saline crater lakes.

Lake	Mean ±SD of the different physico-chemical parameters					
	Depth (m)	D.O (mg l ⁻¹)	pH	Temp. (°C)	Salinity(g l ⁻¹)	Cond (µS cm ⁻¹)
Katwe	2.1±0.7	2.6±0.2	9.9±0.1	27.9±0.3	180±67.8	104.5±6.4
Munyanyange	0.2±0.1	1.7±0.4	10.8±0.4	34.4±2.4	101.0±7.1	59.7±8.2
Nyamunuka	0.2±0.2	2.6±0.3	11.5±1.0	30.5±3.1	205.0±15.3	10.5±0.6
Murumuri	0.2±0.0	1.7±0.5	11.1±1.3	32.0±0.8	162.8±34.2	106.3±3.5
Bunyampaka	0.2±0.1	2.20±0.6	9.6±0.1	30.33±1.5	199.50±16.4	103.90±4.3
Bagusa	1.9±0.5	3.2±0.8	10.5±0.4	32.1±2.0	199.5±16.4	103.9±4.3
Maseche	1.3±0.2	2.9±0.4	10.9±0.4	30.0±0.7	92.3±7.6	71.2±1.3
Kikorongo	2.3±0.3	6.0±1.0	10.4±0.0	28.9±0.4	0.0±0.0	18.6±0.1

Lakes Munyanyange, Nyamunuka, Murumuri, and Bunyampaka were found to be relatively much shallower (depth<0.3m) at the time of sampling. Lake Kikorongo was found to be the

deepest, with an average depth of 2.3m. The highest measured dissolved oxygen (DO) was 6.0 ± 1.0 mg/l in Lake Kikorongo while lakes Munyanyange and Murumuri had the lowest and second lowest DO of 1.7 ± 0.4 and 1.7 ± 0.5 mg/l respectively. All the lakes were found to be alkaline with pH ranging between 9.58 ± 0.1 and 11.5 ± 1.0 , measured in lakes Bunyampaka and Nyamunuka respectively. The highest measured temperatures during the time of sampling in the studied lakes ranged between $28.9 \pm 0.4^\circ\text{C}$ and $34.4 \pm 2.4^\circ\text{C}$. Salinity among the studied lakes ranged between 0 g/l and 205.0 ± 15.3 g/l in lakes Kikorongo and Nyamunuka respectively. The conductivity in all the studied lakes ranged between 10.5 ± 0.6 $\mu\text{S}/\text{cm}$ and 106.3 ± 3.5 $\mu\text{S}/\text{cm}$ which was the lowest and highest conductivities recorded in lakes Nyamunuka and Murumuri respectively (Table 4.3.1).

4.3.2 *Phytoplankton diversity*

A total of twenty nine (29) phytoplankton species were found in the eight study lakes. Out of this nineteen (19) belonged to Cyanophyceae, commonly known as cyanobacteria (blue-green algae (BG)) which are predominantly photosynthetic prokaryotes containing a blue pigment in addition to the chlorophyll (WHO, 1999); Six (6) belonged to Chlorophyceae commonly referred to as Chlorophyta (green algae (G)); four (4) belonged to Bacillariophyceae, commonly referred to as diatoms (D). Lakes Maseche ($912,347 \mu\text{g}/\text{L}$) and Bagusa ($210,290 \mu\text{g}/\text{L}$) were found to have the highest and second highest algal biomass respectively. These were followed by lakes Katwe and Murumuri which had algal biomass of $90,653 \mu\text{g}/\text{L}$ and $86,240 \mu\text{g}/\text{L}$ respectively. Lakes Katwe and Bunyampaka showed the highest algal biodiversity while lakes Maseche and Nyamunuka had the lowest biodiversity. Cyanophyceae (BG) dominated the algal composition of all the studied lakes. With the exception of Lake Munanyange where no Chlorophyceae species (G) were found, the rest of the other studied lakes were found to have some species belonging to

Chlorophyceae and Bacillariophyceae species (D) in relatively small abundances in comparison to the Cyanophyceae species. With the exception of Lake Bagusa where *Anabaena circinalis* was found to be the dominant phytoplankton, Bunyampaka and Nyamunuka were no *Spirulina platensis* was found, the rest of the sampled lakes in this study had *Spirulina platensis* as the dominant phytoplankton (Table 4.3.2).

Table 4.3. 2. Mean Wet biomass ($\mu\text{g/L}$) concentrations of the different phytoplankton taxon in the selected Alkaline, saline lakes considered in this study

Mean Wet biomass ($\mu\text{g/L}$) in different Alkaline, saline lakes									
Taxa Group	Taxa	Bagusa	Bunyampaka	Munyanyange	Maseche	Katwe	Kikorong	Nyamunuk	Murumuri
Blue-green	<i>Aphanocapsa</i> spp	522	274	548	-	342	-	365	730
	<i>A. anabilium</i>	-	-	-	456	-	91	-	183
	<i>Anabaena</i> spp	15,649	219	-	-	3,286	-	-	-
	<i>A. flos-aquae</i>	-	-	-	-	-	-	-	365
	<i>A. circinalis</i>	157,273	11,867	9,859	5,112	6,207	548	38,085	913
	<i>A. tanganyikae</i>	-	55	-	-	-	-	-	-
	<i>Chroococcus</i> spp	391	137	-	365	-	23	-	-
	<i>C. limnetica</i>	-	183	365	-	274	137	342	297
	<i>C. disperses</i>	-	-	-	-	-	68	-	-
	<i>Coelosphaerium kuetzingianum</i>	-	-	-	-	1,826	-	-	4,747
	<i>Merismopedia tenuissima</i>	-	-	-	-	-	-	-	292
	<i>Oscillatoria tenuis</i>	-	-	-	17,253	-	-	-	-
	<i>Planktolyngbya limnetica</i>	15,845	2,773	-	8,216	7,343	-	-	7,805
	<i>P. circumcreta</i>	440	411	1,438	-	-	-	205	411
	<i>P. undulate</i>	-	-	-	-	103	-	-	-
	<i>Pseudo anabaena</i>	-	411	411	-	103	-	308	-
	<i>Romeria</i> spp	-	55	-	-	-	-	-	-
	<i>Spirulina platensis</i>	12,324	-	25,880	879,909	66,856	43,133	-	69,012
	<i>Tiny blue green</i> (un identified)	-	101	151	-	-	228	-	-
	<i>Ankistrodesmus falacius</i>	-	-	-	-	103	-	-	-
Green Algae	<i>Closterium acerosum</i>	-	-	411	411	205	205	411	-

<i>Kirchneriella</i> <i>subsolitaria</i>	162	-	-	-	-	1,506	-	-
<i>Monoraphidium</i> <i>contortum</i>	-	-	-	-	-	-	-	22
<i>Sitochococcus</i> spp	7580	338	274	479	3252	-	-1575	1404
<i>Centric diatoms</i>	104	292	37	146	730	-	292	37
<i>Cyclostephanodiscus</i> spp	-	-	-	-	-	37	-	-
<i>Navicula gastrum</i>	-	-	-	-	-	856	-	-
<i>N. acicularis</i>	-	274	-	-	23	-	137	23
Total	210,290	17,488	39,372	912,347	90,653	48,065	44,698	86,240

4.3.3 Zooplankton diversity

Three taxa (Rotifera, Copepoda, and Cladocera) were encountered. Among the studied lakes it was only lakes Kikorongo, Maseche, and Katwe which were found to have zooplanktons belonging to the Rotifera. Lakes Maseche and Kikorongo which had zooplanktons belong to genus Copepoda and none of the studied lakes had Cladocera. Lakes Munyanyange, Maseche, Murumuli, Katwe and Nyamunuka had some small cysts which could not be identified. Water samples from lakes Bunyampaka and Bagusa had neither zooplankton nor cysts (Table 4.3.3).

Table 4.3. 3. Mean zooplankton abundance (Individuals per litre) in the different studied saline crater lakes

Mean zooplankton recorded abundances (individuals per litre)								
Lake	Munyayange	Kikorongo	Maseche	Murumuli	Bunyampaka	Katwe	Bagusa	Nyamunuka
Rotifera								
<i>Brachionus calyciflorus</i>	-	+++	-	-	-	+	-	-
<i>Brachionus angularis</i>	-	-	+	-	-	-	-	-
<i>Lecane luna</i>	-	+	-	-	-	-	-	-
<i>Tricorpea cylindrica</i>	-	-	-	-	-	-	-	-
<i>Syncheata</i> sp.	-	+	-	+	-	-	-	-
Copepoda								
Nauplii	-	+	-	-	-	-	-	-
Cyclopoid copepodite	-	-	+	-	-	-	-	-
Cladocera								
<i>Moina micrura</i>	-	-	-	-	-	-	-	-
Un identified cysts	+	-	+	+	-	+	-	+

+ = 1 to 10 individuals /L, ++ = 10 to 50 individuals/L, +++ = 50 to 100 individuals/L, - = 0 individuals/L

4.3.4 Fish Biodiversity

None of these lakes was found to have fish at the time of sampling. Communities reported Lake Kikorongo to sometimes have African catfish (*Clarias gariepinus*) more especially during the rainy season.

4.4 Discussion

4.4.1 Phytoplankton

Blue-green (cyanobacteria) algae were dominant in all the lakes, with *Spirulina* being the most dominant phytoplankton (Table 4.3.2). Earlier studies in alkaline, saline crater lakes also suggested cyanobacteria *Spirulina platensis* to be the most dominant (Hecky & Kilham, 1997) while diatoms dominate anthroposaline lakes in Romania and Bolivia (Stenger-Kovács et al., 2014). The dominance of cyanobacteria in these harsh environments could be attributed to their ability to withstand extremely high water temperatures, pH and salinity. Some Cyanobacteria species have got special adaptations such as ultraviolet absorbing sheath pigment which increases their fitness in relatively unfavourable environmental conditions like high salinity, brackish or fresh water, in cold or hot springs and in environments where no other microalgae occur (WHO, 1999). Allelopathy can be another factor to explain the dominance of Cyanobacteria in these lakes. Freshwater cyanobacterium *Oscillatoria* sp releases exudates which can inhibit green alga *Chlorella vulgaris* (Leão et al., 2010). In the same way cyanobacteria in these saline crater lakes might be influencing the algal biodiversity through allelopathy. Matagi (2004) reported Cyanobacteria *Spirulina* (*Arthrospira fusiformis*) to be the most successful algae in colonizing alkaline saline lakes in the Eastern Rift valley. Lake Lonar, an inland alkaline saline crater lake in India has its phytoplankton biomass dominated by *Spirulina platensis* (Satyanarayan, Chaudhari, & Dhadse, 2007; Siddiqi, 2007; Yannawar & Bhosle, 2013). Lake Nakuru, Bogoria and Elmenteita which are alkaline-saline lakes in Kenya were characterized by mass growth of cyanobacteria including *Arthrospira fusiformis* (Ballot et al., 2004; Harper et al., 2003). The Presence of cyanobacteria in these alkaline-saline lakes is in conformity with the

findings of this study where *Spirulina* dominates the phytoplankton biomass. Jones and Grant (1999) reported *Spirulina spp* to be the main contributors to primary production in moderately saline lakes, while Hadgembes (2006) documented *Spirulina* to be one of the unique cyanobacteria occurring in East African saline lakes. The extreme inhospitable conditions in alkaline, saline crater lakes means that the biodiversity in these systems is limited to organisms with special adaptation to survive such extreme conditions (Matagi, 2004). Primary production in lakes with Flamingo of East Africa, which are also alkaline and saline in nature, was reported to be dominated by *A. fusiformis* with *Ectothiorhodospira sp* also reported to sometimes play a key role (Jones & Grant, 1999; Matagi, 2004). *Nitzschia sp*, and *Navicula sp* were some of the other algal species found in these lakes, also reported by Matagi (2004) in other highly alkaline and saline environments. *Chroococcus spp* is another species of cyanobacteria recorded in these lakes, which was reported by Jones and Grant (1999) to play a key role in primary production in soda lakes of East Africa. In studies to reconstruct relationships between diatoms assemblages with salinity, it was reported that salinity might not be the primary cause of the shift in the diatom assemblage but a factor highly related to drivers of species shift (Saros & Fritz, 2000). In the same way salinity might be playing a key role in determining the cyanobacteria species occurring in the alkaline saline environments but other possible drivers should not be over looked. In a study to determine the extent to which salinity influences community structures in saline systems, it was reported to play a less significant role in the determination of composition, species richness and biodiversity (William, 1998). Lakes Katwe and Bunyampaka having the highest algal biodiversity with lakes Maseche and Nyamunuka having the least algal biodiversity might be attributed to the daily anthropogenic disturbances experienced by lakes Katwe and Bunyampaka during the process of salt extraction while lakes Maseche and Nyamunuka are

among those lakes with no daily anthropogenic disturbance. It should be noted that communities around lakes Katwe and Bunyampaka continuously extract salt from them on a daily basis and such disturbances might be impacting various algal species differently.

4.4.2 Zooplankton

All the studied lakes had very low zooplankton biodiversity, with only *Brachionus calyciflorus* present in lakes Kikorongo and Katwe; *Brachionus angularis* in Lake Maseche, only *Synchaeta* (Rotifera) present in lakes Kikorongo and Murumuli (Table 3). *Brachionus plicatilis* and *Paradiaptomus africanus* were reported in earlier studies to be characteristic zooplankton of alkaline saline crater lakes (Hecky & Kilham, 1973). Matagi (2004) reported *Lovanula africana*, *Brachionus didiatus*, *B. plicatilis* and Chironomids as the dominant macro-invertebrates in the highly alkaline, hypersaline flamingo lakes of East Africa. All of these were conspicuously absent in the studied alkaline and saline lakes. Nauplii in Lake Kikorongo and cyclopoid copepodites in Lake Maseche were the only Copepoda members present. Other macrozooplanktons like cladocerans, nekton fauna, and crustacean decapods were absent in all the studied lakes. This can be attributed to the high alkalinity, salinity and pH in these lakes. Lake Bogoria in Kenya, an alkaline saline lake had no macro-zooplankton with the only grazer being the lesser Flamingo, *Phoeniconaias minor* which occasionally visit these lakes in high numbers (Harper et al., 2003). The absence of crustacean decapods in this limnological study of Lake Bogoria and Lonar an alkaline, saline crater lakes in India was attributable to pH shock ($\text{pH} > 10-11$, alkaline death point) (Siddiqi, 2007). An inverse proportionality between biodiversity and salinity (Dolan, Prepas, & Herbert, 2003; Larson & Belovsky, 2013; Ríos-Escalante, 2013) might be the explanation for the slightly high zooplankton biodiversity in Lake Kikorongo, since it was found to be fresh with zero salinity (Table 4.3.1). In field and laboratory experiments designed to

examine the consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes, severe disturbances in zooplankton community structure and abundance were found to be caused by even very small salinity changes, with even slight increments in salinity capable of leading to biodiversity depletion (Schallenberg, Hall, & Burns, 2003). Similarly, the low zooplankton biodiversity in these lakes might be attributed to the high salinity levels (Table 4.3.1).

The unidentified cysts found in the different studied lakes might be dormant stages of zooplanktons released as a mean of surviving extreme conditions. It is possible that these dormant stages shall return to life on the set of suitable conditions. Certain zooplanktons more especially members of order Anostroca like *Artemia* release dormant embryo in form of cysts which aid them in withstanding harsh conditions. All *Artemia* strains reproduce either ovoviviparously by releasing live nauplii or oviparously by releasing dormant embryos in form of cysts that can withstand harsh unfavorable environmental conditions (Ben Naceur, Jenhani, & Salah, 2012 ; Ghomari, Selselet, Hontaria, & Amat, 2011).

4.4.3 Fish biodiversity

Although some fish species like *Oreochromis alcalicus graham*, *O. amphimelas*, and *O. alcalicus* were reported to inhabit lakes Magadi and Natron both of which are alkaline saline in nature (Matagi, 2004), no fish were found in these lakes. Hecky and Kilham (1973) also reported cichlid fish like *Tilapia grahami* Boulenger to inhabit alkaline, saline lakes. The absence of fish in the studied lakes might be attributed to their extreme environmental conditions like the high temperatures, and the high salinity (Chessman & Williams, 1974) and alkalinity. The complete absence of fish in alkaline, saline Lake Lonar coincided with extreme levels of environmental

physico-chemical parameters (Siddiqi, 2007). These lakes being shallow, they do experience very high variations in volume and surface area, with some evaporating to dryness during extreme dry seasons (Nkambo et al., 2015). This makes it difficult for them to have fish. Fish cannot survive the dry seasons when these lakes depth become too shallow, with some evaporating to dryness. The absence of appropriate fish food organisms in form of zooplankton could be another probable reason for the absence of fish in the lakes. They had very low zooplanktons, with the exception of Lake Kikorongo which had the highest zooplankton biodiversity having a rotifer *Brachionus calyciflorus* as the most abundant ranging between 50 to 100 individuals /litre (Table 3). *Brachionus plicatilis* widely reported to be suitable food for fish larvae was conspicuously absent in all the studied lakes. Rotifers are living food capsules transferring nutrients to fish larvae (Lubzens, Tandler, & Minkoff, 1989). Their total absence in some of the studied lakes and presence in very small quantities in other lakes could be a probable reason for the absence of fish in these lakes.

Some of the Cyanobacteria like *Anabaena* sp, *Anabaenopsis* and *Oscillatoria*, can release cyanotoxins with lethal effects on mammals (Lyra et al., 2001; WHO, 1999). Although studies on cyanotoxins have focused on humans and other livestock (Leão et al., 2010), it is possible that they have similar toxic effects on aquatic organisms, including fish. Some *Anabaena* species release anatoxin-a which is a neurotoxin with lethal effects when tested on *Cyprinus carpio* larvae (Oswawald, Rellan, Carvalho, Gago, & Vasconcelos, 2007). The presence of toxic cyanobacteria might be hindering fish occurrence in the lakes. With the exception of Lake Kikorongo which sometimes receives flood waters from the neighboring Lake George (Hecky & Kilham, 1973; Mungoma, 1990; Nkambo et al., 2015), the rest of the lakes considered under

study are located in closed basins with no connections to other lakes and river systems. This implies that these lakes have no possibilities of being seeded with fish by inflowing waters from other natural systems. The reported occurrence of African Catfish (*Clarias gariepinus*) in Lake Kikorongo in the rainy season may be a result of flood waters from Lake George. Nkambo et al (2015) found that incoming flood waters from Lake George cause a reduction in the salinity of Lake Kikorongo, rendering it habitable to fish during the rainy season. It is possible that catfish (*Clarias gariepinus*) brought along with floods remains in the lake during the rainy season and dies in the dry season as the environmental conditions like salinity become extreme.

4.5 Conclusions

These findings are in agreement with earlier studies by Hecky and Kilham (1973), Matagi (2004), Satyanarayan et al (2007), Siddiqi (2007) and Yannawar and Bhosle (2013) which reported cyanobacteria to dominate algal biomass in saline systems; and are contrary to earlier studies by Stenger-Kovács et al. (2014) which reported diatoms to dominate the algal biomass in saline lakes. Lakes considered under this study had very low zooplankton abundance and diversity, with Lake Kikorongo which had the highest zooplankton biodiversity having a rotifer, *Brachionus calyciflorus* as the most abundant, ranging between 50 to 100 individuals /litre. None of the study lakes had fish.

The information on the fish and plankton biodiversity in these alkaline, saline systems provides the ecological basis for their development and management. The low plankton densities and abundance together with absence of fish which are potential predators for *Artemia* are indications that these lakes have a high potential for *Artemia* production. The dominant zoo and

phytoplanktons in these systems can be used as bio-indicators in assessing the ecological as well as the impact of climate these unique systems.

4.6 Recommendations

Further comprehensive studies are needed to capture the effect of season variability occurrence and plankton biodiversity in these saline lake systems.

4.7 References

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CHAPTER FIVE

Artemia occurrence, salinity and ionic rates in saline crater lakes of western Uganda

5.0 Abstract

Salinity and ionic composition being among the many factors affecting *Artemia* Occurrence, growth and survival, it was important that salinity and ionic rates of these lakes were studied. Lakes Katwe, Munyanyange, Nyamunuka, Murumuri, Bunyampaka, Bagusa, and Kikorongo were investigated for *Artemia* occurrence, salinity and ionic rates in the wet and dry season. Cyst-like materials along the lake shores and foam suspected to have *Artemia* cysts from the surface of each of these lakes were collected in separate plastic sample bottles for examination in the laboratory for cysts using the density separation technique. In addition, the collected water samples were analysed for other zooplankton and ionic composition in the laboratory. In the laboratory, collected water samples were analysed following APHA (1990) standard operating procedures. All the studied lakes had neither *Artemia* biomass nor *Artemia* cysts at the time of sampling both in dry and wet season. Both in the dry and wet season, the salinity in all the study lakes was significantly higher than that of sea water while the ionic rates of $\text{Cl}^-/\text{SO}_4^{2-}$, Cl^-/Na^+ , K^+ , and $\text{Mg}^{2+}/\text{Ca}^{2+}$ in all the study lakes were all significantly less than that of sea water. The salinity levels during the dry season for all the studied lakes was found to be significantly higher than the salinity in the wet season (t -value = 0.0205). There were no significant seasonal differences in the ionic rates of $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ in the studied lakes with t -values of 0.5665, 0.4082 and 0.4523 respectively. There is need for continuous long term monitoring of these saline lakes for the presence of *Artemia* resources since many other factors like temperature, the presence of wading migratory birds which are dispersion agents for *Artemia* were witnessed.

Keywords: *Artemia* occurrence, ionic rates, saline lakes

5.1 Introduction

Aquaculture production is one route suggested to bridge the gap between the ever increasing demand and the declining capture fisheries production (Namatovu et al., 2018). One of the requirements for sustainable aquaculture industry is reliable source of fish seed. Fish seed requires readily available source of live food, especially at the larval stage and this has been one of the bottle necks for the development of Uganda's aquaculture (Mwanja, Rutaisire, Ddungu, and Aruho, 2015). Although *Artemia* has been reported to be of great importance as part of live food chain in culture of fish larvae (Ogello, Kembenya, Githukia, Nyonje, and Munguti, 2011; Soundarapandian & Saravanakumar, 2009; Triantaphyllidis, Abatzopoulos, & Sorgeloos, 1998), the high cost of imported *Artemia* cysts which ranges between US\$50 to 100 per Kg (FAO, 2011; Ogello et al., 2014), and sold at a market price of between US\$ 150-250 (Mwanja Waitsira Wilson – per comm.) has prohibited its wide use in Uganda's hatcheries. This has resulted in very low survival rates in the majority of Uganda's hatcheries (Kemigabo, Kang'ombe, Jeyaraj, Sikawa, and Masembe, 2016). Uganda being endowed with hypersaline (salt lakes) environments which are among the reported natural habitats for *Artemia* (Castro-Mejía, Castro-Mejía, Castro-Barrera, De Lara-Andrade, & Monroy, 2013; Pinto, Amat, Almeida, & Vieira, 2011; Triantaphyllidis et al., 1998), there was need to investigate whether there is naturally occurring *Artemia* resources. Occurrence of local *Artemia* resources would offer a cheaper option for seed production compared to expensive imported cysts.

Inland saline systems are unique, widely spread all over the world (Brauner, Gonzalez, and Wilson, 2012), most especially in endorheic basins in semi-arid regions (Alcocer & Escobedo,

1993). They are potential sources of natural resources, including salts, aquatic organisms like algae, and invertebrates, such as *Artemia* that are harnessed as starter feeds for fish fry worldwide. In Uganda, with the exception of the salt mining done in lakes Katwe, Kibiro and Bunyampaka (Kasedde, Kirabira, Bähler, Tilliander, & Jonsson, 2014; Kirabira, Kasedde, & Semukuuttu, 2013; Matagi, 2004; Nkambo, Bugenyi, Naluwairo, Nayiga, & Waswa, 2015), the rest of the other reported natural resources in these saline lakes are not yet harnessed. Exploration of occurrence of local *Artemia* resources in these saline lakes systems provides useful information on plausible occurrence of vital natural resources to the aquaculture industry and in the region which can be harvested.

Ionic composition and rates are key factors affecting salinity levels in lakes, therefore affecting the occurrence of various organisms in these systems including fish and *Artemia* (Brauner et al., 2012). Having an understanding of the ionic composition and rates in these saline lakes provides useful information which might explain the occurrence or absence of various organisms in these systems. These inland saline systems have been generally classified into carbonate and bicarbonate ($\text{CO}_3^{2-} + \text{HCO}_3^-$, sulphate (SO_4^{2-}) and chloride (Cl^-), depending on the dominant anion (Alcocer & Escobar, 1993; Mungoma, 1990). *Artemia* resources occur in all these three categories of saline systems (Shadrin, Anufrieva, & Galagovets, 2012; Triantaphyllidis et al., 1998) with a preference for waters with chlorides as the dominant anions (Camargo et al., 2005).

5.1.2 *Artemia* distribution

Artemia are primitive arthropods with segmented bodies and broad leaf-like appendages known as thoracopods (FAO, 2011). They are micro-zooplankton belonging to anostracan crustaceans

occurring on all continents except in the Antarctica (Ben Naceur, Jenhani, & Romdhane, 2012; Ogello et al., 2014; Triantaphyllidis, Abatzopoulos, & Sorgeloos, 1998). They naturally occur in saline lakes with salinities as high as 340g/l (Sorgeloos & Tackaert, 1991; Ogello, Nyong'u, & Van Stappen, 2014; Ogello et al., 2014), at varying ionic compositions, and in different climatic conditions and altitude (Ben Naceur, Jenhani, & Romdhane, 2012). World-wide, over 100 *Artemia* sites have been discovered and more effort is required to explore other sites and identify more species and or subspecies (Abatzopoulos et al., 2009; Ben Naceur et al., 2012; Ogello et al., 2014). *Artemia* has both sexually and parthenogenetically reproducing strains. A number of environmental parameters more especially salinity are responsible for the switch between growing live young ones and production of diapausing cysts (Kaiser, Gordon, & Paulet, 2006). It is the diapausing cysts that can withstand the harsh conditions (Abatzopoulos et al., 2009), stay viable for several years and are able to hatch into live nauplii on return of favorable conditions (Kaiser et al., 2006).

Although *Artemia* has a wide geographical distribution, very little is known about its distribution in Sub Saharan Africa (Van Stappen, 2002). Due to favorable climatic conditions, most African countries are considered to have a huge potential for *Artemia* occurrence. Much of the information about *Artemia* distribution in Africa has been on *A. franciscana*, *A. salina* and a parthenogenetic strain in North African countries like Morocco, Egypt, Tunisia, Libya and Chad sites (Ben Naceur et al., 2012; Ogello et al., 2014). *Artemia* resources have also been reported in the East African region at the coast in Tanga, Tanzania and in Kenya with more speculated in the saline lakes in Western Uganda (Ogello et al., 2014). This further adds to the importance of

study to explore the plausible occurrence of *Artemia* resources in the saline lakes of western Uganda.

5.1.3 *Artemia* importance

Artemia as live food within the aquaculture industry dates back to the 1930s after it was discovered to be of high nutritional value to the new hatched fish larvae (Bengtson, Léger, & Sorgeloos, 1991; FAO, 2011). Owing to its high nutritional value and convenience in use, *Artemia* is acknowledged world-wide as one of the most important starter feed within hatcheries of shell and fin fish. Despite the various efforts to find a suitable starter feed to replace *Artemia* because of cyst shortage associated with the high costs of production of *Artemia* cysts and varying levels of yield (FAO, 2011), up to date no suitable artificial starter feed to replace *Artemia* has been found (Ogello et al., 2014). Over 2000 tons of *Artemia* dry cysts are used annually within the aquaculture industry world-wide (FAO, 2011; Ogello et al., 2014) and its demand is projected to increase with the ever increasing demand for fish leading to increased aquaculture production. It is this projected growth in aquaculture production that has led to increase in demand for *Artemia* as a starter feed leading to numerous efforts for search for alternative starter feeds, as well as new sites for production (FAO, 2011).

Through bio-encapsulation and enrichment, *Artemia* is used as a medium to supply essential nutrients like vitamin, high unsaturated fatty acids (FAO, 2011), medication and antibiotics to shrimp and fish larvae within the aquaculture industry (Kaiser et al., 2006; Osinga et al., 2008; Roiha, Otterlei, & Samuelsen, 2010). Besides its use in aquaculture industry, it has also used to improve salt quality because of its capacity to filter algae from the salt pans (Ruebhart, Cock, &

Shaw, 2008). Ogello et al., (2014) reported salt production companies to have agreed *Artemia* production is vital in controlling the algal blooms within the salt production pans.

5.2 Materials and Methods

5.2.1 Study Area

The studied lakes, i.e. Katwe (029.87033°E, 00.13217°S), Munyanyange (029.88591°E, 00.13513°S), Nyamunuka (029.98743°E, 00.09344°S), Bagusa (030.17958°E, 00.09793°S), Murumuri (029.99186°E, 00.07323°S), Bunyampaka (030.12819°E, 00.03765°S) and Kikorongo (030.01228°E, 00.01190°S) are found in western Uganda and belong to Katwe – Kikorongo volcanic field. With the exception of Kikorongo which is sometimes fresh, other lakes were found to be alkaline saline in nature (Nkambo, Bugenyi, Naluwairo, Nayiga, & Waswa, 2015) with an average depth range between <1 to 6m (Kirabira, Kasedde, & Semukuutu, 2013).

5.2.2a *Artemia* Cysts Occurrence

Data collection was carried out over a period of one year between 26th of February, 2014 and 25th February, 2015. Since this area experiences a bimodal rainfall pattern (Jassogne, Laderach & Van Asten, 2013; Kasedde et al., 2014), data collection considered the two wet seasons (March – May and September – December) and the two dry seasons (December-February and June-August). In the field cyst-like materials along the lake shores and foam suspected to have *Artemia* cysts from the surface of each of the studied saline crater lakes were collected in plastic sample bottles for further laboratory analysis. In the laboratory, at Kajjansi Aquaculture Research and Development Center (KARDC), cyst-like materials and foam suspected to contain *Artemia* cysts were tested for *Artemia* cysts using the density separation technique. The collected materials were washed in a strong brine solution (above 45g/l), followed by fresh water (0g

and finally rinsed in a strong brine solution (above 45mg l^{-1}). *Artemia* cysts float in strong brine solution, sink when washed in fresh water and float again when washed in a strong brine solution. Any cyst-like materials which floated in strong brine solution, sunk in fresh water solution and floated again on washing in strong brine solution were taken for further microscopic observation for confirmation as to whether it was *Artemia* cysts. Any collected materials that behaved contrary to the above mentioned observation for cysts under the density separation technique were considered not to be *Artemia* cysts.

5.2.2b *Artemia* biomass occurrence

Four litres of surface water were collected and concentrated by filtering it through a $50\mu\text{m}$ zooplankton net. The plankton net sieve was rinsed with distilled water after filtering the four litres of the collected lake water and the rinsed water together with the residue collected in a plastic bottle. This was fixed by adding ethanol (90%) equal to a tenth of the total volume of the sample collected in the plastic sample collection bottle. In the laboratory at KARDC, the collected water samples were observed under a dissecting microscope for any *Artemia* biomass present.

5.2.3 Ionic composition and rates determination

Surface water samples for chemical composition analysis of each of the studied lakes were collected using a Van Dorn water sampler from three geo-referenced sampling points and stored in pre-rinsed Nalgene bottles for analysis in the laboratory. In the field all water samples collected in pre-rinsed Nalgene bottles were kept in a cooler box containing dry ice and later transferred to the National Fisheries Resource Research Institute (NaFIRRI) laboratory in Jinja. In the laboratory the collected water samples were analysed for the following parameters:

alkalinity, bicarbonate (HCO_3), carbonate (CO_3), Sulphate (SO_4), Silicates (SiO_2), Chloride (Cl), Fluoride (F), Sodium (Na), Magnesium (Mg), Calcium (Ca), Potassium (K), and Hydrogen Sulphide (H_2S), Sodium, Magnesium, Calcium, Potassium, Chloride (Cl), and silicon followed APHA 1975 standard Operating procedures. The measured salinities in the dry and wet seasons as well as the ionic rates were compared with those of sea water. Seasonal differences in salinity and ionic rates within the studied lakes were tested for significance using a two tailed, paired t-test.

5.3 Results

5.3.1 *Artemia* occurrence

All the studied lakes had neither *Artemia* cysts nor their biomass at the time of sampling in both the dry and wet season.

5.3.2 *Salinity and Ionic rates*

The $\text{Cl}^-/\text{SO}_4^{2-}$ ionic rates in this present study ranged between 0.5 and 1.73 observed in Lake Bagusa and Murumuri respectively and these are far less than the 7.16 of sea water.

Table 5.3.2. 1: Salinity and ionic rates of the different studied lakes in the dry and wet season

Lakes	Salinity (g l^{-1})		Ionic rates					
	Dry	Wet	$\text{Cl}^-/\text{SO}_4^{2-}$		$\text{Cl}^-/\text{Na}^+ + \text{K}^+$		$\text{Mg}^{2+}/\text{Ca}^{2+}$	
			Dry	Wet	Dry	Wet	Dry	Wet
Bagusa	67.29	67.93	0.50	0.62	1.28	1.29	0.28	0.28
Bunyampaka	88.12	72.4	0.77	0.88	0.84	1.05	0.23	0.23
Katwe	116.67	97.07	0.78	0.82	0.69	0.99	0.36	0.36
Murumuli	87.2	72.15	1.73	0.81	1.30	1.16	0.23	0.23
Kikorongo	15.97	0	0.06	-	0.09	-	0.25	-
Nyamunuka	-	100	-	0.55	-	0.71	-	0.38
Munyanyange	-	19.2	-	0.69	-	0.95	-	0.28
Sea water	35		7.16		1.73		3.18	

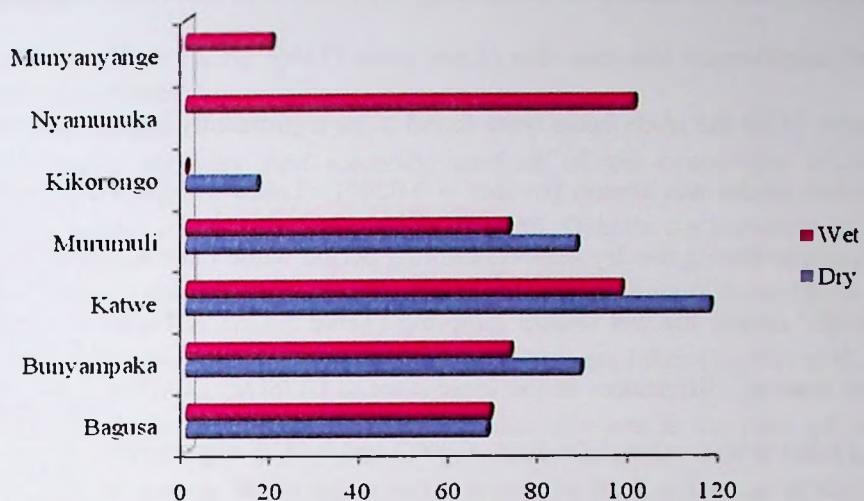


Figure 5.3.2. 1: Dry and wet season salinity changes in the studied saline lakes

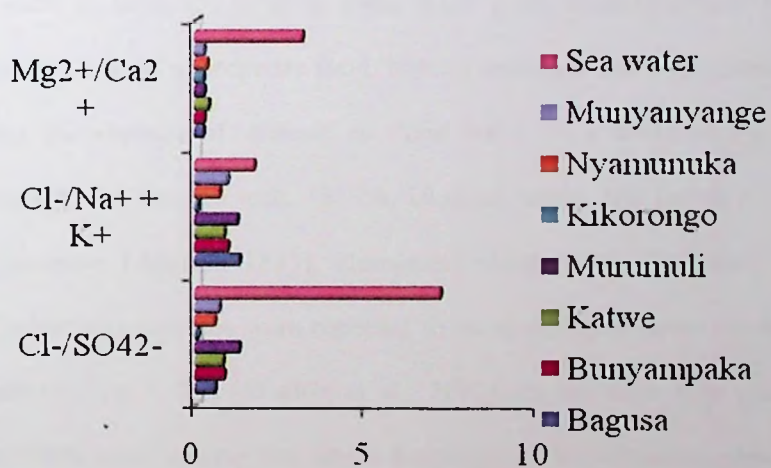


Figure 5.3.2. 2: Ionic rates of the different studied lakes in comparison with sea water

Both in the dry and wet season, the salinities of all the study lakes were significantly higher than that of sea water while the ionic rates of $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ in all the study lakes were all significantly less than that of sea water (Table 5.3.2.1 & Figure 5.3.2.2). The dry season salinities of all the study lakes were found to be significantly higher than the salinities of these same lakes in the wet season (t -value = 0.0205). Lakes Munyanyange and Nyamun were dry and empty during the dry season sampling period while Lake Kikorongo was fresh with a salinity of 0mg l^{-1} during the wet season sampling (Table 5.3.2.1 & Figure 5.3.2.1). There were no significant seasonal differences in the ionic rates of $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ in the studied lakes with t -values of 0.5665, 0.4082 and 0.4523 respectively.

5.4 Discussion

5.4.1 *Artemia* occurrence

This study constitutes the very first scientific analysis of the occurrence of local *Artemia* resources in the alkaline saline lakes of western Uganda. Despite the presence of the flamingo birds which are among the dispersion agents for brine shrimp eggs (Castro-Mejía et al., 2013) and the speculation of occurrence of *Artemia* resources in these lakes (Ogello et al., 2014), none of the study lakes was found to have *Artemia* biomass or cysts at the time of sampling both during the dry and wet season. Wind and wading migratory birds are some of the main vectors for the spread and maintenance of gene flow between distant *Artemia* populations (Abatzopoulos et al., 2009; Kaiser et al., 2006; Shadrin, Anufrieva, & Galagovets, 2012). The presence of migratory birds such as flamingo birds in these lakes gives an anticipation of *Artemia* being inoculated. Predation, lack of appropriate food, habitat activities and other abiotic factors might be responsible for the absence of *Artemia* in these lakes. In a study of the distribution and historical biogeography of *Artemia* lech, 1819 in Ukraine, adults and larvae of some Coleoptera (*Hygrotus ennegrammus* (Ahrens, 1833), Hemiptera (Coricidae), Ostracods *Eucypris inflata* (Sars 1903) and other invertebrates were reported to have probably eaten all the *Artemia* in the lakes with salinities below 110g/l (Shadrin et al., 2012). In the same way zooplanktons, other invertebrates and birds most notably the lesser flamingos (*Phoeniconaias minor*) which live in these saline crater lakes might be eating all the *Artemia* inoculated in these lakes. Although *Artemia* can occur at salinities as high 340g/l (Shadrin et al., 2012), other abiotic factors like dissolved oxygen may become limiting factors at such high salinity. Dissolved oxygen

significantly decrease with increasing salinity (Shadrin et al., 2012) and this could be a reason to explain the absence of *Artemia* in these hypersaline lakes.

Planktons serve as the primary source of food for different groups of aquatic animals (Said Balough, Jahangir, Baloch, & Lashari, 2014). The zooplankton biodiversity decreases with increasing salinity (Larson & Belovsky, 2013; Medina-Junior & Riezler, 2005), with only a few species able to withstand the harsh conditions becoming dominant. Although some bacterial strains are potential diets for *Artemia* (Van Stappen, Huynh, Van Hoa, Sui, Deng, & Bost, 2014), phytoplankton constitute the main natural *Artemia* diet (Ruebhart, Cock & Shaw, 2014). Because the studied lakes had very high salinities, it is expected that their plankton biodiversity is very low, hence the lack of appropriate food organisms for *Artemia*. This is further supported by findings from a study on feeding biology of parthenogenetic *Artemia* in shallow seasonally stratified hypersaline lake in Western Australia, where limited plankton primary productivity was reported (Savage & Knott, 1998). Although salts are part of integral biochemistry of life in aquatic environments, at very high concentration they have toxic effects on organisms (Dunbar, McGregor, & Horrigan, 2005). *Artemia* being non selective filter feeders (FAO, 2011; Savage & Knott, 1998; Van Stappen et al., 2014), it would require too much energy to filter large volumes of water so as to capture the very few existing plankton. Much as habitat activities play a role in creating new habitats for *Artemia*, anthropogenic activities, such as mining have destructive effects on the old existing habitats (Shadrin et al., 2012). This very explanation might be extended for the absence of *Artemia* in lakes like Katwe and Bunyampaka, where salt mining constitutes the main commercial activities.

5.4.2 Salinity, ionic composition and rates

It was found that ionic rates of the saline systems are different from those of sea water. Alcocer and Escobar (1993) reported that the majority inland saline systems have different ionic composition from that of sea water. Ionic composition in the inland systems is largely determined by the incoming waters from inflow and rainfall, dissolved material from the soils, rocks, and sediment of the drainage and lake basin (Brauner, Gonzalez, & Wilson, 2012). Climate, geography, and topography of the drainage basin are other key factors which affect ionic composition in saline waters (Alcocer & Escobar, 1993; Stenger-Kovács et al., 2014). Climate and hydrological inputs from precipitation, surface runoffs, groundwater inflow and outflows such as evaporation are determinants of ionic content in brine (Gajardo, Sorgeloos and Beardmore, 2006). Worldwide ionic composition and ionic rates of saline waters vary but with regional consistence (Brauner et al., 2012). This regional consistence is witnessed in this study where all the studied lakes had $\text{Cl}^-/\text{SO}_4^{2-}$ rates less than 2, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$ less than 1.5, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ less than 0.5 both in the dry and wet sampling season (Table 1). In a study of fluoride concentration and mineralogical composition of East African Magadi (trona), Lake Magadi in Kenya, and Natron in Tanzania were found to have very similar mineral composition (Nelson, 1999). This further emphasizes the regional consistency in ionic composition in these saline water bodies. From the ionic rates in this study, it indicates that sea water has more chloride ions than any of the current studied lakes but this cannot be used to explain the absence of *Artemia* in the saline lakes of western Uganda, since they have been reported in hypersaline environments (Ben Naceur, Jenhani, & Romdhane, 2012) other than the sea. The ability of *Artemia* to occur in environments of diverse ionic concentrations is well documented and is one of the factors responsible for diversity in phenotypes within the *Artemia* strains (Ben Naceur et al., 2012).

Nonetheless, it should be noted that the majority of the study lakes are carbonate-sulphate dominated in terms of anion composition (Nkambo et al., 2015), yet a number of science articles have reported the brine shrimps to have a preference for carbonate-chloride dominated waters (Camargo et al., 2005). This might be pointed to as an explanation for the absence of *Artemia* resources in these studied lakes.

5.5 Conclusion

No *Artemia* resources were found in saline lakes in western Uganda. The salinity and ionic rates of the lakes considered under this study were found to be different from that of sea water. There is need for long term monitoring of these saline lakes for the presence of *Artemia* resources, since many other factors like temperature, the presence of wading migratory birds which are their dispersion agents were witnessed.

5.6 References

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CHAPTER SIX

Hatchability of commercial *Artemia* strains in natural waters of five saline crater lakes of western Uganda

6.0 Abstract

The hatchability of commercial strains of *Artemia* in waters from different habitats was examined with a major objective of identifying the best performing strain, as well as the best suited saline crater lakes in western Uganda for production, based on hatchability. The tested commercial strains were *Artemia franciscana* from Great Salt Lake (GSL) in USA, *A. franciscana* from salt ponds in Vinh Chau (VC), China, the Parthenogenetic *Artemia* from Kazakhstan (TUZ) and the Parthenogenetic *Artemia* strain from Siberia (PAS). The tested waters were from lakes Katwe, Murumuri, Bunyampaka, Bagusa and Maseche in Western Uganda. *Artemia* cysts were hatched under conditions described by Van Stappen (1996) in fabricated hatching cones at a salinity of 40ppm, temperature of $27\pm 2^{\circ}\text{C}$, and light of 2000lux. Hatchability was monitored after 24hour and 48hour of incubation. *Artemia franciscana* (VC) had significantly higher hatching percentage than all tested strains ($p < 0.05$). Lakes Katwe, Bunyampaka and Maseche waters presented the highest hatching percentages and were considered as the best suited for *Artemia* production in western Uganda.

Key words: *Artemia*, Hatching percentage, saline crater lakes

6.1 Introduction

Artemia is commonly referred to as a brine shrimp and belongs to the order Anostraca (Ben Naceur, Jenhani, & Romdhane, 2012). They are micro crustaceans reported to inhabit extremely

saline water environments, including inland water bodies and coastal solar salt works (Gajardo & Beardmore, 2012; Ogello, Kembanya, Githukia, Nyonje, & Munguti, 2014; Sui, Deng, Wang, Sorgeloos, & Van Stappen, 2014). The use of these micro crustaceans as an important larval food for fish dates back to the 1930s (Bengtson, Leger, & Sorgeloos, 1991). *Artemia* is an excellent live food in the aquaculture industry both in fish and crustacean culture (Makridakis & Vatnium, 1999; Marini, 2002). Its high nutritive value, ease in handling (Bengtson et al., 1991), adaptability to inhabit diverse environments and geographical regions, short generation time, high fecundity, and ability to grow at high densities are among the key factors put forward for its wide use (Lavens, Tackaert, & Sorgeloos, 1986; Soundarapandian & Sarwanakumar, 2009). Uganda is one of the African countries experiencing poor survivals in fish hatcheries (Mwanja, Rutaisire, Constatine, Ddungu, & Aruho, 2015). Lack of a suitable starter feed is one of the probable factors for the low survivals in Ugandan fish hatcheries. A few commercial hatcheries have used *Artemia* as a live starter feed in Ugandan hatcheries with good levels of success in improving hatchery survival as it has been in many other countries world-wide. The current biggest impediment to its use in Ugandan hatcheries is the high cost of imported cysts (Nkambo, Bugenyi, Naluwairo, Nayiga, & Waswa, 2015). The cost of imported *Artemia* cysts has continued to increase since the 1950s and by the mid seventies its prices were reported to range between US\$ 50-100 per kg (Bengtson et al., 1991). Many small to medium hatcheries in developing countries can hardly afford expensive imported *Artemia*. Uganda being endowed with a number of hypersaline lakes which are suspected habitats for *Artemia* (Ben Naceur et al., 2012; Gajardo & Beardmore, 2012), this makes exploration of possibilities for production of local *Artemia* resources to availing cheap affordable *Artemia* resources for use by Ugandan fish hatcheries a noble idea. If successfully produced, affordable local *Artemia* resources could result

in its frequent use hence lead to improved survival in hatcheries and enhanced aquaculture production. In a drive towards exploring possibilities for production of local *Artemia* resources, several saline crater lakes were surveyed with no local resources being found (Nkambo, Bugenyi, & Nayiga, 2015).

Artemia being highly osmotolerant (Ben Naceur et al., 2012), it is able to adapt to a diverse range of extreme environmental conditions (Gajardo & Beardmore, 2012). In the absence of local strains, domestication of commercial strains using waters from local hypersaline environments as has been successfully done elsewhere offers realistic option for production of cheaper *Artemia* resources. A classical example of such domestication is *A. franciscana* being artificially produced in salt ponds in Vietnam (Kappas, Abatzopoulos, Hoa, Sorgeloos, & Beardmore, 2004; Sui et al., 2014). Its controlled inoculation in salt ponds is reported to play a role in management of salt pond ecosystems (Sui et al., 2014). *Artemia* populations have also been inoculated in salt pans as a biological control measure for algal blooms, thereby resulting into improved salt quality (Hajirostamloo & Pourrabbi, 2011). Use of waters from hypersaline environments in Uganda to break diapauses and cause hatching of selected commercial *Artemia* strains is one of the crucial steps towards identifying suitable strains for domestication. Therefore, the purpose of this study was to identify the best performing commercial *Artemia* strain in terms of hatchability, as well as the best suited lake among the selected saline crater of western Uganda for production. In this study, a strain found to show the highest hatchability were considered the best performing, while the lake whose waters were used in a hatching experiment which showed the highest hatchability was considered the best suited lake. The hatchability of *Artemia* cysts was

defined as the proportion in percentages which were able to break diapauses to give live nauplii in comparison to the total number of cysts incubated.

6.2 Materials and Methods

An *Artemia* hatching unit was fabricated using conical chicken drinkers which were modified to have a tap at the bottom for controlling the water volumes. These were supported in metallic rings directly below lumination of approximately 2000lux (Figure 6.2.1).

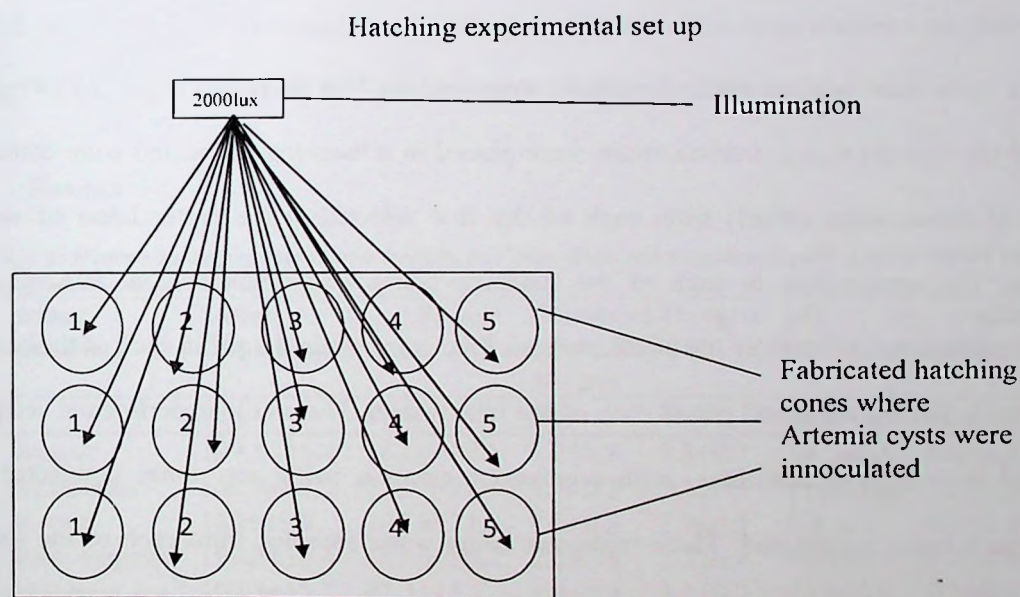


Figure 6.2. 1; Experimental setup showing placement of hatching cones during the hatchability experiment.

All the water samples used in the hatchability experiments were filtered using a series of sieves of 200 μ m, 100 μ m and 50 μ m to remove the thick algal biomass and other possible predators to the *Artemia* nauplii. *Artemia* is reported to be defenseless in nature, prone to destruction by a wide range of predators and therefore can only occur where predators are excluded (Jennings &

Whitaker, 1941; Kumar & Babu, 2015). The salinity in all the used water samples was reduced to 40g/l by diluting using filtered under ground fresh water from Kajjansi (salinity of 0g/l). The pH of the different water samples after dilution was recorded.

The tested *Artemia* strains (*A. franciscana* from Great Salt Lake (GSL), artificially produced from salt ponds in Vinh Chau, Vietnam (VC), Chinese strain (Chinese), TUZ Parthenogetic *Artemia* from Kazakhstan (TUZ) and Parthenogenetic *Artemia* strain from Siberia (PAS)) were obtained from the Reference Center at Ghent University, Belgium, Salt Research Institute of China of China Salt Industry Corporation, China and Can Tho University, Vietnam, respectively.

Artemia cysts were hatched under conditions described by Van Stappen (1996). 2g of cysts of each of the five (5) tested *Artemia* strain were placed in a hatching fabricated cone containing 2litres of saline water (40g/l) from each of the five selected saline crater lakes of western Uganda. The temperature in each of the hatching cones was maintained at $27\pm 2^{\circ}\text{C}$ using thermostat heaters (220-240V, 200W, 30cm, Sera Precision) while the pH in each of the hatching cone was left at the measured pH of each of the lake waters. The whole experimental setup was supplied with light at 2000luxs, with continuous aeration using air stones connected to a compressor being maintained. Three replicates using water samples from each of the selected alkailine saline Crater Lake were made for each *Artemia* strain. After a 24hour and 48hour incubation period, three (3) sub-samples of 0.25ml were taken from each cone to monitor the hatchability. Hatchability in this study was used to refer to the hatching percentage. The number of nauplii, embryos and un-hatched cysts of the pooled sample (1ml) were counted under a binocular light microscope (Leica DM750). The hatching percentage was calculated as follows:

Hatching Percentage (%) = Nauplii/ (Nauplii+embryos+ un-hatched cysts) X100%.

Because water quality parameters including dissolved oxygen, alkalinity and pH, which affect hatchability (Sui et al., 2014) were found to vary with seasons in these lakes (Nkambo, Bugenyi and Nayiga, 2015), for this reason the hatchability experiments were done using waters collected in both the dry and wet seasons.

Statistical analysis was conducted on the hatching percentages of the different strains using water samples collected from the lakes in the different seasons using Ms Excel 2007. Significant differences among the water sample treatments were determined using a two- sample t-test, at $P < 0.05$. The p-values were obtained using the calculated t-values from student t-test distribution tables.

6.3 Results

Table 6.3. 1: Water quality physical characteristics (salinity, dissolved oxygen and pH) of the collected waters samples from the selected lakes

water sample	Salinity Dilution(g/l)		Dissolved Oxygen after dilution to 40 (g/l)		pH (-) after dilution to 40g/l	
	Dry	Wet	Dry	Wet	Dry	Wet
Bagusa	199.5±16.4	187.6±17.1	3.2±0.8	3.3±0.7	10.5±0.4	9.8±1.2
Murumuri	162.8±25.5	158.9±22.4	1.7±0.5	2.1±0.6	11.1±1.3	10.1±1.8
Katwe	180±25.9	167.8±23.5	2.6±0.2	2.9±0.1	9.9±0.1	9.1±0.3
Bunyampaka	199.3±15.4	189.6±16.2	2.2±0.6	2.3±0.4	9.6±0.1	9.2±0.4
Maseche	102.3±11.7	92.3±11.7	2.9±0.4	3.1±0.2	10.9±0.5	9.9±0.4

Values given in the table are means and standard deviations of the given parameters.

Both in the dry and wet seasons, water samples from lakes Bagusa and Bunyampaka were found to have the highest conductivity. Lake Bagusa dry and wet season water samples were found to have the highest dissolved oxygen levels, while Lake Murumuri dry and wet season samples had the lowest levels. All the collected water samples remained alkaline after dilution with dry and

wet season water samples from lakes Murumuri and Maseche having the highest pH ranges (Table 6.3.1).

Unlike water samples from Bunyampaka, Murumuri and Maseche which formed much foam after 12hours of aeration, water samples from lakes Bagusa and Katwe had little foam. On observation under a light microscope, no nauplii were observed in all the hatching cones for all the cyst strains after 12hours of aeration and incubation. On sampling after 48hours, all the hatching cones were found to have nauplii being dominated by nauplii with 3 thoracopods (Instar I) with some ciliates (Table 6.3.2).

Table 6.3. 2: *Physical observations taken from the water samples of the different lakes in the hatching cones after 12 and 48hours of aeration and incubation after inoculation of Artemia cysts.*

	Bagusa	Bunyampaka	Katwe	Murumuri	Maseche
After 24hrs	-Little foam formed with cysts well mixing -no nauplii-microscopic examination	- foam which hinder cysts mixing -no nauplii-microscopic examination	-Little foam cysts well mixing -no nauplii-microscopic examination	-foam which hinder cysts mixing -no nauplii -microscopic examination	- foam which hinder cysts mixing -no nauplii-microscopic examination
After 48hrs	-Nauplii -3 thoracopods (Instar I) -Some ciliates	Nauplii -3 thoracopods (Instar I) -Some ciliates	Nauplii -3 thoracopods (Instar I) -Some ciliates	Nauplii - 3 thoracopods (instar I) -Some ciliates	Nauplii -3 thoracopods (Instar I) -Some ciliates

Table 6.3. 3: Hatching Percentage of the different *Artemia* strains using the different water samples collected in dry and wet season after 48hrs of inoculation, aeration and incubation.

Artemia Strain	water sample	Hatching percentage (%) at 48hrs	
		Season of collection	
		Dry	Wet
GSL	Bagusa	3.1±0.3	9.8±1.2*
	Murumuri	4.5±2.7	4.8±2.4*
	Katwe	26.8±9.8	30.0±9.9*
	Bunyampaka	29.0±3.6	27.8±4.0*
	Maseche	29.3±4.3	28.7±4.9*
PAS	Bagusa	2.1±0.3	2.3±0.8
	Murumuri	0.3±0.6	0.2±0.3
	Katwe	1.3±1.5	0.7±0.6
	Bunyampaka	3.3±0.7	1.7±2.0
	Maseche	2.9±0.2	2.5±0.7
TUZ	Bagusa	1.0±1.0	1.3±0.3
	Murumuri	0.7±1.3	0.7±0.6
	Katwe	0.7±1.2	1.5±0.5
	Bunyampaka	0.4±0.7	0.7±0.6
	Maseche	1.1±1.1	1.8±0.4
V.C	Bagusa	93.9±29.4	79.8±15.2***
	Murumuri	65.1±10.0	70.5±6.6***
	Katwe	89.3±6.9	92.7±3.6***
	Bunyampaka	79.5±19.9	82.2±11.4***
	Maseche	83.4±10.7	83.5±9.1***
Chinese	Bagusa	8.8±10.8	12.1±7.3*
	Murumuri	9.8±5.7	9.9±4.9*
	Katwe	33.0±8.2	33.3±9.4*
	Bunyampaka	26.5±17.4	26.5±17.4*
	Maseche	26.5±17.8	26.1±16.6*

Values are means and standard deviation of the three replicates. *Indicates significant difference in hatching percentages between *Artemia* strains ($p < 0.05$).

For all the tested *Artemia* strains there was no significant difference in the hatching percentages between water samples collected in the dry and wet season ($p > 0.05$). In all the collected water samples from all the selected lakes in both the dry and wet season, *A. franciscana* from salt pond in Vinh Chau, Vietnam (VC) was found to have a significantly higher hatching percentage in

comparison to all the other tested strains ($p < 0.05$). Although less than VC, *A. franciscana* from the Great Salt Lake (GSL) and Chinese *Artemia* strains (Chinese) were found to have significantly higher hatching percentages than Parthenogenetic *Artemia* from Siberia (PAS) and Parthenogenetic *Artemia* from Kazakhstan (TUZ). There was no significant difference in the hatching percentages of *A. franciscana* from the Great Salt Lake (GSL) and Chinese *Artemia* strain when tested in the different collected water samples. Parthenogenetic *Artemia* from Siberia (PAS) and Parthenogenetic *Artemia* from Kazakhstan (TUZ) were found to have the lowest hatching percentages. With the exception of the trials with Parthenogenetic *Artemia* from Siberia (PAS) and Parthenogenetic *Artemia* from Kazakhstan (TUZ), water samples from lakes Katwe, Bunyampaka and Maseche generally had higher hatching percentages (Table 6.3.3).

6.4 Discussion

The physico-chemical parameters in the different sampled lakes were comparable to those reported in earlier limnological studies of the saline crater lakes of western Uganda by Nkambo et al. (2015a). The foam observed in all the hatching cones (Table 2) during the incubation and aeration process is probably attributed to the used lake water samples being high in dissolved organic matter resulting from the thick algal biomass in these lakes (Nkambo et al., 2015a). Unique cyanobacteria and algal blooms occur in these alkaline saline lakes (Hadgembes, 2006; Jones & Grant, 1999; Matagi, 2004; Mungoma, 1990). The lack of hatched nauplii after the first 24 hours in all the hatching cones is probably attributed to slow cyst hydration caused by the foam in the different hatching cones. The foam formed on aeration in the different hatching cones meant that many of the inoculated cysts were held up in the foam before getting in contact with water hence took long to be well hydrated. Critical hydration levels have been reported to

limit metabolic activities in *A. franciscana* cysts (Drinkwater & Crowe, 1991) and this in effect prevents hatching by ensuring *Artemia* cyst diapause is not deactivated until after the cysts are well hydrated. *Artemia* cysts swell and become spherical after 1-2 hours after inoculation in seawater. After 12-20 hours of hydration, cyst shells burst leaving the embryo in its hatching membrane visible (FAO, 1996). On rehydration diapauses are deactivated with cysts suddenly undergoing embryonic development (Truchan & Deyrup-Olsen, 1993; Drewes, 2006; Podrabsky & Hand, 2015). It is reported that active metabolisms resume in the cyst after 60% hydration (FAO, 1996). In studies of the effect of hydrogen peroxide treatment in *Artemia* cysts of different geographical origins, hydration levels were found to have an effect on hatching of San Francisco bay (*A. franciscana*) cysts (Van Stappen, Lavens, & Sorgeloos, 1998). The observed ciliates after 48 hours of incubation might have been eggs of zooplanktons which were able to go through the filters and hatched during the incubation process.

Salinity, temperature, food availability and ionic composition play a vital role in determining the occurrence of *Artemia* (Gajardo & Beardmore, 2012; Sui et al., 2014). Since salinity and temperature were regulated and kept the same in all cones in this present experiment, this left ionic composition as one of the factors which might have affected hatchability in this experiment. Effective reproductive isolation in some *Artemia* populations has been reported to be due to the different tolerance to ionic compositions (Munoz, Amat, Green, Figuerola, & Gomez, 2013). In a study of *Artemia* occurrence, salinity and ionic rates in saline crater lakes of western Uganda by Nkambo et al. (2015b), there were no significant differences in the ionic rates of the water samples in the dry and wet season. This could be the reason explaining the lack of significant difference in the hatching percentages between the collected wet and dry season water

samples from all the studied lakes. Although *Artemia* has been reported to be extremely tolerant to environmental parameters like salinity and pH, among others (Ben Naceur et al., 2012; Gajardo & Beardmore, 2012), it is relatively intolerant to certain ions like potassium (Gajardo & Beardmore, 2012; Jennings & Whitaker, 1941). *Artemia franciscana* Kellogg (1906), the most widely distributed brine shrimp (Camargo et al., 2005; Munoz et al., 2013), is adapted to live in a broad diversity of isolated water bodies with varying ranges of water chemistry (Munoz et al., 2013). Genetic and morphological variations have been reported in *A. franciscana* populations with an ability to adapt to local ionic compositions of their environments (Munoz et al., 2013). It has proven to be a good inoculum in many new environments (Sui et al., 2014). These might be the very reason explaining the significantly higher hatching percentage of *A. franciscana* from salt pond in Vinh Chau, Vietnam (VC), and the GSL strain compared to the other tested strains. *A. franciscana* from salt pond in Vietnam (VC) having a significantly higher hatching percentage than the GSL strain, despite both being strain of *A. franciscana* could be because *A. franciscana* from salt pond in Vietnam (VC) is already adapted to salt pond water environments.

Water samples from Lake Murumuri, which is a carbonate-chloride dominated lake (Nkambo et al., 2015a) presented some of the lowest hatching percentage, yet *Artemia* is reported to prefer such waters (Camargo et al., 2005). Lakes Katwe, Bunyampaka, and Maseche despite being carbonate-sulphate dominated (Nkambo et al., 2015a), presented the highest hatching percentages. Even though alkaline conditions are the preferred pH conditions for hatching, extreme alkaline conditions might not be suitable for *Artemia* cyst hatching. In an experiment relating pH with hatching success, cysts of *Triops cancriformis*, a Crustacean Branchiopod were not able to hatch at pH values of 9 and above (Schonbrunner & Eder, 2006). A pH range of 8-8.5

has been reported as the range for optimum *Artemia* cyst hatching (Ben Naceur, Jenhani, & Romdhane, 2009; FAO, 1996; Marini, 2002; Zheng et al., 2015). A similar pH range of 7-8.5 is reported to be the optimum range where maximum hatchability levels were observed in diapausing freshwater shrimp *Streptocephalus dichotomus* (Baird) (Amutha & Subramanian, 2008). The low hatching percentages in water samples from Lake Murumuri might have been caused by the pH of these waters being way above the optimal hatching limit. Alkalinity has been reported as one of the key environmental parameters which affect growth and survival of *Artemia* under culture conditions (Soundarapandian & Saravanakumar, 2009).

6.5 Conclusion

Based on hatching percentages from the present study, *A. franciscana* from salt pond in Vinh Chau, Vietnam (VC), followed by *Artemia franciscana* from the Great Salt Lake (GSL) and the Chinese strain in that order were the best performing strains. Water samples from lakes Katwe, Bunyampaka and Maseche presented the highest hatching percentages giving an indication that waters samples from these lakes might be best suited for *Artemia* production.

6.6 Recommendation

It is recommended that survival and growth performance assessment be included among the different parameters used in assessing performance of the different *Artemia* strains in the subsequent trials. Since decapsulation has been reported to increase hatchability (Hammer, 1986), similar trials should be done using decapsulated cysts.

6.8 References

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CHAPTER SEVEN

Performance of selected *Artemia* strains under bucket (*ex-situ*) and pond (*in-situ*) culture using saline waters from Lake Katwe

7.0 Abstract

Artemia is reported to be an irreplaceable live feed for larval fish rearing. In an attempt to find out if *Artemia* could be cultured using waters from saline crater lakes of western Uganda, experiments were done in buckets at salinities of 40, 60, 80 and 100g/l using waters from Lake Katwe. The waters were filtered through a series of 500, 200, and 100µm sieves to fend off potential *Artemia* predators. Kyebamba spring fresh waters were filtered using a 100µm sieve and used to make the above salinity dilutions. Using light of 2000lux and Lake Katwe waters of salinity of 40g/l, with temperatures maintained between 28 and 30°C using thermostat heaters, GSL and VC *Artemia* strains were hatched by putting 2g of cyst per litre in the hatching bucket. After hatching *Artemia* nauplii were inoculated in culture buckets at densities of 100 nauplii per litre for an 8day culture period. For the pond cultures, waters from both Lake Katwe and Kyabamba spring were filtered using a 50µm net and the hatched nauplii were stocked at the same density of 100nauplii per litre at salinities of 60 and 80g/l. Half of the culture waters were exchanged every after 2 days as means of reducing metabolite accumulation in the culture buckets. In the culture ponds, water losses due to evaporation were replaced on a daily basis. Survival and growth performance were monitored every after 2days for both bucket and pond culture experiments. In the culture experiments, both GSL and VC strains had the best survivals at salinities of 40g/l and 60g/l, while low survivals were recorded at salinities at 80g/l and 100g/l. Although the GSL strain showed better survival than the VC strain, none of these stains survived longer than 8days under bucket cultures. Both GSL and VC strains were able to survive up to

day 10 after inoculation in *Artemia* culture ponds in both treatments. There was no significant difference in the growth performance of both strains based on length increment in the salinity treatment of 60g/l, despite GSL strain having been significantly longer during inoculation. GSL strain grew significantly longer than the VC strain after 6 days at salinity of 80g/l despite the no significant difference in the first 6 days of culturing. The survival decreased for both *Artemia* strains with increasing culture period in all treatments with none of the two strains being able to survive longer than 10 days after inoculation in ponds. Both GSL and VC strains were unable to attain reproductive maturity. More research is needed to establish the cause of the high mortalities after day 8 and day 10 of culturing in buckets and ponds, respectively before attempting commercial production. These results show that it possible to culture *Artemia* using waters from these alkaline saline crater once right bio-manipulation are done.

Key words; Bio-manipulation, Hatchability, *Artemia* survival and Growth performance

7.1 INTRODUCTION

Aquaculture is championed for blue revolution, as a means of providing animal protein to feed the world on a sustainable basis (Kumar & Babu, 2015). It is the fastest growing food production system globally with a reported 9% increase in animal crop per year since 1985 (FAO, 2007). In all the various forms of fish production under controlled environments like fresh water fish production, brackish water aquaculture and mari-culture, production of good quality seed is a major bottleneck (Kumar & Babu, 2015; Nabulime, Rutaisire, Ssekaayi, Smith, & Kabasa, 2015). Successful aquaculture depends on availability of good quality seed (Krishnakumar, Asha, Sivagnanam, & Munuswamy, 2014) and feed (Kumar & Babu, 2015; Mechaly, Angeletti,

De Los Rios, & Cervellini, 2013). To produce quality fish seed effective control over the environmental parameters (Saraswathy, Muralidhar, Sundaray, Natarajan, & Periyamuthu, 2015) is required. This is attributed to the developing larvae being fragile and physiologically undeveloped at the time of hatching (Carlos & Ribeiro, 2012; Rønnestad et al., 2013). It is important to have a feed that is small enough to be ingested by the larvae, containing digestive enzymes to trigger enzymatic activity of the undeveloped gut of the larvae and soft enough to be handled by the delicate gut (Rønnestad et al., 2013). Formulated feeds do not meet these requirements and often result in poor survival and growth in hatcheries (Kumar & Babu, 2015). It is for these reasons that live food organisms are the preferred feeds and the most used starter feeds in the aquaculture industry. *Artemia* is reported to be an irreplaceable live feed for fish larval rearing (Sorgeloos, Dhert, & Candreva, 2001). So no formulated diet has been able to completely replace live feeds as a starter (John, Abatzopoulos, & Marian, 2004; Krishnakumar et al., 2014; Liu et al., 2014; Sorgeloos et al., 2001). *Artemia* is the most important live food organism for shell and fin fish hatcheries (Krishnakumar et al., 2014; Kumar & Babu, 2015; Mechaly et al., 2013; Shanmugasundaram, Rajendran, & Thangasamy, 2017). It is very nutritious containing both macro and micronutrients required for both fish and crustacean larvae (Mechaly et al., 2013). Because of their highly efficient osmo-regulatory systems, *Artemia* are among the very few organisms which can inhabit hypersaline environments (Abatzopoulos, Kappas, Bossier, Sorgeloos, & Beardmore, 2002; Castro, Gajardo, Castro, & Castro, 2006; Gajardo & Beardmore, 2012; John et al., 2004; Mechaly et al., 2013; Vignatti, Cabrera, Pilati, & Echaniz, 2014). Salinity and temperature are among the various factors affecting the survival and growth of aquatic organisms (Browne & Wanigasekera, 2000; Castro-Mejía et al., 2011; Kumar

& Babu, 2015). *Artemia* is reported to have a salinity tolerance range as high as 300 g/l (Brown & Wanigasekera, 2000; Saji, Al Dhaheer, Shah, & Soorae, 2016).

Artemia being one of the most highly demanded for live feed for a successful aquaculture industry (Asem, Rastegar-Pouyani, & Rios-Escalante, 2010; Krishnakumar & Munuswamy, 2014; Mohebbi et al., 2016), and in view of its shortage on the market, deliberate efforts should be made to enhance commercial exploitation, as well as development of new resources (Abatzopoulos, Agh, Van Stappen, Razavi-Rouhani, & Sorgeloos, 2006; Anh, 2014; Lavens & Sorgeloos, 2000). Among the proposed interventions for increasing *Artemia* resources is the natural harvesting from new sites, such as those reported in France, China, Columbia, Argentina, and Canada, among others (Anh, 2014). Although *Artemia* cysts can be dispersed by natural agents like wind and birds (Ben Naceur, 2017; Gajardo & Beardmore, 2001), deliberate inoculation techniques in salt ponds with non-endemic *Artemia* nauplii with a target of culturing for cyst production has been used with relative success in Thailand, Philippines, Vietnam and other countries (Anh, 2014). In East Africa deliberate inoculations of *Artemia franciscana* along the Kenyan coastal has resulted in its successful adaptation in the Kenyan environmental conditions (Ogello, Kembenya, Githukia, Nyonje, & Munguti, 2014). Understanding the response and performance of the commercial *Artemia* strains in waters from the saline crater lakes of western Uganda is one of the major steps required before attempting deliberate inoculations in saline pans of Uganda. It was therefore the aim of this study to understand the performance of *A. franciscana* from the Great Salt Lake (GSL strain) and from Vinh Chau (VC strain) under bucket (*ex-situ*) and pond (*in-situ*) experiments using waters from Lake Katwe, a saline crater in western Uganda. In addition, such a big range as reported in nature of up to

340g/l (Castro et al., 2015) is difficult to use under controlled *Artemia* production conditions; hence this study also aimed at identifying the salinity ranges suitable for its production using waters from Lake Katwe.

7.2 MATERIALS AND METHODS

7.2.1 *Water sample collection*

From Kyebamba fresh water spring, 220 litres (L) were collected and stored in a container fabricated with a tap at its bottom to allow easy removal of the stored water during filtration. Using similar systems, 220 L of salt water were also collected from Lake Katwe. The 220 L collection container was also fabricated with a tap at the bottom to ease removal of the collected waters. The 220 L containers having fresh and salt water from Kyebamba fresh water spring and Lake Katwe respectively were placed on support to ensure that they were raised at least 1 metre (m) above the ground to allow for ease removal of the stored water during filtration by opening the tap at the bottom of the 220 L containers.

7.2.2 *Filtration of the collected water samples*



Figure 7.2.2. 1; *The filtration process before making the different salinity dilutions.*

Using a 500 μ m sieve, followed by one of 200 μ m, then a 100 μ m sieve, the collected salt water samples from Lake Katwe were filtered and stored in 40 L buckets. Freshwater samples were also filtered using a 100 μ m sieve and also stored in a different 40 L bucket. 500 and 200 μ m sieves were used to remove the bigger solid particles and possible *Artemia* nauplii predators which could have been present in the collected water samples (Figure 7.2.2.1).

7.2.3 Making culture dilutions

Using a 2 L and a 1 L calibrated water jar for collecting and mixing freshwater from Kyabamba spring with salt water from Lake Katwe in a 20 L bucket, dilutions of salinities 40, 60, 80 and 100g/l were made. A portable refractometer (WZ-211, Salinity/ATC 0~100g/l) was used to measure the salinities when making the dilutions.

7.2.4 Preparation of culture buckets

A total volume of 15 L were made for each salinity dilution and was distributed into three culture buckets by introducing 5 L in each. In total, twelve 10 L plastic buckets were used for culturing the selected *Artemia* strains (*A. franciscana* from the Great Salt Lake (GSL strain) and *A. franciscana* from Vinh chao Vietnam (VC strain)) at different salinities (40, 60, 80 and 100g/l) as shown (Figure 7.2.4.1);

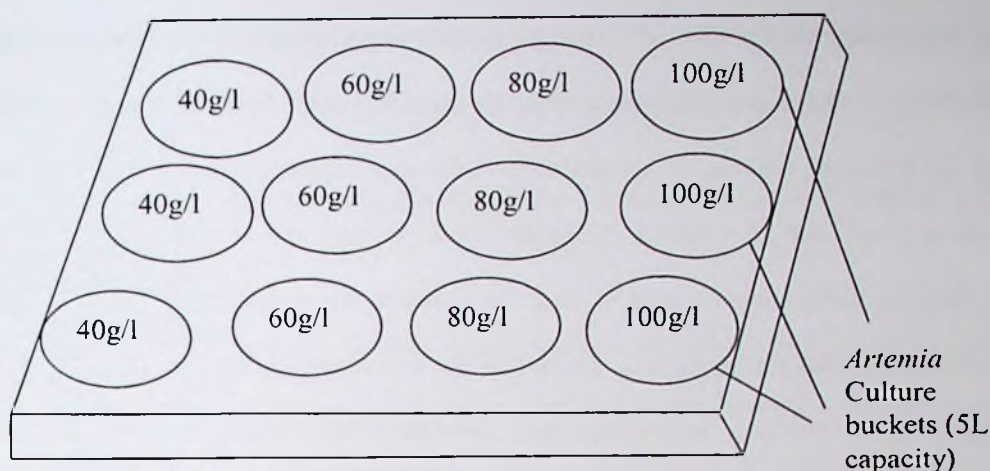


Figure 7.2.4. 1; *Bucket culture experimental set-up*

The big blue 20 L bucket on top just below the fluorescent tubes was the hatching bucket, while the twelve 10 L buckets below were for culturing *Artemia*. Air was continuously supplied to the culture buckets using air stones connected to a compressor using silicon tubes (Figure 7.2.4.1).

As a measure of preventing rain from diluting the bucket cultures, the whole experimental set up was housed in a shade and the culture buckets were covered every time before the rains. A power generator was used as a source of electricity whenever the electricity supply was off.

7.2.5 Hatching of the cysts for inoculation into the buckets (ex-situ) and Pond (In-situ) culture

A calibrated 20 L bucket was filled up to 18 L with filtered water that had been diluted to a salinity of 40g/l and used for hatching the cysts that were inoculated in the buckets (*ex-situ*) and

pond (*in-situ*) for culture. The water in the 20 L hatching bucket was aerated using air stones connected to an air compressor using silicon tubes. The water temperatures in the hatching bucket were maintained at 28 - 30°C using a water heater and thermostat. Fluorescent tubes were placed directly above the hatching bucket to provide bright light at approximately 2000lux during the hatching process. After attaining stable conditions in the hatching bucket, *Artemia* cysts were inoculated at a density of 2g dry cysts per litre and monitored for nauplii after 12 hours. In case there were no nauplii after the 12hours, the process was left to continue while being monitored every after 2hours. The pH of the waters used in hatching was 9.9 ± 0.1 . Neither antifoam which prevents excessive foam formation nor antimicrobial products used for minimizing pathogenic bacteria activity during *Artemia* hatching were used in this experiment. On hatching, the cyst shells together with the unhatched cysts were decanted off by gently transferring the water from the hatching bucket to a transparent 10 L bucket. Carefully the nauplii were siphoned into another clean bucket where they were well bubbled with air using air stones to keep them in suspension before drawing the innoculum using a dropper.



Figure 7.2.5. 1; Separating nauplii from un-hatched cysts and cyst shells, drawing the inocula to determine the density per ml

Using a 5ml plastic dropper, 1ml of the well circulated water sample was taken into a counting vial to determine the number of nauplii per ml (Figure 7.2.5.1). This was done in triplicates and the average nauplii density (nauplii/ml) was used to determine the total volume of the inocula that was introduced to the 10 L buckets and ponds for *ex-situ* and *in-situ*, culturing respectively.

7.2.6 Inoculation of the Hatched Artemia Nauplii in Bucket (*ex-situ*) and Pond (*in-situ*) for culturing

After determination of the nauplii density (nauplii/ml), each culture bucket and pond were inoculated with a 100nauplii/liter. In total 500nauplii were inoculated in 5L of water in each of the culture bucket (Figure 2) while 520,000 nauplii were inoculated in 5200L in each pond. These were then cultured for 10 and 12days for bucket (*ex-situ*) and Pond (*in-situ*), culture respectively.



Figure 7.2.6. 1: Water mixing in Artemia culture ponds before sampling and in-situ measurement of physical chemical parameters

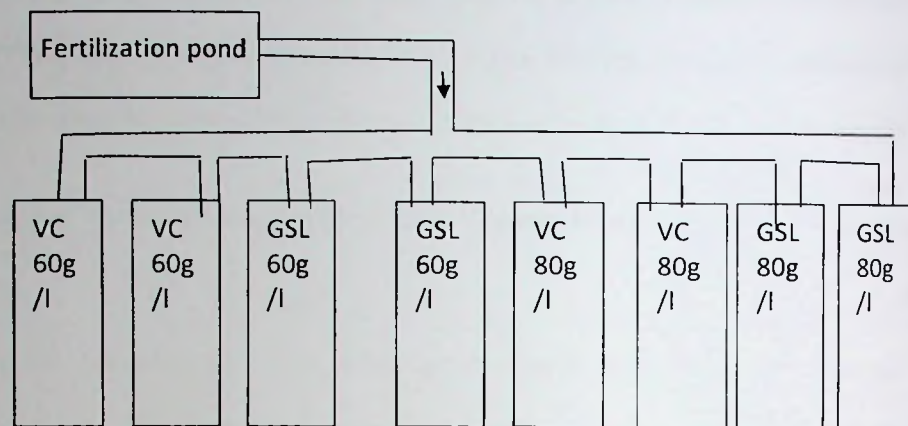


Figure 7.2.6. 2; *Set-up of Artemia pond culture experiment*

A total of 8 ponds (2X4X0.65m) were used in the *Artemia* pond culture experiments. These ponds were set parallel to each other adjacent to Lake Katwe, between Kyabamba freshwater spring and Katwe saltlake. One of the ponds was used for mixing fresh water from Kyabamba freshwater spring with salt water pumped from Lake Katwe, thereby making dilution of 60 and 80g/l which were used for culturing the selected best performing *Artemia* strains from the bucket cultures. The waters in the second pond were fertilized using chicken droppings and cow dung to generate phytoplanktons which was used for feeding *Artemia* nauplii in the culture ponds. It is the fertilized waters which were used to replace the lost water due to evaporation from each of the culture ponds. Then the other 6 ponds were used for culturing *Artemia*, 3 used for culturing *Artemia* at salinity of 60g/l in triplicates and the other 3 used also used for culturing *Artemia* at 80g/l in triplicates.

7.2.7 Daily water exchange and measurement of physical – chemical parameters

Measurements of pH, temperature (°C) and Salinity (g/l) were done twice a day (at 08:00hrs and 14:00hrs) in each culture bucket and pond. Temperature and pH measurements were taken using an OAKTON pH Tester 30, while salinity was measured using a portable refractometer (WZ-211, Salinity/ATC 0~100g/l).

Every day at 10:00am, 50% of the water in the culture bucket was exchanged as a means of introducing more food and reducing on the released toxic waste. This was done by filtering 50% of the total volume of water in the culture bucket using a 50µm sieve. The *Artemia* nauplii trapped on the sieve were washed back into the culture bucket. The 50% volume removed during the sieving was replaced with the same volume of filtered water at the same salinity.

For pond cultures, a white wooden meter ruler was placed in the middle of each *Artemia* culture pond to give a measure of the estimate of the evaporated waters. Every day at 4pm, the water loss due to evaporation from each pond was replaced by pumping an equal volume of water from the fertilization ponds through a 100µm sieve to the *Artemia* culture pond.

7.2.8 Sampling

Every after 2 days at 08:00hrs, a 1L of sample water was collected from each of the culture buckets while a 4 L were collected from each pond. The collected samples were filtered using a 100µm sieve so as to collect *Artemia* nauplii from the different buckets and ponds to assess survival and growth performance in each bucket and pond. *Artemia* nauplii collected on the sieve were washed off into a petri-dish for counting under an inverted microscope so as to determine the percentage survival and growth performance (mean total length) after a given culture period.

7.2.9 Growth and survival determination



Figure 7.2.9. 1; A photograph a 4day old nauplii (*A. franciscana*) with a visible gut during length measurement.

Growth was determined by taking the length measurements of the *Artemia* nauplii in the filtered water sample every after 2days (Figure 7.2.9.1). To determine the growth rate, the length measurements were plotted against time (days). Survival was determined by counting the number of live *Artemia* nauplii found in every liter of the culture water sampled every 2 days. The percentage survival was determined by dividing the number of surviving nauplii per liter by the stocking density per liter, multiplied by 100.

7.3 Statistical Analysis

Stastical analysis was done using IBM SPSS statistics 20 software. Since the data was found not to be normally distributed, it was transformed using natural log (Ln). The mean total length of VC *Artemia* strain nauplii across treatments was compared using one-way ANOVA. Non-parametric test was used to compare growth variations across treatments after the same culture

periods. Independent- samples Kruskal-Wallis test was used to evaluate the growth variations across treatments after the same culture period.

7.4 RESULTS

7.4.1a Bucket culture experimental findings

Table 7.4. 1a; Summary statistics for survival and growth performance of the GSL and VC Artemia strains under Bucket (ex-situ) cultures using saline waters from Lake Katwe

Cultured period	Treatment	N	GSL Artemia strain		N	VC Artemia Strain	
			Total Length $\pm$ SD	Mean Survival $\pm$ SD		Total Length $\pm$ SD	Mean Survival $\pm$ SD
Day0	g/140g/l	90	382.4 $\pm$ 22.8	100 $\pm$ 0	90	405.9 $\pm$ 42.6	100 $\pm$ 0
	g/160g/l	90	380.5 $\pm$ 22.1	100 $\pm$ 0	90	404.3 $\pm$ 40.11	100 $\pm$ 0
	g/180g/l	90	382.4 $\pm$ 24.4	100 $\pm$ 0	90	404.4 $\pm$ 41.8	100 $\pm$ 0
	g/1100g/l	90	384.6 $\pm$ 24.6	100 $\pm$ 0	90	405.9 $\pm$ 42.6	100 $\pm$ 0
	Total	360	382.5 $\pm$ 23.5	100 $\pm$ 0	360	405.1 $\pm$ 41.6	100 $\pm$ 0
Day2	g/140g/l	85	537.7 $\pm$ 77.5 ^a	62 $\pm$ 20 ^a	80	487.2 $\pm$ 55.0 ^a	19 $\pm$ 13
	60g/l	87	524.1 $\pm$ 91.4 ^a	55 $\pm$ 7 ^a	60	442.6 $\pm$ 32.4 ^{ab}	6 $\pm$ 5
	80g/l	79	450.7 $\pm$ 73.3 ^b	64 $\pm$ 7 ^a	37	437.0 $\pm$ 39.7 ^{ab}	1 $\pm$ 2
	100g/l	66	413.7 $\pm$ 32.7 ^c	49 $\pm$ 16 ^a	4	423.5 $\pm$ 11.0 ^c	0 $\pm$ 0
	Total	317	486.5 $\pm$ 89.1	56 $\pm$ 13	181	460.7 $\pm$ 50.5	7 $\pm$ 10
Day4	40g/l	29	596.3 $\pm$ 68.4 ^a	19 $\pm$ 15 ^b	17	526.7 $\pm$ 46.3 ^a	5 $\pm$ 3
	60g/l	30	587.4 $\pm$ 54.5 ^a	53 $\pm$ 16 ^a	21	470.4 $\pm$ 42.3 ^{ab}	13 $\pm$ 12
	80g/l	18	504.8 $\pm$ 62.9 ^b	50 $\pm$ 14 ^a	8	442.8 $\pm$ 7.8 ^{ab}	5 $\pm$ 8
	100g/l	34	443.6 $\pm$ 34.2 ^c	22 $\pm$ 9 ^b	3	436.3 $\pm$ 6.4 ^c	1 $\pm$ 1
	Total	111	532.3 $\pm$ 86.0	36 $\pm$ 20	49	483.3 $\pm$ 51.2	6 $\pm$ 8
Day6	40g/l	6	676.5 $\pm$ 82.5 ^a	2 $\pm$ 1 ^b	1	550.00 ^a	3 $\pm$ 2
	60g/l	17	619.2 $\pm$ 33.6 ^{ab}	20 $\pm$ 5 ^a	8	501.9 $\pm$ 38.5 ^a	6 $\pm$ 5
	80g/l	15	547.1 $\pm$ 29.8 ^c	12 $\pm$ 4 ^a	0		0 $\pm$ 0
	100g/l	13	474.7 $\pm$ 74.0 ^c	3 $\pm$ 2 ^b	0		0 $\pm$ 0
	Total	51	567.9 $\pm$ 85.5	9 $\pm$ 8	9	507.2 $\pm$ 39.5	3 $\pm$ 4
Day8	40g/l	2	660.0 $\pm$ 0.0 ^{ab}	1 $\pm$ 1 ^c	6	528.0 $\pm$ 30.3 ^a	1 $\pm$ 1
	60g/l	4	695.8 $\pm$ 59.2 ^a	2 $\pm$ 2 ^c	1	506.00 ^a	1 $\pm$ 2
	80g/l	2	561.0 $\pm$ 15.6 ^c	2 $\pm$ 2 ^c	3	465.7 $\pm$ 16.8	0 $\pm$ 0
	100g/l	11	495.0 $\pm$ 25.1 ^c	2 $\pm$ 1 ^c	0		0 $\pm$ 0
	Total	19	561.6 $\pm$ 93.4	2 $\pm$ 2	10	507.1 $\pm$ 37.9	1 $\pm$ 1

GSL *Artemia* strain presented better survivals than the VC *Artemia* strain under bucket culture conditions using saline waters from Lake Katwe. For both GSL and VC *Artemia* strains, the percentage survivals decreased across all treatments with increase in the culture days. The highest observed percentages survival for GSL strain under bucket culture was 64 ± 7 observed on day 2 of sampling at salinity of 80g/l. VC *Artemia* strain was found to have much lower survival with the highest being 19 ± 13 observed on day 2 of sampling at salinity of 40g/l. Percentage survival for both the GSL and VC *Artemia* strains greatly decreased after day 6 of culturing. On day 8 of sampling, percentage survival of both the GSL and VC *Artemia* strains across all treatments had reduced less than 4 nauplii/litre, with no nauplii found at salinities of 80 and 100g/l for the VC *Artemia* strain (Table 7.4.1a). Although VC *Artemia* strain nauplii inoculated for bucket culturing were bigger than the GSL *Artemia* strain nauplii at day 0, across all treatments the GSL *Artemia* nauplii were found to have grown to a high mean total length after 2days of culturing and remained with a significantly higher mean total length across all treatments throughout the experiment period (Table 7.4.1a).

7.4.2a Survival of the VC *Artemia* strain in Bucket cultures using waters collected from Lake Katwe

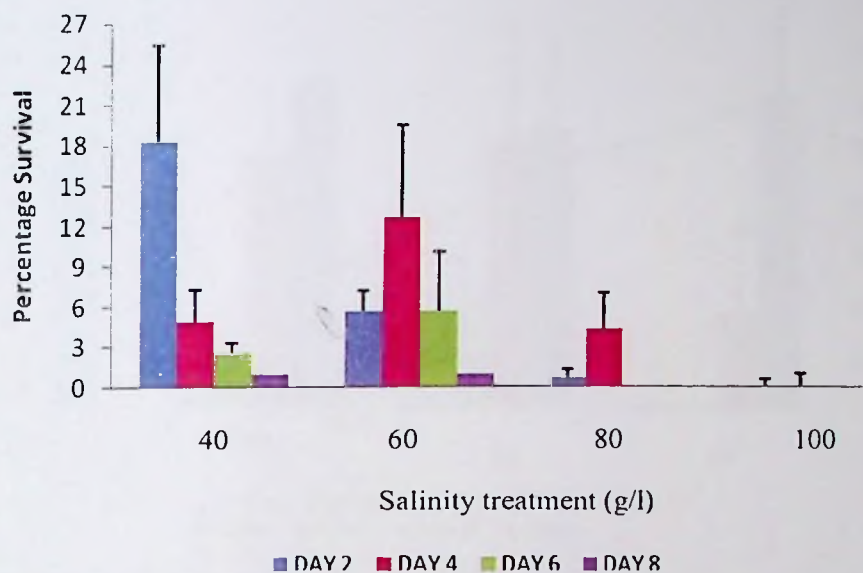


Figure 7.4. 1a; Percentage survival of the VC *Artemia* strain under bucket (ex-situ) culture using saline waters from Lake Katwe

Among the different treatments, there was no significant difference in the survival of the VC strain after the same culture periods. The mean survival decreased with increasing culture days across all treatments with only a few individuals after day 6 of culturing (Figure 7.4.1a).

7.4.3a Survival of the GSL *Artemia* strain in Bucket cultures using waters collected from Lake Katwe

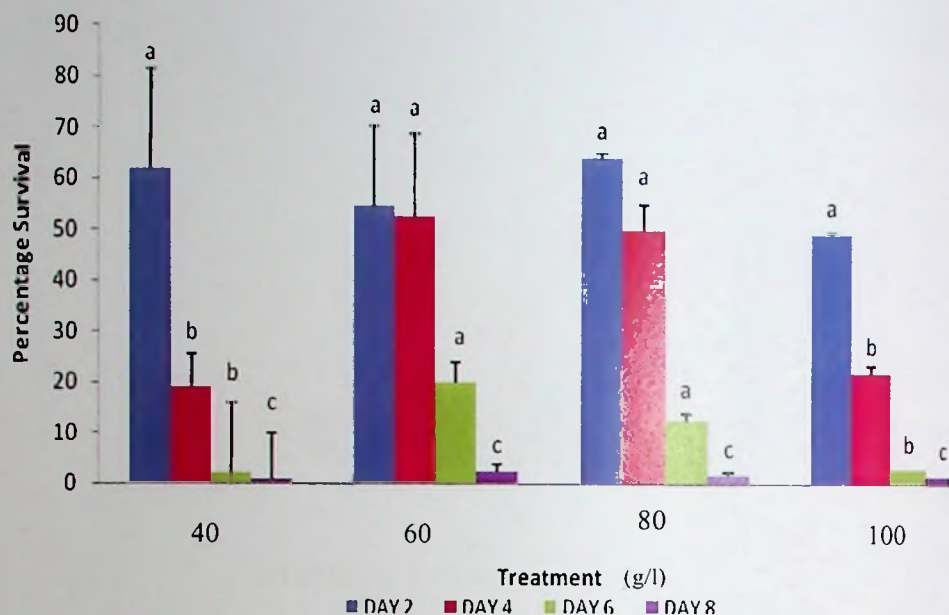


Figure 7.4. 2a; Percentage survival of GSL *Artemia* strain under Bucket (Ex-situ) culture using saline waters from Lake Katwe

The observed percentage survival decreased with increasing culture period across all treatments. The highest percentage survival was observed at salinity of 40 and 80g/l after 2days of culturing while the lowest percentage survival was observed after 8days of culturing at salinities of 40 and 100g/l. No significant difference was observed in the percentage survival across treatments after 2days of culturing the GSL *Artemia* strain ($\text{sig} \leq 0.551$). The percentage survival of the GSL *Artemia* strain cultured at salinities of 60 and 80g/l were found to be significantly higher than the percentage survival of those cultured at 40 and 100g/l after 4 and 6days of culturing with significance levels of 0.033 and 0.001 respectively. After 8days of culturing, no significant difference was found in the percentage survival of the GSL *Artemia* strains across all treatments ($\text{sig} \leq 0.410$) (Figure 7.4.2a).

7.4.4a Growth performance of the VC *Artemia* strain in Bucket cultures using water samples collected from Lake Katwe.

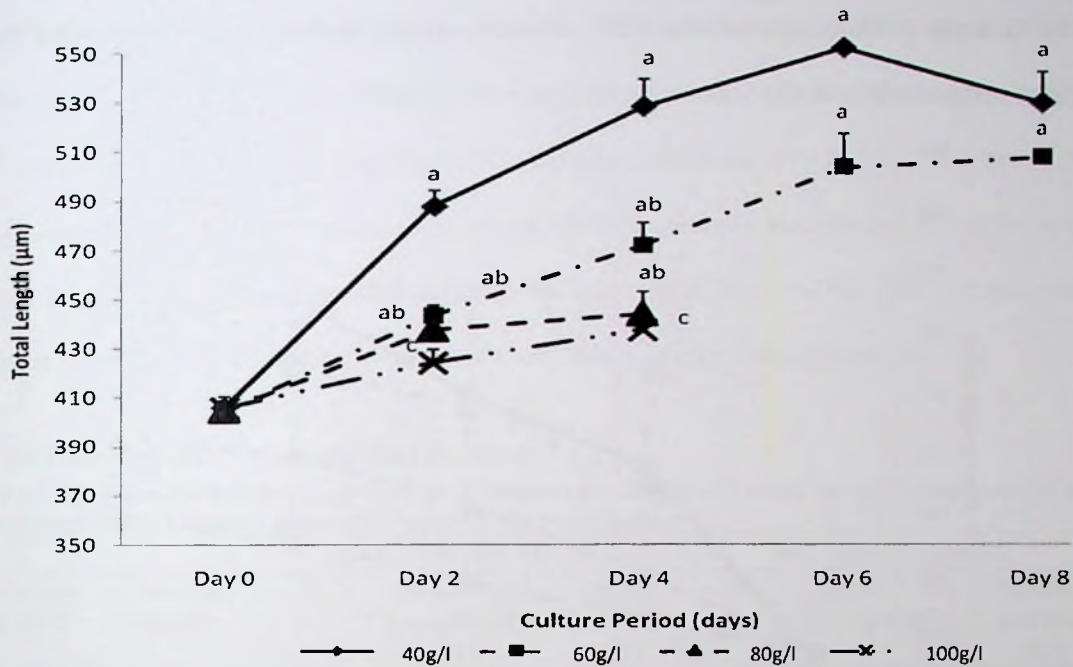


Figure 7.4.4. 1a; Growth performance of the VC *Artemia* strain under bucket (ex-situ) culture using saline waters from Lake Katwe

After the first 2 and 4 days of culturing in buckets, VC *Artemia* nauplii cultured at salinity of 40g/l was found to have significantly higher growth rate (length increment) than those at salinities of 60g/l, 80g/l and 100g/l (Asymptotic sig (2-sided tested) ≤ 0.000). Although nauplii cultured at salinities 60g/l and 80g/l had significantly higher growth rates compared to nauplii cultured at salinity of 100g/l (Asymptotic sig (2-sided tested) ≤ 0.000), the difference in growth performance between nauplii at salinities of 60g/l and 80g/l was not significant after 2days and 4days of culturing. Although *Artemia* nauplii cultured at salinity of 40g/l had a higher growth

rate than those at salinity 60g/l at 6 and 8 days of culturing, the difference in the growth performance was found not to be significant. No nauplii were able to survive beyond day 4 of culturing at salinities 80g/l and 100g/l (Figure 7.4.4.1).

7.4.5a Growth performance of the GSL *Artemia* strain in Bucket cultures using water samples collected from Lake Katwe.

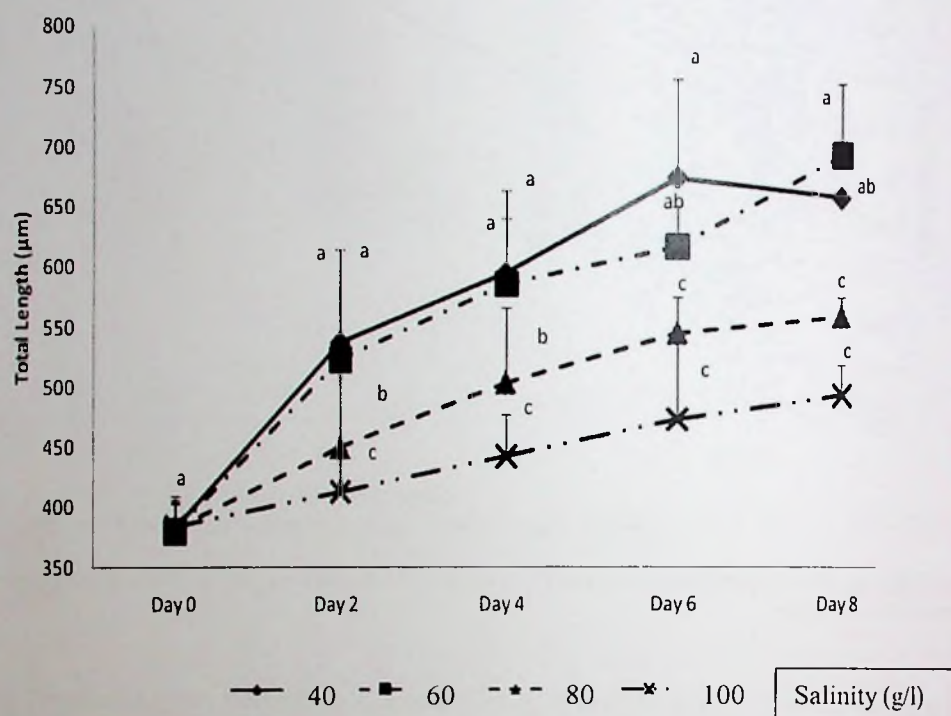


Figure 7.4.5. 1a; Growth performance of GSL *Artemia* strain under bucket (ex-situ) culture using saline waters from Lake Katwe

Nonparametric test was used to compare growth variations (mean total length) of the GSL *Artemia* strain across treatments after the same culture period. Using Independent- samples Kruskal-Wallis test, with the exception of day 0 where there was no significant difference in the mean total length across all treatments (Asymptotic sig (2-sided tested) ≤ 0.694), the the mean total lengths across the different treatment after the same culture period were found to be

significantly different. Throughout the experimentation period, the mean total length of GSL *Artemia* nauplii cultured at salinities of 40 and 60g/l were found to be significantly higher than that cultured at salinities of 80 and 100g/l. Although no significant difference was found in the mean total length of GSL *Artemia* nauplii culture at 40 and 60g/l, the mean total length of GSL *Artemia* nauplii cultured at 40g/l remained slightly higher than that cultured at 60g/l upto day 6 when *Artemia* nauplii cultured at 60g/l took over with a faster growth rate. During day 2 and day 4 of sampling the mean total length of GSL *Artemia* nauplii cultured at 80g/l was found to be significantly higher than the mean total length of GSL *Artemia* nauplii cultured at salinity of 100g/l. There was no significant difference in the mean total length of the GSL *Artemia* nauplii cultured at salinity of 80g/l and 100g/l after 6 and 8days of culturing (Figure 7.4.5.1a).

7.4.1b Pond culture experimental findings

Table 7.4. 2b; Summary statistics for growth performance and survival of the GSL and VC *Artemia* strains under pond culture (in-situ) cultures using saline waters from Lake Katwe

Culture Period	Treatment /Salinity	GSL <i>Artemia</i> Strain			VC <i>Artemia</i> strain		
		N	Mean Total Length(μ m) $\pm$ SD	Mean Survival $\pm$ SD	N	Mean Total Length $\pm$ SD	Mean Survival $\pm$ SD
Day0	60g/l	89	443.0 $\pm$ 35.8 ^a	100 $\pm$ 0	90	404.8 $\pm$ 25.6 ^b	100 $\pm$ 0
	80g/l	90	444.5 $\pm$ 36.5 ^a	100 $\pm$ 0	90	417.5 $\pm$ 34.6 ^a	100 $\pm$ 0
Day2	60g/l	82	742.8 $\pm$ 80.4 ^a	89 $\pm$ 8	82	800.6 $\pm$ 46.8 ^a	100 $\pm$ 7
	80g/l	59	691.9 $\pm$ 89.5 ^b	66 $\pm$ 26	32	746.9 $\pm$ 81.4 ^b	54 $\pm$ 31
Day4	60g/l	46	750.6 $\pm$ 48.1 ^a	46 $\pm$ 22	19	841.7 $\pm$ 68.6 ^a	28 $\pm$ 10
	80g/l	26	709.1 $\pm$ 62.2 ^b	26 $\pm$ 21	26	742.8 $\pm$ 49.2 ^b	30 $\pm$ 15
Day6	60g/l	2	810.0 $\pm$ 43.8 ^a	22 $\pm$ 11	23	870.0 $\pm$ 77.2 ^a	8 $\pm$ 5
	80g/l	1	712.00 ^b	17 $\pm$ 11	21	801.4 $\pm$ 57.5 ^b	8 $\pm$ 9
Day8	60g/l	2	854.0 $\pm$ 60.8 ^a	10 $\pm$ 6	12	871.6 $\pm$ 87.9 ^a	4 $\pm$ 2
	80g/l	1	751.00 ^a	8 $\pm$ 2	15	654.1 $\pm$ 71.6 ^b	4 $\pm$ 4
Day10	60g/l	1	921.0000 ^a	2 $\pm$ 1	2	923.0 $\pm$ 91.9 ^a	3 $\pm$ 3
	80g/l	1	789.0000 ^a	1 $\pm$ 1	1	699.0000 ^b	2 $\pm$ 3

While the mean total length generally increased with increasing culture period for both the GSL and VC *Artemia* nauplii cultured at salinities of 60 and 80g/l, the percentage mean survival decreased with increasing cultured period. The highest mean total lengths were 921.0 μ m and 923.0 μ m for the GSL and VC *Artemia* nauplii respectively both of which were observed at salinities of 60g/l after 10days of culturing. No *Artemia* nauplii were found to survive upto day 12 of culture at both salinities of 60 and 80g/l for both the GSL and VC *Artemia* strain (Table 7.4.2b).

7.4.2b Survival for selected *Artemia* strains cultured using saline waters in pond (*in-situ*) adjacent to Lake Katwe

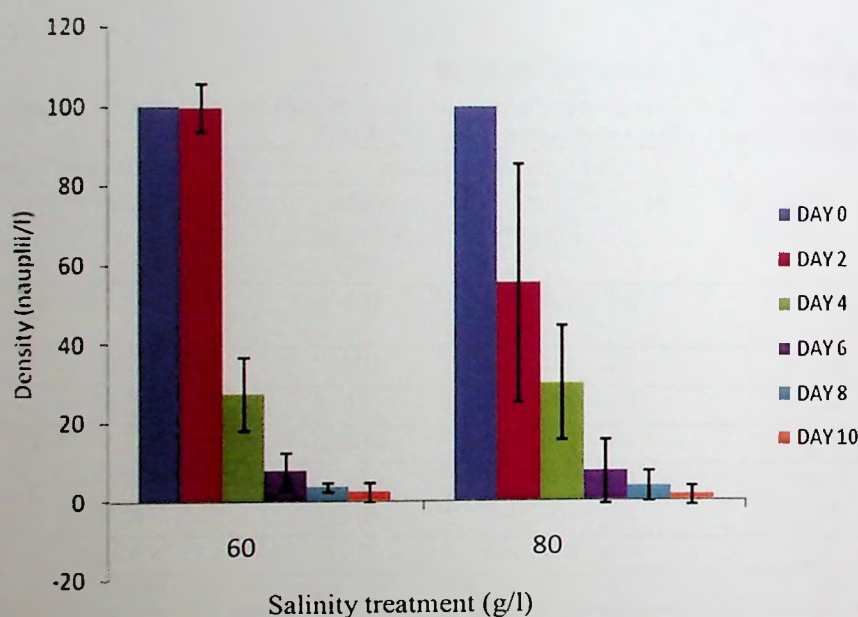


Figure 7.4. 3b; Survival of the VC *Artemia* strain cultured in Ponds (*in-situ*) using saline waters from lake Katwe

Using Independent- samples Kruskal-Wallis test, the mean survival of the VC *Artemia* nauplii did not significantly vary across the treatments (60 and 80g/l) after the same culture period in the salt ponds (Asymptotic sig (2-sided tested) ≥ 0.200) (figure 7.4.3b).

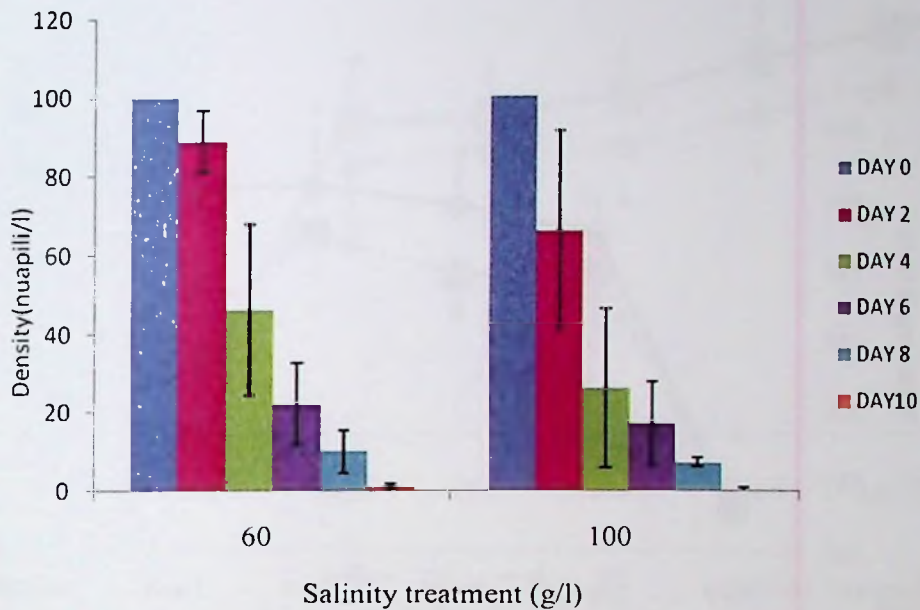


Figure 7.4. 4b; Survival of the GSL *Artemia* strain cultured in Ponds (in-situ) using saline waters from lake Katwe

Using Independent- samples Kruskal-Wallis test, the mean survival of GSL *Artemia* nauplii cultured in salt ponds did not significantly vary across the different treatments after the same culture period (Asymptotic sig (2-sided tested) ≥ 0.05) (Figure 7.4.4b). In all pond culture experiments of both the VC and GSL *Artemia* strains, no *Artemia* nauplii survived after day 10 of culturing.

7.5 DISCUSSION

7.5.1 *Ex-situ (bucket) Culture Experiments*

Artemia growth, survival and reproduction are dependant on environmental conditions like dissolved oxygen, temperature, salinity and food (Mechaly et al., 2013; Saji et al., 2016). Whereas salinity is one of the crucial factors that control growth and survival of *Artemia* (Saji et al., 2016), different strains are reported to have different levels of salinity and temperature tolerance (Abdullah, Salman, & Ali, 2015; John et al., 2004). Adaptation to harsh environmental conditions such as salinity and temperature takes place from individual levels to populations level (Gajardo & Beardmore, 2012). GSL *Artemia* strain having a significantly higher mean survival at the different salinities could be explained by the reported high ability of *Artemia franciscana* to colonise new environments (Anh, 2014; Castro-Barrera et al., 2006; Gajardo & Beardmore, 2012; Vignatti et al., 2014; Redón, Green, Georgiev, Vasileva, & Amat, 2015). *A. franciscana* has high phenotypic plasticity associated with its very wide distribution (Browne & Wanigasekera, 2000). It has been sucessfully introduced in Asia (Anh, 2014), Africa, and Europe (Browne & Wanigasekera, 2000; Ogello et al., 2014). *A. franciscana* is the most dominant species in the world (Mechaly et al., 2013). Genetic variation causes changes that can influence physiological differences which may lead to differences in the salinity and temperature torelance levels (Abdullah et al., 2015).

The lack of significant difference in the growth performance could be explained by the fact that despite the VC strain being domesticated in Vinh Chau, Vietnam, it is originally from San Francisco Bay, USA (Anh, 2014). Earlier studies of the effect of temperature and salinity on growth and survival of *A. franciscana* reported no significant difference in survival in the

different treatments at temperature of 25°C (Abdullah et al., 2015). The absence of *Artemia* survivals in all experiments after 8days in bucket culture and 10days in pond cultures could be attributed to the depletion of food for *Artemia* as the culture period increased. Although Lake Katwe waters are reported to have *Spirulina spp* a reported food organism for *Artemia* as the one of the dominant algae (Nkambo, Bugenyi, Naluwairo, Naiga, & Waswa, 2015), its densities might have been insufficient to support these cultures for more than eight days. Intensive culturing of live feed organisms requires provision of food for these organisms at high concentrations (Marques, Francois, Dhont, Bossier, & Sorgeloos, 2004). Although *Artemia* has diverse geographical distribution, the anionic composition of the different water environment affects its occurrence (Abatzopoulos, Kappas, Bossier, Sorgeloos, & Beardmore, 2002). The anionic composition of the waters used in this study might have affected *Artemia* survival in this experiment. Chemical substances like ammonia, nitrites, nitrates, phosphates and total organic carbon have also been reported to affect *Artemia* biomass and cyst production (Saji et al., 2016).

At Salinities of 80 and 100g/l, no VC *Artemia* strain was able to survive beyond day four of culture in all the different treatments. This might be an indication that the VC strain has a narrow salinity range for survival in Lake Katwe waters. Salinity tolerance in some *Artemia* strains is limited, like in the case of *Artemia* population from Thamaraiikulam in India whose salinity tolerance is restricted to 35g/l (John et al., 2004). GSL *Artemia* strain had significantly higher survival at salinities of 40 and 60g/l. This is contrary to the survival and reproductive performance estimators which are reported to consistently give 24°C and 180g/l as the optimal niche for *Artemia* (Browne & Wanigasekera, 2000). Mexican *A. franciscana* were reported to have died in different experimental cultures where salinity was 60g/l (Castro-Mejía et al., 2015).

From a study of *Artemia* sites in Iran, it was reported that they can survive at salinities as low as 10g/l (Abatzopoulos et al., 2006) and as high as 340g/l (Post & Youssef, 1977). Castro-Mejia et al. (2011) reported Mexican *Artemia* strains to have the best survival and growth at salinities between 100 - 120g/l. Much as natural *Artemia* populations have been reported at salinities as low as 33g/l, their possibility of completing life cycle at such low salinities is still not clearly known (Browne & Wanigasckera, 2006).

7.5.2 In-situ (Pond) Culture Experiments

The problem of predation is well documented in cases where *Artemia* is cultured in open ponds (Soundarapandian & Saravanakumar, 2009). In this study, predation was reduced by use of liners which prevented fresh water from entering the culture ponds via infiltration. In so doing, the lowering of the salinity levels in the culture ponds was prevented. Both the VC and GSL *Artemia* nauplii showed higher growth rates when cultured at salinity of 60g/l. Findings from this study were close to earlier studies which reported the best *A. franciscana* survival and growth at salinity of 55g/l with least survival at 95g/l (Dawood, Salman, & Malik, 2015). Despite the VC nauplii cultured at salinity of 80g/l being bigger than those cultured at 60g/l at the start of the pond culturing, they were able to over take those cultured at salinity of 80g/l after two days of culturing. Although there was no significant difference in the total length of GSL *Artemia* nauplii at start of culture experiments at salinity of 60 and 80g/l, GSL *Artemia* nauplii culture at salinity of 60g/l were found to have a significantly higher total length than those cultured at 80g/l during day 2 and day 4. After day four of culturing there were no significant difference in the growth performance between nauplii culture at 60 and 80g/l. This is contrarary to previous studies regarding the effect of salinity on *A. franciscana* population from Mexico where the highest mortalities were experienced at salinities under 60g/l with the best survivals and growth at

salinities of 100 – 120g/l (Castro-Mejía et al., 2011). Saji et al. (2016) reported populations of *A. tibetiana* in the wild environment to be the most abundant at salinities of 121g/l. Under studies of combined effect of salinity and temperature on survival and reproduction of *Artemia*, it was reported that over 90% of the nauplii were able to survive to maturity at the range of 20-28°C and salinity of 100-170g/l (Browne & Wanigasekera, 2000). This might be due to the fact that even though *Artemia* is a euryhaline organism with ability to survive at salinities as high as 300mg/l (Soundarapandian & Saravanakumar, 2009), it is more comfortable when exposed to optimum salinity ranges. Salinity is an important factor in influencing growth and survival of aquatic organisms (Castro-Mejía et al., 2011). Salinities above 80g/l are important in fending off *Artemia* predators (Abdullah et al., 2015; Krishnakumar et al., 2014; Van Hoa, 2014; Vignatti et al., 2014) and food competitors cannot survive at such high salinities (Brown & Macdonald, 1982). Total absence of nektonic fauna – crustaceans, decapods and fish in extreme environments has been reported to be due to the extreme physico-chemical conditions of these environments (Saddiqi, 2007). Ogello et al. (2014) reported *Artemia* to occur in ponds on the Kenyan coast at salinities ranges of 8-140g/l with cyst production taking place at salinities of 80-250g/l. From field studies carried out by sampling reservoir, evaporation and crystallisation ponds, *Artemia* populations were found to be abundant at salinity range of 70-200g/l, temperatures of 24.5°C to 35.5°C and dissolved Oxygen levels of 0.8 to 6.1mg/l (Krishnakumar et al., 2014). Much as *Artemia* is reported to reach reproductive maturity after day 20, 17 and 14 when cultured in freshwater, brackish water and sea water respectively (Soundarapandian & Saravanakumar, 2009), in this particular study, no nauplii was able to survive for more than 10 days. The absence of surviving *Artemia* after day 10 of culturing can be attributed to predation reported for *Artemia* cultures in ponds (Soundarapandian & Saravanakumar, 2009). Much as

efforts were taken to prevent predation through dam lining of the *Artemia* culture ponds and filtering of water to be used in pond cultures, it is very difficult to completely get rid of predators in these pond cultures. It is also possible that there were no food left in the green culture waters after 10 days of culturing, yet the culture of these live feed organisms at high densities requires high concentrations of live feeds (Marques et al., 2004). Additional factors like food quality and quantity, and dissolved oxygen levels can also have an effect on growth and survival of *Artemia* (Browne & Wanigasekera, 2000). *Artemia* being a continuous non-selective filter feeder (Marques et al., 2004), *Chlorella*, *Dunaleilla*, *Tetraselmis*, *Spirulina*, *Scenedesmus*, other microalgae plus other agricultural waste such as wheat bran, yeast based feed (LANSY) have commonly been used as their feeds in intensive cultures (Atashbar, Agh, & Kmerani, 2010; Browne & Wanigasekera, 2000; Saygi & Demirkalp, 2002; Marques et al., 2004; Soundarapandian & Saravanakumar, 2009; Mohebbi et al., 2016). Intensive culture of live feeds requires feeds at high concentrations (Marques et al., 2004). This study was not able to isolate the unicellular algae as food for *Artemia*. Since the study targeted generating cheap and easy-to-adopt *Artemia* production technology, greenwater cultures were instead generated by fertilizing the waters using chicken droppings. Although algae play a key role as food for *Artemia*, its composition affects their growth, density and other characteristics (Mohebbi, 2010). *Artemia* populations under natural conditions are also reported to be affected by temperature, feeding conditions, and salinity (Naceur, Jenhani, & Romdhane, 2011). Dissolved oxygen level is among the other key factors affecting occurrence of *Artemia* in the different geographical locations (Vignatti, Cabrera, & Echaniz, 2017). Changes in dissolved oxygen levels in shallow waters drastically change with high salinities and temperatures because of the reduced diffusion of atmospheric oxygen which would under normal circumstances compensate for the reduction in

oxygen levels due to respiration in these water bodies (Browne, Sorgeloos, & Trotman, 1990). Once oxygen becomes critically low, it leads to mortality of many of the aerobic organisms in these environments. Since shallow ponds were used, with green water cultures to provide food for *Artemia*, it is possible that dissolved oxygen became critically low in the cultures with increasing culture days, hence leading to mortalities after 10 days of culturing. Algal blooms in *Artemia* culture ponds deprive them of dissolved oxygen (Khoi, 2006). Growth performance and survival decreased with increasing temperature, while growth increased with increasing temperature in a study of TUZ *Artemia* (Saygi & Demirkalp, 2002). It took 30 days for TUZ *Artemia* to reach adult stage at 18°C, 20days at 24°C and 15days at 30°C. Increase in mortality was reported at temperatures of 30°C-32°C, with no larvae observed to survive at day 30 at 30°C. It is also possible that the high temperature in the region of $28.0 \pm 3.2^\circ\text{C}$ (Mungoma, 1990; Nkambo et al., 2015) and in the waters could have been the cause of mortalities which lead to no *Artemia* nauplii being able to survive after day 10.

Both the GSL and VC *Artemia* strain showed the best growth performance at salinity of 60g/l might be an indication that despite survival and growth performance being strain dependant (Saygi & Demirkalp, 2002), the salinity range for the best performance of both *A. franciscana* (GSL and VC strains) when cultured in Lake Katwe waters was the same range as *A. salina* cultured at 30 to 80g/l (Gozan, Rahman, & Hermawan, 2017; Lavens & Sorgeloos, 1991). Egyptian *Artemia* population were reported to have the best growth performance at a salinity of 80g/l (El-Bermawi, 2004), while a salinity of 100-120g/l were reported as the best range for the growth and survival of *A. franciscana* populations from Mexico (Castro-Mejía et al., 2011). These studies concurred that survival and growth performance in *Artemia* is strain dependant. It is

also a possibility that these experiments might have been attacked by pathogenic bacteria, like *Vibrios*. Gram-negative bacterium *Vibrio harveyi* has been reported to cause mass mortalities in penaeid shrimps with an ability to affect almost all types of cultured animals (Crab, Lambert, Defoirdt, Bossier, & Verstraete, 2010).

Although none of the two tested *Artemia* strains was able to survive beyond 10 days of culture, VC and GSL *Artemia* strains were reported to survive and even reproduce in the *Artemia* reference Center in Belgium using waters from Lake Bunyampaka which is a saline crater lake in Western Uganda (Sserwadda, Kagambe, & Van Stappen, 2018). This study highlights the possibility of culturing *Artemia* using waters from saline crater lakes of western Uganda. It is important that more research is conducted to find the causes of the observed high *Artemia* mortalities before attainment of reproductive maturity.

7.6 Conclusion

7.6.1 Ex-situ (Bucket) Culture Experiments

While GSL strain had some surviving *Artemia* even after 8 days of culturing in all treatments, significantly higher survivals were observed at salinity of 40 and 60g/l. No VC strain was able to survive beyond day four in all the different salinity treatment. These findings point to GSL strain as more commercially appropriate for domestication using waters from Lake Katwe, with the preferred culture salinity being 60g/l. Although both the VC and GSL strains showed good survival and growth performance at salinity of 40g/l, this level should be avoided, since at such salinities the inoculated *Artemia* becomes prone to predation.

7.6.2 *In-situ (Pond) Culture Experiments*

Much as previous studies have reported salinity of 100–120g/l as the best for culturing *A. franciscana* (Castro-Mejia et al., 2011), it was possible to culture both *A. franciscana* from the Great Salt Lake (GSL) and from Vinh chau, Vietnam (VC strain) using waters from Lake Katwe at salinities of 60 – 80g/l. Exception at the start in the treatment for 60g/l where GSL nauplii were significantly longer, at day 8 and 10 in treatment of salinity 80g/l, there was no significant difference in growth performance across the treatments of 60 and 80g/l during the same culture period.

7.7 Recommendation

Since it was found that at salinities of 60-80g/l, it was possible to culture *Artemia* in ponds using waters from Lake Katwe, it is recommended that more research trials are done to establish what prevented *Artemia* strains from attaining reproductive maturity and encysting before deliberate commercial production is done using waters from these saline crater lakes.

Involvement of salt miners in trials using salt ponds should be encouraged. Such was the approach used by countries like Vietnam where local scientists were supported by the *Artemia* Reference Center (Ghent University, Belgium) in the early 1980's in the establishment of its commercial industry in Vietnam (Van Hoa, 2014). Also in Kenya, a similar approach has been adopted with *Artemia* being first introduced at the Kenyan coast via a collaborative project between Kenya Marine & Fisheries Institute (KMFRI) and the Belgian Agency for Development Cooperation (BADC) (Ogello et al., 2014).

It is important that predator control measures are put in place more especially in the rainy season when salinities in the ponds are greatly reduced. It is important green water culture ponds are prepared for each production systems as a source of food for *Artemia* under culture (Van Hoa, 2014). Lake Katwe waters are reported to contain lots of impurities like organic matter and suspended solids (Kirabira, Kasedde, & Semukuutu, 2013). Having *Artemia* cultured in salt production ponds could significantly reduce the organic matter impurities which mainly originate from the high algal bloom concentrations from these salt ponds as was the case in Kenyan salt ponds between 1992 and 1993 (Ogello et al., 2014). Improved salt quality, production of *Artemia* biomas and cysts using the same labour and production facility are among the key benefits of integrating *Artemia* production in salt ponds.

7.9 References

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CHAPTER EIGHT

General Discussion

8.0 General Discussion

The aim of this study was to manipulate the waters from the selected saline crater lakes for *Artemia* production. World-wide, *Artemia* plays an important role as the most suitable starter feed in hatcheries (Mechaly, Angeletti, De Los Rios, & Cervellini, 2013; Krishnakumar, Asha, Sivagnanam, & Munuswamy, 2014; Kumar & Babu, 2015; Shanmugasundaram, Rajendran, & Thangasamy, 2017) and is the most widely used live feed in aquaculture (Tarekegn, 2015). No artificial diet has so far been able to replace the need for live feeds as starter diets (Sorgeloos, Dhert, & Candreva, 2001; John, Abatzopoulos, & Marian, 2004; Krishnakumar, Asha, Sivagnanam, & Munuswamy, 2014). Artificially formulated feeds do not meet the nutritional requirements of fish in their early stages of development (Naceur, Jenhani, & Romdhane, 2011; Liu et al., 2014; Kumar & Babu, 2015). *Artemia* is very nutritious, containing both macro and micronutrients required for both fish and crustacean larvae (Mechaly et al., 2013). Although most continents have local *Artemia* resources and often export to other continents, Africa is mainly dependent on imports from Europe, Asia or the USA at prices as high as 200% of the cost in the international market. It is the high cost of *Artemia* that has severely limited its wide use in Ugandan hatcheries and has contributed to the observed poor survival rates in Ugandan hatcheries (Mwanja et al., 2015). Since *Artemia* naturally occurs in hypersaline environments (Abatzopoulos, Kappas, Bossier, Sorgeloos, & Beardmore, 2002; Gajardo & Beardmore, 2012; Hammer, 1986a; Vignatti et al., 2014), these environments being similar to that of the saline crater lakes in western Uganda, it offers a possibility of culturing these organisms using waters from these saline crater lakes which if successful will help mitigate the high cost of imported *Artemia*. Information generated from this study on manipulation of waters from the various

saline crater lakes of western Uganda provides the first information towards the use of the saline crater lakes of western Uganda for *Artemia* production. As a first step towards understanding the waters of these saline craters lakes before manipulation for *Artemia* production, it was important that their limno-chemistry is studied and understood (Nkambo et al., 2015a, herein).

All the study lakes were confirmed to be alkaline and saline in nature as per previous studies, except Lake Kikorongo which was found to be totally fresh, contrary to previous studies by Mungoma (1990). Lake Kikorongo was dropped from the study, having established it to be a freshwater lake, and having had so many zooplankton species and other organisms which would prey on the inoculated *Artemia*, hence rendering it as not suitable for further evaluation for its production. In alkaline and saline waters, the high monovalent chloride (Cl^-) anions and sodium cations influences the saline biota and prevents the occurrence of nekton fauna including crustaceans, decapods and fishes (Saddiqi, 2007). Decreasing water levels and increasing salinity can lead to elimination of organisms like fish in saline systems (Hammer, 1986a). Salinity of these studied lakes was found to be significantly higher than that of sea. It was found to vary with seasons, the high salinities being observed in the dry season. Salinity plays a key role in influencing growth and survival of aquatic organism (Castro-Mejía et al. 2011; Hammer, 1986a). Salinities above 80g/l are important in fending off *Artemia* predators (Abdullah, Salman, & Ali, 2015; Krishnakumar et al., 2014; Van Hoa, 2014; Vignatti et al., 2014) and food competitors cannot survive at such high salinities (Brown & Macdonald, 1982). In most saline lakes multicellular life is limited to the brine shrimps, *Artemia* sp (Bowman & Sachs, 2008). Therefore, apparently alkaline and saline conditions are natural protection against *Artemia*

predators. The absence of *Artemia* predators is crucial during the identification of an *Artemia* production site.

With the exception of Lake Murumuli which was found to be a carbonate-chloride in nature, the rest were found to be carbonate-sulfate, contrary to earlier studies which reported lakes Katwe and Kikorongo to be carbonate-chloride lakes (Nkambo et al., 2015a, Herein). *Artemia* biomass and cysts have been reported to usually occur naturally in Sodium Magnesium Sulfate hypersaline lakes in British Colombia, Alberta and Saskatchewan, and sometimes in sodium chloride lakes in other parts of the world (Hammer, 1986a). Although crustaceans were previously thought to be excluded from high carbonate waters, some *Artemia salina* populations were reported to occur in soda lakes which do have high carbonate waters (Cole & Brown, 1967). Under laboratory studies, *A. franciscana* that feed on yeast was reported to thrive in media containing sodium, chloride and sulfates with smaller amounts of magnesium, potassium, calcium with either borate or carbonate as buffer (Bowen, Buoncristini, & Carl, 1988). This gives an indication that despite these lakes being classified as carbonate-sulfate lakes, they can be used for *Artemia* production.

Whereas *Artemia* occurs in both permanent and seasonal lakes, it only flourishes year round in the permanent lakes (Gajardo & Beardmore, 2012). *Artemia* resources in seasonal lakes dry-out in sometimes unpredictable circumstances (Gajardo & Beardmore, 2012), it is for this reason that lakes Munyanyange, and Nyamunuka which were found to be seasonal, with no major water inflows were not considered for further hatchability and *Artemia* culture trials. Lakes Katwe, Bagusa, Murumuli, Maseche and Bunyampaka were found to be permanent with fresh water

inflow found within their catchments that were considered for hatchability and *Artemia* culture experiments.

Although salinities of all the study lakes were higher than that of sea both in the dry and wet season, the $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ ionic rates of the studied lakes were found to be lower than those of sea with no significant difference in the $\text{Cl}^-/\text{SO}_4^{2-}$, $\text{Cl}^-/\text{Na}^+ + \text{K}^+$, and $\text{Mg}^{2+}/\text{Ca}^{2+}$ ionic rates in the different studied lakes. *Artemia*, known to occur in hypersaline environments (Hammer, 1986a; Liu et al., 2014), with sea being one of its common habitats and salinity being the key driver of its survival, growth (Castro-Mejía et al., 2011; Saji, Al Dhaheri, Shah, & Soorae, 2016) and distribution (Gajardo & Beardmore, 2012; Saji et al., 2016), it was therefore important to compare the salinity and ionic composition of the studied lakes with that of sea water (Nkambo et al., 2015b, herein). Ionic composition and biotic interactions play a key role in *Artemia* distribution (Kappas, Abatzopoulos, Van Hoa, Sorgeloos, & Beardmore, 2004). This difference in the ionic rates and salinity might be the explanation for the absence of *Artemia* in these studied lakes. Much as some studies have pointed to presence of *Artemia* on the shores of some of the saline crater lakes of western Uganda (Ogello, Kembenya, Githukia, Nyonje, & Munguti, 2014), neither their cysts nor biomass were found in all the studied lakes throughout the dry and wet seasons of the three year sampling regime.

Blue-Green algae (Cynobacteria) were found to be the dominant phytoplankton in these saline crater lakes, in agreement with other studies which have reported primary production to be from photosynthesis with cynobacteria being the dominant contributor (Leboulanger et al., 2017). Saline Soda lake systems have been reported to be more selective than other aquatic systems

with cyanobacteria reported to be often dominant in these extreme environments (Leboulanger et al., 2017). Although a positive correlation is reported between the pH and salinity of these alkaline-saline lakes (Leboulanger et al., 2017), the same Cyanobacteria was reported to be dominant in lakes Saka, Mburo, George (Dumont & Tundisi, 2013), Edward and in Murchison bay plus Napoleon gulf, Lake Victoria (Okello & Rainer, 2018). High populations of cyanobacteria which are dominant in Lake Abaita were reported as the probable reason for the absence of cladocerans in this lake (Dumont & Tundisi, 2013). It might be the dominance of cyanobacteria that caused the absence of cladocerans in all lakes considered in this study. Although Lake Mahega was not considered in this study, Melack and Kilham (1972) reported presence of cyanobacteria in Lake Mahega which is another highly saline lake in western Uganda (Hammer, 1986b). A negative relationship between cladocerans and cyanobacteria was reported in Lake Langano and a positive relationship between cyanobacteria and rotifer densities (Dumont & Tundisi, 2013). Rotifera were the only zooplankton found in Lake Katwe, while Lakes Maseche and Kikorongo had both Rotifera and Copepoda. Cladocera densities have a positive relationship with chlorophyte densities, while Rotifera densities are positively correlated with cyanobacteria densities (Dumont & Tundisi, 2013).

With the exception of Lake Kikorongo where catfish was found during the rainy season, none was found to have fish during the entire study period. Lake Kikorongo was reported to receive water from Lake George in the rainy season which sometimes comes with fish (Mungoma, 1990). The extreme salinity and alkalinity might be the probable reason to explain the absence of fish in these studied lakes. The lack of fish in Lake Katwe was reported to be caused by salt (George, 2017). The fish brought to Lake Kikorongo with floods from Lake George were

reported to slowly die off as the salinity increase in the dry season (Mungoma, 1990). Some cyanobacteria species release cyanotoxins which have deleterious effects on tissues and organs of aquatic and terrestrial animals (Kateregga, Babu, Abuine, & Ndukui, 2014). Presence of cyanotoxin producing cyanobacteria threaten ecosystems health (Kateregga et al., 2014) and therefore might have been the cause of absence of fish plus other vertebrates in these lakes. A classical example is the number of lesser flamingo which has been reported to increase with the increase in blue-green algae *Arthrospira fusiformis* (*Spirulina platensis*). Flamingo densities decline with declining water levels, which lead to low phytoplankton densities and dominated by toxic cyanobacteria which could be one of the causes of flamingo mortalities (Ndeti & Muhandiki, 2005). In alkaline-saline lakes in Kenya, microcystins and anatoxin-a have been found in stomach and liver samples of dead flamingos. This pointed to cyanotoxins being the probable cause of the flamingo mortalities were these extracts were found. The toxins have been associated with human illnesses, aquatic mammals and birds' morbidity and mortality as well extensive fish kills (Kotut, Ballot, & Krienitz, 2006).

Artemia cysts undergo a state of prolonged dormancy as a means of surviving stressful unfavorable environmental conditions (Kappas et al., 2004; King, Toxopeus, & MacRae, 2014; Podrabsky & Hand, 2015; Robbins et al., 2010). This state of dormancy is broken with resumption of embryonic development once the cysts are exposed to favorable environmental conditions (Dai et al., 2011; Van Stappen, Sorgeloos, & Lavens, 1998). Exposure to adverse environmental conditions give reliable cues to the soon coming environmental conditions like light/photoperiod or food quality/quantity (Podrabsky & Hand, 2015), desiccation (King et al., 2014), and cold are some of the cues that induce encysting and reduced metabolism while

dehydration/rehydration, freezing, cold storage, de-capsulation and exposure to hydrogen peroxide induce diapauses termination and embryonic development (Podrabsky & Hand, 2015; Robbins et al., 2010). Encysting and diapauses termination in *Artemia* is reported to vary with *Artemia* population and the environment condition of the habitat (Robbins et al., 2010). Waters from lakes Katwe, Bunyampaka, Maseche, and Bagusa were able to break diapauses in *Artemia franciscana* from Great Salt Lake (GSL), *A. franciscana* from salt ponds in Vinh Chau (VC), Chinese strain (Chinese), TUZ Parthenogenetic *Artemia* from Kazakhstan (TUZ) and Parthenogenetic *Artemia* strain from Siberia (PAS) (commercial strain). Waters of these lakes being able to break diapause was a good indication that they are favorable for *Artemia* production despite the absence of local strains. *A. franciscana* (VC) had significantly higher hatching percentage than all tested strains ($p < 0.05$), followed by *A. franciscana* (GSL strain) from the Great Salt Lake give an indication of these two strains being the most suited for production in these lakes. *A. franciscana* exhibits high level of phenotypic plasticity which enables it adapt to different environmental conditions (Kappas et al., 2004). Studies of comparisons of genetic and reproductive differentiation of *A. franciscana* from the San Francisco Bay (SFB) and *A. franciscana* introduced in Vietnam at Vinh Chau (VC), the VC strain was found to be adapted to high temperatures based on reproductive outputs (Kappas et al., 2004). This could be the probable explanation for the high hatching percentage presented by *A. franciscana* (VC and GSL strains). Lakes Katwe, Bunyampaka and Maseche waters have presented the highest hatching percentages. This gave an indication that these lakes were the best suited for *Artemia* production.

Both GSL and VC strains having best survival rates at salinities of 40g/l and 60g/l and very low survivals at salinities at 80g/l and 100g/l could be explained by the fact that although *Artemia* survive in hypersaline environments with salinities as high as 300g/l, a range of 30 – 80g/l is reported as the best in terms of growth performance (Lavens & Sorgeloos, 1991). An even lower range of 32 to 65g/l was reported as suitable for the best physiological performance for both nauplii and adult *Artemia salina* in a study of the effect of salinity and densities on Magnesium and calcium impurity level in salt production (Gozan, Rahman, & Hermawan, 2017). In this study the GSL strain survived longest in all the different treatments, even though its survival at salinity treatments of 80 and 100g/l were very low. The survival of the GSL strain throughout the entire 8 day culture period in all treatments could be attributed to the reported high phenotypic plasticity of *A. franciscana* (Kappas et al., 2004).

In this study salinities of 60 and 80g/l were used for pond cultures despite *A. franciscana*, (VC) strain having significantly high growth performance at 40g/l in the bucket culture. Salinities of 60 and 80g/l were adopted for pond cultures because of the need to have high enough salinities to fend off predators in the culture ponds. This is supported by earlier studies which reported salinity ranges below 80g/l being the best for growth and physiological performance in *Artemia* (Gozan et al., 2017; Lavens & Sorgeloos, 1991). Salinity of 100g/l and above was not used in the pond cultures because of the high mortalities observed in the bucket cultures at salinity treatment of 100g/l. Both GSL and VC strains were able to survive up to day 10 after inoculation in *Artemia* culture ponds under both treatments.

The lack of difference in the growth performance of both strains based on length increment in the salinity treatment of 60g/l despite GSL strain having been significantly longer at the start could be an indication that at salinity of 60g/l the VC strain acclimatized faster to these waters than the GSL strain and therefore gained in length faster to catch up with the GSL strain. Since a salinity range of 30 to 80g/l was reported as a suitable range for *Artemia* (Gozan et al., 2017; Lavens & Sorgeloos, 1991), it is possible that the VC strain has got better acclimatization traits when cultured at optimal salinity ranges.

GSL strain gaining significantly more in length than the VC strain after 6days at salinity of 80g/l despite the no significant difference in the first 6days of culturing might be an indication that the GSL strain acclimatizes better and faster than the VC strain at high salinities. The survival decrease for both *Artemia* strains with increasing culture period in all treatments with none of the two strains being able to survive longer than 10days after inoculation in ponds meant that all strains were unable to attain reproductive maturity. Temperature, feeding conditions, and salinity are reported to be the major parameters influencing *Artemia* populations in the natural environment (Khoi, 2006; Naceur et al., 2011). In addition to temperature and salinity, dissolved oxygen concentrations are among the factors affecting occurrence of *Artemia* in the different geographical locations. The dissolved oxygen levels in shallow waters is reported to change quickly at high salinities and temperatures with the slow diffusion of oxygen from air being unable to compensate for the reduction in oxygen levels due to oxygen consumption by the living organisms (Browne et al., 1990). This leads to the oxygen demand by the living organisms in these environments exceeding the supply, resulting into mortality of many of the aerobic organisms. Shallow ponds having been used in these cultures, with green water cultures being

used to provide food for *Artemia*, it is possible that the dissolved oxygen levels in these ponds went on decreasing with increasing phytoplankton densities during the culturing with oxygen levels becoming critical after day 10 of culturing. This might have meant that none of the two *Artemia* strains was able survive beyond day 10 of culturing because of critical dissolved oxygen levels. Where algae have used as a food source for *Artemia*, its composition has been reported to affect its growth and population densities (Mohebbi, 2010). Algae have been reported to deprive *Artemia* of dissolved oxygen (Khoi, 2006). Temperature affects growth performance and survival of *Artemia* with survival decreasing with increasing temperature and growth increasing with increasing temperature (Saygi & Demirkalp, 2002). In a study of temperature effects on the growth performance of *Artemia* from TUZ Lake in Turkey, it took 30 days to reach adult stage at 18°C, 20 days at 24°C and 15 days at 30°C. Fast increase in mortality was reported at temperatures of 30°C to 32°C, with no larvae observed to survive at day 30 at 30°C. It is also possible that the very high temperature in the region of $28.0 \pm 3.2^\circ\text{C}$ (Mungoma, 1990; Nkambo et al., 2015a) and the waters could have been the cause of mortalities which led to no *Artemia* nauplii being able to survive after day 10.

Survival, growth and cyst diameters have also been reported to be strain dependant (Saygi & Demirkalp, 2002). Both the GSL and VC *Artemia* strain showing the best growth performance at salinity of 60g/l might be an indication that despite survival and growth performance being reported to be strain dependant, the salinity range for the best performance of both *Artemia franciscana* (GSL and VC strains) when cultured in lake Katwe waters is the same range as *Artemia salina* of 30 to 80g/l (Gozan et al., 1991). Egyptian *Artemia* population were reported to have the best growth performance at a salinity of 80g/l (El-Bermawi, 2004) while a salinity of

100-120g/l were reported as the best ranges for the best growth and survival of *A. franciscana* populations from Mexico (Castro-Mejia et al., 2011). All these point to survival and growth performance in *Artemia* being strain dependant. The survival of *Artemia* for the 10 culture days is an indication of possibilities for *Artemia* production in these waters.

8.1 Conclusions and Recommendations

- Contrary to reports of the occurrence of *Artemia* in the saline crater lakes of Western Uganda (Ogello et al., 2012), no local *Artemia* resources were found in the studied saline crater lakes considered in this study.
- With the exception of lake Kikorongo which was found to be fresh contrary to reports by Mungoma, 1990, the rest of the studied lakes were found to be alkaline-saline, very high primary production and low in zooplankton biodiversity (50 to 100 individuals/litre) in their bio-physico-chemical nature.
- It was possible to break *Artemia* diapause using waters from the selected saline crater lakes of Western Uganda (from Hatchability experiments) with *A. franciscana* (VC) and *A. franciscana* of GSL strain presenting the highest hatching percentages.
- Although it was not possible to cause encysting in this study, it was possible to culture *Artemia* both *ex-situ* (buckets) and *in-situ* (ponds), using waters from Lake Katwe at a salinity range of 60 to 80g/l.
- More research and trials are recommended to establish what prevents *Artemia* strains from attaining reproductive maturity and encysting before deliberate commercial production is done using waters from the saline crater lakes.

8.2 References

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APPENDIX (Published Papers)

Artemia Occurrence, Salinity and Ionic Rates in Saline Crater Lakes of Western Uganda

Mujibu Nkambo^{1*}, Fred W. Bugenyi¹, Sinda Nayiga²

¹Agriculture Research and Development Center - Kyaransi, National Fisheries Resources Research Institute (NaFIRRI), Kampala, Uganda

²Department of Biological Sciences, College of Natural Sciences (CONAS), Makerere University, Kampala, Uganda

³Faculty of Law Islamic University in Uganda, Mbale, Uganda

Abstract Seven selected saline crater lakes in Western Uganda were surveyed for *Artemia* occurrence, salinity and ionic rates both in the wet and dry season. The studied lakes included lakes Katwe, Munyanyange, Nyanzindika, Mummuri, Bwanyanga, Bagasa, and Kikorongo. In the field cyst-like materials along the lake shores and foam suspected to have *Artemia* cysts from the surface of each of the studied saline Crater Lake were collected in a plastic sample bottle. Presence of cysts was examined using the density separation technique. A zooplankton net of 50µm was used to filter water samples to be used for analysis of presence of *Artemia* biomass. Water samples for ionic composition analysis were taken from at least three geo-references points on each of the studied lakes. *In-situ* measurements of salinity were taken at each point of sample collection in each of the studied lakes. In the laboratory, collected water samples were analysed following APHA standard operating procedures. Seasonal differences in salinities and ionic rates within the studied lakes were tested for significance using a two tailed, paired t-test. All the studied lakes had neither *Artemia* cysts nor biomass at the time of sampling both in the dry and wet season. Both in the dry and wet season, the salinity in all the study lakes was much higher than that of sea water while the ionic rates of Cl^{-}/SO_4^{2-} , $Cl^{-}/Na^{+} + K^{+}$, and Mg^{2+}/Ca^{2+} in all the study lakes were all less than that of sea water (table 1 & figure 2). The dry season salinity of all the study lakes was found to be significantly higher than the salinity in the wet season (t -value = 0.0005). There were no significant seasonal differences in the ionic rates of Cl^{-}/SO_4^{2-} , $Cl^{-}/Na^{+} + K^{+}$, and Mg^{2+}/Ca^{2+} in the studied lakes with t -values of 0.5665, 0.4082 and 0.4523 respectively. There is need for continuous monitoring of these saline lakes for the presence of *Artemia* resources since many other factors like temperature, the presence of washing migratory birds reported to be the dispersion agent for *Artemia* were witnessed in these lakes.

Keywords: *Artemia* Occurrence, Ionic rates, Saline lakes

1. Introduction

Aquaculture production is one sure route suggested to bridge the gap between the ever increasing fish demand and the declining capture fisheries production. One of the requirements for sustainable aquaculture industry is reliable source of fish seed. Fish seed requires readily available source of live food especially at the larval stage and this has been one of the bottle necks for the development of Uganda's aquaculture. Although *Artemia* has been reported to be of great importance as part of live food chain in culture of fish larvae [13], [31], [21], the high cost of imported *Artemia* cysts which ranges between US\$50 to 100 per Kg [10], [21] has prohibited its wide use in Ugandan hatcheries hence the very low survival rates in the majority of Uganda's hatcheries. Despite Uganda being endowed with hypersaline (salt lakes) environments which are among the reported

natural habitats for *Artemia* [33] [8], [24], a comprehensive survey to explore the occurrence of local *Artemia* resources and possibility for *Artemia* production in these lakes is still lacking. The purpose of this study is therefore to explore the occurrence of local *Artemia* resources and ionic rates of these saline lakes systems as baseline information for commercial *Artemia* production in Uganda. This information on occurrence of local *Artemia* resources and ionic rates of these saline lakes is very useful in guiding the development of a commercial *Artemia* production industry in Uganda, which would offer a cheaper option for the currently very expensive imported *Artemia* resources.

Past studies on these saline lakes in Western Uganda have focused on limnology [18], [20] and salt production in these lakes [13], [14]. Although there has been speculation about occurrence of *Artemia* in saline lakes of Uganda [21], Currently there is no grey literature on the occurrence of *Artemia* resources and ionic rates of these saline crater lakes in western Uganda. To guide this study, the following hypotheses were used; (1) There are no locally occurring *Artemia* resources in the saline crater lakes of Western Uganda. (2) Ionic rates of the saline crater lakes of western

* Corresponding author:
mujibu@yaho.co.uk (Mujibu Nkambo)
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Uganda do not favor *Artemia* occurrence.

Artemia distribution

Artemia are primitive arthropod having segmented bodies with broad leaf-like appendages known as thoracopods [10]. *Artemia* are commonly referred to as brine shrimps. They are micro-zooplankton belonging to monstracum crustaceans with a reported occurrence on all continents except in the Antarctica [33], [4], [22]. They are reported to naturally occur in salt lakes [30] and hypersaline environments with salinities as high as 340 g l^{-1} , a wide geographical distribution [21], [22], varying ionic compositions, climatic conditions and altitude [3]. The geographical distribution of *Artemia* was reported to be world-wide with the exception of the Antarctica [1] [3], [22] and it is related to the different types of climate. World-wide over 600 *Artemia* sites are reported to have been discovered with more efforts on going to identify more sites [1] [3] [21]. *Artemia* is reported to have both sexually and parthenogenetically reproducing strains with a number of environmental parameters most notably salinity being reported to be responsible for the switch between giving live young ones and production of diapausing cysts [12]. It is the diapausing cysts that can withstand the harsh adverse conditions [1], stay viable for several years and are able to hatch into live nauplii on return of favorable conditions [12].

Much as *Artemia* is reported to have a wide geographical distribution, very little is known about its distribution in Sub Saharan Africa [34]. Due to favorable climatic conditions most African countries are considered to have a huge potential for *Artemia* occurrence. Much of the information about *Artemia* distribution in Africa has been on *Artemia franciscana*, *Artemia salina* and the parthenogenetic strain in North African countries like Morocco, Egypt, Tunisia, Libya among others [3], [22]. Recently *Artemia* resources have also been reported in the east African region at the coast in Tanga, Tanzania and in Kenya with no concrete information on its occurrence in Uganda.

Artemia importance

Artemia as live food within the aquaculture industry dates back to the 1930s after it was discovered to be of high nutritional value to the new hatched fish larvae [5], [10]. Owing to its high nutritional value and convenience in use, *Artemia* is acknowledged world-wide as one of the most important starter feed within hatcheries of shell and fin fish. Despite the various efforts to find a suitable starter feed to replace *Artemia* because of cyst shortage and its high costs yielding varying levels of success [10], up to date no suitable artificial starter feed to replace *Artemia* has been found [21]. Currently over 2000 tons of *Artemia* dry cysts are reported to be used annually within the aquaculture industry world-wide [10] [21] and its demand is projected to increase with the ever increasing demand for fish leading to increased aquaculture production. It is this projected increment in demand for *Artemia* as a starter feed which has led to numerous search for alternative starter feeds as well as new sites for *Artemia* production [10]. Through bio-encapsulation

and enrichment, *Artemia* is currently being used as a medium to supply essential nutrients like vitamins, high concentrated fatty acids [10], medication and antibiotics to shrimp and fish larvae within the aquaculture industry [12], [23], [25]. Besides its use in aquaculture industry, it has also been reported to improve salt quality because of its capacity to filter algae from the salt pans [26]. Ogello et al., (2014) reported salt production companies to have agreed that *Artemia* production is vital in controlling the algal blooms within the salt production pans. Having natural occurring *Artemia* resources would play a vital role in improve the salt quality in these saline lakes where commercial salt extraction is currently being carried out.

Ionic rates

Ionic composition and ionic rates are among the factors affecting salinity levels in these lakes and therefore affecting the occurrence of various organisms in these systems including fish, *Artemia* and others [6]. Having an understanding of the ionic composition and ionic rates in these saline lakes provides useful information which might explain the occurrence and absence of various organisms in these systems. These inland saline systems have been generally classified into carbonate and bicarbonates ($\text{CO}_3^{2-} + \text{HCO}_3^-$), sulphate (SO_4^{2-}) and chloride (Cl) depending on the dominant anion [18], [2] with *Artemia* population reported to occur in all these different categories [33], [28] with a reported preference for waters with chlorides as the dominant anions [7].

2. Materials and Methods

Study Area

All the studied Lakes Katwe (029.87033°E, 00.13217°S), Munyamunga (029.88591°E, 00.13513°S), Nyamunika (029.98743°E, 00.09344°S), Bagasa (030.17958°E, 00.09793°S), Murumuri (029.99186°E, 00.07323°S), Bonyampaka (030.12819°E, 00.03785°S) and Kikorongo (030.01228°E, 00.01190°S) are found in western Uganda and belong to Katwe – Kikorongo volcanic field. With the exception of Kikorongo which is sometimes fresh, all the studied lakes are alkaline saline in nature [20], with a reported average depth ranging between <1 to 6m [14].

Artemia Cysts Occurrence

Data collection in this study was carried out over a one year sampling period between the 26th of February, 2014 and 25th February, 2015. Since this area has been reported to receive a bimodal rainfall pattern [11], [13] during data collection the two wet seasons (March – May and September – December) and the two dry seasons (December-February and June-August) were considered. In the field cyst-like materials along the lake shores and from suspected to have *Artemia* cysts from the surface of each of the studied saline Crater Lake were collected from at least four sites, put in plastic sample bottles before being carried to the laboratory for further analysis. In the laboratory at Kajjansi Aquaculture

Research and Development Center (KARDC), cyst-like materials and foam suspected to contain *Artemia* cysts were tested for *Artemia* cysts using the density separation technique. Under the density separation technique, cyst-like materials and foam suspected to contain *Artemia* cysts were washed in a strong brine solution (above 45mg/l⁻¹), followed by washing in fresh water (0mg/l⁻¹) and finally washing in a strong brine solution (above 45mg/l⁻¹). *Artemia* cysts float in a strong brine solution, sink when washed in fresh water and float again when washed in a strong brine solution. Any cyst-like materials which floated in strong brine solution, sunk in fresh water solution and floated again on washing in strong brine solution were taken for further microscopic observation to be confirmed as *Artemia* cysts. Any collected material that behaved contrary to the above mentioned observation for cysts under the density separation technique were considered to have no *Artemia* cysts present.

Artemia biomass occurrence

Four litres of surface water were collected and concentrated by filtering it through a 50µm zooplankton net. The plankton net sieve was rinsed with distilled water after filtering the four litres of the collected lake water and the rinsed water together with the residue collected in a plastic bottle. This was fixed by adding ethanol (90%) equal to a sixth of the total volume of the sample collected in the plastic sample collection bottle. In the laboratory at KARDC, the collected water samples were observed under a dissecting microscope for any *Artemia* biomass present.

Salinity, Ionic composition and rates

Surface water samples for chemical composition analysis of each of the studied lakes were collected using a Van Dorn water sampler from three geo-referenced sampling points and stored in pre-rinsed Nalgene bottles for analysis in the laboratory. In the field all water samples collected in pre-rinsed Nalgene bottles were kept in a cooler box containing dry ice and later transferred to the National Fisheries Resource Research Institute (NaFIRRI) laboratory in Jinja. In-situ measurements of salinity were taken using

hand-held refractors (0- 160mg/l⁻¹). In the laboratory the collected water samples were analysed for the following parameters: bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), Sulphate (SO₄²⁻), Chloride (Cl⁻), Fluoride (F⁻), Sodium (Na⁺), Magnesium (Mg²⁺), Calcium (Ca²⁺), Potassium (K⁺), and Sodium, Magnesium, Calcium, Potassium, and Chloride (Cl⁻) following APHA 1975 standard Operating procedures. The measured salinities in the dry and wet season as well as the ionic rates were compared with those of sea water. Seasonal differences in salinities and ionic rates within the studied lakes were tested for significance using a two tailed, paired t test.

3. Results

Artemia occurrence

All the studied lakes had neither *Artemia* cysts nor biomass at the time of sampling in both the dry and wet season.

Salinity and Ionic rates

The Cl⁻/SO₄²⁻ ionic rates in this present study ranged between 0.5±0.2 and 1.73±0.7 observed in lakes Bagesa and Murumuri in the dry season and these far less than the 7.16 of sea water.

Both in the dry and wet season, the salinity in all the study lakes was much higher than that of sea water while the ionic rates of Cl⁻/SO₄²⁻, Cl⁻/Na⁺ + K⁺, and Mg²⁺/Ca²⁺ in all the study lakes were all less than that of sea water (table 1 & figure 2). The dry season salinity of all the study lakes was found to be significantly higher than the salinity in the wet season (t-value = 0.0205). Lakes Muryanyange and Nyanamuka were found to be dry and empty during the dry season sampling period while Lake Kikorongo was found to be fresh with salinity of 0mg/l⁻¹ during the wet season sampling (table 1 & figure 1). There were no significant seasonal differences in the ionic rates of Cl⁻/SO₄²⁻, Cl⁻/Na⁺ + K⁺, and Mg²⁺/Ca²⁺ in the studied lakes with t-values of 0.5665, 0.4082 and 0.4523 respectively.

Table 1. Salinity and ionic rates of the different studied lakes in the dry and wet season.

Lakes	Salinity (mg/l ⁻¹)		Ionic rates					
	Dry	Wet	Cl ⁻ /SO ₄ ²⁻		Cl ⁻ /Na ⁺ + K ⁺		Mg ²⁺ /Ca ²⁺	
			Dry	Wet	Dry	Wet	Dry	Wet
Bagesa	67.3±17.2	68.0±8.2	0.50±0.2	0.62±0.08	1.28±0.8	1.25±0.2	0.28±0.8	0.2
Buryanyanga	88.1±14.6	72.4±11.2	0.77±0.1	0.88±0.7	0.84±0.1	1.03±0.6	0.23±0.7	0.29±0.1
Katun	116.7±8.3	97.1±10.1	0.78±0.3	0.82±0.4	0.69±0.6	0.99±0.4	0.36±0.3	0.44±0.3
Murumuri	87.2±19.4	72.2±1.7	1.73±0.7	0.83±0.9	1.30±0.7	1.16±0.8	0.23±0.9	0.34±0.7
Kikorongo	16.0±12.6	0.0±7.0	0.06±0.01	-	0.08±0.7	-	0.25±0.1	-
Nyanamuka	-	100.0±1.0	-	0.53±0.3	-	0.71±0.3	-	0.36±0.3
Muryanyanga	-	19.2±3.3	-	0.60±0.7	-	0.95±0.3	-	0.27±0.4
Sea water	35		7.16		1.73		3.18	

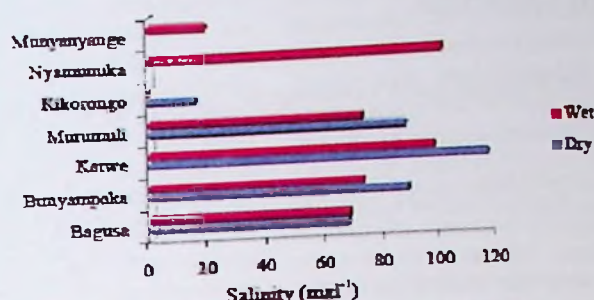


Figure 1. Dry and wet season salinity changes in the studied saline lakes

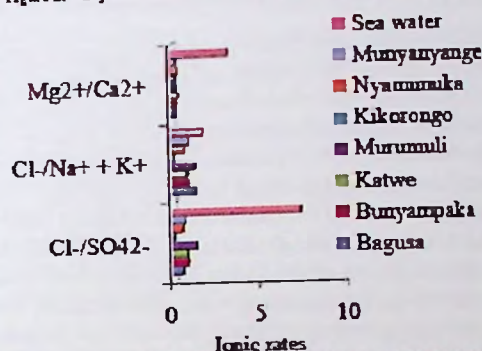


Figure 2. Ionic rates of the different studied lakes in comparison with sea water

4. Discussion

Artemia occurrence

This study confirms the very first comprehensive survey of the occurrence of local *Artemia* resources in the saline lakes of western Uganda. Despite the presence of the flamingos which are among the report dispersion agents for brine shrimp eggs [8] and the speculation of occurrence of *Artemia* resources in these lakes [21], none of the study lakes was found to have *Artemia* biomass or cysts at the time of sampling both during the dry and wet season. Wind and wading migratory birds have been reported as the main vectors for the spread and maintenance of gene flow between distant *Artemia* populations [12], [1], [28]. The presence of migratory birds such as flamingos in these lakes gives an anticipation of *Artemia* being inoculated in these lakes by these birds. Predation, lack of appropriate food, habitat activities and other abiotic factors might be responsible factors responsible for the absence of *Artemia* in these studied lakes. In a study of the distribution and historical biogeography of *Artemia* lech, 1819 in Ukraine, adults and

larvae of some Coleoptera (*Hydrotus anisogrammus* (Ahrens, 1833), Hemiptera (Corixidae), Ostracoda *Emysia inflata* (Sars 1903) and other invertebrates were reported to have probably eaten away all the *Artemia* in the lakes with salinities below 110mg l⁻¹ [28]. In the same way zooplanktons, other invertebrates and birds most notably the lesser flamingos (*Phoenicopatris minor*) which live in these saline crater lakes might be eating away all the *Artemia* inoculated in these lakes. Although *Artemia* has been reported to exist at salinities higher than 340mg l⁻¹ [28], the very high salinities might not be the only limiting factor. Other abiotic factors like dissolved oxygen might also be limiting. Dissolved oxygen is reported to significantly decrease with increasing salinity [28] and this could be another reason to explain the absence of *Artemia* in these hypersaline lakes in this current study.

Planktons serve as the primary source of food for different groups of aquatic animals [29], with their biodiversity reported to decrease with increasing salinity [17], [15]. Although some bacteria strains have been reported as potential diets for *Artemia* [35], phytoplanktons are reported to be the main constituent of natural *Artemia* diets [26].

Planktonic and Fisheries biodiversity of Alkaline Saline crater lakes of Western Uganda

Mujibu Nkambo¹*, Fredrick W. Bugenyi², Janet Naluwayiro¹, Sauda Nayiga³, Vicent Kiggundu¹, Godfrey Magezi¹ & Waswa Leonard⁴

¹Aquaculture Research and Development Center-Kajjansi, National Fisheries Resources Research Institute (NaFIRRI), Uganda

²Department of Biological Sciences, College of Natural Sciences (CONAS), Makerere University, Kampala, Uganda

³Islamic University In Uganda (IUIU)

⁴Katwe Kabatoro community, Uganda

*Corresponding author, email: mnkambo@yahoo.co.uk

ABSTRACT

Eight (8) selected saline crater lakes in Western Uganda were sampled for fish biodiversity. Water samples collected from each of these lakes were analysed for zoo- and phytoplanktonic composition and abundance. In situ, physico-chemical parameters including average depth, salinity, temperature, conductivity, Dissolved Oxygen and pH were taken at each sample collection point. The Mean $\pm$ SD of the different parameters ranged between 0.2 $\pm$ 0.0 m and 2.3 $\pm$ 0.3 m for average depth, 0.0 $\pm$ 0.0 mg l⁻¹ and 205.0 $\pm$ 15.3 mg l⁻¹ for salinity, 27.9 $\pm$ 0.3°C and 34.4 $\pm$ 2.4°C for temperature, 18.6 $\pm$ 0.1 mscm-l and 106.3 $\pm$ 3.5 mscm-l for conductivity, 1.7 $\pm$ 0.4 mg l⁻¹ and 6.0 $\pm$ 1.0 mg l⁻¹ for Dissolved Oxygen and 9.6 $\pm$ 0.1 and 11.5 $\pm$ 1.0 for pH. With the exception of the Lakes Bagusa, where *Anabaena circinalis* Rabenhorst ex Bornet et Flahault was found to dominate the algal biomass, and Bunyampaka and Nyamunuka where no *Spirulina platensis* (Nordstedt) Gomont was found, the rest of the studied lakes had *S. platensis* dominating their algal biomass. All lakes showed very low zooplankton abundances and biodiversity, with Lake Kikorongo (the one with the highest zooplankton biodiversity) having *Brachionus calyciflorus* Pallas, 1766 as the most abundant, only ranging between 50 to 100 individuals/litre. None of the lakes had fish at the time of sampling.

KEY WORDS

Zooplankton; Phytoplankton; Fish; saline; alkaline; lakes.

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INTRODUCTION

Minute free-floating organisms found in various water bodies can be referred to as planktons and have been reported to be the main food for fish (Lind, 1965). Planktons have been reported to play pivotal roles in the biosphere in terms of both primary and secondary production (Boero et al., 2008). Plant-like minute organisms continuously drifting in the water are referred to as phytoplankton while the minute animal-like organisms, unable to syn-

thesize food are referred to as zooplankton. Planktons are not only food organisms for fish fry, fingerlings and adult fish but also influence key abiotic features in aquatic systems (Joshi, 2009).

Saline systems have been reported to have a generally low biodiversity (Hammer, 1986), with diatoms being more dominant among algal biomass in alkaline saline systems (Stenger-Kovács et al., 2014). Rotifera, Cladocera, Copepoda and Anostraca species generally are the dominant zooplankton in saline systems with their biodiversity decreasing

with increasing salinity (Hammer, 1993). In particular, East African saline lakes have been reported to show more rotifers in their zooplankton assemblages than either Copepods or Cladocerans, with the dominant species of Rotifera, Copepoda and Cladocera reported to change with the salinity gradient (Green, 1993). Alkaline saline crater lakes are considered very productive environments (Harper et al., 2003; Grant, 2006), with prokaryotic photosynthetic primary production suggested to be the driving force behind nutrient recycling in these systems (Jones & Grant, 1999). Community evenness decreases with increasing nutrient concentration, with the few favored species being dominant (Harper et al., 2003). Abundance of certain species like *Dunaliella* sp. dominate the saline waters of Utah lake in the USA (Larson & Belovsky, 2013), while '*Spirulina*' *Arthrospira fusiformis* (Voronikhin) Komárek et J.W.G.Lund has been reported to be dominant in lake Bogoria which is a hypersaline lake in Kenya, east Africa (Harper et al., 2003; Matagi, 2004) with no macro-zooplankton and lesser flamingo, *Phoeniconaias minor* (E. Geoffroy Saint-Hilaire, 1798), as the only grazers (Harper et al., 2003).

Several fish species more especially amphihaline species have been described to have physiological mechanisms which enable them to migrate between freshwater and sea water, with many other species with ability to tolerate, adapt or even acclimate to salinity, alkalinity and ionic compositions levels outside the conventional freshwater and seawater conditions (Brauner et al., 2013). Whereas both native and exotic species were found in waters with salinities less than 30 mg l⁻¹ in a study of fish distribution in inland saline waters in Victoria, Australia, no inland fish species were found at salinities above 30 mg l⁻¹ (Chessman & Williams, 1974). Flamingo lakes with salinity levels below 20 mg l⁻¹ are reported to have fish species of commercial value (Hadzembes, 2006). Several species were said to tolerate salinities as high as 15,000 mg l⁻¹ with only the nine-spined stickle back resisting at salinities of 20,000 mg l⁻¹ (Rawson & Moore, 1944). *Oreochromis alcalicus alcalicus* (Hilgendorf, 1905), *O. alcalicus grahami* (Trewavas, 1983), and *O. amphimelas* (Hilgendorf, 1905), have been reported to be endemic in lakes Magadi and Natron which are among the East African saline lakes (Matagi, 2004).

A number of environmental factors including salinity and nutrients in hypersaline systems may be potential factors which do affect biodiversity in saline environments (Larson & Belovsky, 2013). Saline lakes show limited species complement in micro-organisms contrary to the considerable biodiversity in micro-organisms (Harper et al., 2003). Larson & Belovsky (2013) reported salinity and nutrient concentration in hypersaline lakes as among the strong determinants of phytoplankton diversity, with species richness decreasing with increasing salinity and increasing with increasing nutrient concentration. Despite the inverse proportionality between saline and aquatic biodiversity, the relationship between salts is still not well understood (Derry et al., 2003; Rios-Escalante, 2013). Contrary to the numerous fish and planktonic biodiversity studies in fresh water systems, very little of such studies has been conducted in these unique saline systems (Jones & Grant, 1999; Larson & Belovsky, 2013). The aim of this study is, therefore, to investigate fish and planktonic biodiversity in selected saline crater lakes of western Uganda as a way of providing more information on fish and planktonic biodiversity in saline systems.

MATERIAL AND METHODS

Study area

Lakes considered in this study are small unique water bodies found in Katwe-Kikorongo volcanic field in western Uganda. Lake Katwe (029.87033°E, 00.13217°S), is the largest among these lakes with an average area of 2.5 km² (Nixon et al., 1971). Other lakes considered in this study were Katwe Munyanyange (029.88591°E, 00.13513°S), Nyamunuka (029.98743°E, 00.09344°S), Bagusa (030.17958°E, 00.09793°S), Murumuri (029.99186°E, 00.07323°S), Maseche (030.19019°E, 00.09355°S), Bunyampaka (030.12819°E, 00.03765°S) and Kikorongo (030.01228°E, 00.01190°S). Among the studied lakes, Bagusa and Kikorongo were at the lowest and highest altitude, 884 m and 939 m, respectively, above sea level (a. s. l). The majority of these lakes are alkaline and saline in nature with dominant anions being carbonates and sulphates (Nkambo et al., 2015). These lakes exhibit consid-

erable temporal variations in volume and surface area, with their total depth ranging between <1–6 m (Kirabira et al., 2013).

Zooplankton and phytoplankton diversity

Data collection in this study was done between the 26th of February and 3rd of March, 2014, a period towards the end of the dry season in this region. A Global Positioning System (GPS) unit (GARMIN 12XL) was used to take GPS coordinates and the Altitude / elevation above sea level of the different sampling points.

Zooplankton samples were obtained by filtering four liters of water collected from every set geo-referenced sampling point through a 50 μ m mesh zooplankton net. The samples obtained after filtration were preserved in 95% ethanol and carried to the National Fisheries Resources Research Institute (NaFIRRI) laboratory, Jinja, for identification to the lowest possible taxonomic level and counted under an inverted microscope. Using a Van Dorn water sampler, water samples for phytoplankton analysis were collected at a depth of 0.5 m in lakes whose average depth was more than 1 m. For the very shallow lakes (depth < 0.5 m), surface water samples were collected for zoo and phytoplankton analysis. 500 ml of the collected water samples were preserved using Lugol's solution in pre-rinsed Nalgene bottles which were kept in a cooler box containing dry ice and later transferred to the National Fisheries Resource Research Institute (NaFIRRI) laboratory in Jinja. In the laboratory, phytoplanktons in the collected water samples were identified to the lowest possible taxonomic level and the wet biomass of each of the identified group determined.

Selected physical and chemical parameters (depth, temperature ($T^{\circ}\text{C}$), dissolved oxygen concentration (DO), pH, Conductivity (Cond) and salinity) were also measured in-situ at the lake surface and bottom. Where lakes were too shallow, physico-chemical measurements were taken at the lakes surface. Water temperature, dissolved oxygen concentration and conductivity were measured using a YSI oxygen/temperature/conductivity meter (Model YSI 550A), pH was determined using an OAKTON pH Tester 30, while salinity was measured with a refractometer. The depth was determined using a portable depth finder (Hondex PS-7).

Fish diversity

All the study lakes, deeper than 0.5 m were sampled for fish by setting gill nets and seine nets in the evening at 5 pm and removing them in the following morning at 7 am. In addition we also asked to the people belonging to the communities around each of the studied lakes whether they have ever seen or got any fish from these lakes.

RESULTS

Physico-chemical parameters

Lakes Munyanyange, Nyamunuka, Murumuri, and Bunyampaka were found to be very shallow (depth < 0.5 m) at the time of sampling, whereas, lake Kikorongo was the deepest. The highest measured dissolved oxygen (DO) was $6.0 \pm 1.0 \text{ mg l}^{-1}$ in Lake Kikorongo while Munyanyange and Murumuri had the lowest and second lowest DO (1.7 ± 0.4 and $1.7 \pm 0.5 \text{ mg l}^{-1}$, respectively). All the sampled lakes were found to be alkaline with pH ranging between 9.58 ± 0.1 (lake Bunyampaka) and 11.5 ± 1.0 (Nyamunuka). The highest temperatures ranged between $28.9 \pm 0.4^{\circ}\text{C}$ and $34.4 \pm 2.4^{\circ}\text{C}$. Salinity was between 0 mg l^{-1} (lake Kikorongo) and $205.0 \pm 15.3 \text{ mg l}^{-1}$ (Nyamunuka) Conductivity ranged between $10.5 \pm 0.6 \text{ mscm}^{-1}$ (Nyamunuka) and $106.3 \pm 3.5 \text{ mscm}^{-1}$ (Murumuri) (Table 1).

Phytoplankton diversity

A total of twenty nine (29) phytoplankton species were found in the eight study lakes. Out of them, nineteen (19) belonged to Cyanophyceae, commonly known as Cynobacteria (blue-green algae (BG)) which are predominantly photosynthetic prokaryotes containing a blue pigment in addition to the chlorophyll (WHO, 1999). Six (6) belonged to Chlorophyceae commonly referred to as Chlorophyta (green algae (G)). Four (4) belonged to Bacillariophyceae, commonly referred to as diatoms (D). Lakes Maseche ($912,347 \mu\text{g L}^{-1}$) and Bagusa ($210,290 \mu\text{g L}^{-1}$) were found to have the highest and second highest algal biomass. These were followed by lakes Katwe and Murumuri which had algal biomass of $90,653 \mu\text{g L}^{-1}$ and $86,240 \mu\text{g L}^{-1}$, respectively. Lakes Katwe and

Lake	Depth (m)	D.O (mg l ⁻¹)	pH	Temp. (°C)	Salinity (mg l ⁻¹)	Cond (mScm ⁻¹)
Katwe	2.1±0.7	2.6±0.2	9.9±0.1	27.9±0.3	180±67.8	104.5±6.4
Munyanyange	0.2±0.1	1.7±0.4	10.8±0.4	34.4±2.4	101.0±7.1	59.7±8.2
Nyamunuka	0.2±0.2	2.6±0.3	11.5±1.0	30.5±3.1	205.0±15.3	10.5±0.6
Murumuri	0.2±0.0	1.7±0.5	11.1±1.3	32.0±0.8	162.8±34.2	106.3±3.5
Bunyampaka	0.2±0.1	2.20±0.6	9.6±0.1	30.33±1.5	199.50±16.4	103.90±4.3
Bagusa	1.9±0.5	3.2±0.8	10.5±0.4	32.1±2.0	199.5±16.4	103.9±4.3
Maseche	1.3±0.2	2.9±0.4	10.9±0.4	30.0±0.7	92.3±7.6	71.2±1.3
Kikorongo	2.3±0.3	6.0±1.0	10.4±0.0	28.9±0.4	0.0±0.0	18.6±0.1

Table 1. Mean±SD of the selected measured physico-chemical parameters in the selected studied saline crater lakes.

Bunyampaka showed the highest algal biodiversity while lakes Maseche and Nyamunuka had the lowest biodiversity. Cyanophyceae (BG) dominated the algal composition of all the studied lakes. With the exception of Lake Munyanyange, where no Chlorophyceae species (G) were found, the other lakes showed Chlorophyceae and Bacillariophyceae (D) species in relatively small abundances in comparison to Cyanophyceae. With the exception of Lake Bagusa where *Anabaena circinalis* was found to be dominant, and Bunyampaka and Nyamunuka where no *Spirulina platensis* was found, in the rest of the lakes *S. platensis* was dominant (Table 2).

Zooplankton diversity

Lakes Kikorongo, Maseche, and Katwe were found to have zooplanktons belonging to Rotifera. Lakes Maseche and Kikorongo showed also zooplanktons belonging to Copepoda and none of the studied lakes was found to have cladocerans at the time of sampling. Lakes Munyanyange, Maseche, Murumuli, Katwe and Nyamunuka had small cysts which could not be identified. Water samples from lakes Bunyampaka and Bagusa had neither zooplankton nor un-identified cysts (Table 3).

Fish diversity

None of the selected saline crater lakes considered in this study had fish at the time of sampling.

With the exception of Lake Kikorongo, in which the African catfish, *Clarias gariepinus* (Burchell, 1822), was observed (sometimes) during the rainy season, none of the other studied lakes was reported to have fish, ever.

DISCUSSION

Phytoplankton diversity

In the present study, blue-green (Cyanobacteria) algae are dominant in all the lakes, with *Spirulina* P.J.F. Turpin ex M. Gomont being the most dominant phytoplankton in the majority of them (Table 2). This is in agreement with earlier studies done in alkaline, saline crater lakes which reported *Spirulina platensis* to be the most dominant (Hecky & Kilham, 1973) in contrast with the reported dominance of algal biomass by diatoms in anthroposaline lakes in Romania and Bolivia (Stenger-Kovács et al., 2014). The dominance of Cyanobacteria in these harsh environments can be attributed to their ability to withstand extreme water conditions like very high temperatures, pH and salinity. Some Cyanobacteria species have got special adaptations like ultraviolet absorbing sheath pigments which increase their fitness in relatively exposed environments; indeed they have been reported to occur in waters that are salty, brackish or fresh, in cold or hot springs and in environments where no other

Taxa Group	Taxa	Bagusa	Bunya-ampaka	Munya-nyange	Museche	Katwe	Kikorongo	Nyamunuka	Murumuri
BG	<i>Planktolynghya limnetica</i> (Lemmermann) Komárková-Legnerová et Cronberg	15845	2,773	-	8,216	7,343	-	-	7,805
BG	<i>Aphanocapsa</i> sp.	522	274	548	-	342	-	365	730
BG	<i>Spirulina platensis</i> (Nordstedt) Gomont	12,324	-	25,880	879,909	66,856	43,133	-	69,012
BG	<i>Anabaena</i> sp.	15,649	219	-	-	3,286	-	-	-
G	<i>Stichococcus</i> sp.	3,912	96	-	-	787	-	-	-
D	Centric diatoms	104	292	37	146	730	-	292	37
BG	<i>Planktolynghya circumcreta</i> (G.S. West) Anagnostidis et Komárek	440	411	1,438	-	-	-	205	411
BG	<i>Chroococcus</i> sp.	391	137	-	365	-	23	-	-
BG	<i>Anabaena circinalis</i> Rabenhorst ex Bomet et Flahault	157,273	11,867	9,859	5,112	6,207	548	38,085	913
G	<i>Stichococcus</i> sp.	3,668	342	274	479	2,465	-	1,575	1,404
G	<i>Nephrochlamys rostrata</i> Nygaard, Komárek, J. Kristiansen et O.M. Skulberg	162	-	-	-	-	1,506	-	-
BG	Tiny blue green	-	101	151	-	-	228	-	-
BG	<i>Anabaenopsis tanganyikae</i> (G.S. West) Woloszyńska et V.V. Miller	-	55	-	-	-	-	-	-
BG	<i>Pseudanabaena</i> sp.	-	411	411	-	103	-	308	-
BG	<i>Anacystis limnetica</i> (Lemmermann) Drouet et Daily	-	183	365	-	274	137	342	297
BG	<i>Romeria</i> sp.	-	55	-	-	-	-	-	-
D	<i>Nitzschia acicularis</i> (Kützinger) W. Smith	-	274	-	-	23	-	137	23
G	<i>Closterium acerosum</i> Ehrenberg ex Ralfs	-	-	411	411	205	205	411	-
BG	<i>Oscillatoria tenuis</i> C. Agardh ex Gomont	-	-	-	17,253	-	-	-	-
BG	<i>Aphanocapsa nubila</i> Komárek et H.J. Kling	-	-	-	456	-	91	-	183
BG	<i>Coelosphaerium kuetzingianum</i> Nägeli	-	-	-	-	1,826	-	-	4,747
BG	<i>Planktolynghya undulata</i> Komárek et H. Kling	-	-	-	-	103	-	-	-
G	<i>Ankistrodesmus falcatus</i> (Corda) Ralfs	-	-	-	-	103	-	-	-
BG	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	-	-	-	-	-	68	-	-
D	<i>Cyclotephanodiscus</i> sp.	-	-	-	-	-	37	-	-
D	<i>Navicula gastrum</i> (Ehrenberg) Kützinger	-	-	-	-	-	856	-	-
BG	<i>Merismopedia tenuissima</i> Lemmermann	-	-	-	-	-	-	-	292
G	<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová	-	-	-	-	-	-	-	22
BG	<i>Aphanizomenon flos-aquae</i> Ralfs ex Bomet et Flahault	-	-	-	-	-	-	-	365
	Total	210,290	17,488	39,372	912,347	90,653	48,065	44,698	86,240

Table 2. Mean Wet biomass ($\mu\text{g/l}$) concentrations of the different phytoplanktons in the selected Alkaline, saline lakes considered in this study. BG = Blue green algac, G = green algac, D = Diatoms.

microalgae occur (WHO, 1999). Allelopathy can be another factor to explain the dominance of *Cyanobacteria* in these lakes. Freshwater *Cyanobacteria* like *Oscillatoria* sp. have been reported to have exudates which can inhibit green alga *Chlorella vulgaris* Beyerinck [Beijerinck] (Leão et al., 2010). In the same way *Cyanobacteria* in these saline crater lakes might be influencing the algal biodiversity through allelopathy. Matagi (2004) reported *Spirulina* (*Arthrospira fusiformis*) to be the most successful algae in colonizing alkaline, saline lakes found in the Eastern Rift valley. Lake Lonar, an inland alkaline saline crater lake in India was reported to have its phytoplankton biomass dominated by *Spirulina platensis* (Satyanarayan et al., 2007; Siddiqi, 2007; Yannawar & Bhosle, 2013). Lakes Nakuru, Bogoria and Elmenteita, which are alkaline-saline lakes in Kenya, were characterized by mass growth of *Cyanobacteria* including *Arthrospira fusiformis* (Harper et al., 2003; Ballot et al., 2004). The Presence of *Cyanobacteria* in these

alkaline-saline lakes is in conformity with the findings of the present study where *Spirulina* dominates the phytoplankton biomass. Jones & Grant (1999) reported *Spirulina* spp. to be among the main contributors to primary production in moderately saline lakes while studies by Hadgembes (2006) documented *Spirulina* to be one of the unique *Cyanobacteria* occurring in East African saline lakes.

The extreme inhospitable conditions in alkaline, saline crater lakes mean that the biodiversity in these systems is limited to organisms with special adaptations to survive such extreme conditions (Matagi, 2004). Primary production in Flamingo lakes of East Africa was reported to be dominated by *A. fusiformis* with *Ectothiorhodospira* sp. sometimes playing a key role (Jones & Grant, 1999; Matagi, 2004). *Nitzschia* sp. and *Navicula* sp. are some of the other algal species found in these lakes (present study) or in other highly alkaline and saline environments (Matagi, 2004). *Chroococcus* sp. is another species of *Cyanobacteria* recorded in this

	Munya-yange	Kiko-rongo	Maseche	Murumuli	Bunyam-paka	Katwe	Bagusa	Nyamunuka
ROTIFERA								
<i>Brachionus calyciflorus</i> Pallas, 1766	-	+++	-	-	-	+	-	-
<i>Brachionus angularis</i> Gosse, 1851	-	-	+	-	-	-	-	-
<i>Lecane luna</i> (Müller, 1776)	-	+	-	-	-	-	-	-
<i>Trichocerca cylindrica</i> (Imhof, 1891)	-	-	-	-	-	-	-	-
<i>Syncheata</i> sp.	-	+	-	+	-	-	-	-
COPEPODA								
nauplii	-	+	-	-	-	-	-	-
cyclopoid copepodite	-	-	+	-	-	-	-	-
CLADOCERA								
<i>Moina micrura</i> Kurz, 1874	-	-	-	-	-	-	-	-
unidentified cysts	+	-	+	+	-	+	-	+

Table 3. Mean zooplankton abundance (Individuals per litre) in the different studied saline crater lakes.
+ = 1 to 10 individuals/l, ++ = 10 to 50 individuals/l, +++ = 50 to 100 individuals/l, - = 0 individuals/l.

study which was reported by Jones & Grant (1999) to play a key role in primary production in soda lakes of East Africa. In studies aimed at reconstructing relationships between diatoms assemblages with salinity, it was observed that salinity might not be the primary cause of the shift in the diatom assemblage but a factor highly related to drivers of species shift (Sáros & Fritz, 2000).

In the same way salinity might be playing a key role in determining the Cyanobacteria species occurring in the alkaline saline environments, but other possible drivers should not be overlooked. In a study to determine the extent to which salinity influences community structures in saline systems, salinity was reported to play a less significant role in the determination of composition, species richness and biodiversity (William, 1998). For example, the highest algal biodiversity observed in lakes Katwe and Bunyampaka and the least algal biodiversity (in lakes Maseche and Nyamunuka) might be attributed to the daily anthropogenic disturbances experienced by the first two lakes during the process of salt extraction, while the other two are lakes with no daily anthropogenic disturbance. It should be noted that communities around lakes Katwe and Bunyampaka continuously extract salt from these lakes on a daily basis and such disturbances might be impacting various algal species differently.

Zooplankton diversity

All the studied lakes showed a very low zooplankton biodiversity, with only *Brachionus calyciflorus* in lakes Kikorongo and Katwe; *Brachionus angularis* in Lake Maseche, and *Syncheata* sp. in lakes Kikorongo and Murumuli (the only species of the phylum Rotifera present, see Table 3). *Brachionus plicatilis* (Müller, 1786) and *Paradiaptomas africanus* (Daday, 1910) (= *Lovenula africana*) have already been reported to be characteristic zooplankton in alkaline saline crater lakes (Hecky & Kilham, 1973). Matagi (2004) listed *Lovenula africana* (Daday, 1910), *Brachionus dimidiatus* (Bryce, 1931), *B. plicatilis* (Müller, 1786) and chironomids as the dominant macro-invertebrates in the highly alkaline, hypersaline flamingo lakes of east Africa. All of these were absent in our study. Nauplii in Lake Kikorongo and cyclopoid copepodites in Lake Maseche were the only Copepoda members observed.

Other macro-zooplanktons like cladocerans, neck-ton fauna, and crustacean decapods were conspicuously absent, probably due to the highly alkaline pH. Lake Bogoria in Kenya which is an alkaline, saline lake was reported to have no macro-zooplankton with lesser Flamingo, *Phoeniconaias minor* as the only grazer occasionally visiting these lakes in high numbers (Harper et al., 2003). The absence of crustacean decapods in a limnological study of lake Lonar an alkaline, saline crater lakes in India was attributed to a pH shock (pH > 10–11, alkaline death point) (Siddiqi, 2007). An inverse proportionality between biodiversity and salinity was reported by Derry et al., (2003); Larson & Belovsky (2013), and Rios-Escalante (2013); this might be the explanation for the slightly high zooplankton biodiversity in Lake Kikorongo since its salinity was found to be very low at the time of sampling (Table 1). In field and laboratory experiments designed to examine the consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes, severe disturbances in zooplankton community structure and abundance were caused by even very small salinity changes, with even very small increments in salinity capable of leading to biodiversity depletion (Schallenberg et al., 2003). Similarly, the low zooplankton biodiversity in these lakes might be attributed to the high salinity levels (see Table 1).

The unidentified cysts found in some of the lakes might be dormant stages of zooplanktons released as a mean of surviving extreme environmental conditions. These dormant stages return to life on the set of suitable conditions within the lakes. Some zooplanktons, particularly members of the order Anostroca like *Artemia* Leach, 1819, have been reported to release dormant embryos in form of cysts. Indeed, all *Artemia* species and strains reproduce ovoviviparously (by generating live nauplii) under favorable conditions, and oviparously when dormant embryos are released in form of cysts to withstand the harsh unfavorable environmental conditions (Ghomari et al., 2011; Ben Naceur et al., 2012).

Fish diversity

Although some fish species like *Oreochromis alcalicus alcalicus*, *O. alcalicus grahami*, and *O. amhimelas* were reported to inhabit lakes Magadi

and Natron which are both alkaline and saline in nature (Matagi, 2004), no fish were found in these lakes during the study. Hecky & Kilham (1973) also reported cichlid fish like *Alcolapia grahami* (Boulenger, 1912) to occur in some of the alkaline, saline lakes. The absence of fish in the different studied lakes at the time of sampling can be attributed to the extreme environmental conditions like the very high temperatures, salinity and alkalinity. Previously, the complete absence of fish in Lake Lonar was reported to be correlated with extreme environmental physico-chemical parameters (Siddiqi, 2007). Many lakes, being shallow, experience very high variations in volume and surface area with some of these lakes reported to evaporate to dryness during the extreme dry seasons (Nkambo et al., 2015). This makes difficult, if not impossible, for fish to survive during the dry seasons. Moreover, the absence of appropriate food organisms in form of zooplankton (i.e. *Brachionus plicatilis*) could be another probable reason for the absence of fish (see Table 3).

Some of the Cyanobacteria found in these lakes like *Anabaena* sp., *Anabaenopsis* V.V. Miller, 1923 and *Oscillatoria* Vaucher ex Gomont, 1892, have been reported to release cyanotoxins with lethal effects on mammals (WHO, 1999; Lyra et al., 2001). Although several research works on cyanotoxins have focused on humans and other livestock (Leão et al., 2010), it is possible that these cyanotoxins have similar toxic effects on aquatic organisms including fish. *Anabaena* sp. have been reported to release anatoxin-a which is a neurotoxin reported to have lethal effects when tested on *Cyprinus carpio* Linnaeus, 1758 larvae (Oswawald et al., 2007). The presences of toxic Cyanobacteria might be hindering fish occurrence in these lakes considered under this study. With the exception of Lake Kikorongo, which sometimes receives flood waters from the neighboring lake Gorge (Hecky & Kilham, 1973; Mungoma, 1990; Nkambo et al., 2015), the rest of the lakes considered under this study are located in closed basins with no connections to other lakes or rivers. This implies that these lakes have no possibilities of being seeded with fish by inflowing waters from other natural systems. The reported occurrence of the African Catfish, *Clarias gariepinus*, in Lake Kikorongo in the rainy season might be due to flood waters from Lake Gorge. In fact, Nkambo et al., (2015) reported incoming flood waters from Lake Gorge to cause a

reduction in the salinity of Lake Kikorongo. A reduction in salinity might make this lake conducive for fish survival during the wet season. It is possible that catfish brought along with floods remain in this lake during the rainy season and die off in the dry season as the water conditions become extremely unbearable.

CONCLUSIONS

Our findings are in agreement with earlier studies by Hecky & Kilham (1973), Matagi (2004), Satyanarayan et al., (2007), Siddiqi (2007) and Yanawar & Bhosle (2013) which reported Cyanobacteria to dominate algal biomass in saline systems, in contrast with earlier studies by Stenger-Kovács et al., (2014), which reported diatoms to dominate the algal biomass in saline lakes in Romania and Bolivia. Lakes considered under this study had very low zooplankton abundance and diversity with Lake Kikorongo, which had the highest zooplankton biodiversity, showing the rotifer *Brachionus calyciflorus*, the most abundant, ranging only between 50 to 100 individuals /litre. None of the study lakes had fish at the time of sampling.

In our opinion, this information on fish and planktonic biodiversity in these alkaline, saline systems is very useful in providing the ecological basis for the management of the lakes. Data on dominant zoo and phytoplanktons in the lakes can be used as bio-indicators in assessing the ecological status, as well as the impact of climate change on these unique systems.

Recommendations

Further comprehensive studies are needed to assess the effect of season variability on fish and planktonic biodiversity. Detailed studies of the daily anthropogenic disturbances on the algal and zooplankton biodiversity in these lakes during the salt extraction process are required to give a better understanding of the changes in planktonic composition, species abundance and biodiversity due to anthropogenic disturbances.

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Limnological Survey of the Alkaline, Saline Crater Lakes of Western Uganda

Mujibu Nkambo^{1*} Fredrick W. Bugenyi² Janet Naluwayiro¹ Sauda Nayiga¹ Waswa Leonard⁴
1.Aquaculture Research and Development Center- Kalljansi, National Fisheries Resources Research Institute (NaFIRRI)

2.Department of Biological Sciences, College of Natural Sciences (CONAS), Makerere University

3.Islamic University In Uganda (IUIU)

4.Katwe Kabatoro community

Email of the corresponding author: mnkambo@yahoo.co.uk

Abstract

Eight alkaline, saline crater lakes in western Uganda were surveyed between 26th February and 3rd March, 2014 with an aim of providing information on the physico-chemical features of these less known unique habitats and provide a basis for comparison with similar environments in other parts of the world. Lakes considered for this study included: Katwe, Kikorongo, Murumuli, Nyamunuka, Munyange, Bunyampaka, Bagusa and Maseche. Surface and Bottom measurements of physico-chemical parameters were measured in-situ from at least three geo-referenced points. Water samples for alkalinity, nutrients and detailed ionic analysis were taken from each of the points. In the laboratory, collected water samples were analysed following APHA standard operating procedures. All the studied lakes were alkaline and saline in nature as reported in the previous studies except Lake Kikorongo which was found to be fresh with a salinity of zero in this present study. With the exception of Lake Murumuli which was found to be a Carbonate-chloride lake, the rest of the sampled lakes are carbonate-sulphate lakes which is contrary to earlier studies which reported lakes like Katwe and Kikorongo to be Carbonate-chloride lakes.

Keywords: Alkaline, saline, carbonate-chloride, carbonate-sulphate, lakes

Introduction

The Western part of Uganda is richly endowed with crater lakes many of which are both alkaline and saline. They are small unique water bodies occurring in basin associated with the Western Rift valley. These lakes being small, alkaline and saline in nature this makes them have very unique physical and chemical characteristics in comparison to the big fresh water systems like Lake Victoria, Edward, Albert, Kyoga and George. Lakes Katwe, Munyanyange, Nyamunuka, Bagusa, Murumuli, Maseche, Bunyampaka and Kikorongo, all of which belong to the Katwe-Kikorongo volcanic field in western Uganda have been considered for this study. Lake Katwe with an average area of 2.5km² is the largest among these lakes (Nixon, Morton *et al.*, 1971; Kirabira, Kasedde *et al.*, 2013). This area has got volcanic soils, exhibits a relatively hot and dry climate and experiences a bimodal rainfall pattern with March – May and September – November being the rain seasons. This area has been reported to receive a mean annual rainfall range of 60 – 70cm with a mean monthly temperatures ranging between 19.0±2.8°C and 28.0±3.2°C. The dominant vegetation is mainly savannah grassland and dry woodland mosaic (Mungoma, 1990). The majority of these lakes are alkaline in nature and exhibit considerable temporal variations in volume and surface area. The total depth of the lakes have been reported to range between <1 – 6m (Kirabira, Kasedde *et al.*, 2013). Stable alkaline conditions in previously studied alkaline systems have been attributed to a unique combination of climate, geological and topographical conditions (Grant, 2006). These lakes have been categorized by earlier studies into three group: carbonate-chloride, sulphate-chloride or chloride lakes depending on the dominant anion(s) (Mungoma, 1990; Camargo, Durán *et al.*, 2005) while Matagi (2004) in a biodiversity assessment of the flamingo lakes of East Africa reported highly alkaline, hypersaline environments to be referred to as soda lakes (Matagi, 2004). Soda lakes have been described as the earth's most stable high pH environments (Grant, 2006). These lakes were reported to have a pH above 9.6 (Matagi, 2004; Grant, 2006). These lakes have been reported to be associated with low Mg²⁺, Ca²⁺ geology with very high evaporation rates often exceeding their water inflows (Grant, 2006). Other areas other than the East African Rift Valley where such environments have been reported to occur include arid and semi-arid areas of tropical and / or subtropical rain-shadow deserts like in North America and continental Interiors of Asia (Grant, 2006). The ionic composition and concentration in these lakes plays an integral role in determining their limnochemical characteristics and resource exploitation by the surrounding communities.

In Uganda, it is only lakes Katwe and Bunyampaka which are commercially exploited for common salt (NaCl) by the local communities (Matagi, 2004). The majority of these other alkaline, saline lakes have not been put to use.

In the past these lakes attracted a lot of attention because of their unique physico-chemical characteristics. In comparison to fresh water systems and other inland water bodies, the limnology and

hydrobiology of saline systems have not been given as much attention in the recent studies (Stenger-Kovács, Lengyel *et al.*, 2014). Despite the vast research done on these lakes which dates back from Beadle (1932, 1974), Holmes & Harwood (1932), von Knorring & DuBois (1961), Arad & Morton (1969), Kilham (1971a, b), Melack & Kilham (1972), Hecky and Kilham (1973), Melack (1976, 1978), Melack *et al.*, (1982) and so on, none of these research focused on the limnology of these lakes.

It is therefore the aim of this present study to conduct a limnological survey in selected alkaline, saline crater lakes in Western Uganda to provide information on the physico-chemical features of these less known unique habitats and provide a basis for comparison with similar environments in other parts of the world.

Materials and Methods

Study Area



Figure 1; Map showing the selected Alkaline saline crater lakes of Western Uganda considered in this study

Lakes	Coordinates		Altitude (H a.s.l)	lake Surface Area (Km ²)	Average Depth (mean±SD) (M)
	Long. E	Lat. N			
Katwe	029.87033°E	00.13217°S	897	2.45	2.1±0.7
Munyanyange	029.88591°E	00.13513°S	915		0.2±0.0
Nyamunuka	029.98743°E	00.09344°S	929		0.2±0.0
Murumuri	029.99186°E	00.07323°S	887		0.2±0.0
Bunyampaka	030.12819°E	00.03765°S	893	0.43	0.20±0.1
Bagusa	030.17958°E	00.09793°S	884		1.9±0.5
Maseche	030.19019°E	00.09355°S	918		1.3±0.2
Kikorongo	030.01228°E	00.01190°S	939	1.03	2.3±0.3

Table 1: Main geomorphology and hydrological characteristics of the studied lakes

General assessment of the lake environments

Data collection in this study was done between the 26th of February and 3rd of March, 2014, a period towards the end of the dry season in this region. A Global Positioning System (GPS) unit (GARMIN 12XL) was used to take GPS coordinates and the Altitude / elevation above sea level (H. a.s.l) of the different points of sample collection at each of the sampled lake. Visual observation of water color, number of fresh water inflows, and percent

vegetation cover were made at each of the studied lake. Fringing vegetation in the littoral zone around each of the studied lake was characterized and the major plant species identified where possible.

Determination of physico-chemical parameters

At each lake, selected physical and chemical parameters (depth, temperature ($T^{\circ}\text{C}$), dissolved oxygen concentration (DO), pH, Conductivity (Cond) and salinity) were measured at the Lake Surface and bottom *in situ*. Where lakes were too shallow, measurement were done at the lakes surface. Water temperature, dissolved oxygen concentration and conductivity were measured using a YSI oxygen/temperature/conductivity meter (Model YSI 550A), pH was determined using an OAKTON pH Tester 30, while salinity was measured with a refractometer. From each of the selected sampling point, Depth was determined using a portable depth finder (Hondex PS-7). The depth finder was lowered just below the water surface, while pointing to the lake bottom, the button was pushed and the depth reading was taken from the backlit screen in meters.

Surface water samples for chemical and nutrient characteristics of each of the studied lakes were collected using a Van Dorn water sampler from geo-referenced sampling points and stored in pre-rinsed Nalgene bottles for analysis in the laboratory. In the field all water samples in pre-rinsed Nalgene bottles were kept in a cooler box containing dry ice and later transferred to the National Fisheries Resource Research Institute (NaFIRRI) laboratory in Jinja. In the laboratory the collected water samples were analysed for the following parameters: alkalinity, bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), Sulphate (SO_4^{2-}), Silicates (SiO_2), Chloride (Cl^-), Fluoride (F), Sodium (Na), Magnesium (Mg), Calcium (Ca), Potassium (K), and Hydrogen Sulphide (H_2S). Sodium, Magnesium, Calcium, Potassium, Chloride (Cl), Fluoride (F), and silicon following APHA 1975 standard Operating procedures.

Determination of Nutrients characteristics

Water samples for dissolved nutrients; soluble reactive phosphorus (SRP), ammonia-nitrogen (NH_3N) and nitrite-nitrogen (NO_2N) were, filtered through 47mm pore Whatman GF/C filter papers and analysed by spectrophotometric methods following procedures by Stantoin *et al.*, 1977. Water samples were also analysed for total suspended solids (TSS).

Results and Discussion

Lakes Munyanyange, Murumuri and Nyamunuka were reported by the locals to be seasonal lakes and sometimes evaporate to dryness during the dry seasons. Lakes Katwe, Bunyampaka, Bagusa and Maseche are permanent lakes with very high variations in water volumes between the dry and wet seasons reported by the locals (Table 2). Lakes Katwe, Bunyampaka, and Maseche waters were found to be pea-nut soup green in color (figure 3) while lakes Nyamunuka and Murumuri waters were dark green in color. Lakes Kikorongo and Munyanyange waters were greenish in color. Lake Bagusa waters were pinkish along the shore and reddish brown offshore (figure 2).



Figure 2; *Cyprus lavigatus* with the Pinkish waters near the shores of lake Bagusa which turns reddish brown towards the middle of the lake.



Figure 3: Pea-nut soup green waters from Lake Katwe

Lakes Katwe and Munyanyange had the lowest vegetation cover, estimated to be 35% and 45% respectively. This was followed by Lake Bunyampaka with an estimated vegetation cover of 60%. The rest of the other studied lakes had a vegetation cover estimates of 75% and above (Table 2). Lakes Katwe, Bagusa and Maseche were found to have freshwater wetlands surrounding them in some parts. *Cyperus lavigatus*, savannah woodland dominated by *Acacia* woodland and *Euphorbia candelabra* were the dominant vegetation surrounding these lakes.

Table 2; Selected environmental characteristics of the selected alkaline, saline Lakes of Western Uganda

Lake	General environment characteristics			
	Seasonality	Waters	% Vegetation cover	Fringing vegetation
Katwe	Permanent but experience variations in water volume	Thick, slimy & Peanut soup green in color	35	Acacia woodland, <i>Euphorbia candelabra</i> , savanna grassland and <i>Cyperus lavigatus</i> on the shoreline
Munyanyange	Seasonal, sometimes evaporates to dryness in dry season	Slimy & green in color	45	Savannah grassland, scattered thorny shrubs, <i>Euphorbia candelabra</i> , <i>Cyperus lavigatus</i>
Nyamunuka	Seasonal, sometimes evaporates to dryness in dry season	Thick & Dark in color	80	Savannah grassland Woodland and scattered shrubs
Murumuli	Seasonal, sometimes evaporates to dryness in dry season	Thick & Dark in color	90	Acacia woodland
Bunyampaka	Permanent but experience variations in water volume	Thick slimy & Peanut soup green	60	Woodland dominated by <i>Euphorbia candelabra</i> . Other plant species include: <i>Acacia siberiana</i> , wild palms, <i>Caparis tomentosa</i> . Shoreline has wetland dominated with <i>Cyperus lavigatus</i> and wild palms.
Bagusa	Permanent but experience variations in water volume	Thick, slimy, Pinkish on shore and Reddish-brown offshore	80	Grassland, woodland, wild palms. Shoreline dominated by <i>Cyperus lavigatus</i>
Maseche	Permanent but experience variations in water volume	Thick slimy & Peanut soup green in color	75	Grassland, woodland, wild palms. Shoreline dominated by <i>Cyperus lavigatus</i>
Kikorongo	Permanent but experience variations in water volume	greenish in color	80	Grassland, shrubs and woodland.

Physical characteristics of selected alkaline, saline lakes

Table 3; Mean±SD of *in-situ* measured of selected physical characteristics of the study alkaline, saline crater lakes of Western Uganda

Lake	Mean ±SD					
	Depth (m)	D.O (mg/l ¹)	pH(-)	Temp. (°C)	Salinity(mg/l ¹)	Cond (mScm ⁻¹)
Katwe	2.1±0.7	2.6±0.2	9.9±0.1	27.9±0.3	180±67.8	104.5±6.4
Munyanyange	0.2±0.1	1.7±0.4	10.8±0.4	34.4±2.4	101.0±7.1	59.7±8.2
Nyamunuka	0.2±0.2	2.6±0.3	11.5±1.0	30.5±3.1	205.0±15.3	10.5±0.6
Murumuri	0.2±0.0	1.7±0.5	11.1±1.3	32.0±0.8	162.8±34.2	106.3±3.5
Bunyampaka	0.20±0.1	2.20±0.6	9.58±0.1	30.33±1.5	199.50±16.4	103.90±4.3
Bagusa	1.9±0.5	3.2±0.8	10.5±0.4	32.1±2.0	199.5±16.4	103.9±4.3
Maseche	1.3±0.2	2.9±0.4	10.9±0.4	30.0±0.7	92.3±7.6	71.2±1.3
Kikorongo	2.3±0.3	6.0±1.0	10.4±0.0	28.9±0.4	0.0±0.0	18.6±0.1

With the exception of Kikorongo whose average depth was above 2m, the rest of the sampled lakes were found to have an average depth less than 2m. The highest measured dissolved oxygen (DO) was 6.0±1.0mg/l measured in Lake Kikorongo, followed by lakes Bagusa and Maseche which had D.O levels of 3.2±0.8 and 2.9±0.4mg/l respectively. In all the sampled lakes the pH was high above neutral ranging between 9.58±0.1 and 11.5±1.0, measured in lakes Bunyampaka and Nyamunuka respectively. The highest measured temperatures were 34.4±2.4°C followed by 32.1±2.0°C and 32.0±0.8°C measured in lakes Munyanyange, Bagusa and Murumuri respectively. The lowest measured temperatures were 27.9±0.3°C and 28.9±0.4°C measured in lakes Katwe and Kikorongo respectively. Of all the sampled lakes it was only Lake Kikorongo which had fresh waters. The rest of the sampled lakes were found to be hypersaline with salinities ranging between 92.3±7.6mg/l and 205.0±15.3mg/l measured in lakes Maseche and Nyamunuka respectively. The conductivity in all the studied lakes ranged between 10.5±0.6mS/cm and 106.3±3.5mS/cm which were the lowest and highest conductivities recorded in lakes Nyamunuka and Murumuri respectively (Table 3).

Chemical composition of selected chemical parameters in the Alkaline, saline crater lakes

Lakes Katwe (110205.0±7.1mg/l), Nyamunuka (101254.5±6.4mg/l), and Maseche (76909.1±7.0mg/l) had the highest alkalinities while lakes Kikorongo (12534.4±1.6mg/l) and Munyanyange (12767.2±329.3) had the lowest alkalinities. All the studied lakes were found to have carbonate and bicarbonates as the dominant anions with lakes Nyamunuka and Kikorongo having the highest and lowest carbonate concentrations respectively. Calcium was found to be the dominant cation in all the studied lakes. Lakes Bunyampaka, Katwe and Maseche had the highest sodium concentrations while lakes Murumuri, Bunyampaka and Katwe respectively had the highest Chloride concentration (table 4).

Table 4; Mean ±SD of selected chemical parameters and ionic composition of the waters of the different lakes considered in this study.

Parameter	Mean±SD of the concentration of the different chemical parameters in each lake							
	Bagusa	Bunyampaka	Katwe	Murumuri	Nyamunuka	Munyanyange	Kikorongo	Maseche
Alkalinity (mg/l ₁)	66161.7±3.5	65191.7±500.3	110305.0±7.1	66050.5±71.4	101254.5±6.4	12767.2±329.3	12534.4±1.6	76909.1±7.0
Calcium (mg/L ⁻¹)	147.7±17.2	674.5±77.1	1275.7±1.4	368.5±0.7	677.5±0.7	124.0±5.7	111.0±1.4	631.3±6.9
Carbonates (mg/L ⁻¹)	46395.3±142.6	59151.0±8.5	31905.0±7.1	37250.5±0.7	67804.0±5.7	8315.2±21.4	8333.5±0.5	43628.0±7.1
Chloride (mg/L ⁻¹)	164.3±8.5	203.1±55.9	202.3±0.0	1140.0±0.7	102.0±2.1	134.0±8.5	84±0.5	183.4±4.6
Bicarbonates (mg/L ⁻¹)	19518.7±319.4	6999.8±20.0	78302.0±2.8	28753.5±4.9	34451.0±1.4	4650.0±70.7	4205.5±7.8	33241.2±56.6
Sulphates (mg/L ⁻¹)	293.0±7.3	243.9±20.6	244.9±0.6	143.0±4.2	181.7±0.3	194.0±14.1	154.0±28.3	233.9±3.2
Sodium (mg/L ⁻¹)	84.5±4.7	135.9±1.4	134.6±2.1	67.8±0.2	92.6±0.7	89.9±0.4	62.2±1.4	106.6±1.9
Magnesium (mg/L ⁻¹)	33.8±0.1	176.4±45.8	557.3±2.8	114.5±7.1	246.5±0.7	32.1±4.1	27.4±0.4	226.4±2.0
Potassium (mg/L ⁻¹)	43.1±1.6	78.6±5.7	65.1±3.5	31.0±1.4	48.6±0.1	45.4±7.0	30.2±0.3	56.7±1.0
Hydrogen sulphide (mg/L ⁻¹)	15.0±0.2	1.2±0.1	13.8±1.4	4.3±0.7	7.4±0.2	1.6±0.1	0.4±0.1	7.7±0.6
Total silica (mg/L ⁻¹)	21.8±1.1	10.6±5.5	28.8±1.5	16.2±0.7	19.3±0.2	10.0±0.3	11.3±0.1	19.9±1.0
Fluoride (mg/L ⁻¹)	0.4±0.0	0.7±0.0	0.8±0.1	0.3±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.9±0.4

Table 5: Mean±sd of selected nutrients concentrations in the selected Alkaline, saline lakes considered in this study

Lake	Mean ± SD for Nutrients and TSS				
	NH ₃ -N mg l ⁻¹	NO ₂ -N mg l ⁻¹	NO ₃ -N mg l ⁻¹	SRP mg l ⁻¹	TSS mg l ⁻¹
Katwe	8.03±0.28	1.48±0.05	0.19±0.04	64.93±2.91	281.00±30.29
Bunyampaka	7.78±2.55	1.68±0.10	0.29±0.11	32.90±13.68	3103.00±2122.09
Maseche	7.20±1.44	1.30±0.10	0.17±0.03	32.47±0.61	8462.85±5782.53
Munyanyange	6.53±0.68	1.83±0.15	0.24±0.01	15.53±1.76	1984.32±176.88
Bagusa	13.45±1.89	1.98±0.13	0.47±0.04	12.23±1.34	5241.86±1305.56
Murumuri	6.13±0.03	1.31±0.27	0.23±0.04	14.15±7.99	35175.23±93.96
Kikorongo	0.04±0.01	0.15±0.07	0.21±0.01	9.40±0.71	90.03±10.20
Nyamunuka	7.7±1.21	1.7±0.11	0.23±0.04	82.01±0.91	91833.33±186.23

Lake Kikorongo had the lowest concentrations of NH₃-N, NO₂-N, NO₃-N, SRP and TSS. Among the sampled Lakes, Bagusa had the highest NH₃-N, NO₂-N, and NO₃-N concentrations while Lake Nyamunuka had the highest SRP and TSS (Table 5 above).

Discussion

General Environment characteristics of selected Alkaline, saline lakes

In this study, communities confirmed Lake Munyanyange and Murumuri in addition to Lake Nyamunuka to evaporate to dryness during the extreme dry seasons. These lakes having no major inflow and the intense solar heating they experience are the main reason why these lakes evaporate to dryness in the extreme dry seasons. Although soda lakes are characterized by high evaporation rates, the presence of many fresh water inflows around Lakes Katwe, Bunyampaka, Bagusa and Maseche ensures that these lakes do not evaporate to dryness even in the dry season hence making them permanent lakes (Musinguzi and Katikro, 2000). The high variations in the volume of these lakes can be attributed to the very high evaporation rates experienced in saline lakes (Jones and Grant, 1999). Melack (1978) reported Lake Nyamunuka to have evaporated to dryness for a period of two weeks in May 1971. This could have been mainly because lake Nyamunuka has few and very small fresh water inflows which are not enough to maintain the water in the lakes during the dry season.

The peanut soup green, dark green and greenish color in lakes Katwe, Bunyampaka and Maseche, Nyamunuka and Murumuri, Kikorongo and Munyanyange respectively observed in this study might be attributed to the cyanobacteria and algal blooms which have been reported in alkaline, saline lakes (Mungoma, 1990; Jones and Grant, 1999; Matagi, 2004). Lakes Kikorongo and Maseche waters were reported to be peanut soup green in color while Lake Bagusa waters were reported to be brown in color in the earlier limnological studies done on the lakes (Mungoma, 1990). East African Saline lakes have been reported to have unique cyanobacteria community (Hadgembes, 2006). With the exception of Lake Kikorongo whose waters were not thick and slimy, the waters in the rest of the other sampled lakes were found to be thick and slimy. Previous studies by Matagi (2004) attributed the thickness and slimy nature of the waters in alkaline, saline the high algal abundance and high evaporation rates in hypersaline lakes which leave behind lots of organic matter (Jones and Grant, 1999; Grant, 2006).

Lakes Katwe, Munyanyange and Bunyampaka are experience severe impacts of overgrazing and deforestation. Deforestation and overgrazing have been reported to be among the major drivers of reduced vegetation in the hypersaline rift valley lakes in Tanzania and Ethiopia (Matagi, 2004). A lot of vegetation has been cleared around lakes Katwe and Bunyampaka by salt miners who use in re-enforcing salt crystallization pans during their construction. It is also used to cover the extracted salt as a way of protecting it from rain during storage while waiting for the buyers. The high vegetation cover in the other studied lakes (Murumuri, Nyamunuka, Bagusa, Maseche and Kikorongo) could be because the lakes are found in Queen Elizabeth National park (QENP) where there are protected from encroachment by Uganda Wild Life Authority (UWA). The freshwater wetlands around Lakes Katwe, Bagusa and Maseche are maintained by ground water and seasonal streams flowing from highlands surrounding these lakes (Grant, 2006). Musinguzi *et al* (2000) reported lakes Katwe, Munyanyange and Nyamunuka to be surrounded by pure strands of *Cyperus lavigatus*. Salinity has been reported to have a significant impact on the emergent vegetation in the littoral zones of saline lakes (William and Fawn, 2009). This explains why *Cyperus lavigatus* a species that is adapted to such saline environments was found to be surrounding many of the lakes in this study. In a biodiversity assessment of the flamingo lakes of East African, savannah woodland, bush land and shrub land were reported to be the dominant vegetation in the alkaline rift valley lakes of East Africa. *Euphorbia candelabra* was reported in most highland forests surrounding these lakes while *Cyperus lavigatus* dominated the surrounding wetland vegetation (Matagi,

2004). *Cyperus lavigatus* and *Cyperus rotundus* are among plants reported to be tolerant to difficult conditions in arid and semi-arid areas (Alsherif, Ayesh *et al.*, 2012). This region having hot and dry climate with high evaporation rates (Mungoma, 1990), it is mainly the plants like *Cyperus lavigatus*, and *Euphorbia candelabra* with ability to survive in such harsh conditions that abound to be the dominant vegetation.

Physical characteristics of selected alkaline, saline lakes

The studied lakes being shallow can be attributed to the mode of their formation. All these studied lakes were formed by water filling a series of shallow depressions which were formed by tectonic activities during the formation of the great East African rift valley (Matagi, 2004; Grant, 2006). Endorheic, saline have been described to be typically small (<50ha) and shallow (<1m) (Stenger-Kovács, Lengyel *et al.*, 2014). Previous limnological studies of alkaline, saline lakes in Uganda reported these lakes to be shallow with only lakes Kikorongo and Mahega being the only lakes reported to have a depth exceeding 5m (Mungoma, 1990). This remains true as per the findings of this present study.

With the exception of Lake Kikorongo, the rest of the sampled lakes had very low dissolved oxygen (DO) levels (<3.5mg/l). The low DO could be explained by the high decomposition rates of dead algae since these lakes are reported to be amongst the most productive aquatic systems in the world (Grant, 2006). Earlier studies have reported dissolved oxygen levels (less 1mg/l) to be very low in these alkaline, saline crater lakes (Mungoma, 1990). Alkaline, saline lakes have been reported to generally have high pH above 9.6 (Matagi, 2004; Grant, 2006). Study finding from this study are in agreement with the past studies, with the current studied lakes having pH ranging between 9.58 ± 0.1 and 11.5 ± 1.0 . With the very high evaporation rates experienced in these lakes, the dissolved minerals are concentrated into alkalis, with pH ranging from 8.5 in dilute lakes to around 12 in highly concentrated lakes (Grant, 2006). Other East African Alkaline, saline lakes like Nakuru (10.5), Elmenteita (9.4), Manyara (10.2), Reshitani (9.2) and Big Momela (10.4) have been reported to have similarly high pH ranges (Melack and Kilham 1974).

The highest and lowest measured temperatures of $34.4 \pm 2.4^\circ\text{C}$ and $27.9 \pm 0.3^\circ\text{C}$ measured in lakes Munyanyange and Katwe respectively were higher than the reported the reported mean monthly temperatures of this region $19.0 \pm 2.8^\circ\text{C}$ and $28.0 \pm 3.2^\circ\text{C}$ from previous studies by Mungoma (1990). This could be because the survey was carried out in the dry season with high atmospheric temperatures. This area experiences a hot and dry climate compared to the surrounding highlands (Mungoma, 1990). Such climates are characterized by high atmospheric temperature. These alkaline saline lakes being small and shallow with no major out flow, there are very sensitive to weather and climate changes. Small changes have been reported to cause large and sometimes irreversible changes in the nature of saline lakes (Hammer, 1990). Shallow closed-basin saline lakes of Northern Great Plains have been reported to be very sensitive to changes in precipitation and evaporation (Evans and Prepas, 1996). Like the shallow closed-basin saline lakes of the Northern Great Plains, the alkaline saline lakes of Uganda are closed with no major outflow. This implies that they are also very sensitive to weather and climate variations.

Lake Kikorongo was found to be fresh with a salinity of $0.0 \pm 0.0 \text{mg l}^{-1}$. This is contrary to previous literature which reported lake Kikorongo to be saline (Mungoma, 1990; Hadgembes, 2006). The rest of studied lakes were hypersaline. With the exception of Lake Maseche whose salinity was slightly below 100mg l^{-1} , the rest of the sampled lakes had their salinities above 100mg l^{-1} (Table 3). Such hypersaline lakes with high pH above 10 have been referred to as soda lakes (Matagi, 2004). These lakes have been reported to be experiencing temporal variations in salinities as high as 50mg l^{-1} (Mungoma, 1990). Lake Kikorongo has been reported to receive fresh water from floods coming from the neighboring Lake George during the rain seasons (Hecky and Kilham, 1973; Mungoma, 1990). This together with precipitation which has been reported to affect salinity in closed lakes (Saros and Fritz, 2000), could be responsible for the reduction in salinity observed in lake Kikorongo. From studies of potential effect of climate change on ion chemistry and phytoplankton communities in prairie saline lakes, precipitation and evaporation were reported among the factors affecting salinity in these lakes (Evans and Prepas, 1996). Evans *et al.*, (1996) also reported local difference in surface and ground water to have an effect on brine concentration and composition in closed saline lakes. This together with addition waters received by Lake Kikorongo from precipitation might be some of the factors responsible for the reduction in salinity levels in this lake.

Conductivity has been reported as a useful indicator for the levels of available inorganic constituents in water and play an important role in determining the availability of aquatic organisms in certain environments (Gupta and Mrinal, 2013). The observed high conductivity in these lakes under the current study is due to the high concentration of inorganic ions in these lakes.

Chemical characteristics of selected alkaline, saline lakes

The low alkalinities, salinities and conductivities in Lake Munyanyange can be attributed to it being a seasonal lake and had just received its waters from rain only a few days ago at the time of sampling. It usually requires

some residence time for the lake waters to infiltrate down to the salt rich parent rock which leads to diffusion of salts from the parent rock to the lake waters. As the rain waters spend more time in the lake, the salinity, alkalinity and conductivity increase. The low alkalinity, salinity and conductivity in Lake Kikorongo can be explained by dilutions with fresh water which is reported to come from the neighboring Lake George when it floods during the rainy season (Mungoma, 1990).

In this present study, all lakes were found to have carbonates as the dominant anions, followed by sulphates. This is in agreement with earlier studies where carbonates were reported to be the major dissolved anions in soda lakes of East Africa (Jones and Grant, 1999). Modern and Holocene carbonate sedimentology of two saline volcanic maar lakes in Southern Australia reported carbonates to be among the common components in sediments of modern salt lakes (Last and De Deckker, 1990). The high carbonate concentrations in these lakes waters might be caused by incoming waters into these lakes dissolving the carbonates from the sediments of these lakes. Jones and Grant (1999) reported the high concentration carbonates as major dissolved anions to be caused by evaporative concentration. Contrary to the current study findings, previous studies by Mungoma (1990) reported lakes Katwe and Kikorongo to have more chlorides than carbonates. Chloride concentrations in lake Katwe might have reduced due to the excessive salt extraction while for Kikorongo it might be the dilution cause by rains and the reported floods from lakes George (Hecky and Kilham, 1973; Mungoma, 1990). With the exception of SiO₂ and F which are lower, all the other chemical compounds considered in this current study are higher than those reported earlier studies by Mungoma in the 1990s. The high concentration of nitrogenous compounds including NH₃-N, NO₂-N, and NO₃-N in these lakes (table 5) can be attributed to the high algal biomass found in these lakes where upon decay and subsequent nitrification lots of nitrogenous compound like nitrates are released in these highly productive lakes. Lake Kikorongo having the lowest concentrations of NH₃-N, NO₂-N, NO₃-N, SRP and TSS can be attributed it being the least productive among the different studied lakes. Total phosphorous concentrations were reported to be frequently highly in saline lakes in the Canadian prairies. The high phosphorous concentration was associated with the conservation of phosphorous in these lakes (Evans, Arts et al., 1996). In the same way, phosphorous might be conserved in these saline crater lakes of western Uganda like it is in the phosphorous-rich saline lakes of the Canadian prairies since these lakes are also found in areas experiencing high evaporation rates exceeding precipitation rates and in closed drainage basins (Jones and Grant, 1999) as is the case for Canadian prairies saline lakes.

Conclusions

All the studied lakes were alkaline and saline in nature as reported in the previous studies except Lake Kikorongo which was found to be fresh with a salinity of zero in this present study. With the exception of Lake Murumuli which was found to be a Carbonate-chloride lake, the rest of the sampled lakes are carbonate-sulphate lakes which is contrary to earlier studies which reported lakes like Katwe and Kikorongo to be Carbonate-chloride lakes.

Climate change and human activities such as rock salt extraction, water diversion to salt pans, deforestation, and livestock grazing are threatening the ecological status of these lakes more especially those outside the protected areas like Katwe and Munyanyange. Could the observed increment in water temperatures in the study lakes and a reduction in salinity more especially in Lake Kikorongo be a sign of climate change? For this to be confirmed, it requires routine and continuous monitoring in these lakes. Although the majority of these lakes are found in Queen Elizabeth National Park (QENP) which is a protected area, there is need to offer additional protection to these unique systems if these systems are to be protected from human induced climate change. Alkaline saline lakes in Carpathian Basin which are under ex. lege Protection (by law) (Stenger-Kovács, Lengyel et al., 2014) can be useful examples.

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