A COMPARATIVE ANALYSIS OF THE QUANTITY AND QUALITY OF OIL EXTRACTED FROM FIVE COMMERCIALLY IMPORTANT FRESHWATER FISH SPECIES IN KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Limnology of Egerton University

EGERTON UNIVERSITY

MAY, 2021

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted in part or whole for an award of a degree in any institution.

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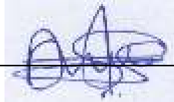
Signature:  Date: ___21.05.2021___

Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

I dedicate this work to my mom Ruth Wanjiru Njenga and my family members; Faith Mwangi, Moses Ndichu and Peter Njenga for their immense spiritual, emotional and material support during the entire study period.

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ABSTRACT

Fish is an important source of relatively cheap protein. The Kenyan fisheries and aquaculture sector contributes about 0.8% of the Gross Domestic Product (GDP) and this has created direct employment opportunities to over 500,000 people.Generally, there is a huge fish procesing industry that generate huge amount of solid waste and by-product.Some of the solid waste and by-products can be a valuable source for extraction of health promoting fish oil which is rich in polyunsaturated fatty acids (PUFA), especially omega-3 (n-3) fatty acids. Therefore, this study aimed at increasing avenues for value addition of fish products and byproducts through oil exploitation, thus reducing fish waste in landing sites and increasing the overall percentage contribution of fisheries to the Country's GDP. This study compared the quantitative yields and quality (oxidative stability) of oil extracted from five inland freshwater fish species namely: Nile perch *Lates niloticus,* Common carp *Cyprinus carpio*, African catfish *Clarias gariepinus*, Marbled lungfish *Protopterus aethiopicus*, and the Nile tilapia *Oreochromis niloticus*. Basic biological attributes (feeding habits and condition factor) of the five selected species were also assessed prior to the commencement of oil extraction. Fish oil from the various body parts (head, frame, fillet, tail and body cavity) in all the five fish species was extracted using the conventional method of cooking, pressing and centrifugation to determine quantitative yield. The extracted oil was subjected to a composition test (iodine value) and quality tests which included both a hydrolytic degradation test (acid value) and oxidative stability tests (peroxide value and p-anisidine value). Iodine value, acid value, and peroxided value tests were done using titrimetric methods and p-anisidine value test performed using colorimetric method. The results of biological attributes indicated that the exponent 'b' (slope) of all the fives species was significantly less than three ($b<3$, $P<0.05$) therefore exhibiting negative allometric growth patterns. Relative condition factor 'Kn' was greater than one and did not differ significantly between species (Kruskal-Wallis test, $p>0.05$) indicating that all species were in good condition in their natural habitats. Results of fish oil yields showed that in all the species the body cavity had the highest yield except for *P. aethiopicus* which yielded more oil from the tail. The quality test results were significantly within their respective set limits, except for *C. gariepinus* acid value that was higher than the set limit of 7–8 mg KOH/g. This study concluded that three fish species; *L. niloticus*, *C. gariepinus* and *C. carpio* have the potential for oil exploitation from their body cavities while *P. aethiopicus* can yield commercially viable oil from both the body cavity and tail.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION ii

DEDICATION iv

ACKNOWLEDGEMENTS v

ABSTRACT vi

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF ABBREVIATIONS AND ACRONYMS xii

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background information 1

1.2 Statement of the problem 2

1.3 Objectives 3

1.3.1 General objective 3

1.3.2 Specific objectives 3

1.4 Hypotheses 3

1.5 Justification 3

CHAPTER TWO 5

LITERATURE REVIEW 5

2.1 Historical background on the exploitation of fish oil 5

2.2 Basic chemistry and classification of fish oil 5

2.3 Fish oil in relation to human health and disease control 9

2.4 Factors affecting oil production in fish 10

2.5 A brief description of the five proposed fish candidates and fish oil in fresh water species 10

2.5.1 Fulton condition factor 11

2.5.2 Gut content analysis 12

2.6 Fish oil extraction 12

2.7 Quality test and standards associated with fish oil 14

2.7.1 Peroxide value test (PV) 14

2.7.2 p-Anisidine value (AV) 14

2.7.3 The total oxidation value (TOTOX value) 15

2.7.4 Free fatty acid and Acid value test ... 15
2.7.5 Iodine value test ... 15
2.7.6 Fish oil autoxidation preventive measures ... 16

CHAPTER THREE ... 17
MATERIALS AND METHODS ... 17
3.1 Sample collection ... 17
3.2 Determination of basic biological attributes of fish ... 17
3.2.1 Fish condition factor (K) ... 17
3.2.2 Gut content analysis ... 18
3.3 Fish oil extraction and yield determination ... 19
3.4 Quality analysis of extracted oil ... 19
3.4.1 Acid value test ... 19
3.4.2 Iodine value test ... 20
3.4.3 Peroxide value test ... 20
3.4.4 p-Anisidine value test ... 21
3.5 Data analysis ... 22

CHAPTER FOUR ... 23
RESULTS ... 23
4.1 Length weight relationship ... 23
4.2 Gut content analysis ... 26
4.3 Fish oil quality ... 32

CHAPTER FIVE ... 34
DISCUSSION ... 34
5.1 Length weight relationship ... 34
5.2 Gut content analysis ... 34
5.3 Fish oil yield ... 36
5.4 Fish oil quality ... 36

CHAPTER SIX ... 40
CONCLUSIONS AND RECOMMENDATIONS ... 40
6.1 Conclusions ... 40

6.2 Recommendations....40

REFERENCES....42

APPENDICES....52
Appendix 1: A photograph showing *Cyprinus carpio*....52
Appendix 2: A photograph showing *Protopterus aethiopicus*....53
Appendix 3: A photograph showing *Lates niloticus*....53
Appendix 4: A photograph showing *Clarias gariepinus*....54
Appendix 5: A photograph showing body cavity fatty deposit in *Cyprinus carpio*....54
Appendix 6: A photo showing *Oreochromis niloticus*....55
Appendix 7: A photograph showing body cavity fatty deposit in *Lates niloticus*....56
Appendix 8: A photograph showing Tail fatty deposit in *Protopterus aethiopicus*....57
Appendix 9: A photograph showing body cavity fatty deposit in *Clarias gariepinus*....57
Appendix 10: A photograph showing body cavity fatty deposit in *Clarias gariepinus*....58
Appendix 11: A photograph showing *Protopterus aethiopicus* tail oil....59
Appendix 12: A photograph showing *Cyprinus carpio* body cavity oil....60
Appendix 13: A photograph showing *Lates niloticus* body cavity oil....61
Appendix 14: A photograph showing *Clarias gariepinus* body cavity oil....62
Appendix 15: A photograph showing *Protopterus aethiopicus* body cavity oil....63
Appendix 16: SPSS ANOVA output for oil yield between body parts....64
Appendix 18: Publication....66

LIST OF TABLES

Table 1: A summary of fish oil quality parameters..15

Table 2: Weight Length relationship (WLRs) for 5 commercially important fish species in Kenya..23

Table 3: Degree of Stomach Fullness (%) in the five selected fish species......................27

Table 4: Prey occurrence (%) in the five selected fish species..27

Table 5: Tukey grouping of percentage oil yield from the various body parts of *P. aethiopicus*..29

Table 6: Tukey grouping of percentage oil yield from the various body parts of *C. carpio*..29

Table 7: Tukey grouping of percentage oil yield from the various body parts of *C. gariepinus*..29

Table 8: Tukey grouping of percentage oil yield from the various body parts of *O. niloticus*..30

Table 9: Tukey grouping of percentage oil yield from the various body parts of *L. niloticus*..30

Table 10: Tukey grouping of percentage oil yield from all the five species and their various body parts..31

Table 11: The quality of oil from the five commercially important species in Kenya.......33

LIST OF FIGURES

Figure 1: Examples of polar and non- polar lipids 6
Figure 2: Some members of Family ω-3 fatty acids. 7
Figure 3: Metabolic pathway of omega-6 and omega-3 fatty acid synthesis....8
Figure 4: A Map showing the three lakes in Kenya where fish samples were collected; Lake Victoria, Lake Naivasha and Lake Baringo 17
Figure 5: Parabolic relationship between length and weight of African catfish *Clarias gariepinus* 24
Figure 6: Parabolic relationship between length and weight of Marbled lungfish *Protopterus aethiopicus* 24
Figure 7: Parabolic relationship between length and weight of Common carp *Cyprinus carpio* 25
Figure 8: Parabolic relationship between length and weight of Nile perch *Lates niloticus* ...25
Figure 9: Parabolic relationship between length and weight of Nile tilapia *Oreochromis niloticus* 26

LIST OF ABBREVIATIONS AND ACRONYMS

ALA	α-linolenic Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemist's Society
AV	Acid Value
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CABI	Centre for Agriculture and Bioscience International
DAG	Diacylglycerols
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
ESP	Economic Stimulus Programme
FAO	Food and Agricultural Organization
FFA	Free Fatty Acid
GDP	Gross Domestic Product
GOED	Global Organization for EPA and DHA
IAFMM	International Association of Fish Meal Manufacturers
MAG	Monoacylglycerols
PL	Phospholipids
PUFA	Polyunsaturated Fatty acids
SPSS	Statistical Package for the Social Sciences
TAG	Triacylglycerols
TBHQ	Tert-Butylhydroquinone
TOTOX	Total oxidation value
4-HHE	4-hydroxy-2-hexenal
4-HNE	4-hydroxy-2-nonenal

CHAPTER ONE
INTRODUCTION

1.1 Background information

The Kenyan fisheries and aquaculture sector contributes about 0.8% of the Gross Domestic Product (GDP), providing direct employment opportunities to over 500,000 people and supporting over two million people indirectly (KMFRI, 2017). The reported value of fish exports from Kenya as per the year 2013 is approximately Ksh 6.29 billion (FAO, 2016). Total fishery and aquaculture production in the year 2013 amounted to approximately 186,700 tonnes with 83% of this production coming from inland capture fisheries of which Lake Victoria contributed about 90% (FAO, 2016). Marine fisheries, on the other hand, produce less than 9,000 tonnes per year. The reported increase in farmed fish production in the year 2013 of 23,501 tonnes was mainly contributed by the nationwide fish farming campaign launched by the government through the Economic Stimulus Programme in the year 2009 (FAO, 2016). This campaign led to an increase in the total area of fish farms from 220 ha to 468 ha through the construction 7,760 new ponds. The major fish species cultured in Kenya include Nile tilapia, African catfish, Common carp and Rainbow trout.

Fish is an important source of relatively cheap protein recommended by medical practitioners world wide. The huge industry of fish processing accommodates diverse production processes than include filleting, curing, salting, smoking and canning. These processes generate huge amount of solid waste and by-product which often represent more than 50% of the total fish weight (Shahidi, 2007). Some of the solid waste and by-products can be a valuable source for extraction of health promoting fish oil which is rich in polyunsaturated fatty acids (PUFA), especially omega-3 (n-3) fatty acids (Chow, 2008).

Grimm and Loyd (2003) reported a significant demand for high-quality fish oils with the potential of a highly profitable business endeavour given the availability of high-quality raw materials. Fish oil processing and production is an important value addition practice that significantly increases the value of fish and fish by-products. Exploitation of fish for oil has been done in many marine fish species for several decades with little effort in freshwater candidates (Grimm & Loyd, 2003). This has led to the over-exploitation of most marine oily fish species for example anchovy salmon, tuna, herrings, cod and menhaden (Graham et al., 2007). One of the core components of fish oil that is of significance to human health is the

presence of long chain polyunsaturated Omega-3 fatty acids specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Anandganesh et al*.,* 2016).

The American Heart Association recommends at least twice servings of fish in a week for prevention and control of cardiovascular diseases. Some of the associated health benefits of the long chain polyunsaturated omega 3 fatty acids include atherosclerosis prevention, reduction in blood pressure, reduction of chances of developing pulmonary diseases, cystic fibrosis symptoms alleviation, increment of survival chances for cancer patients, reduction of symptoms in asthma patients, protection against manic-depressive illness and improvement in learning ability (Ramakrishnan et al., 2013).

The amount of oil extracted usually depends on several factors that include the fish species, size of the fish, body part from which the oil is extracted from, natural diet of the fish or the fish feed composition, and occasionally the geographical location of the fish (Maqsood et al., 2012). Approximately 2-25% of the fish body weight is fat, 15-30% is protein and the remaining 50-80% is water (Deepika et al., 2014). Ramakrishnan et al. (2013) reported that about 50% of fish body weight is usually wasted during and after fish processing with specific discards being trimmings (gut, fins, tails), skin, and frame.

This study focused on extraction of fish oil, assessment of the quantities, physical and oxidative quality of the oil, and a comparison between five inland fish species (the Nile perch *Lates niloticus*, Common carp *Cyprinus carpio*, African catfish *Clarias gariepinus*, Marbled lungfish *Protopterus aethiopicus*, and the Nile tilapia *Oreochromis niloticus*) that are commercially important in Kenya.

1.2 Statement of the problem

The demand for high-quality fish oil has led to the over-exploitation of most marine species commonly processed for their fish liver oil. Population decline of these fish species has led to the need of finding alternatives and sustainable sources of commercially viable fish oil. There are few studies on the quantity and quality of oil from freshwater fish species. Furthermore, there is an urgent need of developing value addition avenues for freshwater fish products and by-products through oil exploitation since little effort in terms of research on their alternative use has been done. The health promoting attributes of fish oil related to cardiovascular benefits,

brain development and reduction in obesity among others contributes to the high commercial value of fish oil posing a dire need of finding alternative cheap sources of fish oil.

1.3 Objectives

1.3.1 General objective

To assess the quantity and quality of oil extracted from inland fish species that are of commercial importance in Kenya.

1.3.2 Specific objectives

i. To determine the basic biological attributes (feeding habits and condition factor) of *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus.*

ii. To determine the quantitative yield of oil extracted from the various parts (head, frame, fillet, tail and body cavity) of *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus*.

iii. To determine the quality of oil extracted from *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus*.

1.4 Hypotheses

H_{O1}: There is no significant difference in the basic biological attributes (feeding habits and condition factor) of *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus.*

H_{O2}: There is no significant difference in the quantitative yield of oil extracted from the various body parts (head, body cavity, frame, and fillet) and between *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus*.

H_{O3}: There is no significant difference in the quality of oil extracted from *Lates niloticus*, *Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus* and *Oreochromis niloticus*.

1.5 Justification

This study aimed at increasing avenues for value addition of fish products and byproducts through oil exploitation, thus reducing fish waste in landing sites and increasing the overall percentage contribution of fisheries to the Country's GDP. Ecologically through gut content

analysis, the study was able to track changes in the fish food chain. Additionally, the potential of this study to contribute to the commercial production of fish oil has other benefits in socio-economic dimension including employment, higher income in the fishery sector and the overall livelihood improvement of the local fishermen. Furthermore, this study focused to address SDG number 1 (End poverty in all its forms everywhere) and 2 (End hunger, achieve food security and improved nutrition and promote sustainable agriculture) and one of Kenya's big four agenda on manufacturing new products.

CHAPTER TWO
LITERATURE REVIEW

2.1 Historical background on the exploitation of fish oil

The use of cod liver oil for medicinal purposes dates back to early 1840 (Butler, 1948). The source of this oil was mainly attributed to the English, Norwegian and Newfoundland fisheries. The high interest in oils by the pharmaceutical industries predisposed the gradual development of techniques related to raw material selection, processing, and refining of fish oils. During the early 20th century, chemists established that the most crucial health benefiting attribute of fish liver oil was the presence of vitamins A and D (Butler, 1948). The establishment of this fact led to the innovative search of more commercially viable fish candidates specifically in the marine fishery. After the realization that Atlantic halibut had a higher vitamin A and D content than cod liver oil, other fish species such as Tuna, sablefish, lingcod, rockfish and soupfin shark from the Pacific coast flooded the fish oil industry (Rice & Ismail, 2016).

With the increase in demand and public awareness on the significance of vitamins A and D, more sophisticated fishing gears and liver handling techniques by fishermen and dealers were developed to ensure long shelf life and bulk production of fish oils. With the advent of synthetic vitamin D from activated ergosterol and activated 7-dehydrocholesterol, these sources of natural vitamin D became less important (Butler, 1948). The substantive demand for cod liver oil at this time only focused on the per gram activity of vitamin A since vitamin D could easily be synthesized. With the realization of the health benefits associated with omega 3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) a new demand has now focused on not only fish liver oil but also fish oils extracted from fatty fish such as mackerel and herring that tend to accumulate oil in fatty layers of flesh rather than the liver (Ellie, 2013).

2.2 Basic chemistry and classification of fish oil

Lipids can be classified into two major groups that include, neutral (non-polar) and polar lipids. Neutral lipids comprise of Monoacylglycerols (MAG), Diacylglycerols (DAG), Triacylglycerols (TAG) and sterols (Deepika et al*.,* 2014). Polar lipids comprise of Free Fatty Acids (FFA), phospholipids (PL) and sphingolipids as illustrated in figure 1. Fish tissue is mainly composed of triacylglycerols which are present in hydrophobic aggregates and contain fatty acids of varying chain lengths and different degree of unsaturation (Fahy et al., 2011).

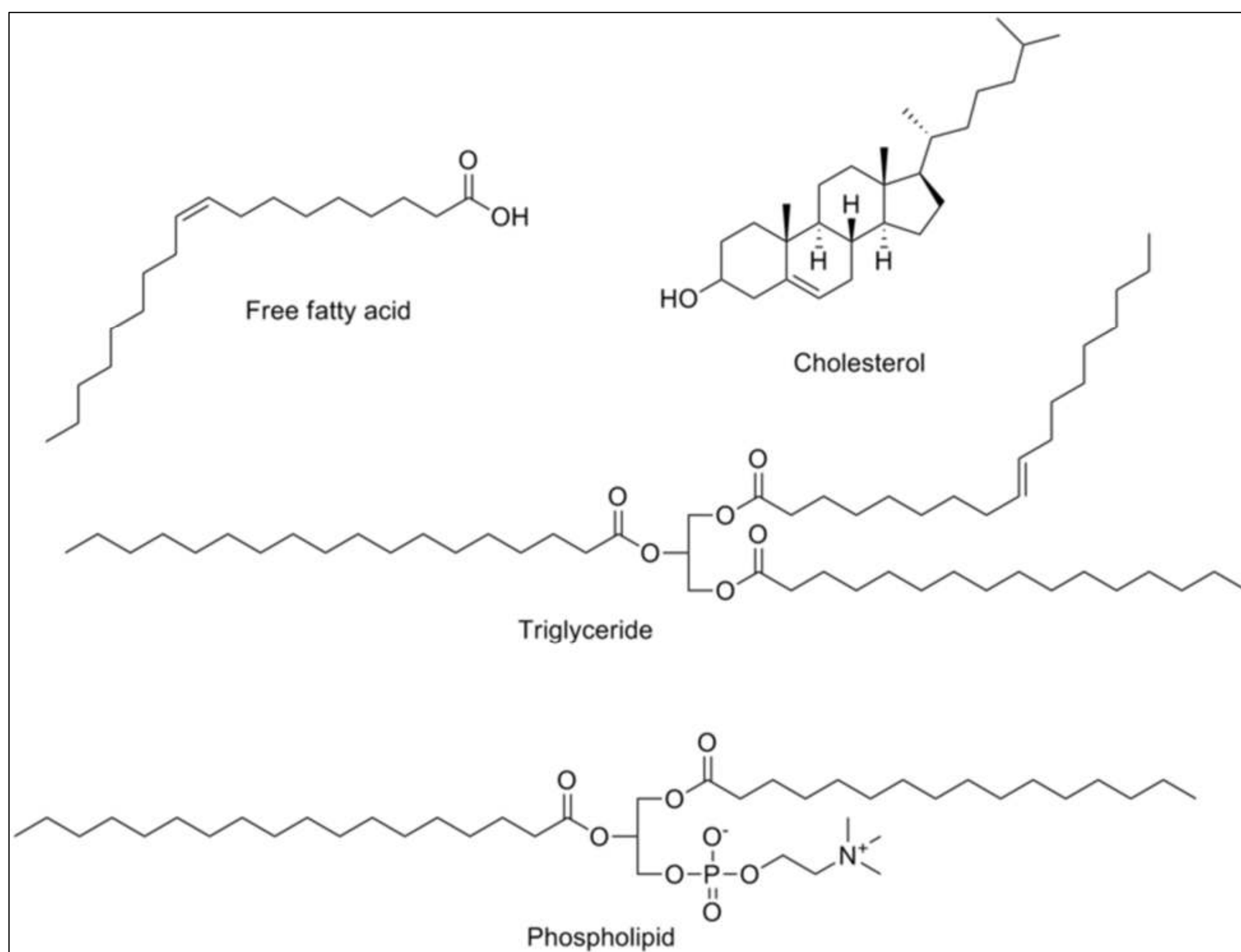

Figure 1: Examples of polar and non- polar lipids adopted from Bettelheim et al. (2009).

The classification of Polyunsaturated fatty acids (PUFAs) is done on the basis of the position of the double bond either from the carboxyl end or the methyl end (figure 2). Usually, the counting in “Omega” or “n” convention is done by locating the position of the double bond from the methyl group.

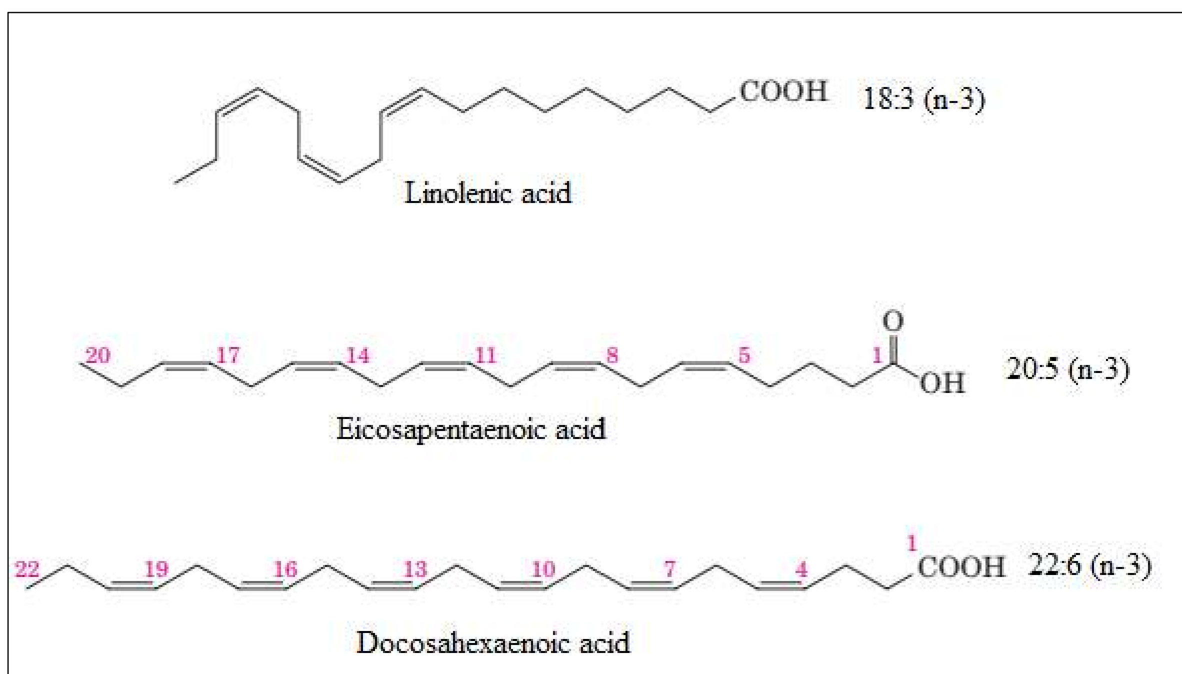

Figure 2: Some members of Family ω-3 fatty acids adopted from Bettelheim et al. (2009).

Most of PUFAs that are of biological importance belong to the groups ω-6 (arachidonic acid) and ω-3 (eicosapentaenoic acid). The first double bond of ω-3 fatty acids starts at the third carbon atom from the methyl end. Examples of fatty acids belonging to this family include eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (DPA, 22: 5), docosahexaenoic acid (DHA, 22:6 ω-3), and α-linolenic acid (ALA, 18:3). These molecules are characteristically crumpling and tend to bend at the site of the double bond due to the presence of nonconjugate (methylene interrupted) cis-type double-bond configuration) (Sahena et al., 2009). Ideally, the precursors of n-3 and n-6 fatty acids are ALA (alpha-linolenic acid) and linoleic acid respectively (illustrated in figure 3) and EPA and DHA occur as a constituent of fish triglycerides and phospholipids.

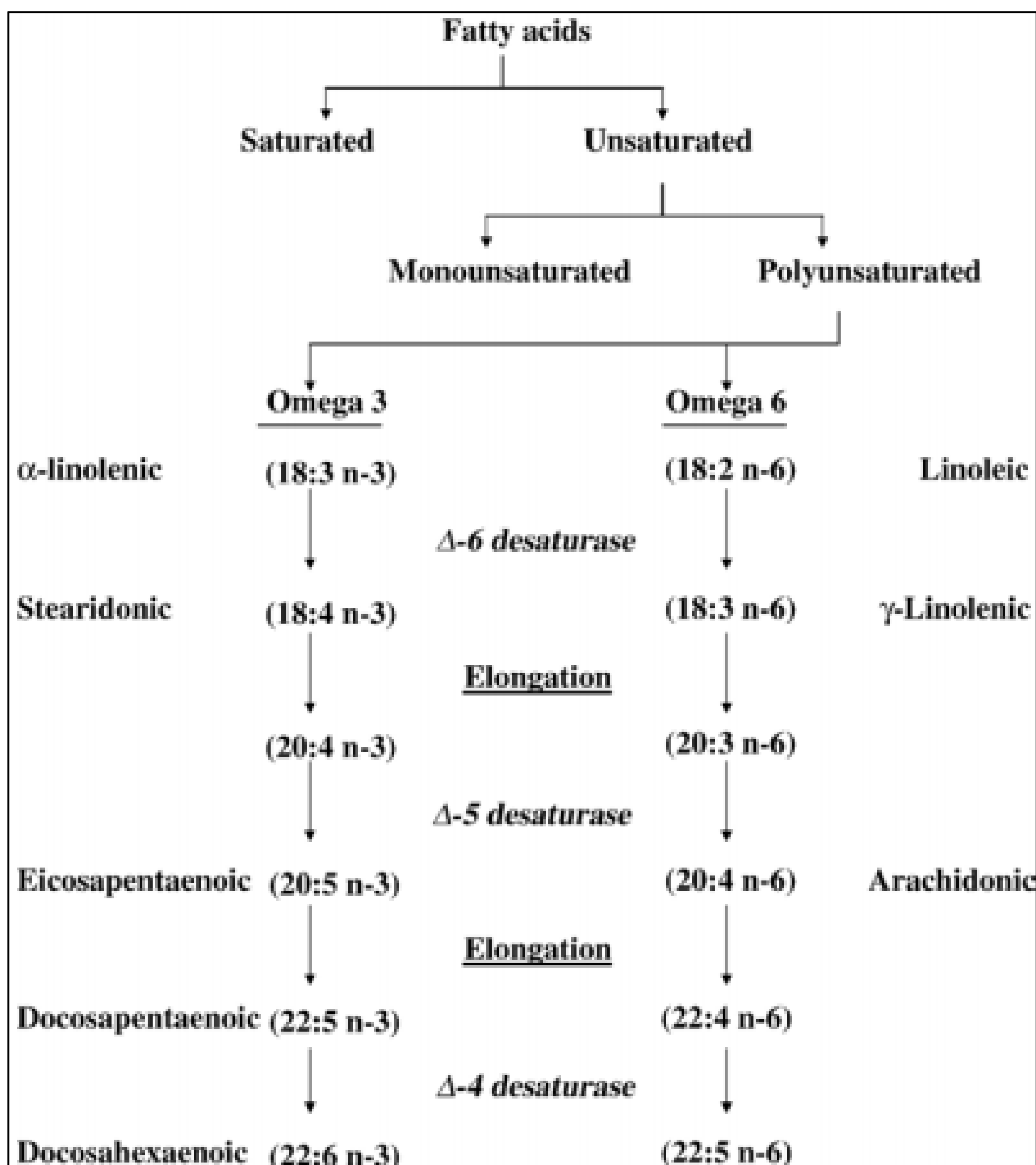

Figure 3: Metabolic pathway of omega-6 and omega-3 fatty acid synthesis adopted from Siddiqui et al. (2007)

The source of these fatty acids as reported by Sahena et al. (2009) are seaweeds and unicellular phytoplankton that tend to accumulate in fish. The composition and content of fatty acids among most studied marine species often vary with species used, reproductive status, age, size, sex, season and geographical location (Pigott & Tucker, 1990). PUFAs in fish are characteristically responsible for maintaining cell structure, function, growth, and development (Cejas et al., 2004).

2.3 Fish oil in relation to human health and disease control

Besides the previous recognition of vitamins, A and D as the main purpose of consumption and sale of fish liver oils, the importance of PUFA intake has also been greatly recognized in the past decade (Sahena et al., 2009). A 10% to 20% PUFAs of total lipid intake have now been recommended by health organizations. The significance of PUFAs consumption to diseases progress is not yet clear compared to other determinants such nutritional factors, environmental influence and genetic factors. Epidemiological works of Dyerberg and HO (1979) on the possible correlation of low heart diseases to consumption of seafood are the pioneers of many other studies related to the role of ω-3 fatty acids in human health and diseases. In a study done by Pamela (2001), EPA and DHA were identified to have treatment and preventive effect on various disorders and diseases such as asthma, cancers, diabetes, coronary heart disease, rheumatoid, arthritis among others. Other beneficial effects reported by Weaver and Holub (1988) included positive effects in cardiovascular diseases, various inflammations, and autoimmune disorders. These beneficial effects of EPA and DHA on disease control have been attributed to Zicosanoid metabolism in the circulatory system leading to production of prostaglandins considerably weaker in inducing platelet aggregation than those produced by other fatty acids such as ω-6 fatty acids.

The physiological effects attributed to ω-3 fatty acids include decreased blood pressure; reduction of inflammatory arthritis, psoriasis, asthma; decreased viscosity of blood; lowered of plasma triglycerides and reduction in tumours. Sahena et al. (2009) relates potential of application of PUFAs in therapeutics, foods and nutrition. Some of the structural and functional benefits to human health associated with PUFAs of both 3-series and 6-series include regulation of architecture, dynamics, phase transitions and permeability of membranes. Some of the membrane-bound proteins such as ATPase, histocompatibility complexes, and transport membranes are also regulated by PUFAs. Furthermore, the activity of some genes attributed to the coding for sodium channel proteins, fatty acid synthase, and nitric-oxide synthase are also regulated by PUFAs. This, in turn, has an overall effect on cellular biochemistry, cell-stimulus response and transport processes that finally contribute to cold adaptation, improved immune response, and prevention of cardiovascular disease. Lagarde (2008) reported DHA as an abundant component of brain phospholipids therefore significant in brain development and functionality.

2.4 Factors affecting oil production in fish

There are several factors that affect the amount of oil in a fish. Some of these factors include the fish species, sex, environmental conditions, and season. By virtue of morphology and physiology, it is clear that different species have different amounts of oil stored in different parts of the body. Generally, fish species that store their lipids in the liver are characterized as lean while those that store their lipids in fat cells distributed in other body tissues as fatty fish. Examples of lean fish are bottom dwellers such as cod, saithe and hake while fatty fish include pelagics such as herring, mackerel and sprat. Some species such as barracuda, mullet and shark are characterized as semi-fatty species since they store, their lipids in limited parts of their body or in lower quantities than typical fatty fish (Ellie, 2013).

Feeding is another critical factor that plays a vital role in dictating the amount of oil within the same species. Some of the physiological and behavioural changes in fish that significantly affect feed intake in fish include migratory swimming and sexual changes in relation to spawning (Love, 1970). During these periods of migration and spawning, the fish often starves itself while concentrating on migration and spawning. It is at this point that such fish species rely heavily on energy reserves such as lipids. Migration coupled with spawning often utilizes both lipids and protein reserves, therefore, affecting the general biological condition of the fish (Stansby & Hall, 1967). Fish species will often have the lowest amount of oil during these physiological (spawning) and behavioural changes (migration).

Furthermore, plankton feeders such as herrings often experience a variation of oil content due to other environmental factors affecting plankton productivity. Size is another important factor that affects oil content in fish. Watanabe (1971) observed that oil content in fish species varied with size where larger fish contain about 1 % more oil than smaller ones.

2.5 A brief description of the five proposed fish candidates and fish oil in fresh water species

The five selected candidates for this study were Nile perch *Lates niloticus*, Common carp *Cyprinus carpio*, African catfish *Clarias gariepinus*, Marbled lungfish *Protopterus aethiopicus*, and the Nile tilapia *Oreochromis niloticus*. Nile perch *Lates niloticus* is one the largest fresh water species native to Congo, Nile, Senegal, Niger, and Lake Chad, Volta, Lake Turkana and other river basins, and is now widespread through tropical Africa. The fish has a maximum length of 2 metres with the highest recorded weight of up to 200 kg (Ohwayo, 1994).

It is fierce predator that feeds on fish including its own species, crustaceans and insects. The juveniles feed on zooplankton. The fish was introduced in Lake Victoria in 1962 and has caused the decline or extinction of an estimated 200 cichlid fish (Ohwayo, 1994).

African catfish *Clarias gariepinus* is widely distributed in Africa freshwater systems such as lakes, rivers and swamps. The fish is an opportunistic omnivore ingesting a variety or food items including algae, macrophytes, insects, fish prey, detritus, amphibians, sand grains and zooplanktons (Tesfahun, 2018). The diet varies within season and spatial environmental conditions. Marbled lungfish *Protopterus aethiopicus* is a native fish of central and east Africa. It is an obligate air breather that relies on lungs for aerial respiration. The fish is mostly found in extreme environmental conditions such as anoxia and severe desiccation. The fish primarily feeds on molluscs, insects, crustaceans, worms, amphibians and fish (CABI, 2018a). Nile tilapia *Oreochromis niloticus* on the other hand is a freshwater cichlid native to the Nile basin. It has been introduced to over fifty countries all over the world except Antarctica for the purpose of farming. It is a tropical species with lower and upper lethal temperatures of 11°-12° and $42^{o}C$ respectively (CABI, 2018b). The fish is an omnivores mouth brooder that feeds on periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films.

For a long time, the belief that freshwater species have relatively low omega-3 (n-3) fatty acids have deterred studies on quality and quantity analysis of fish oil on potential freshwater candidates. This argument has been greatly disapproved in works of Nair and Gopakumar (1978) with recommendations of freshwater fish which possess high omega-3 oil content such as the channel catfish, black bass, carp, lake trout and lake herring. Additionally, studies by Deepika et al. (2014) have focused on finding alternative methods of low yield fish oil extraction techniques with potential methods identified are supercritical fluid extraction and enzymatic extraction.

2.5.1 Fulton condition factor

There are a number of direct and indirect indices that are used to provide a simplified assessment of the nutritional status and relative health of fishes. Some of the direct methods include gonado-somatic index, hepatosomatic index and visceral somatic index. These indices provide information on historical traits, nutritional status and the response of fish to environmental effects (Michael & Murphy, 1991). The most commonly used indirect indices include condition factor and relative weight. These indices also known as morphometric indices

are mostly based on external measures of length and weight relationships. Their applicability arises from the assumption that a heavier fish of a given length has greater energy reserves and consequently is in a better condition (Bolger & Connolly, 1989). One of the most frequently used morphometric indices is the Fulton's condition factor (K-factor), expressed as the ratio of body mass and the cube of length (Nash et al., 2006). Studies of Herbinger and Friars (1991) and Chellappa et al. (1995) have confirmed the strong positive relationship between the K-factor and total lipid content of fish and assigned K-factor as a simple proxy of energy reserves in a fish body.

2.5.2 Gut content analysis

Fish gut content analysis provides an integral insight of fish feeding patterns. Lagler (1949) noted that the gut content analysis only indicates what the fish would feed on. Accurate description of fish diet and feeding habits also provides the basis of understanding trophic interactions in aquatic food webs. Diets of fish represent an integration of many important ecological components that include behaviour, condition, habitat use, energy intake and intra/interspecific interactions. A food habit study might be conducted to determine the most frequently consumed prey or to determine the relative importance of different food types to fish nutrition. It might also be used to quantify the consumption rate of individual prey types. Thus, this study determined the relative importance of difference food items through frequency of occurrences.

Methods of describing feeding habits in fish can be broadly categorized into qualitative and quantitative techniques (Zacharia & Abdurahim, 2012). Qualitative analysis involves the complete identification of the various organisms eaten by the fish in the gut. This technique requires extensive experience, with the aid of good references to identify broken, digested and finely comminute materials. The quantitative technique, on the other hand, incorporates three methods which include numerical, gravimetric and volumetric methods. The important information to note about the fish prior to gut content analysis includes sex, body weight, body condition, among others (Baker et al., 2013).

2.6 Fish oil extraction

The conventional method of fish oil extraction involves cooking, pressing and centrifugation although it is associated with several challenges such as relatively low oil yield and thermal degradation (Pigott, 1967). Despite the above challenges this is the most preferred method of

oil extraction since it is cheaper, easier and quicker and does not require highly qualified personnel to perform. Furthermore, this method is also recommended for oil grades used for both human consumption and animal feeds since no organic chemicals are used (Liyanage, 1999).

The second method is solvent extraction which is commonly used in the isolation of lipids from food samples including fish (Sahena et al., 2009). This method is based on the principle that lipids are soluble in organic solvents (acetone and methanol) and insoluble in water hence can easily be isolated from water-soluble compounds such as carbohydrates, minerals, and proteins. The efficiency of this method of extraction depends on the polarity of both sample and the solvent whereby polar lipids such as phospholipids and glycolipids are more soluble in polar solvents such as alcohol compared to non-polar solvents such as hexane. A good example of a technique that uses the principle behind solvent extraction is Soxhlet extraction (McClements, 2005).

Enzymatic hydrolysis is the third potential method of fish oil extraction that has received attention in the work of Deepika et al. (2014). Many enzymes have been clinically tried but the most promising one is the enzyme alcalase. Usually, this process of oil extraction is carried out under mild condition and yields relatively high oil content than other methods within 30-120 minutes (Deepika et al., 2014).

Several studies in the past decade have also focused on extraction and fractionation of fish oil using supercritical fluid. A supercritical fluid is defined as a condensed gas that is compressible and thus expands to completely fill its container as opposed to normal liquids that take the shape of the container (Weideman & Liescheski, 2004). Carbon dioxide is the most commonly used gas due to its relatively high availability in pure forms and low toxicity. Some of the major determining factors that affect oil yields in this method of extraction include temperature, CO_2 rate, and pressure (Sahena et al., 2009). However, the method of extraction that was used in this study was the conventional method of oil extraction since it is relatively cheaper, recommended for oils grades to be used for human consumption and does not require highly qualified personnel.

2.7 Quality test and standards associated with fish oil

Due to the high content of long-chain polyunsaturated fatty acids, fish oil autoxidation is relatively higher than that of any other oil. The two major pathways of fish oil spoilage described by Cmolik and Pokorny (2000) are oxidative spoilage and hydrolytic spoilage. Undesirable flavours and odors are some of the effects of both hydrolytic and oxidative spoilage. Products of fish oil autoxidation are highly dynamic but mostly relate to alcohols, fatty acid peroxides and aldehydes. Specifically, products of peroxidation include a variety of isoprostanes, 4-hydroxy-2-hexenal (4-HHE) and 4-hydroxy-2-nonenal (4-HNE). The presence or absence of these compounds is used to test for oxidative stress in fish oils. Oxidation of polyunsaturated fats is often not a single reaction and involves complexation. Besides physical quality assessment of fish oils, recommended oxidation tests include peroxide value test, iodine value test, acid value test and a p-Anisidine value (AOCS, 2003).

2.7.1 Peroxide value test (PV)

This test simply determines the level of peroxide in an oil sample. It is based on the principle of assessing primary oxidation whereby the first products of fatty acid oxidation are hydroperoxides. Titrimetric method is the commonly used method of measuring peroxide value according to AOCS (2003) method. Generally, the lower the peroxide value the better the quality of the oil though this value can also decrease due to further secondary oxidation of peroxides (primary oxidation products). The allowable peroxide value limit set by FAO (Food and Agricultural Organization) and GOED (Global Organization for EPA and DHA) is ≤ 5 milliequivalents/kg.

2.7.2 p-Anisidine value (AV)

It is a test for secondary oxidation products such as aldehydes of α- and β-unsaturation. The process of secondary oxidation after primary oxidation as the initially formed peroxides are further oxidized to short chain aldehydes and alcohols. These products of secondary oxidation are responsible for the rancid smell in oils and generally, a low anisidine value is an indication of high-quality oil. This test is done through a colorimetric method whereby a mixture of test oil, acetic acid, and para-anisidine absorbance is measured at 350 nm wavelength. The principle behind this method is the measurement of 2-alkenals and 2,4-alkadienals that react with para-anisidine in acidic conditions and turn yellow. The allowable limit set by FAO (Food and Agricultural Organization) and GOED (Global Organization for EPA and DHA) as cited in Deepika et al. (2014) is ≤ 20.

2.7.3 The total oxidation value (TOTOX value)

The overall oxidation state of oil is determined through TOTOX value. The value represents both primary and secondary oxidation products i.e., both hydroperoxides and aldehydes. It is calculated by the addition of anisidine value to the product of two peroxide value (AV+2PV). The allowable limit set by FAO (Food and Agricultural Organization) and GOED (Global Organization for EPA and DHA) as cited in Deepika et al. (2014) is ≤ 26.

2.7.4 Free fatty acid and Acid value test

Both Free fatty acid and Acid value test are fairly representative tests of the quality of raw materials used for oil extraction since they reveal enzymatic activities associated with microbes in the raw material. Fish tissues pose a relatively high risk of autolytic activities of lipolysis and oxidation due to high level of polyunsaturated fatty acid (Aryee et al., 2009). The allowable limit of free fatty acid for unrefined oil is 1-7% (Miller, 2010). Acid value, on the other hand, represents the acidity of the oil. The principle behind this test relates to the amount of Potassium hydroxide (mg) required to neutralize one gram of fat or oil. The acceptable limit for this test is 7-8 mg/KOH g (AOCS, 2003). All the fish oil quality parameters mentioned above are summarized in Table 1.

Table 1: A summary of fish oil quality parameters

Characteristics	**Acceptable value**	**Source**
Acid value test	7-8 mg/KOH g	(GOED, 2013)
Peroxide value test (PV)	≤ 5 milliequivalents/kg	(GOED, 2013)
p-Anisidine value (AV)	≤ 20	(GOED, 2013)
TOTOX value	≤ 26	(GOED, 2013)

2.7.5 Iodine value test

This test measures the number of reactive double bonds present in an oil. Ideally, this test is not a quality indicator but rather a composition indicator (Miller, 2010). A higher iodine value indicates more double bonds (unsaturation) inferring a high caution in terms of measures to reduce or slow down autoxidation. This value can range from 94-120 for rapeseed oil and up to 185 for fish oil.

2.7.6 Fish oil autoxidation preventive measures

Some of the contributing factors of autoxidation can be controlled to reduce the speed and level of oxidation in fish oils. These factors include temperature whereby it should be reduced to the lowest point possible, reduction in oxygen exposure, light exposure, and moisture level preferably to less 0.2%, and limit contact time with transition metals like iron and copper that are pro-oxidants (GEOD, 2013). Antioxidants on the other hand, are compounds that slow down oxidation of oils by scavenging free radicals such as lipid alkyl radicals or lipid peroxy radicals, controlling transition metals or inactivating sensitizers (Choe & Min, 2006). In other word, antioxidants slow down oxidation by being oxidized themselves. Antioxidants can donate hydrogen atoms to free radicals and convert them into more stable nonradical products (Decker, 2010). Common synthetic oil antioxidants include BHT- butylated hydroxytoluene, BHA-butylated hydroxyanisole, TBHQ-tert-Butylhydroquinone, Propyl gallate and Ethoxyquin. Some natural extracts used as antioxidants include flavonoids, rosemary and spice extracts, Tea catechins and seaweed (Decker, 2010).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Sample collection

Thirty samples of each of the five species (*L. niloticus*, *C. carpio*, *C. gariepinus*, *P. aethiopicus* and *O. niloticus*) were bought (mature table size fish) from different landing sites of lakes in Kenya namely; Dunga beach (Lat. 0° 7' 0.77" S; Long. 34° 44' 52.36" E) in Lake Victoria (for *L. niloticus* and *O. niloticus*), Kamere (Lat. 0° 49' 18.95" S; Long 36° 19' 13.02" E) in Lake Naivasha (for *C. carpio* and *C. gariepinus*) and Kambi ya Samaki (Lat. 0° 37' 3.94" N; Long. 36° 04' 60.00" E) in Lake Baringo (for *P. aethiopicus*) as shown in figure 4. The samples were then transported in cool boxes to Egerton University for further analysis.

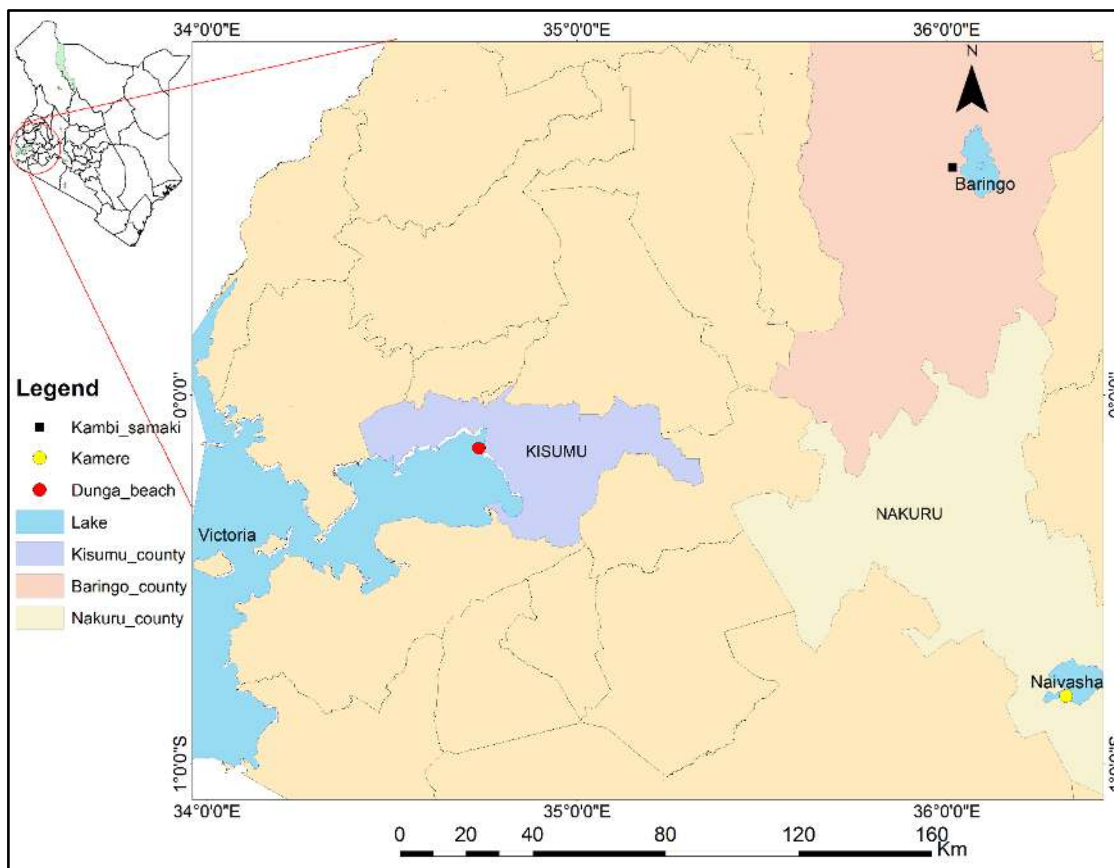

Figure 4: A Map showing the three lakes in Kenya where fish samples were collected; Lake Victoria, Lake Naivasha and Lake Baringo (Source: DIVA-GIS shapefiles modified using ArcMap)

3.2 Determination of basic biological attributes of fish

3.2.1 Fish condition factor (K)

Fish total length (cm) was measured using a measuring board as described by Ricker (1971) and fish weight measured using A&D weighing balance (model: GF.3000, manufacturer:

Mettler Toledo, USA). The length-weight relationship was determined as per cube law given by Le Cren (1951) using the formulae.

$$W = aLb \qquad (1)$$

Where, W is the weight of fish in grams; L is the total length of fish in centimetres; a is exponent describing the rate of change of weight with length (the intercept of the regression line on the Y-axis) and 'b' is the slope of the regression line (also referred to as the Allometric coefficient).

The length-weight relationship parameters a and b where consequently estimated using the least square method based on logarithms:

$$Log\ (W) = log\ (a) + b\ log\ (L) \qquad (2)$$

The values of exponent b play as explained by Morey et al. (2003) provides vital information on fish growth. A value of $b = 3$ infers that the weight increase in fish is isometric. When b is not equal to three, the weight increase is allometric whereby positive allometry is when $b>3$ and negative allometry if $b<3$. To test for significance difference of the b-value obtained from that of the isometric value 3, a t-test was performed according to Sokal and Rohlf (2009) following the equation;

$$ts = \frac{b-3}{Sb} \qquad (3)$$

where t_s is the t-test value; b is the slope and s_b is the standard error of slope (b). Relative condition factor was then calculated according to Le Cren equation (1951):

$$Kn=W/aL^b \qquad (4)$$

Where; W is the weight of the fish in grams and L is the total length of the fish in centimetres and a and b are parameters of length-weight relationship.

3.2.2 Gut content analysis

Prior to gut content analysis, the preliminary sample preparation involved individual removal of the gut samples through dissection, and categorization of intestine content based on the degree of fullness (estimated as the proportion of the maximum stomach volume occupied by prey items) as either full (4/4), three quarter full (3/4), half full (2/4), a quarter full (1/4) and empty (0/4) (Iromini, 2018; Manko, 2016). Examination involved mixing 2ml of distilled water to the gut samples in a petri-dish for easy separation and identification. Fish gut content analysis was determined using numeric (frequency of occurrence) method as laid out by Zacharia and Abdurahiman (2012) whereby, stomach contents were examined and the

individual food organisms sorted and identified. Frequency of occurrence was calculated using the following formulae:

$$Oi=\frac{Ji}{P}\times 100 \quad (5)$$

Where; Oi is the frequency of occurrence; Ji is the number of fish containing prey i and P is Number of fish with food in their stomach.

3.3 Fish oil extraction and yield determination

The fish samples were thoroughly washed in running tap water, scaled and separated into fillet, head, frame, body cavity and tail. The samples were then minced (for homogeneity) using a generic meat mincer No. 12. The samples were weighed (using Sartorius weighing balance model: ED4202S) and oil extraction from a weighed sample (either fillet, head, frame, tail or body cavity) was done using the conventional method (Pigott, 1967). In this method the homogenized fish samples were then taken in a muslin bag and kept in steam boiler at 70-80ºC for 30 minutes to facilitate protein coagulation and cell membrane disruption (Anandganesh et al., 2016). The cooked fish sample was screw pressed using an oil extractor (Piteba oil extractor) for the separation of liquid (dissolved dry matter and oil) from the solid (press cake). The liquid (dissolved dry matter and oil) was then heated at 90-95ºC (temperature maintained using an automated water bath) prior to centrifugation at 2000rpm for 15 minutes (to remove settleable impurities). Oil yield for the various parts was calculated using the following formulae by Immanuel and Palavasam (2009):

$$\text{Yield (\%)} = \frac{\text{crude oil weight (g)}}{\text{weight of sample (g)}} * 100 \quad (6)$$

3.4 Quality analysis of extracted oil

3.4.1 Acid value test

Free Fatty Acid content (%FFA) and the acid value (AV) were determined according to the titrimetric method described by AOCS (2003). A well shaken oil sample (7.05 ± 0.05 g) was weighed into a 250 ml conical flask and 75 ml of hot neutralized 95% ethanol and 2 ml of 1% phenolphthalein indicator solution added to the oil sample. The mixture was heated for 15 minutes in a water bath (75-80ºC) after which it was titrated while hot against 0.25N sodium hydroxide with vigorous shaking. The endpoint was determined as a colour change of the phenolphthalein indicator from colourless to light pink (persisting for 15 secs). Percentage Free Fatty Acid content (%FFA) and acid value were calculated using the following Equations by AOCS (2003).

$$FFA (\%) = \frac{\text{ml of alkali} * N * 28.2}{W} \quad (7)$$

Where:

N= Normality of NaOH solution

W= weight of oil (g)

28.2= concentration conversion coefficient

Acid value (mgKOH/g) =1.99*FFA (%)

3.4.2 Iodine value test

The Iodine value was determined using Hanus method laid out by Liyanage (1999), whereby 10 ml of chloroform was added in a 250 ml conical flask containing approximately 0.25 g of the extracted oil. 30 ml of Hanus iodine solution (prepared by dissolving 18.2 g of iodine in 1L of glacial acetic acid and then 3 ml of bromine water added to increase the halogen content) was then added and the mixture kept in the dark for 30 minutes with periodic shaking. Ten millilitres of 15% potassium iodide was added with gentle shaking and 100 ml of distilled water added. This solution was then titrated against 0.1 N Sodium thiosulfate solution until the yellow colour turned to almost colourless. At this point, 2-3 drops of starch indicator were added and titration continued until the blue colour disappeared. For blank test, the same procedure was repeated without the oil sample. Iodine value was calculated using the following Equation by Liyanage (1999):

$$\text{Iodine value} = \frac{(B - S) * N * 12.69}{\text{weight of sample (g)}} \quad (8)$$

Where:

B= ml thiosulphate for blank

S=ml thiosulphate for sample

N= normality of thiosulphate solution

12.69= concentration conversion coefficient

3.4.3 Peroxide value test

The Peroxide value was determined using the method by AOCS (2003) whereby the extracted oil was filtered using Whatman No. 40 filter paper (to remove moisture and impurities). Five grams of the filtered oil was weighed into a 250ml conical flask and 30 ml of 3:2 acetic acid-chloroform added and swirled to mix well. This was followed by addition of 0.5 ml of saturated potassium iodide solution and allowed to stand for 1 minute. Thirty millilitres of distilled water was then added and swirled to mix and titrated against 0.1 N sodium thiosulfate until the yellow

iodine colour disappeared. Subsequently, starch indicator (2 ml) was added and titration continued until the blue colour disappeared. Blank samples were simultaneously prepared and their titration did not exceed 0.1 ml (Razak et al., 2000). The peroxide value was determined using the formulae by AOCS (2003) as follows:

$$\text{Peroxide Value (milliequivalents peroxide/1000 g sample)} = \frac{(S-B)*N*1000}{W} \quad (9)$$

Where:

S= volume of titrated sample (ml)

B= volume of titrated blank (ml)

N= Normality of sodium thiosulfate solution

W= weight of oil (g)

3.4.4 p-Anisidine value test

p-Anisidine value was determined using the method laid out by IAFMM (1981) whereby the oil sample was filtered using Whatman No. 40 filter paper to remove moisture and impurities. Then 0.5g-4g of the extracted oil sample was weighed in a 25-ml volumetric flask and consequently dissolved using 25 ml iso-octane (test solution). The absorbance (AB) of the oil sample (test solution) was then measured at 350 nm using a spectrophotometer (Thermo Fisher Scientific model: Genesys 10-S) against pure iso-octane (blank). A 5 ml sample of oil (test solution) was pipetted into one test tube and 1 ml of p-Anisidine reagent added. Subsequently, 5 ml iso-octane was added to another test-tube with 1 ml of p-Anisidine reagent and used as blank. The p-Anisidine reagent was prepared by adding 0.25g of p-Anisidine to 100 ml of glacial acetic acid. After 10 minutes the absorbance (AS) of the oil sample with the p-Anisidine was measured using a spectrophotometer and the p-Anisidine value calculated as follows:

$$\text{p-Anisidine value} = \frac{25*(1.2AS-AB)}{W} \quad (10)$$

where:

AS= absorbance of the fat solution after reaction with the p-Anisidine reagent

AB= absorbance of fat solution

W=weight of oil (g)

TOTOX value was calculated using the formulae by Deepika et al. (2014) as:

TOTOX= AV + 2PV

Where:

AV= p-anisidine value

PV= Peroxide value

3.5 Data analysis

The Kolmogorov-Smirnov and Levene tests were used to analyze both normality and homogeneity of variance respectively (Zar, 2014). The Kruskal-Wallis test at $p<0.05$ was used to test for significance difference in mean Kn between species since the Kn data was 13not normally distributed. The results of oil yield were expressed as means ± SD. One-way Analysis of variance (ANOVA) was used to test for significance differences in percentage oil yield within body parts (head, body cavity, fillet, frame and tail of each species) and between body parts (head, body cavity, fillet, frame and tail of all species) at $\alpha=0.05$. One-way Analysis of variance (ANOVA) was also used to test for significance deference in the various oil quality tests between the five species. Consequently, Tukey's groupings were conducted for quantitative yield results and single tailed Dunnett t (<limit) test used to compare the various quality tests against their respective set limits (8, 7, 5, 20, 26 for acid value, free fatty acid content, peroxide value, p-Anisidine value and TOTOX value respectively).

CHAPTER FOUR
RESULTS

4.1 Length weight relationship

Five species (*P. aethiopicus, C. gariepinus*, *C. carpio, L. niloticus* and *O. niloticus*) from three different lakes in Kenya (Lake Victoria, Lake Naivasha and Lake Baringo) were sampled with a sample size of (n=30) in each species. Table 2 shows species sampled, the lake from which the fish was caught, the minimum, maximum and mean length (±S.E), the minimum and maximum weight measured. WLRs parameters show exponents *a* and *b* which are the intercept of log-transformed weight and length and the slope of log-transformed weight and length respectively.

Table 2: Weight Length relationship (WLRs) for 5 commercially important fish species in Kenya

Species	Source	*n*	Length characteristic	Weight characteristics	WLRs parameters	
			Mean	**Mean**	***a***	***b***
Lates niloticus	L.Victoria	30	41.75±6.62	785.60±263.08	-0.005	1.782
Cyprinus carpio	L.Naivasha	30	46.35±9.30	1442.23±728.73	-0.354	2.094
Protopterus aethiopicus	L. Baringo	30	87.45±12.80	3827.93±2949.90	-1.455	2.563
Oreochromis niloticus	L.Victoria	30	31.20±5.06	582.93±127.93	0.909	1.240
Clarias gariepinus	L.Naivasha	30	49.25±7.75	880.91±407.90	-0.232	1.862

The relationship between length and weight of different fish species are shown in figures 5,6,7,8 and 9 parabolic scatter plots indicating per cube law formula $W=aL^b$ and the coefficient of determination, R^2 (Le Cren, 1951). The five species sampled exhibited negative allometric growth patterns ($b<3$, $P<0.05$). There was a relatively high degree positive correlation between standard lengths and body weights as shown in the following correlation coefficient (*r*) of 0.742, 0.739, 0.951, 0.898 and 0.949 for *P. aethiopicus, C. gariepinus*, *C. carpio, L. niloticus* and *O. niloticus* respectively at $P < 0.05$.

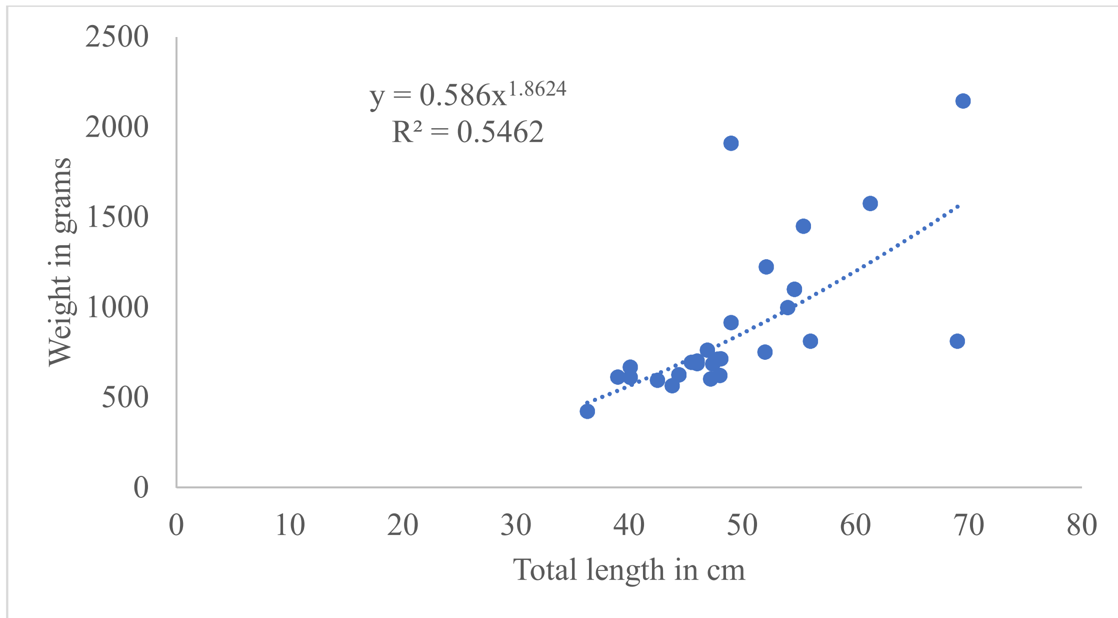

Figure 5: Parabolic relationship between length and weight of African catfish *Clarias gariepinus*

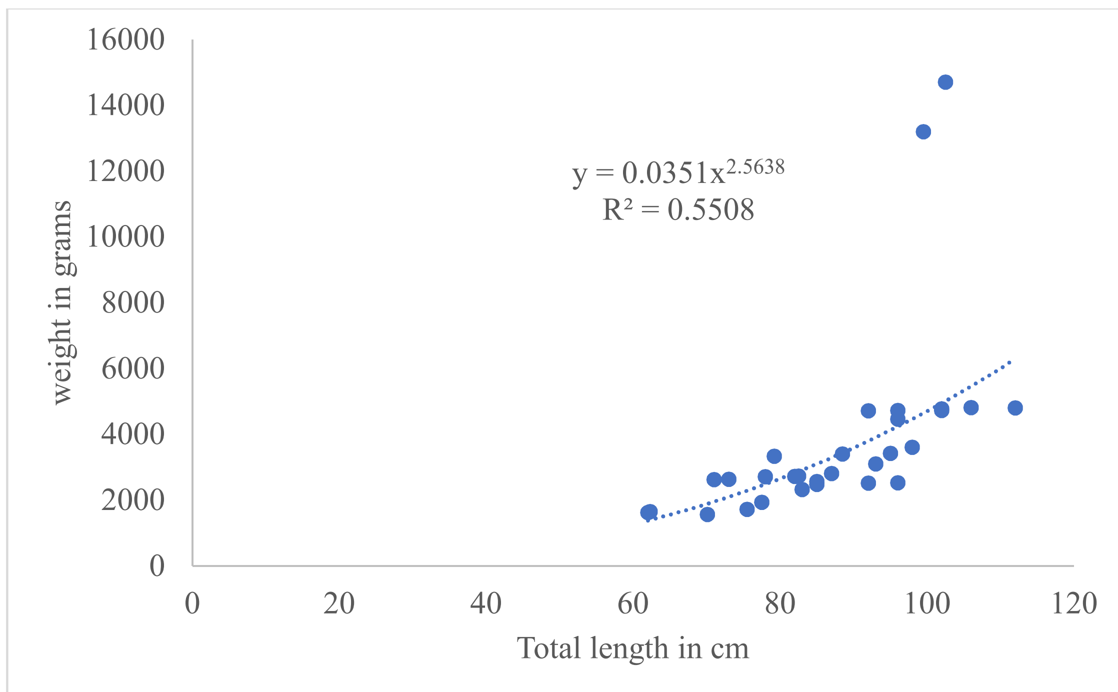

Figure 6: Parabolic relationship between length and weight of Marbled lungfish *Protopterus aethiopicus*

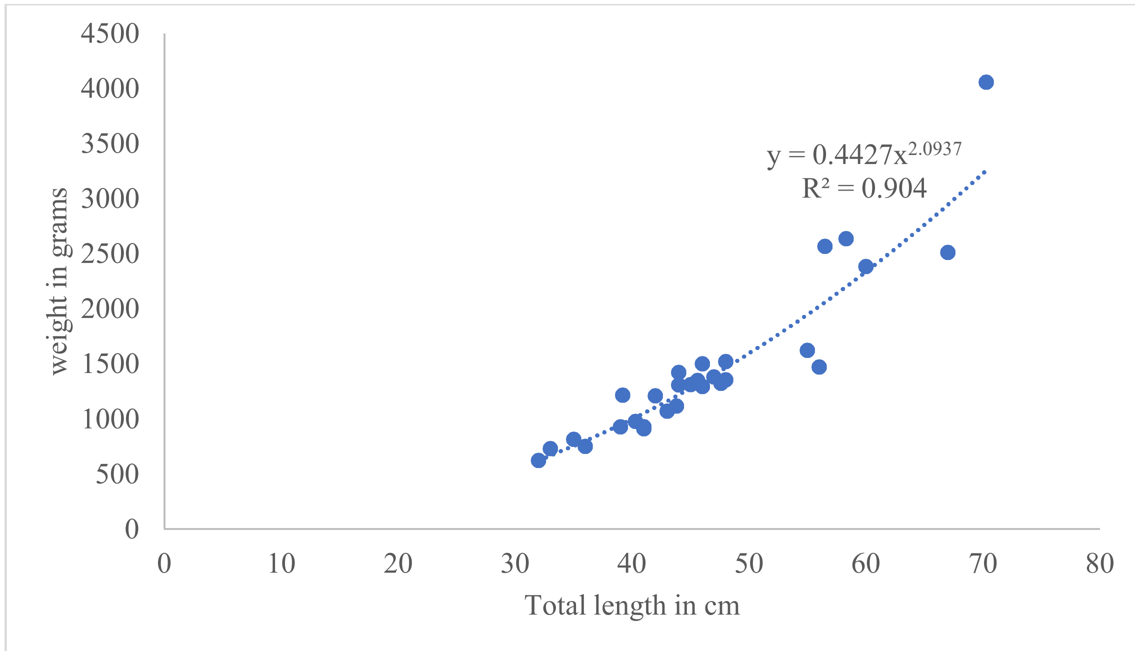

Figure 7: Parabolic relationship between length and weight of Common carp *Cyprinus carpio*

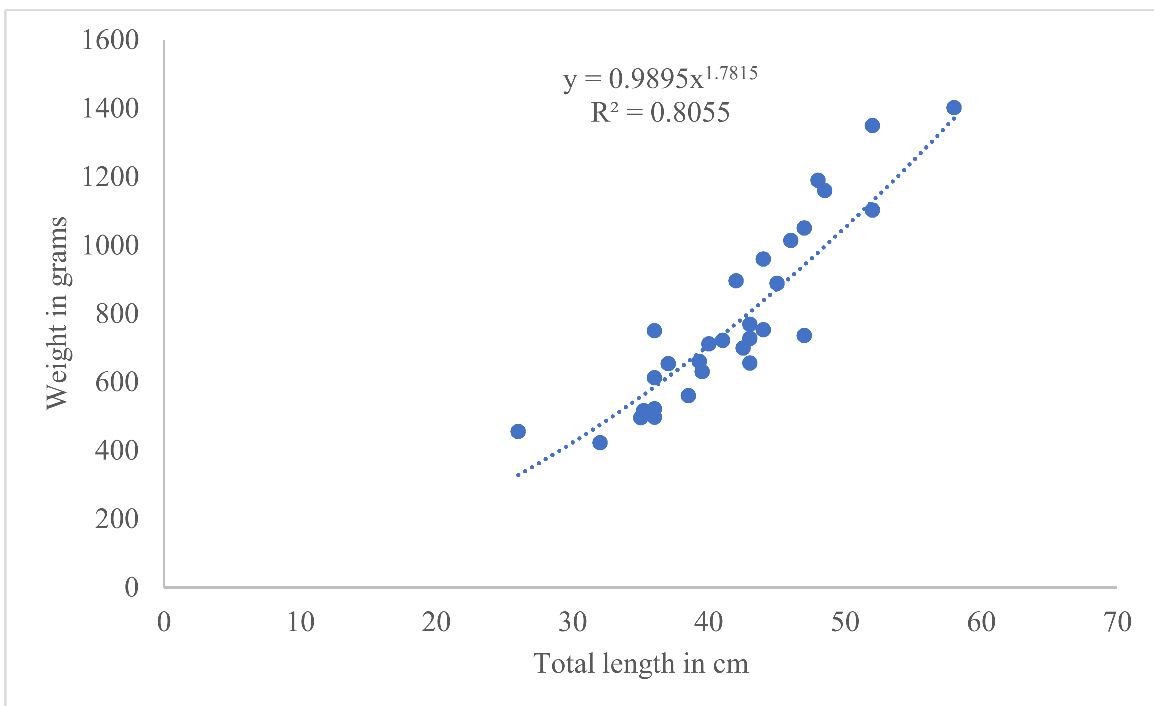

Figure 8: Parabolic relationship between length and weight of Nile perch *Lates niloticus*

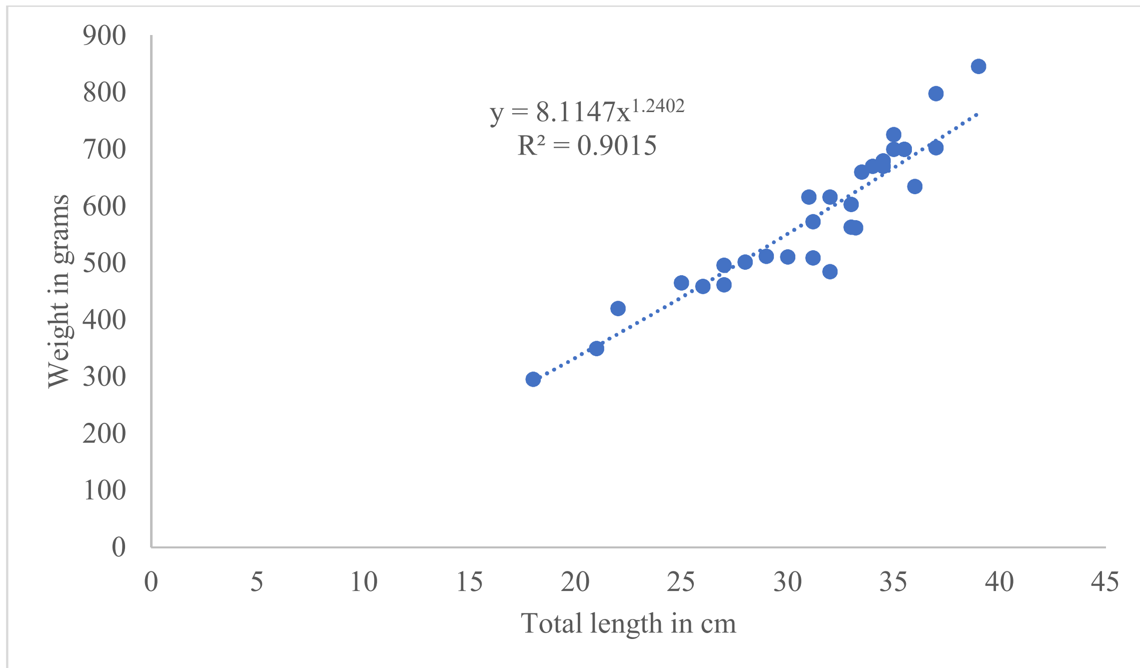

Figure 9: Parabolic relationship between length and weight of Nile tilapia *Oreochromis niloticus*

Relative condition factor (Kn) for the five species were 1.075±0.528, 1.035±0.309, 1.008±0.132, 1.01±0.148, 1.003±0.072 for *P. aethiopicus, C. gariepinus*, *C. carpio, L. niloticus,* and *O. niloticus* respectively and did not differ significantly between species (Kruskal-Wallis test, P>0.05).

4.2 Gut content analysis

As shown in Table 3, most species had a percentage stomach fullness greater than a half. All the food item categories were fairly represented in all samples as Kruskal-Wallis test between the food item categories did not differ significantly (P>0.05).

Table 3: Degree of Stomach Fullness (%) in the five selected fish species

	Degree of Stomach Fullness (%)				
	4/4	**3/4**	**2/4**	**1/4**	**0/4**
Cyprinus carpio	46.67	20	23.33	10	0
Clarias gariepinus	13.33	6.67	53.33	20	6.67
Protopterus aethiopicus	60	10	30	0	0
Oreochromis niloticus	10	26.67	46.67	16.67	0
Lates niloticus	56.67	36.67	6.67	0	0
% Average	37.33±23.97	20.00±12.25	32.00±18.65	9.33±9.25	4.00±8.94

Quantitative gut content analysis resulted to five general categorizations of the food item. These categories included fish, zooplankton, phytoplankton molluscs and organic debris (Table 4).

Table 4: Prey occurrence (%) in the five selected fish species

	Prey occurrence (%) Oi				
	Fish	**Phytoplankton.**	**Zooplankton**	**Mollusc**	**Organic Debris**
Cyprinus carpio	6.7	66.7	46.7	0	46.7
Clarias gariepinus	57.1	85.7	93.3	42.9	28.6
Protopterus aethiopicus	86.7	0	0	100	33.3
Oreochromis niloticus	0	93.3	33.3	0	86.7
Lates niloticus	100	0	0	0	0
% Average	50.1±45.50	51.8±42.60	38.66±35.10	28.58±44.04	29.72±35.45

Some of the identifiable organism from *C. carpio* included cyanobacteria (*Aphanocapsa sp* and *microcystis sp*), green algae (*Pediastrum sp*), diatoms (A*ulacoseira sp, Surirella sp, Gomphonema sp, Synedra sp* and *Nitzschia sp*), and zooplankton (copepods and daphnia). Organic debris mostly constituted of semi-digested macrophytes (twigs and leaves) and only two fish samples had juveniles of unknown fish species in their gut.

Clarias gariepinus gut had a dominance of mostly zooplanktons which included copepods (cyclopoids) shrimps (*Caradina sp*) and daphnia. There were also traces of recognizable phytoplanktons such as diatoms (*Fragilaria sp, Aulacoseira sp, Pinnularia sp, Surirella sp, Cocconeis sp* and *Cymbella sp*), cyanobacteria (*Aphanocapsa sp, Microcystis sp* and *Oscillatoria sp*) and organic debris which constituted semi-digested plant materials.

Fish and mollusc constituted the highest percentage of identified prey item in the gut of *P. aethiopicus*. Identifiable mollusc shells were those of *Melanoides sp* with organic debris constituting mostly twigs and fish remains (bones, fins and scales).

Oreochromis niloticus gut mostly constituted of phytoplankton with recognizable species being of cyanobacteria (*Microcystis sp* and *Aphanocapsa sp*), diatoms (*Fragilaria sp, Aulacoseira sp*) and green algae (*Scenedesmus sp, Pediastrum sp* and *Staurastrum sp*). *L. niloticus* had a dominance of prey item (fish) with a percentage frequency of occurrence of 100%. The identifiable prey item, in this case, was *Haplochromis sp.*

4.3 Fish oil yield

There was a general trend for all the fish species where body cavity had the highest oil yield (Tables 6,7,8,9) except for *P. aethiopicus* that yielded more oil from the tail (Table 5). The order of percentage oil yield from the highest to the lowest was *L. niloticus* (body cavity) > *C. carpio* (body cavity) > *P. aethiopicus* (tail) > *P. aethiopicus* (body cavity) >*C. gariepinus* (body cavity) > *L. niloticus* (head) > *O. niloticus* (head) > *O. niloticus* (body cavity) > *P. aethiopicus* (frame) and *L. niloticus* (fillet) that had 61.73±5.33, 44.08±4.86, 40.89±3.20, 32.41±3.31, 32.06±4.03, 13.31±2.27, 4.63±0.86, 4.58±1.95, 4.43±0.64 and 3.27±1.42 respectively (Table 10). The remaining body parts of the five species had percentage of oil yields below 3%. There were significant differences in the percentage oil yields between the five fish parts (head, body cavity, fillet frame and tail) (ANOVA, $p < 0.05$) for *P. aethiopicus*, *C. carpio, C. gariepinus, O. niloticus,* and *L. niloticus*. In addition, there were significant differences in the percentage of oil yields between all the body parts sampled (ANOVA, $p < .05$).

Within-group Tukey HSD grouping (Table 5, 6, 7, 8 and 9) revealed that there were no significant differences between *P. aethiopicus* head and fillet; *C. carpio* head, frame, fillet, and tail; *C. gariepinus* fillet, head, tail, and frame; *O. niloticus* frame, fillet, and tail; and *L. niloticus* fillet, frame, and tail. Whereas between-group Tukey HSD grouping (Table 10) revealed *L.*

niloticus body cavity having the highest oil yield, followed by *C. carpio* body cavity and *P. aethiopicus* tail although not significant difference. *P. aethiopicus* body cavity and *C. gariepinus* body cavity followed thirdly, while *L. niloticus* head was fourth in oil yield production. *O. niloticus* head and cavity, *P. aethiopicus* frame, and *L. niloticus* fillet did not differ significantly in the fifth position. The remaining body parts of five fish species that yielded less than 1% did not differ significantly.

Table 5: Tukey grouping of percentage oil yield from the various body parts of *P. aethiopicus*

Body parts	Mean	Std. Deviation	Grouping
Tail	40.89	3.20	A
Body cavity	32.41	3.31	B
Frame	4.43	0.64	C
Head	0.55	0.29	D
Fillet	0.27	0.19	D

*Means that do not share a letter are significantly different.

Table 6: Tukey grouping of percentage oil yield from the various body parts of *C. carpio*

Body parts	Mean	Std. Deviation	Grouping
Body Cavity	44.08	4.86	A
Head	0.34	0.19	B
Frame	0.27	0.16	B
Fillet	0.18	0.10	B
Tail	0.10	0.06	B

*Means that do not share a letter are significantly different.

Table 7: Tukey grouping of percentage oil yield from the various body parts of *C. gariepinus*

Body parts	Mean	Std. Deviation	Grouping
Body Cavity	32.06	4.03	A
Fillet	0.15	0.04	B
Head	0.14	0.06	B
Tail	0.13	0.03	B
Frame	0.07	0.04	B

*Means that do not share a letter are significantly different.

Table 8: Tukey grouping of percentage oil yield from the various body parts of *O. niloticus*

Body parts	Mean	Std. Deviation	Grouping		
Head	4.63	0.86	A		
body cavity	4.58	1.95	A		
frame	0.51	0.17		B	
fillet	0.24	0.10		B	
Tail	0.23	0.11		B	

*Means that do not share a letter are significantly different.

Table 9: Tukey grouping of percentage oil yield from the various body parts of *L. niloticus*

Body parts	Mean	Std. Deviation	Grouping		
Body Cavity	61.73	5.33	A		
Head	13.31	2.27		B	
Fillet	3.27	1.42			C
Frame	0.26	0.13			C
Tail	0.17	0.09			C

*Means that do not share a letter are significantly different.

Table 10: Tukey grouping of percentage oil yield from all the five species and their various body parts

Body part	Mean	Std. Deviation	Grouping
L. niloticus (body cavity)	61.73	5.33	A
C. carpio (body cavity)	44.08	4.86	B
P. aethiopicus (tail)	40.89	3.19	B
*P. aethiopicus (*body cavity)	32.41	3.31	C
C. gariepinus (body cavity)	32.06	4.03	C
L. niloticus (head)	13.31	2.27	D
O. niloticus(head)	4.63	0.86	E
O. niloticus (body cavity)	4.58	1.95	E
P. aethiopicus (frame)	4.43	0.64	E
L. niloticus(fillet)	3.27	1.42	E F
P. aethiopicus (head)	0.55	0.29	F
O. niloticus (frame)	0.52	0.17	F
C. carpio (head)	0.34	0.18	F
C. carpio (frame)	0.27	0.15	F
P. aethiopicus (fillet)	0.27	0.19	F
L. niloticus (frame)	0.26	0.13	F
O. niloticus (fillet)	0.24	0.10	F
O. niloticus (tail)	0.23	0.12	F
C. carpio (fillet)	0.18	0.10	F
L. niloticus (tail)	0.17	0.09	F
C. gariepinus (fillet)	0.15	0.04	F
C. gariepinus (head)	0.14	0.06	F
C. gariepinus (tail)	0.13	0.03	F
C. carpio (tail)	0.10	0.06	F
C. gariepinus (frame)	0.07	0.04	F

*Means that do not share a letter are significantly different.

4.3 Fish oil quality

Percent-free fatty acid (as oleic) for the five species was 2.94 ± 0.59, 3.74 ± 0.31, 0.70 ± 0.32, 2.68 ± 0.43, and 2.99 ± 0.63% for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively (Table 11). The obtained acid values for the five species were 5.84 ± 1.16, 7.43 ± 0.61, 1.55 ± 0.63, 5.33 ± 0.86, and 5.96 ± 1.26 mgKOH/g for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively. The peroxide values for the five species were 2.74 ± 1.41, 3.60 ± 1.43, 2.98 ± 1.00, 2.91 ± 1.15, and 3.14 ± 1.57 meq/kg for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively. *p*-Anisidine values for the five species were 1.52 ± 0.44, 0.37 ± 0.24, 7.73 ± 1.93, 5.15 ± 1.17, and 7.68 ± 0.82 for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively. TOTOX values obtained from this study were 7.00 ± 2.69, 7.57 ± 2.82, 13.67 ± 3.30, 10.99 ± 2.92, and 13.96 ± 2.93 for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively. Finally, the results of iodine values obtained in this study were 119.87 ± 7.55, 109.57 ± 5.08, 113.85 ± 13.64, 91.02 ± 11.59, and 100.30 ± 24.29 for *L. niloticus, C. gariepinus, P. aethiopicus, O. niloticus*, and *C. carpio*, respectively.

There was significant difference in all quality tests results (Table 11) between the five fish species for Percentage Free Fatty Acid (as oleic), Acid value, Peroxide value, p-Anisidine value, Iodine value and TOTOX values (ANOVA, $p < 0.05$). Multiple comparison single tailed Dunnett t results showed that most quality tests were significantly lower than their respective set limit except for *C. gariepinus* acid value that was higher than the set limit (-0.57± 0.46, p= 0.32).

Table 11: The quality of oil from the five commercially important species in Kenya

Test	Species	Mean	Std. Deviation	Set limit	Source
FFA%	*L. niloticus*	2.94	0.59	1-7%	(Bimbo 1998; Miller 2010)
	C. gariepinus	3.74	0.31		
	P. aethiopicus	0.78	0.32		
	O. niloticus	2.68	0.43		
	C. carpio	2.99	0.63		
Acid value	*L. niloticus*	5.84	1.16	≤ 8 mg/KOH g	(GOED 2013)
	C. gariepinus	7.43	0.61		
	P. aethiopicus	1.55	0.63		
	O. niloticus	5.33	0.86		
	C. carpio	5.96	1.26		
Peroxide value	*L. niloticus*	2.74	1.41	≤ 5 meq/kg	(GOED 2013)
	C. gariepinus	3.60	1.43		
	P. aethiopicus	2.97	1.00		
	O. niloticus	2.91	1.15		
	C. carpio	3.14	1.57		
p-Anisidine value	*L. niloticus*	1.52	0.44	≤ 20	(GOED 2013)
	C. gariepinus	0.37	0.24		
	P. aethiopicus	7.73	1.93		
	O. niloticus	5.15	1.17		
	C. carpio	7.68	0.82		
TOTOX value	*L. niloticus*	7.00	2.69	≤ 26	(GOED 2013)
	C. gariepinus	7.57	2.82		
	P. aethiopicus	13.67	3.30		
	O. niloticus	10.98	2.92		
	C. carpio	13.96	2.93		
Iodine value	*L. niloticus*	119.87	7.55	No set limit	
	C. gariepinus	109.57	5.08		
	P. aethiopicus	113.85	13.64		
	O. niloticus	91.02	11.59		
	C. carpio	100.30	24.29		

CHAPTER FIVE
DISCUSSION

5.1 Length weight relationship

Oreochromis niloticus had the lowest value of 'b' therefore, exhibiting the highest level of negative allometry. Species with relatively high 'b' values were *Protopterus aethiopicus* and *Cyprinus carpio* having *b* values of 2.563 and 2.094 respectively with growth patterns tending towards isometry. Negative allometric growth pattern indicates that the rate of weight increase is less than that of the cube of the body length (Adeyemi et al., 2009a). Furthermore, Kuriakose (2017) dictates that in practice, fish with thin elongated bodies often tend to have 'b' value less than 3 while fish with thicker bodies have 'b' values of more than three. This analogy concurs with the reported findings especially on *P. aethiopicus* and *C. gariepinus* since they exhibit a more elongated body shape compared to other species (as shown in appendices 2 and 4 respectively). The 'b' value for *O. niloticus* (1.24) was significantly lower than that recorded in other studies such as Anani and Nunoo (2016) who recorded a 'b' of up to 3.1 for farmed tilapia fed on different diets and Silva et al. (2015) who recorded a 'b' value of 3.06 for cage farmed tilapia. The disparity of the current finding from those in previous studies can be greatly attributed to feeding regimes on farmed tilapia which are often aimed at achieving a positive allometric growth. The 'b' value obtained from *L. niloticus* was fairly within the range of expected values from previous studies where Montcho et al. (2009) reported positive allometric growth ($b>3$) between January and June which are periods of low waters and a negative allometric ($b<3$) growth between October to November during of high waters levels. This compares with the sampling period of this study, which was a high-water period. All the Kn values were more than one in all the species implying a good state of well-being for all the species (Adaka et al., 2015). However, it is paramount to note that factors such as size, state of the stomach, illness, sampling methods, sex, age, state of maturity among others affect fish condition and length-weight relationship parameters (Adeyemi et al., 2009a).

5.2 Gut content analysis

The results of C. *carpio* gut content analysis (Table 4) highly coincides with those of previous studies on the gut content analysis of C. *carpio.* A study by Magalhaes (1993) and Dadebo et al. (2015) reported that *C. carpio* consumed a relatively wide range of food items from zooplankton, phytoplankton, insects and macrophytes. The high preference of zooplankton by the fish was also reported by Saikia and Das (2008) where mostly Cladocera, Copepoda and Rotifera were recorded.

There are diverse findings of previous studies of *C. gariepinus* attributing to its opportunistic omnivorous nature. Adewumi et al. (2014) reported a prevalence of several food categories in the gut of *C. gariepinus* including phytoplankton, zooplankton, insects, crustaceans and nematodes. It is also paramount to point out that this is the only species that had at least all the food item categories in the gut. A fundamental explanation of this attribute is perhaps the fact that *C. gariepinus* possess proteases enzyme similar to that found in carnivorous species, starch digestive capabilities similar to those of specialized herbivore and lysozyme and alkaline phosphatase as similar to detritivores (Adeyemi et al*.,* 2009b; Yalcin et al., 2001). This, therefore, gives *C. gariepinus* the physiological capabilities of handling frequent and irregular meals.

A similar study done by Omondi et al. (2013), on the same lake (Lake Baringo) yielded similar results to this study where the gut content of *P. aethiopicus* was primarily dominated by mollusc with a frequency of occurrence of 98.6%, followed by fish with a frequency of occurrence of 39.4%. Other reported food items included detritus, insects and higher plants that had a frequency of occurrence of ≤1%. *P. aethiopicus* feeds by groping the bottom of the lake where molluscs inhabit. The overwhelming success of *P. aethiopicus* in the lake could, therefore, be attributed to the availability of molluscs in the bottom of the lake (Omondi et al*.,* 2013).

Similar to the findings of this finding, Outa et al. (2014) reported that algae contribute the highest proportion in the diet of *O. niloticus* though other prey items included zooplankton, plant materials, detritus, insects and fish part*.* Zaganini et al. (2012) related that size plays a vital role in the diet preference of *O. niloticus* where reported size class 10.1 to 12 prefers feeding on zooplankton such cladocerans. This perhaps explains the low frequency of occurrence of zooplankton in this study compared to phytoplankton because the mean length of our sample was (31.20±5.06). This size class is less planktivorous and with a relatively high preference for phytoplankton (algae) (Bwanika et al*.,* 2006).

These results of this study on *L. niloticus* prey preference are in agreement with those of Dadebo et al. (2005) who reported a frequency of occurrence of 70.44% for fish as the most preferred prey item. It is reported that present-day *L. niloticus* starts piscivory at a relatively smaller size of 2.5cm to 3 cm TL with reported cannibalism at the size of 2 cm TL (Hopson, 1972). The preference on Lake Victoria *Haplochromis sp* can be closely attributed to prey

anatomy. The anatomy of prey directly affects vulnerability by strategically modifying the bites targets. Bursiform soft-rayed prey such as Haplochromis sp are attacked in the mid-body region with high predator success compared to deep-bodied spiny prey that are struck caudally with minimal predator success (Moody et al., 1983).

5.3 Fish oil yield

The results of *L. niloticus* percentage oil yield specifically on body cavity were higher than 42.4 ± 1.38 reported by Masa et al. (2011) on *L. niloticus* adipose tissues oil but lower than 75.8 ± 6.9 reported by Aloo (2014) on *L. niloticus* viscera oil. Moreover, the percentage oil yield obtained from *L. niloticus* fillet was significantly lower than 13.8±0.42 reported by Masa et al. (2011) on *L. niloticus* muscle oil. The reported yields from *L. niloticus* heads were fairly within range of Mbatia *et al*. (2010) who recorded 13.8 ± 0.8 percentage lipid content of *L. niloticus* heads through enzymatic extraction. *C. gariepinus* body cavity yields were within the range of 31.4±0.85 reported in earlier works of Masa et al. (2011) on *C. gariepinus* adipose tissue. *C. gariepinus* fillet yields on the other hand, were relatively lower than 6.25±0.46 reported by Masa et al. (2011) on *C. gariepinus* muscle oil. The reported percentage oil yield of 32.06±4.03 from *C. gariepinus* body cavity was also higher than 25.87% reported by Sathivel et al. (2003) from *C. gariepinus* viscera (liver, digestive tract, gall bladder, and visceral storage fat) using conventional method of oil extraction. The recorded 0.24±0.10 *O. niloticus* fillet oil yield was significantly higher than 5.23 ± 0.47 reported in the works of Masa et al. (2011) on *O. niloticus* percentage muscle oil yield. The five species sampled can be closely categorized as fatty fish especially due to the availability of irregular fat deposits in different body parts (Bennion, 1980; Pigott,1967).

Fish oil specifically from fatty fish (those that store oil in other parts of the body other than the liver) is stored as essential energy reserves therefore, its availability is affected by physiological and behavioural changes associated with swimming, spawning and starvation which explains the disparity between the recorded findings and those previous studies (Love, 1970; Stansby & Hall, 1967). Generally, body cavities of all the species investigated and the tail of *Protopterus aethiopicus* yielded significant quantities of fish oil to be considered in oil exploitation.

5.4 Fish oil quality

Percent-free fatty acids (as oleic) are one of the quality determination criteria used in most fats and oils. It is an imperative test as it assesses the quality of raw materials used for oil extraction

by revealing enzymatic activities associated with microbes in the raw material (De Koning & Mol, 1991). Chantachum et al. (2000) reported that hydrolysis of ester bonds of triglycerides increases with temperature, and therefore, increasing fatty acids (FFA) % acid. The percentage free fatty acid (as oleic) obtained in this study (0.78–3.74) for the five fish species was significantly lower than the set limit of 7% by Bimbo (1998), indicating the superior quality of the extracted oil. Free fatty acids (FFA)% acid (as oleic) of *C. carpio, L. niloticus, P. aethiopicus*, and *O. niloticus* was lower than (3.35 ± 0.02%) reported by Crexi et al. (2010) for crude carp (*C. carpio*) viscera oil but relatively higher than (0.92 ± 0.08) reported in the study of Aloo (2014) for *L. niloticus* viscera oil. However, the reported free fatty acids of all the five species were within the range of 1.67–6.49% reported by Deepika et al. (2014) for oil extracted from the salmon gut. The obtained fatty acid values are within the allowable limit of free fatty acid for unrefined oil of 1–7% (Bimbo 1998), indicating the superior quality of raw materials that can be used for oil extraction.

The acid value test determines the acidity of the oil, as it quantifies the amount of acid present in the oil. The principle behind this test relates to the amount of potassium hydroxide (mg) required to neutralize 1 g of fat or oil (Wrolstad et al., 2004). The obtained acid values for the five species (1.55–7.43) were relatively lower than the acceptable limit of 7–8 mg KOH/g (AOCS, 2003). The result of multiple comparison single-tailed Dunnett t-test revealed that acid value for most sampled species was significantly lower than the set limit of 8% of GOED (2015), except for *C. gariepinus* that was significantly higher than the set limit (-0.57 ± 0.46, $p = .32$). In addition, the reported acid values for *L. niloticus, C. gariepinus, O. niloticus*, and *C. carpio* were significantly higher than 0.33–2.10 mgKOH/ g, reported by Deepika et al. (2014) for oil extracted from salmon head and frame. Several factors have been discussed in the literature that influences oil acid values; for example, an increase in acid value in oil is often directly proportional to an increase in free fatty acid (Barthet et al., 2008). This is because the conversion of triacylglyceride (TAG) into fatty acid and glycerol increases the acidity of oil (Dave et al., 2014). Also, the activity of lipase in microbes and fish tissue has been reported to increase acid value (Boran et al., 2006). Other factors that significantly influence acid value, as reported by Wrolstad et al. (2004), include the method of extraction, sample preparation, the freshness of raw materials, and oil composition. It is, therefore, possible that some of these factors had a significant impact on the reported acid value through strategic measures such as deep freezing of the raw materials were undertaken to maintain the sample freshness.

Peroxide value is an imperative measure of primary oxidation characterized by the presence of hydroperoxides in oil (Dave et al. 2014). The peroxide value for all five species (2.74–3.60) was fairly within the allowable peroxide value limit set by FAO/WHO (2017) and GOED (2013) of ≤5 milliequivalents/kg, indicating superior quality (in terms of primary oxidation state) of the extracted oil. Besides *C. gariepinus*, the remaining species had peroxide values lower than 3.38 ± 0.01 reported by Crexi et al. (2010) for crude carp (*C. carpio*) viscera oil. However, the results in this study were higher than 0.28–2.65 meq/kg reported by Deepika et al. (2014) for oil extracted from salmon head and frame. Generally, the lower the peroxide value, the better the quality of the oil and its preservation state, although this value can decrease due to secondary oxidation of peroxides (primary oxidation products) (O'Brien, 2004). Usually, fish oils contain relatively large amounts of PUFA that often predispose them to both primary and secondary oxidation. Peroxide value is often high during the early stage of oxidation, which is characterized by the reaction of fatty acids with oxygen to form odourless hydroperoxides. Hydroperoxides responsible for the high peroxide value are very unstable under heating, especially in n-3 polyunsaturated fatty acids, and are consequently decomposed to form secondary oxidation products (aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons) (Dave et al*.,* 2014). This, therefore, means that a low peroxide value is not a guarantee of high quality since the oil may still be in an oxidized state. More often, hydroperoxides do not influence the odor or flavor of oil (Kulås & Ackman, 2001). Factors that influence the formation of hydroperoxides in fish oil include oxygen availability, presence of light, temperature, and presence of antioxidants (Ritter, 2012). It has also been noted that the method of extraction significantly affects the formation of hydroperoxide and consequently influences peroxide value. A study by Chantachum et al. (2000), reported that oil extracted through heat treatment of fish samples had a high peroxide value compared to those extracted without heat. Myoglobin (iron containing protein) often denatures during heat treatment, therefore, releasing iron in the catalytic pool. The iron then interacts with the lipid membrane inducing lipid oxidation. Considering that the method of oil extraction used in the current study was heat extraction (steaming), the high temperature could, therefore, be the main contributing factor to the reported peroxide values.

The obtained *p*-Anisidine values for the five species (0.37–7.73) were within the limit set by GOED (2013) of ≤20. Turon et al. (2005) reported *a p*-Anisidine value of 3.7 ± 0.1 for oil extracted from *L. niloticus* heads via chemical oil extraction, which was relatively higher than the values obtained in this study from *L. niloticus* and *C. gariepinus* but lower than those from

P. aethiopicus, O. niloticus, and *C. carpio*. The obtained results were also lower than 19.8 reported by Pak (2005) in the study of oxidative stability of fish oil in a domestic application. Boran et al. (2006) reported *p*-Anisidine values that ranged from 1.74 to 14.09 for oil extracted from garfish, shad, mackerel, and golden mullet under different time schedules and temperature regimes. *p*-Anisidine value is a test for secondary oxidation products such as aldehydes of α- and β-unsaturation (Deepika et al., 2014); thus, the test focuses on the second phase of oxidation where the already formed peroxides are further oxidized into short-chain aldehydes and alcohols. The products of primary oxidation (hydroperoxides) are often very unstable and easily decompose into volatile and non-volatile secondary oxidation compounds, which results in oil rancidity and the development of off-flavours and odours (Aidos et al., 2001). Stabilizing agents (antioxidants) can be used to prevent the development of both primary and secondary products of oil degradation, especially when added immediately after oil extraction (Dave et al., 2014).

TOTOX value represents both primary and secondary oxidation products, i.e., both hydroperoxides and aldehydes. The obtained TOTOX values in this study (7.00–13.96) were lower than 21 reported by Pak (2005) for oxidative stability of fish oil in the domestic application and the range of 8.04 to 35.29 reported by Boran et al. (2006) for extracted oil from garfish, shad, mackerel, and golden mullet. However, pre-oxidant conditions could affect the generation of both primary and secondary products of oxidation such as high temperatures, oxygen, metal compounds, and light, as reported by Chantachum et al. (2000).

The results of iodine values (91.02–119.87) obtained in this study were within the range of 94–120 for rapeseed oil and up to 185 for fish oil. The obtained values (91.02–119.87) were lower than 123, 143, 158, 165, and 130 for Sheepshead, Tullibee, Maria, Alewife, and Atlantic herring oil, respectively, reported by Ackman et al. (1967). On the other hand, Abdulkadir et al. (2010) reported relatively higher iodine values of 174.41, 182.88, 182.88, and 187.11 for oil extracted from *Mormyrops delicious, Bagrus docmac niger, Tilapia dagati*, and *Clarias anguilloris*, respectively. Since the iodine value test measures the number of reactive double bonds present in an oil, a high iodine value indicates more double bonds (unsaturation) providing caution in terms of measures to reduce or slowdown autoxidation. However, Dave et al. (2014) noted that iodine value does not give information on the nature of saturated or unsaturated compounds in the oil, neither is it a quality indicator but rather a composition indicator.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The length-weight relation of the five commercially important species (*P. aethiopicus, C. gariepinus, C. carpio, L. niloticus,* and *O. niloticus*) deviated from the isometric growth pattern (b=3) and did not follow the cube law. Relative condition factor of all the species was above 1 indicating that all species were in good condition in their natural habitat. The study further revealed the importance of the various prey items in the three lakes by percentage frequency of occurrence. Phytoplankton and zooplankton had the highest percentage frequency of occurrence in *C. carpio* and *C. gariepinus.* While mollusc, phytoplankton and fish prey items frequently occurred in *P. aethiopicus, O. niloticus* and *L. niloticus* respectively.

ii. From this study, it is clear that the body cavities of all the species investigated and the tail of *Protopterus aethiopicus* yielded significant quantities of fish oil worthy to be considered in oil exploitation.

iii. There was no significant difference in the fish oil quality from the 5 fish species. The quality test results (free fatty acid content, acid value, peroxide value, *p*-Anisidine value, and TOTOX value) were significantly within their respective set limits, except for *C. gariepinus* acid value that was higher than the set limit of 7–8 mg KOH/g.

6.2 Recommendations

i. The study recommends the conservation of the environment in the three lakes (Lake Victoria, Lake Naivasha and Lake Baringo) in order to maintain the growth and production of prey items of importance for the five species which include phytoplankton, zooplankton, mollusc, phytoplankton, and fish that had the highest percentage frequency of occurrence in *C. carpio, C. gariepinus. P. aethiopicus, O. niloticus* and *L. niloticus* respectively.

ii. Four species (*Lates niloticus, Cyprinus carpio, Clarias gariepinus*, and *Protopterus aethiopicus*,) are highly recommended for oil exploitation from the body cavity, except for *P. aethiopicus* that had significant oil yields from the tail. The percentage of oil yield in the proposed candidates was above the FAO (1986) 3% minimum-required commercial oil yield viability. The exploitation of oil production provides an opportunity for a potential value addition chain for the body cavity, which is often

discarded as waste in most landing sites in Kenya. Consequently, the use of fish body cavity for oil production minimizes competition for fish as food since other fish parts such as fillet are still viable food components. The actualization of this recommendation will provide the potential for improved economic status coupled with food security for the riparian communities of these systems and other related systems.

iii. From quality analysis, four species (*Lates niloticus, Cyprinus carpio, Clarias gariepinus*, and *Protopterus aethiopicus*,) are highly recommended for oil exploitation. Extra care should be taken when handling raw materials of oil extraction from *Clarias gariepinus* due the to their high risk of autolytic activities of lipolysis and oxidation as envisaged in the high acid value. Further purification (degumming, neutralization, bleaching and deodorization) of oil extracted from the recommended species is paramount prior to human consumption which will consequently reduce the acidy of the oil (neutralization).

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APPENDICES

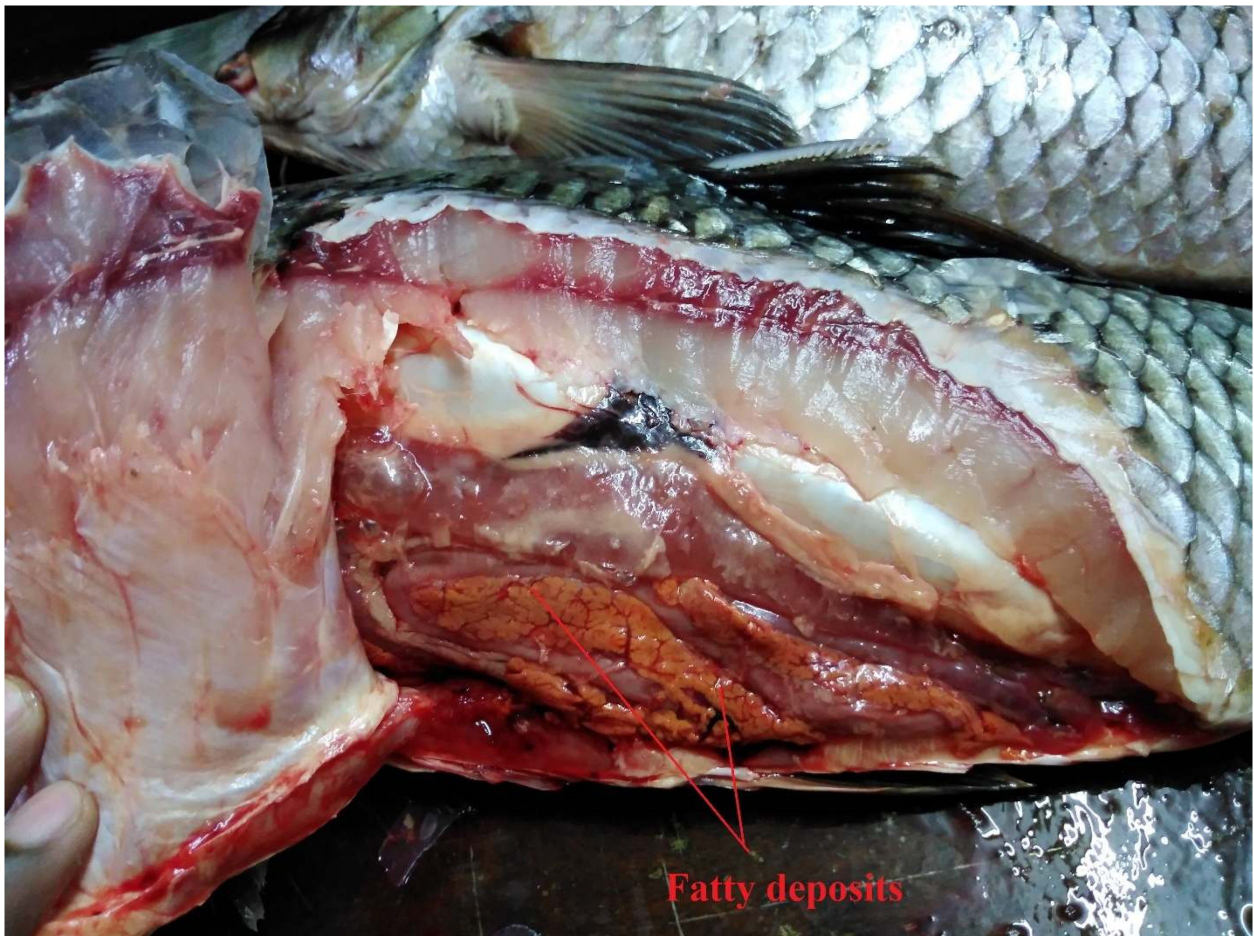
Appendix 1: A photograph showing *Cyprinus carpio*

Appendix 2: A photograph showing *Protopterus aethiopicus*

Appendix 3: A photograph showing *Lates niloticus*

Appendix 4: A photograph showing *Clarias gariepinus*

Appendix 5: A photograph showing body cavity fatty deposit in *Cyprinus carpio*



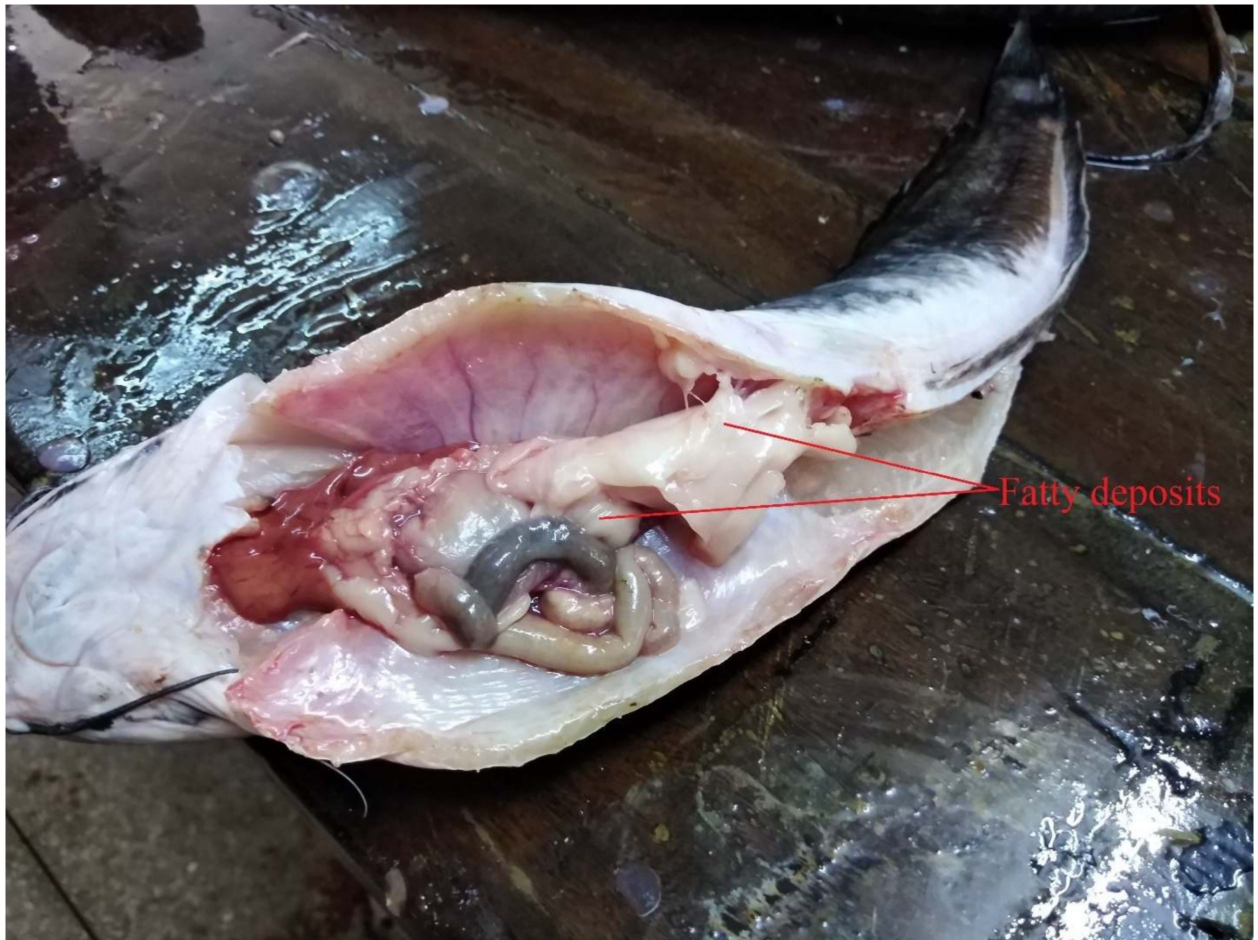
Appendix 6: A photo showing *Oreochromis niloticus*

Appendix 7: A photograph showing body cavity fatty deposit in *Lates niloticus*

Appendix 8: A photograph showing Tail fatty deposit in *Protopterus aethiopicus*

Appendix 9: A photograph showing body cavity fatty deposit in *Clarias gariepinus*

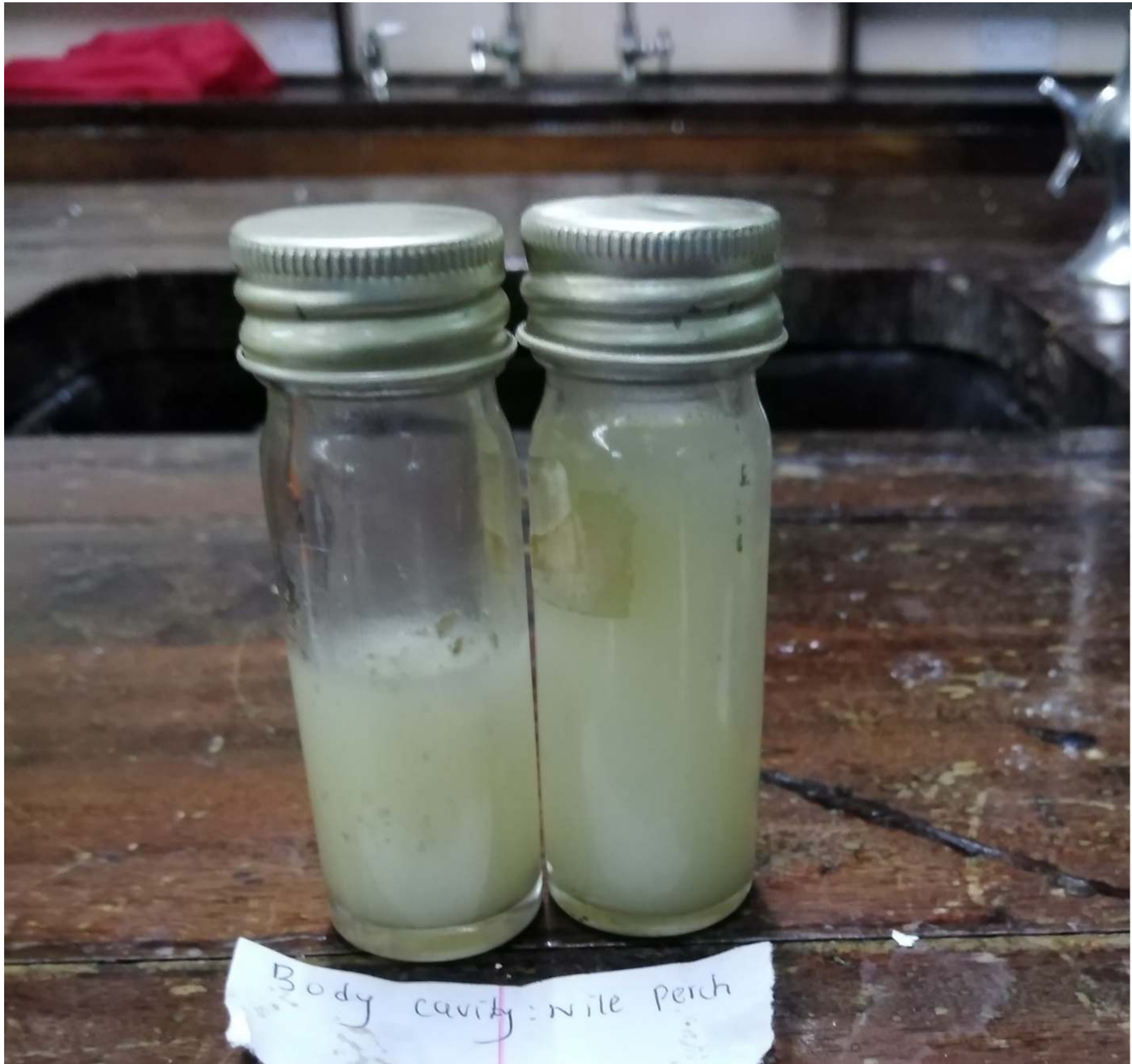
Appendix 10: A photograph showing body cavity fatty deposit in *Clarias gariepinus*



Appendix 11: A photograph showing *Protopterus aethiopicus* tail oil

Appendix 12: A photograph showing *Cyprinus carpio* body cavity oil

Appendix 13: A photograph showing *Lates niloticus* body cavity oil



Appendix 14: A photograph showing *Clarias gariepinus* body cavity oil

Appendix 15: A photograph showing *Protopterus aethiopicus* body cavity oil

Appendix 16: SPSS ANOVA output for oil yield between body parts

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
lungfish_yield	Between Groups	2458.104	4	614.526	369.198	.000
	Within Groups	74.902	45	1.664		
	Total	2533.006	49			
Common_Carp_yield	Between Groups	7523.519	4	1880.880	1073.420	.000
	Within Groups	78.850	45	1.752		
	Total	7602.369	49			
Catfish_yied	Between Groups	8159.366	4	2039.842	627.716	.000
	Within Groups	146.233	45	3.250		
	Total	8305.599	49			
Nile_Tilapia_yield	Between Groups	219.865	4	54.966	59.884	.000
	Within Groups	41.304	45	.918		
	Total	261.169	49			
Nile_perch	Between Groups	10785.557	4	2696.389	8196.317	.000
	Within Groups	14.804	45	.329		
	Total	10800.361	49			

Appendix 17: Research Permit

THIS IS TO CERTIFY THAT:
MR. KIMANI DANIEL MWANGI
of EGERTON UNIVERSITY, 536-20107
Njoro,has been permitted to conduct
research in *Nakuru County*

on the topic: *A COMPARATIVE ANALYSIS OF THE QUANTITY AND QUALITY OF OIL EXTRACTED FROM FIVE COMMERCIALLY IMPORTANT FRESHWATER FISH SPECIES IN KENYA*

for the period ending:
30th April,2020

Permit No : NACOSTI/P/19/22189/29388
Date Of Issue : 2nd May,2019
Fee Recieved :Ksh 1000

....
Applicant's Signature

....
Director General
National Commission for Science, Technology & Innovation

Appendix 18: Publication

JOURNAL OF AQUATIC FOOD PRODUCT TECHNOLOGY
https://doi.org/10.1080/10498850.2020.1856261

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A Comparative Analysis of the Quantity and Quality of Oil Extracted from Five Commercially Important Freshwater Fish Species in Kenya

Kimani Daniel Mwangi, Nzula Kitaka, and Elick Otachi

Department of Biological Sciences, Egerton University, Egerton, Kenya

ABSTRACT

This study investigated the quantities and qualities of fish oil extracted from five freshwater fish species, *Lates niloticus, Cyprinus carpio, Clarias gariepinus, Protopterus aethiopicus*, and *Oreochromis niloticus*, with the aim of determining their commercial viability in oil exploitation. Fish oil from the various body parts (head, frame, fillet, tail, and body cavity) in all the five fish species was extracted using the conventional method of cooking, pressing, and centrifugation to determine quantitative yield. The extracted oil was then subjected to a composition test (iodine value), hydrolytic degradation test (acid value), and oxidative stability test (peroxide value and *p*-anisidine value). The general trend in all the species was that the body cavity had the highest yield, except for *P. aethiopicus*, which yielded more oil from the tail. There was a significant difference in the percentage of oil yields between all the sampled body parts as determined by one-way analysis of variance (ANOVA, $p < .05$). The study concluded that three species (*L. niloticus, C. gariepinus*, and *C. carpio*) have the potential for oil exploitation if the body cavity is utilized, while one species, *P. aethiopicus*, can yield commercially viable oil from both the body cavity and tail.

KEYWORDS
Fish oil extraction; oil yield; quantity; quality; freshwater species; comparative analysis