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Impact of species and environment on the distribution of nutrients in fish

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“What we know is a drop, what we don’t know is an ocean”

Isaac Newton

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List of abbreviations

3-OH LCFA = 3-hydroxy-long-chain fatty acids

3OH-C4 = 3-hydroxybutyryl- carnitine

Asl = above sea level

ATP = adenosine triphosphate

B.P. = boiling point

C0 = free carnitine

C2 = acetyl-carnitine

C3DC = malonyl- carnitine

C4 = butyryl- carnitine

C4-DC = methylmalonyl- carnitine

CF = crude fat

CoA = co-enzyme A

CP = crude protein

DBS = dried blood spot

DM = dry matter

DO = dissolved oxygen

DRI=daily recommended intake

EC = electric conductivity

FAO = Food and Agricultural Organization

GR= Gilgel Gibe Reservoir

ICP_MS = inductively coupled plasma mass spectrometry

IFPRI = International Food Policy Research Institute

LCFA = long-chain fatty acids

LL= Lake Langano

LOD = limit of detection

LZ = Lake Ziway

NH_3 = ammonia

NO_3^-= nitrate

SD = standard deviation

SDG = Sustainable Development Goals

SE = standard error

SEM = standard error of the mean

SIDS = Small Island Developing States

TCA = tricarboxylic acid

USDA = United States department of Agriculture

WHO = World Health Organization

WW = wet weight

CHAPTER 1: General Introduction

1.1 Overview of aquaculture and capture fisheries

Aquaculture (aquatic farming) is the aquatic (freshwater, brackish and marine) equivalent of agriculture or farming on land. It covers the farming of both animals (including finfish, crustaceans and molluscs) and plants (including seaweeds and freshwater macrophytes) with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators (FAO, 2013). Aquatic farming could be under semi-controlled or controlled conditions which implies individual or corporate ownership of the stock being cultivated. Capture fisheries refers to all kinds of harvesting of naturally occurring living resources in aquatic environments (freshwater, marine and brackish) for its food and other economic values (FAO, 1997).

Aquaculture and capture fisheries play a pivotal role in providing food, nutrition, employment, recreation, trade and economic well-being for people throughout the world, for present and future generations (Waite et al., 2014; Kwasek et al., 2020). However, for sustainability, the sectors need a knowledge- and responsibility-based approach. Aquaculture is a relatively young sector compared with terrestrial livestock. It offers the opportunity for technical innovation to further increase resource efficiency.

Aquaculture is the world's fastest growing and most diverse food production sector, contributing to around 17% of human total animal protein consumption globally in 2016 (FAO, 2018). Aquaculture is continuously increasing worldwide to meet the global market demand for fish and fishery products, driven by the increasing human population and over-exploitation of wild capture fisheries (FAO, 2014). In 2017, the contribution of fishery and aquaculture production comprised 50% of total animal protein for human consumption in some countries of Asian, African and Small Island Developing States (SIDS), exceeding beef, pork, and poultry consumption (FAO, 2018). Unlike previous years, capture fisheries have also shown a growth trend: FAO (2020) reported that in 2018 it increased by 5.4 percent from the average previous three years. Recently, aquaculture and capture fisheries are commonly referred to as aquatic food

production (Tacon and Metian et al., 2013; Gephart et al., 2021); the use of this term throughout this thesis is to indicate when both terms are needed to be addressed in common.

1.2 Global fish production, consumption and trade: facts and figures

The average live weight per year of global fish production, consumption and trade for three decades in the period of 1986-1995, 1996-2005, and 2006- 2015 has been summarized and for the past consecutive three years (2016- 2018) presented for each year (FAO, 2000, 2004, 2008, 2012, 2016, 2018) (Table 1. 1). The average live weight of the global total fish production was 101.8 million tonnes in the period of 1986- 1995, 125.6 million tonnes in the period of 1996-2005 and, 149.5 million tonnes in the period of 2006-2015. For the past three consecutive years (2016, 2017 and 2018), the live weight of the world total fish production was 166, 173 and 179 million tonnes respectively.

Generally, the estimated global aquatic food production (from culture and capture fisheries) in 2018 has reached 179 million tonnes. In the same year, the estimated total first sale value from fish has reached 401 billion USD, of which 82 million tonnes, valued at 250 billion USD, came from aquaculture production (FAO, 2020) (Table 1. 1) . Out of the total global fish production (179 million tonnes), 156 million tonnes were used for human consumption and 22 million tonnes were intended for non-food uses which means some amounts were discarded as a by-product and some others were processed for further uses (fishmeal, fish oil and others).

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Table 1.1. The average per year global fish production (million tonnes), consumption (kg/year/capita) and trade (quantity in tonnes, value in USD) in live weight basis

	1986-1995	1996-2005	2006-2015	2016	2017	2018
	Average per year					
	(million tons, live weight)					
PRODUCTION						
Capture						
Inland	6.4	8.3	10.6	11.4	11.9	12.0
Marine	80.5	83.0	79.3	78.3	81.2	84.2
Total capture	86.9	91.4	89.8	89.6	93.1	96.4
Aquaculture						
Inland	8.6	19.8	36.8	48.0	49.6	51.3
Marine	6.3	14.4	22.8	28.5	30.0	30.8
Total aquaculture	14.9	34.2	59.7	76.5	79.5	82.1
TOTAL	101.8	125.6	149.5	166.1	173	178.5
UTILIZATION						
Human consumption	71.8	98.5	129.2	148.2	153	156.4
Non-Food uses	29.9	27.1	20.3	17.9	19.7	22.2
Population (billions)	5.4	6.2	7.0	7.5	7.5	7.6
Apparent consumption (kg/capita)	13.4	15.9	18.4	19.9	20.3	20.5
TRADE						
Fish export – in quantity	34.9	46.7	56.7	59.5	64.9	67.1
Share export– in value (USD billions)	34.3	37.2	37.9	35.8	37.6	37.6
Fish export –in value (USD billions)	37.0	59.6	117.1	142.6	156.	164.1

Excludes aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants. (Source: FAO, 2020)

For over 60 years, global food fish available for human consumption has increased (Figure 1). In per capita terms, fish consumption rose from 9.0 kg (live weight equivalent) in 1961 to 20.5 kg in 2018, at an average rate of about 1.5 percent per year, while total meat consumption grew by 1.1 percent per year in the same period (FAO, 2020). At the global level, since 2016, aquaculture has been the main source of fish available for human consumption, a remarkable increase considering that this share was only 4 percent in 1950, 9 percent in 1980 and 19 percent in 1990. Significant expansion of aquaculture since the mid-1980s has resulted in a sharp increase in the proportion of farmed fish consumed relative to wild-caught alternatives (FAO, 2020). In 2018, aquaculture accounted for 46 percent of the total production and 52 percent of fish for human consumption (Figure 1). However, as indicated on the below figure, from 2008 fish available for human consumption from the wild is showing a decreasing trend, because either natural stocks have been depleted and exceed their maximum sustainable yield (Sampantamit et al., 2020) or more fishermen have shifted to aquaculture (FAO, 2020).

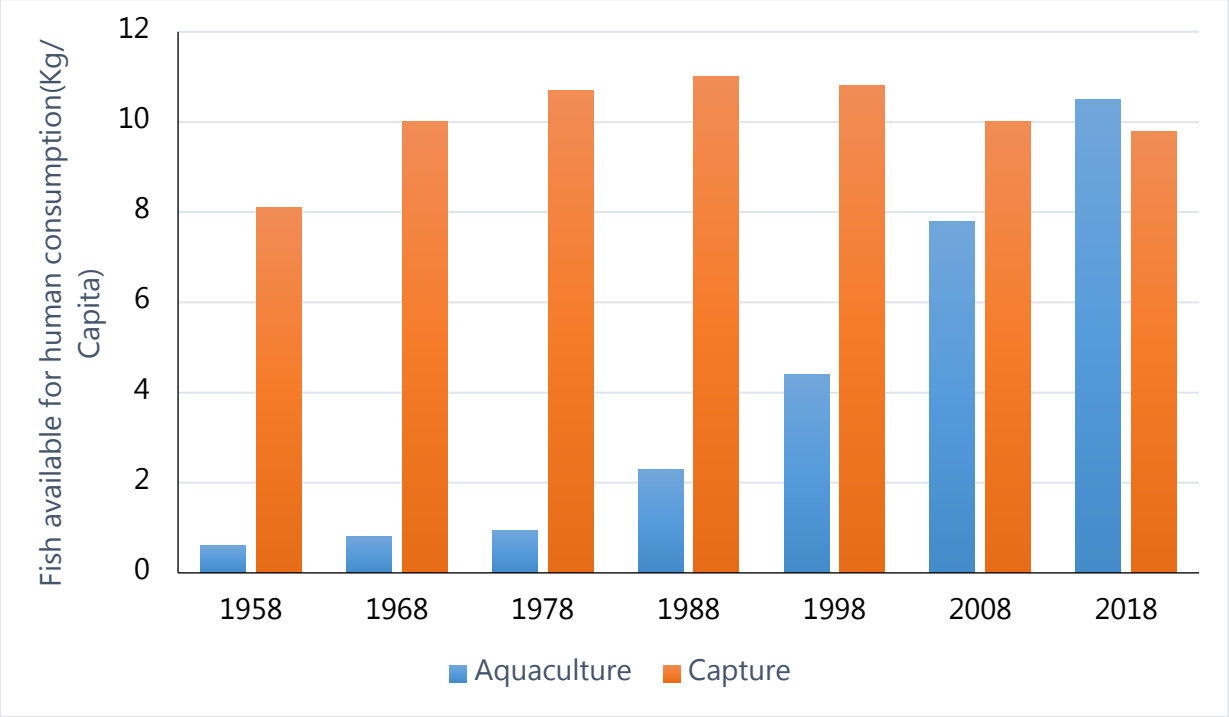

Figure 1.1 Relative contribution of aquaculture and capture fisheries to fish available for human consumption, 1958- 2018 (source: FAO, 2020).

1.3 Aquatic food production in Africa

Aquatic food, which is filling the gap between demand and supply in other regions is practiced mainly as a household livelihood activity in several African countries such as Egypt, Nigeria, Uganda and Ghana. Consequently, its contribution to the continent's fish needs remains marginal (Pant et al., 2014; Golden et al., 2016). The majority of African countries continue to reporting low aquaculture production despite great potential for the sector's development and past government efforts to assist fish farmers (Tran et al., 2019). Aquatic foods offer a life-changing opportunity for the hundreds of millions of undernourished people around the world, particularly in low- and middle-income countries.

Aquatic food production in Africa faces vast challenges; some of the reported challenges are: lack of improved fish breeds, feeds and technical training; weak research capacity; inadequate human and financial resources; weak governance and regulation; poor market infrastructure and access (Brummett et al., 2008). Moreover, climate change, habitat degradation, and postharvest losses is another challenge to the sector development in sub-Saharan Africa. Affognon et al., (2015) indicated that in sub-Saharan Africa a quarter of aquatic food product is lost during post-harvest. These losses are due to the fast perishability of the product combined with underdeveloped cold chains in the region (Chan et al., 2019) and lack of practical knowledge on product shelf-life improvement such as fish smoking, sun drying and salting.

Generally, in Africa, the aquatic food supply is dominated by capture fisheries owing to the continent's endowment of vast fish resources in marine and inland waters. Small-scale fisheries play a fundamental role in the content economies, providing livelihoods for millions of people, especially in remote rural areas, and have been strengthened significantly through the role fish plays in both food security and income generation (Bene et al., 2009). Africa currently produces about 10.2% of the global fish catches (FAO, 2018). According to the recent estimated reports (NEPAD, 2016), nearly half of the African fish stocks are overexploited or fully exploited. Therefore,

aquaculture in the continent is currently perceived as a promising and new frontier for development as aquatic food production sector. For example, about one-sixth of total production of food fish in Africa in 2016 comes from aquaculture (FAO, 2018).

FAO, (2018) reported that in 2016, the continent aquatic food production from aquaculture accounted only for 2.5% of the global aquaculture share, of which Egypt accounted for about two-thirds and Nigeria for one-sixth. Golden et al., (2016), reported that aquaculture has not yet developed significantly in low-income countries where food and technology is in short supply. As can be observed from below figure, aquatic food production in African is unevenly distributed; only few countries have a lion share to the continent (Figure 1.2).

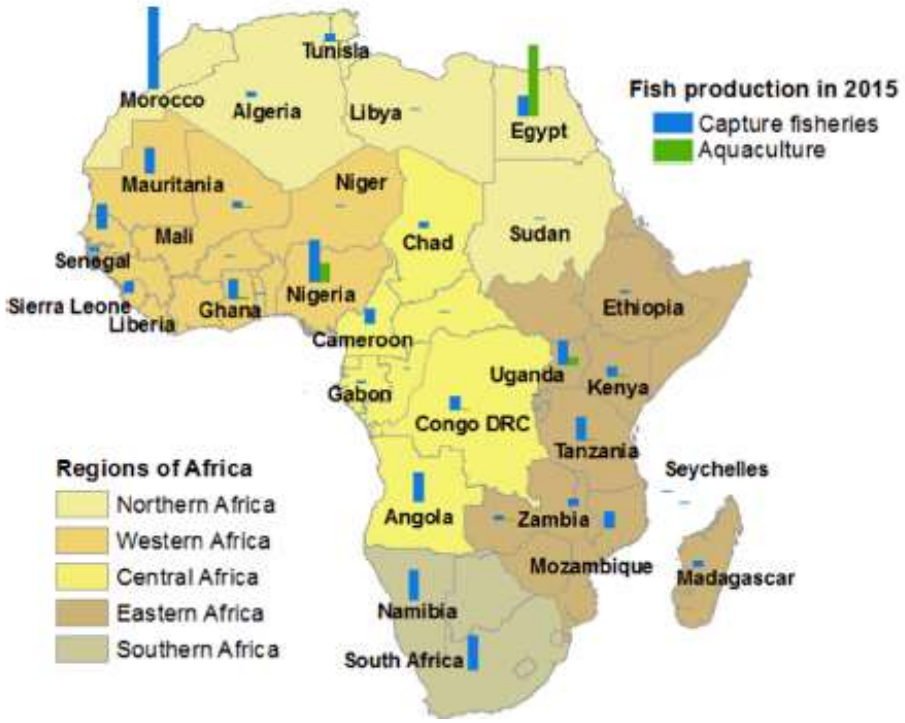

Figure 1.2 African distribution of aquatic food production (capture and culture based fisheries) (source: Chan et al., 2019).

Besides the strengthening of aquaculture in Africa, the rehabilitation of the overexploited wild fishery stocks would also increase the fish supply. This would be via rebuilding overfished or depleted stocks and by ensuring that small-scale fishers receive sufficient resources. The reduction of post-harvest losses also needs attention. Finally, enabling the use of a sufficient portion of small pelagic fish for human consumption would increase the supply (FAO, 2014). Moreover, there would be a diversifying option of fish species used for human consumption. Some African countries have recently started to include the small fish in their traditional diets (Grainger, 2016), small indigenous fish, such as Dagaa (*Rastrineobola argentea*) from Lake Victoria and Kapenta (*Limnothrissa miodon)* in southern Africa, are an important source of important micronutrients (e.g. Ca, P, Zn and Fe) in traditional diets of local communities.

1.4 Aquatic food production in Ethiopia

The practice of aquaculture in Ethiopia dates back to 1955, when a few extremely small experimental ponds were constructed at Dukem (40 km) south of Addis Ababa, for growth observations and introduction of redbelly tilapia *(Tilapia zillii)* into Ethiopia (Lemma, 1987). For a long period of time, its activity was limited to introduction of fish in the existing water body (NADSE, 2009). In recent years, few numbers of farmers have engaged in Nile tilapia (*Oreochromis niloticus*) culture at small scale pond based aquaculture, which accounts for an insignificant amount of total annual aquatic food production in the country (FAO, 2015).

More potential remains for aquaculture in Ethiopia than in actual practice, even though the country's environmental and socio-economic conditions support its development (FAO, 2005, Rothuis et al., 2012). The consumption and demand for fish as an affordable source of protein is on the increase in Ethiopia. However, the aquatic food supply mainly relies on capture fisheries, which is mostly harvested from the major Rift Valley lakes (Chamo, Abbaya, Ziway, Langano, Hawasa), highland lake (Tana), reservoirs (Tekeze, Koka, and Gilgel gibe) and rivers in the country (Janko, 2104). Capture fisheries

based on species that are presently exploited seem to have reached their natural limits (FAO, 1996, Ministry of Agriculture annual report, 2008).

In general, so far aquaculture development in Ethiopia has been only small-scale and no commercial operators exist in the country. The above (under section of Aquatic food production in Africa) mentioned challenges highly affected the development of the sector in the country. Therefore, the country would need the skilled manpower, those who need work on the sector's research and development.

1.5 Aquatic food as a "nutrition-sensitive agriculture"

The Food and Agriculture Organization (FAO, 2014) defines nutrition-sensitive agriculture as "a food-based approach to agricultural development that puts nutritionally rich foods, dietary diversity, and food fortification at the heart of overcoming malnutrition and micronutrient deficiencies". The 2030 Agenda for Sustainable Development has sets aims for the contribution and conduct of fisheries and aquaculture towards food security and nutrition, and the sector's use of natural resources, in a way that ensures sustainable development in economic, social and environmental terms, within the context of the FAO Code of Conduct for Responsible Fisheries (FAO, 2018).

Aquatic food production is one of the candidate sectors recently foreseen as fortification of nutrient-sensitive agriculture. The United Nations Decade of Action on Nutrition for 2016–2025, led by FAO and the World Health Organization (WHO), provides a critical opportunity to raise awareness about the role of fish and to ensure its mainstream incorporation into food security and nutrition policy. Importantly, aquaculture's newfound responsibility comes at a time when the wider food landscape is increasingly leaning toward more sustainable, diverse and resilient food systems and addressing past failings (Gephart et al., 2021).

Aquatic foods are crucial to meet food and nutrition security goals (Thilsted et al., 2016; WHO, 2018; Willett et al., 2019) and to potentially provide more environmentally sustainable animal protein-source foods (Hilborn et al., 2018; Hallstrom et al., 2019).

Fish plays a useful role in a healthy and balanced diet, and its consumption has long been associated with several health benefits (Kwasek et al., 2020). Fish is considered to be among the most nutritious animal- derived foods, due to their content of quality protein (Al Khawli et al., 2019). Moreover, it provides the consumer with a variety of nutrients, balanced essential amino acids, high levels of fat-soluble vitamins (A andD), essential macro- and microminerals and long-chain omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) (Weichselbaum et al., 2013; Al Khawli et al., 2019; Kwasek et al., 2020). Studies have shown that omega-3 fatty acids in fish may reduce the risk of heart disease and stroke and several physicians suggest that eating one to two fish meals each week is helpful in preventing heart disease (Weichselbaum et al., 2013; Al Khawli et al., 2019). Especially the inland aquatic food production systems are still underestimated in its production potential, under-reported in research and under-represented in policy and investment (Mills et al., 2011). In a world where nearly 30% of humanity is suffering from malnutrition and over 70% of the planet is covered with water, aquatic foods represent an essential component of the global food basket to improve the nutrition, health, and wellbeing of all peoples (Kwasek et al., 2020).

Tilapia

Tilapia is the generic name given to a group of freshwater fish species inhabiting streams, ponds, rivers and lakes and less commonly in brackish water. Taxonomically, they are belonging to the class of Actinopterygii under the order Perciformes of Cichlidae family (El-Sayed, 2006). Tilapia originates exclusively from Africa and is widely distributed naturally in Nile River, in most parts of African rivers and lakes as well as in the Middle East (El-Sayed, 2006). Although tilapias are native to Africa, they became one of the most widely farmed fish with an increasing production output worldwide (Prabu et al., 2019).

Tilapias are one of the most important herbivorous fish species reared in aquaculture systems (FAO, 2012). They are naturally adapted to eat plant ingredients in culture condition (El-Sayed, 2006). However, in the wild, Tilapia ingests a wide variety of natural food organisms, including plankton, some aquatic macrophytes, benthic aquatic invertebrates, larval fish, detritus, and decomposing organic matter. They are often considered filter feeders because they can efficiently harvest plankton from the water (Popma and Masser, 1999). Fishery experts have dubbed the tilapia as the "aquatic chicken" because of low-maintenance cultivation, and most importantly its widespread acceptance as a sustainable aquatic food (Prabu et al., 2019). Moreover, unlike most other finfish species, tilapias are extremely hardy fish equally adaptable to a range of culture systems such as pond, cage, raceway and super-intensive culture systems under a wide range of environmental conditions (Costa-Pierce, 2000).

Nile Tilapia *(Oreochromis niloticus)* is a species of tilapia and has been widely introduced outside its natural distribution range because of its global importance in tropical (warm-water) aquaculture and capture fisheries (Ellender et al., 2014). Its large natural distribution area in sub-Saharan Africa and its broad ecological tolerance makes Nile tilapia a successful species in a wide range of aquatic habitats (Zengeya et al., 2020). As a result, more than 90% of all commercially farmed tilapia are Nile tilapia (Popma and Masser, 1999). However, producers both from culture and capture are more focusing on the fillet parts of the fish whereas there is lack of information on the nutritive value of the discarded parts.

Garra

The genus Garra is widely distributed in Ethiopia and contains about 12 species of small barbs that belong to the family Cyprinidae (Froeser and Pauly, 2000; Stiassny and Getahun, 2007). It is mainly found in southeastern Ethiopia, southeastern Eritrea and also on the Arabian Peninsula (Saudi Arabia and Yemen) (Tekle-Giorgis et al., 2016). These species have not been evaluated for their food value yet (Stiassny and Getahun, 2007). The main reason raised by the local fishermen is the small size of the fish. Also

scientifically, there is no study report that indicates the nutritive value of these fish in the country.

However, the genus has ecological important in fisheries, since it is used as a prey fish for African catfish (*Clarias gariepinus)* and *Labeobarbus* (*Labeobarbus intermedius)*, which are commercially important fish species in the country (Elias Dadebo, 2000). The numerous species of genus *Garra* in the country have been overlooked, although they could play a role in aquatic food production in the country. Scientific work is needed to determine its nutritive value and how the species can be brought into aquaculture. The local community should also be made aware if *Garra* has an interesting nutrient profile for the human diet.

Garra quadrimaculata is one the species in the genus *Garra* and indigenous fish, found in many of the Ethiopian water bodies including Gilgel Gibe reservoir (Stiassny and Getahun, 2007; Wakjira and Getahun, 2017). Some *Garra species are* abundantly found in the country's water bodies. For example, a recent report from Ethiopia on fish diversity in the River Debbis showed that *Garra quadrimaculata* and *Garra chebera* are the most dominant species (Urga et al., 2017). It is a benthic, non-migratory, freshwater fish that thrives in tropical climate (Froeser and Pauly, 2000; Tekle-Giorgis et al., 2016). *G. quadrimaculata* is a small fish, grows to a maximum total length of 15 cm and a weight of 40 g (Tekle-Giorgis et al., 2016).

Furthermore, knowledge on proximate composition, macro- and microminerals and toxic trace element sequestration in the body of *G. quadrimaculata* is vital to understand and provide scientific evidence for further integration of this species into aquatic food production systems in the country.

Labeobarbus

Labeobarbus is also a genus in the Cyprinidae family. Its species are widely distributed throughout eastern Africa and southern Africa. The Lake Tana (Ethiopia) is well known

for its *Labeobarbus* species richness and a commonly used term for the lake is *Labeobarbus* flock in which 16 species are reported from (Anteneh et al., 2012).

Labeobarbus intermedius is one of the species in the family and widely distributed in Northern Kenya and most parts of the Ethiopian drainage basin (Dadebo et al., 2013) and dominantly inhabits the Ethiopian Rift Valley basin, Abay basin and the Baro-Akobo basin part of Ethiopia (Vijverberg et al., 2012). The feeding habit of *L. intermedius* from different Ethiopian lakes indicates that the fish is an omnivore, mainly feeding on gastropods, phytoplankton, macrophytes, insects, insect larvae, detritus, nematodes and others (Desta et al., 2006; Dadebo et al., 2013).

L. intermedius is rarely captured and consumed in the country. The fish has been overlooked for production and consumption in the country because of bone fullness (the fish body is composed of less meat and high bone) (Dadebo et al., 2013). However, several studies confirmed that fish bone is a source of micronutrients (Kawarazuka and Béné, 2010). Thus, studying the nutritive value of such fish as whole and fillet part would contribute to the country's aquatic food production diversification and would provide information on the importance of eating whole fish with bone in micronutrient intake.

Fish as a source of macronutrients

Freshwater fish is an important source of macronutrients and micronutrients (Memon et al., 2011). As a macronutrient source, fish and fish products are valued and important for their quality proteins and its derivatives (essential amino acids). However, the macronutrient composition of fish would vary significantly according to species, sources, age, physiological stage and the way in which they are processed and marketed (Table 1. 2) (Jim et al., 2017). The macronutrient composition variability in fish is highly linked to fat deposition and protein composition (Memon et al., 2001), hence the fat content of fish is mainly dependent on biological state of maturity, on nutritional status, age, catching ground and season.

Table 1.2 Summary of mean macronutrient composition of Nile tilapia fillet from different countries and some other freshwater fish species (% in wet weight basis)

Species	Country	MC	CP	CF	Ash	References
Nile tilapia	Ethiopia	79	18.8	0.6	1.4	Geremew et al.,2020
Nile tilapia	Nigeria	76.4	17.6	1.9	3.8	Ayanda et al., 2019
Nile Tilapia	Zimbabwe[1]	79.8	13.9	1.7	1.8	(Jim et al., 2017)
Nile Tilapia	Zimbabwe[2]	75.3	17.1	1.7	3.3	(Jim et al., 2017)
Nile Tilapia	Zimbabwe[3]	77.5	16.5	3.2	2.3	(Jim et al., 2017)
Labeo calbasu	Pakistan	77	18.8	1.0	2.3	(Memon et al., 2010)
Labeo gonius	Pakistan	79.5	19	1.5	0.5	(Memon et al., 2010)
Labeobarbus	Ethiopia	80	15.4	2.4	1.4	(Geremew et al.,2020)
African catfish	Ethiopia	80.5	15.2	1.8	1.3	(Geremew et al.,2020)

MC= moisture, CP= crude protein, CF= crude fat; 1 = from lake Manyame; 2 = from Lake Kariba; 3 = Chivero

Despite macronutrient composition in fish is the most reported part (Memon et al., 2010; Jim et al., 2017; Ayanda et al., 2019 Geremew et al., 2020), some fish species are overlooked in terms of their macronutrient composition. For example, the Garra species has not been reported for its macronutrient composition. To date, no scientific reports exist on macronutrient composition of Garra species. Similarly, these fish species have been overlooked for their nutritive value in the continent. Therefore, generating data concerning the macronutrient composition of such fish is essential to provide information on how consumption of this fish can be safe. This could also positively influence the attitude of the local community towards consuming this fish. Similarly, there is lack of macronutrient composition data on Labeobarbus species.

Nile tilapia is one of the most studied fish species in the world for its nutritive value. Several authors reported on the fillet macronutrient composition of Nile tilapia (Memon et al., 2010; Jim et al., 2017; Ayanda et al., 2019 Geremew et al., 2020). Lately, in some developed countries, scholars started looking on the fish by-products for its

nutritive value. Globally, the expansion of fish processing has resulted in increasing quantities of by-products, which may represent up to 70 percent of processed fish (Al Khawli et al., 2019). Historically, fish by-products were often: thrown away as waste or used directly as feed for aquaculture, livestock, pets or animals reared for fur production; or used in silage and fertilizers. However, other uses of fish by-products have been gaining attention over the past two decades, as they can represent a significant source of nutrition and can now be used more efficiently as a result of improved processing technologies (Al Khawli et al., 2019).

To date, no reports exist from developing countries (e.g. Ethiopia) on the macronutrient composition of Nile tilapia by-products categorizing into various tissue parts. However, in Ethiopia, Nile tilapia capture production contributes to 60% of total capture fisheries and its consumption is only focusing on the fillet part and discarding the by-product to the open field (Figure 1.3). Generally, the post-catch fish losses represent a huge economic and environmental concern occurring in most fish distribution chains, with large amounts of landed fish lost or discarded between landing and consumption (FAO, 2016). During fish processing, a higher percentage fish goes to the wastage of by-product (Al Khawli et al., 2019). Therefore, to transform the by-products to a usable form, there is a need to have insight on the macronutrient and micronutrient concentrations and how these are affected by environmental factors.

Figure 1.3 Fishermen collecting Nile tilapia fillet and throwing it's by- product to the open field at the Lake Langano, Ethiopia, (photo is credited to Tokuma Negisho @2018)

Generally, investigating the macronutrient profiles such as protein, lipids, and moisture content is necessary to evaluate if the overlooked (non-targeted for human consumption in the nation) fish species (*Garra* and *Labeobarbus*) and fish by-products (discarded tissue parts of Nile tilapia) meet the requirements of food regulations and commercial specifications.

Fish as a mineral source

Minerals are inorganic substances required by the organisms (terrestrial and aquatic) in small amounts for a variety of functions (Öztürk et al., 2009). Minerals play a key role in physiology and metabolism of an organisms and they are essential constituents of body fluids and tissues; as components of enzyme systems and for normal nerve function (Öztürk et al., 2009). The functional role and deficiency symptoms of minerals in human nutrition are summarized in Table 1.3. Based on their amount needed for intake, minerals are categorized as macrominerals, microminerals (essential trace elements) and toxic trace elements.

Fish is a good source of both macrominerals (calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P) and microminerals (iron (Fe), zinc (Zn), copper (Cu) selenium (Se), iodine (I), manganese (Mn) (Porto et al., 2016). At the same time, based on the habitat where the fish has been harvested or nutritional composition of the feed that has been fed during aquaculture (culture condition), they would also be a source of toxic trace elements to the consumer (Türkmen et al., 2009). Studies have reported that small species of freshwater fish consumed whole, including bones, heads and gut are a good source of micronutrients (macro and micro-minerals) (Kawarazuka and Béné 2010). Consuming of such small species of freshwater fish therefore have great potential for overcoming micronutrient deficiency which is currently prevalent worldwide, especially in third world countries. For example, in Bangladesh, according to the report of Roos et al (2007), the daily consumption of small fish is estimated to contribute 40% of the total daily requirement of vitamin A and 31% of calcium at the household level. A report from the same country indicated that, small fish such as mola (*Amblypharyngodon mola*) and chanda (*Parambassis baculis*), have high levels of micronutrients (Roos, 2001). Similarly, small indigenous fish species such as chanteas phluk (*Parachela sianensis*) and changwa mool (*Rasbora tornieri*), in Cambodia also reported to contain higher micronutrients (Roos et al., 2007). A comparative study of fillet parts of large size fish species with small fish species eaten whole (Reksten et al.,

2020), resulted that small size fish species are in general significantly more nutrient dense in terms of micronutrients than large fish.

Contrastingly, in Ethiopia small fish are even overlooked for human consumption. Consequently, the country is suffering from undernutrition and micronutrient deficiency (Abebe et al., 2008). Despite the small species of freshwater fish are reported as a good source of micronutrients, in Ethiopia there is no scientific report of the nutrient concentration of small fish species such as *Garra* and *Labeobarbus*.

Table 1.3. A summary on micronutrients (minerals) functions and deficiency symptoms in human

Minerals	Role	deficiency symptoms
Calcium (Ca)	Healthy bones and teeth; integration and regulation of metabolic processes, the control of muscle contraction and blood clotting	In children, results in rickets and, in adults, osteomalacia
Phosphorus (P)	Structure of cell membranes, with calcium involved in formation of bone component, hydroxyapatite.	loss of appetite, anxiety, bone pain
Sodium (Na)	Regulate body water content and electrolyte balance	Weakness, headache
Magnesium (Mg)	Activation of many enzymes (for example enzymes concerned with the replication of DNA and the synthesis of RNA), parathyroid hormone secretion, muscle and nerve function	Muscle weakness and neuromuscular dysfunction
Iron (Fe)	Haemoglobin, enzymes in electron transport chain (e.g succinate dehyrogenase)	Anemia
Copper (Cu)	Ceruloplasmine, and enzymes in oxidative phosphorylation (cytochrome oxidase) and	Myelopathy

	antioxidant system (superoxide dismutase)	("swayback"), anaemia, diarrhoea, depigmentation of hair
Zin (Zn)	Cell replication, several enzyme systems (e.g. alcohol dehydrogenase, lactate dehydrogenase)	Growth retardation, loss of appetite, and impaired immune function
Manganese (Mn)	Pyruvate carboxylase, superoxide dismutase, enzyme activator	Poor growth, skeletal abnormalities
Iodine (I)	Triiodothryonine (T3) and tetraiodothyronine (T4)	Goitre, reproductive abnormalities
Selenium (Se)	A wide variety of enzyme systems, related with antioxidant system (e.g. glutathione peroxidase), hormone synthesis (type I iodothyronine deiodinase)	Foggy mental state Weakened immune system
Chromium (Cr)	Glucose metabolism, possibly lipid synthesis and protein metabolism	Low growth and reproduction

Reviewed from (Institute of Medicine, Food and Nutrition Board, 2001)

Generally, fish has a critical role in providing essential trace element and macrominerals in human nutrition. The dietary essential trace element requirement and recommended intake for human are summarized in Table 1.4. Moreover, the requirement varies according to age, and physiological state (such as state of health and pregnancy).

Table 1.4 Recommendations for human essential trace element intake

	WHO/FAO (2002), RNI[1]		FNB, IM (2000), RDA[2,3]	
	Man	Woman	Man	Woman
Fe mg/d	9-27[a]	20-59[a]	8	18
Cu mg/d	-	-	900	900
Zn mg/d	3-10[a]	3-10[a]	11	8
Mn mg/d[4]	-	-	2.3	1.8
Se µg/d	34	26	55	55
I µg/d	150	150	150	150
Mo µg/d	-	-	45	45

[1]World Health Organization/Food and Agriculture Organization of the United Nations (2002): RNI = recommended nutrient intake,

[2,3] Food and Nutrition Board, Institute of Medicine (2000a,b): RDA = recommended daily allowance

[4]AI= Adequate intake

[a]Range depending on bio-availability of micronutrients

Distribution of minerals in fish tissues

All forms of aquatic animals require minerals for their normal life processes. Contrasting to most terrestrial animals, fish can absorb some minerals not only from their diets but also from their external environment in both freshwater and seawater (Öztürk et al., 2009). Many essential minerals are required in such small quantities that it is difficult to formulate diets and maintain an environment that is low in minerals to demonstrate a mineral deficiency. Despite new developments in the mineral nutrition of fish, most research on mineral has been confined to osmoregulation, toxicity, and related physiological functions.

Tissue mineral distribution of fish is highly governed by habitat and feed type. In aquatic environments, both essential and toxic trace elements are produced from natural and anthropogenic sources and the degree of contamination in fish tissues depend on the pollutant type, fish species, sampling site, trophic level, and their mode of feeding (Allinson, et al., 2009). Fish caught from polluted aquatic environment showed higher mineral accumulation within different tissue parts (Öztürk et al., 2009).

Table 1.6 Summary of mean tissues concentrations of trace elements from various fish species

Fish	Tissue	Fe	Cu	Zn	Mn	Cd	Cr
Nile tilapia	Muscle	65	2.3	24.9	1	0. 5	1.9
	Gill	196	8.3	92	35	<0.01	1.3
	Liver	710	779	116	21	1.70	0.8
Common carp	Muscle	17 ± 7	3.9	-	-	0.2 ±0 .07	1.2 ± 0.7
	Gill	203 ± 106	4 ±1	-	-	0.2 ± 0.2	1.6 ± 0 .7
	Liver	94 ± 55	9.7 ± 4	-	-	0.8 ± 0.3	0.8 ± 0.5
	Heart	118 ± 34	12 ± 6	-	-	0.5 ± 0 .3	1.3 ± 0.4
Pike	Muscle	1.4	0.1	9.4	0.2	-	-
	Gill	24.9	0.4	170	2.3	-	-
	Skin	3.9	0.5	115	5.7	-	-
	Liver	55	2.6	42.5	0.7	-	-

Reviewed from (Allinson**,** et al., 2009; Öztürk et al., 2009; Ayanda et al., 2019); (for Nile tilapia mg/kg dw except Mn (mg/kg ww; for Cyprinus carpio, mean ± SD mg/kg ww; for Pike µg/g ww)

Health risks of aquatic foods

Despite aquatic foods have plenty of health and nutritional benefits, it can accumulate contaminants from the water in which they live or from the food they eat (Jennings et al., 2016). These contaminants may accumulate to levels that can be harmful to the consumers. Moreover, aquatic foods are highly perishable products, and failures in various areas of the value chain, such as storage and distribution, can lead to the food being contaminated, with adverse effects on diets and health. About 80 percent of outbreaks of seafood-borne illnesses are down to biotoxins (ciguatoxin), scombrotoxin or the consumption of raw fish (Huss et al., 2000). A number of biological, chemical and physical hazards are associated with aquatic foods.

The safety of aquatic products varies considerably and is influenced by a number of factors such as origin of the fish, microbiological ecology of the product, postharvest handling and processing practices and traditional preparations (Huss et al., 2000). The aquatic food safety concerns can be biological (due to bacteria, viruses or parasites) or chemical (biotoxins) and can stem from environmental and anthropogenic sources, leading to concerns over the safety of consuming aquatic food products (Jennings et al., 2016).

Nowadays, plastic litter is also becoming a major problem for aquatic environments and microplastic accumulation in fish may pose a risk to the consumer (Thiele et al., 2021). Microplastics are small-sized plastics of less than 5 mm in diameter (Issac, and Kandasubramanian et al., 2021), detected in the gastrointestinal tracts of many commercial fish and shellfish species. Furthermore a laboratory study has already confirmed ingestion of microplastics present in fishmeal in *Cyprinus carpio* (Hanachi et al., 2019). In this case, small fish are commonly eaten whole including gastrointestinal tracts, thus eating whole fish could also be a main source of microplastics.

1.6 Nutrient metabolism in fish

Metabolism is the complex process in living cells, involving a set of chemical reactions that modifies a molecule into another to essentially maintain the living state of a cell or an organism (Kornberg, 2020), and its pathways rely upon nutrients (Da Poian et al., 2010). Like terrestrial animals, metabolism is an important component of fish energy budgets and it can affect the scope for growth in fish by reducing the amount of consumed energy available for growth (Chung et al., 2019). Metabolism has been primarily modeled in terms of fish energy budgets as a function of fish mass (Nordlie, 1978; Hunt von Herbing and White, 2002) and water temperature (Cech et al., 1985; Butler et al., 1992; Chipps et al., 2000).

The metabolic mechanisms in fish are much the same as those in mammals (Chung et al., 2019). The important functional differences between intermediary metabolism in fish and the more completely studied mammals lie in the means by which control is exercised, in the sensitivity of metabolic demand to biotic and abiotic factors, and in the exact roles of tissues and organs (Jeppesen and Kiens, 2012; Jensen and Richter, 2012; Hawley et al., 2014;). Comparatively, little is known for ectotherms and fish generally have a lower ability to use carbohydrates as energy sources, this could be because of low availability of glucose sources (mainly hexose sugars and starch) in aquatic environments and also fish is generally poor in glucoregulators (Hemre et al., 2002). Various factors are responsible to influence metabolism; specifically the aquatic ecosystem is influenced by a wide variety of environmental variables (e.g. temperate and oxygen), and its fluctuations are directly or indirectly influencing the metabolic rate of fishes (Schlaff et al., 2014).

Carbohydrates are the most important fuel for energy production in terrestrial animals (Weber et al., 2016), but as explained above, the history of carbohydrate metabolism in fish is different. Glucose can contribute to energy metabolism in working muscles and is an essential fuel for the nervous system (Mergenthaler et al., 2013). As reported by Wasserman, (2009), to ensure the adequate supply of glucose substrate, mammals and birds need to achieve blood glucose homeostasis to avoid metabolic dysfunction associated with hypoglycemia or the toxic effects of hyperglycemia. However, fish are classically considered as poor glucoregulators, a typical example (Polakof et al., 2012), rainbow trout, normalized glycemia very slowly in glucose tolerance tests and showed limited sensitivity to insulin. This limited sensitivity to insulin is associated with a deficiency in glucose transporters in fish (Wright et al., 1998).

Reports on carbohydrate metabolism in fish lack consistency. For instance, dietary carbohydrate is dispensable for fish (National Research Council, 2011) and has no negative effects on the growth of trout; instead, an improvement of specific growth rate was induced by a carbohydrate-rich diet. Similarly, supplementation of dietary

carbohydrates increased fish growth performance in other studies (Singh et al., 2006; Mohanta et al., 2007). In aquaculture production cost, sparing of dietary protein is crucial. However, a very high level of inclusion of dietary carbohydrates decreases growth performance and induces metabolic disorders in carnivorous fish (Bergot, 1979; Enes et al., 2009; Skiba-Cassy et al., 2013; Kamalam et al., 2017). Dietary carbohydrates not only affect glucose metabolism but also strongly impact the lipid and energy metabolism in liver and muscle of trout (Xuerong et al, 2018).

Lipids are the most crucial substrate for sustained muscle work because they pack more energy per gram than any other fuel (Weber, 2011). Dietary lipids serve a diverse array of purpose in the organism. It constitutes the major macronutrient class required to cover all nutritional and energetic demands in fish along with proteins and to a lesser extent carbohydrates. Therefore, fish muscle is particularly dependent on fat stores to support endurance swimming. Lipid oxidation also plays an important role after swimming to exhaustion, when it provides most of the ATP for white muscle recovery (Richards et al., 2002). Like mammals, excess fat accumulation and metabolic disorders have been a worldwide problem in farmed fish (Du et al., 2008).

Metabolically, proteins play only a trivial role as an energy source in mammalian muscles, but the situation could be quite different in fish metabolism. Mommsen et al. (1980) reported that in sockeye salmon, proteins become the dominant fuel towards the end of migration, when all other substrates reach depletion. However, little is known about the use of proteins as a fuel for muscle work in fish. This could be related to the scarcity of information stemming from the fact that proteins have less importance in mammalian energy production, and research in fish nutrient metabolism is still emerging (Weber et al., 2016).

Generally, fish nutrient metabolism is a growing body of research and the direction of metabolic fish studies changed drastically with the advent of modern aquaculture and a high demand for models to study nutrition, physiology and metabolism (Hancz and Varga, 2017). Despite free ranging fish are challenged by heterogeneous

environmental changes, the available organic nutrient from primary and secondary producers in the aquatic environment impact fish growth and body composition. Hence, the available nutrient type that affect fish metabolism are likely to have a direct impact on the fish growth and tissue nutrient composition (Da Poian et al., 2010).

An aspect that is largely uncovered in studying the nutrition of free-living fish, is the importance of the balance of energy sources in their diet. As mentioned higher, fat is the most efficient energy source, yet this comes with an important condition: fatty acids need to be combusted in the citric acid cycle (Krebs' cycle) through formation of acetyl coenzyme A (Quijano et al., 2016), which can only form citric acid through reaction with oxaloacetate (Figure 1. 4). The latter cannot be produced from fatty acids but needs glucogenic sources.

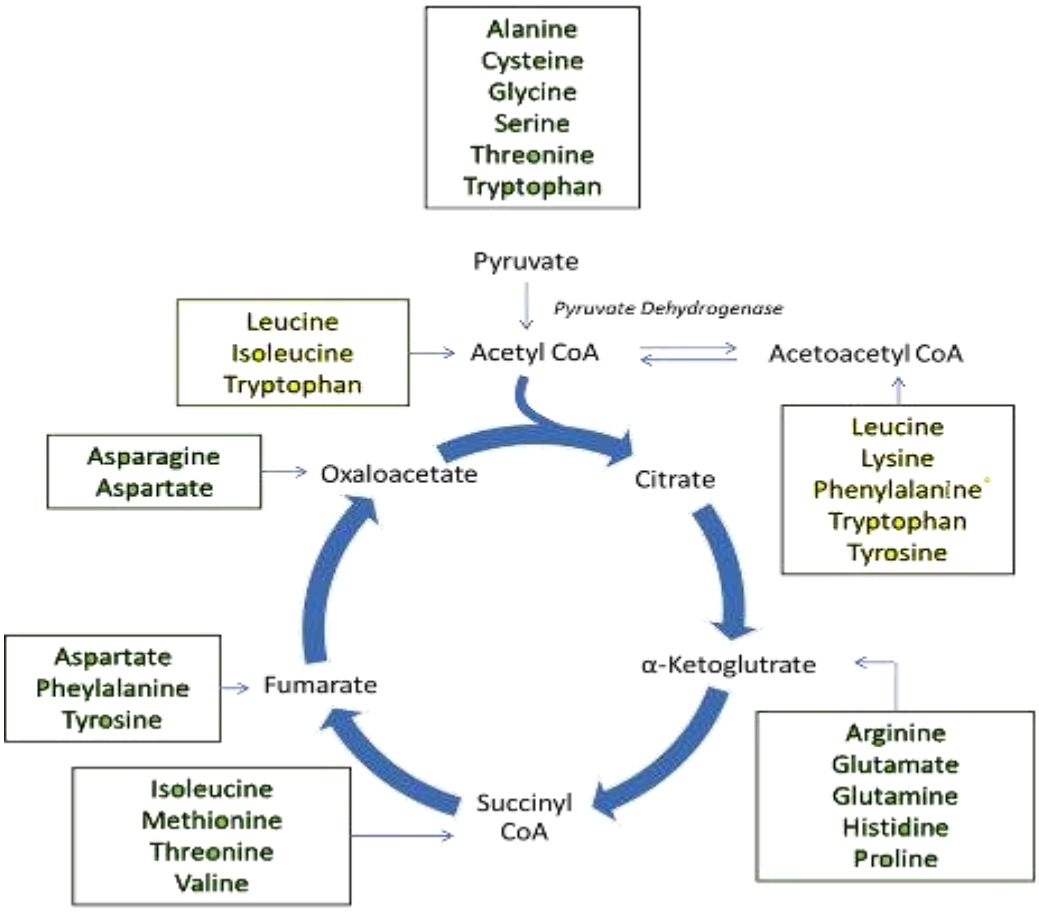

Figure 1.4 Glucogenic amino acids pathway in energy production. Glucogenic amino acids are listed in green boxes and ketogenic amino acids are listed in yellow boxes.

The most obvious glucogenic source is glucose but as described higher, most fish have a limited capacity to digest and metabolize carbohydrates (Hemre et al., 2002). Assuming that propionic acid formation through intestinal fermentation is low in most fish, the only potential glucogenic sources are amino acids from protein. This would mean that a lack of protein as energy source would reduce the efficacy of fat as an energy source. There is one possible pathway to provide oxaloacetate, which is through the breakdown of the exoskeleton of prey items through endogenous chitinase enzymes.

Although chitinase has been studied intensively in fish and other aquatic species (Fujimoto et al., 2002; Banerjee et al., 2016; Gao et al., 2017), little is known about the metabolic fate of digested chitine. The building block of chitine is N-A-acetylglucosamine. Just by its structure, it is logical to assume that it would break down after absorption into the acetyl moiety and glucosamine, which is easily converted into glucose. The above demonstrates that nutrient metabolism in fish still has lingering questions that are important to understand how well fish are using energy sources in particular habitats (whether in aquaculture or free-living).

1.7 The carnitine and free amino acid profile

Carnitine is a quaternary ammonium compound involved in metabolism in most organisms (Figure 1. 5). It plays a crucial biological role in the transporting of a long chain fatty acids into the mitochondria for subsequent β-oxidation, a process that results in the esterification of carnitine to form acylcarnitine derivatives (Bremer, 1983; Bahl; and Bressler, 1987). Moreover, carnitine also plays a role in facilitating the removal of short-chain organic acids from mitochondria, thereby freeing intramitochondrial CoA and acetyl-CoA to participate in the fatty acid β-oxidation, Krebs cycle and other energy metabolism pathways (Marcovina et al.,2013; Jang et al., 2014; Zhang et al., 2014; Novakova et al., 2016).

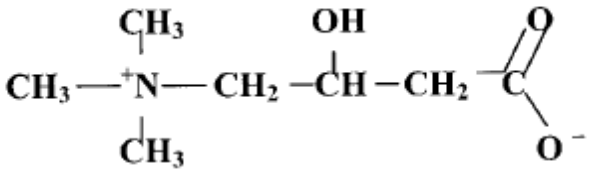

Figure 1.5 Carnitine structural formula (Peluso et al., 2000)

In plasma and in whole blood, total carnitine is present in the form of either free carnitine (non-esterified molecule), or acylcarnitines (esterified form) (Figure 1. 6). The ratio of acylcarnitine to free carnitine is a biomarker to measure the acylated carnitines versus the free carnitines. Measuring tissue concentration of carnitine is crucial to get insight on how an organism copes with the diet available in their environment, because changes in carnitine requirement are correlated with changes in nutritional status (Yu Li et al., 2018).

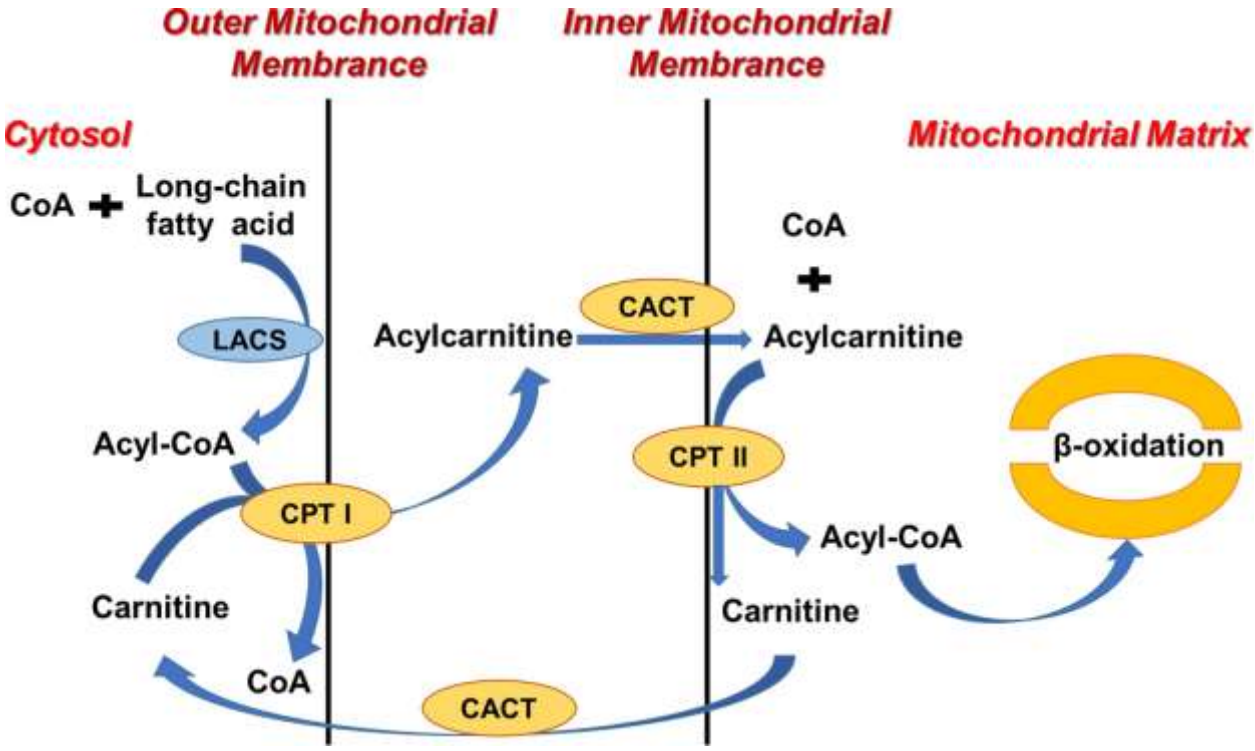

Figure 1.6 Carnitine shuttle. CPT I = carnitine palmitoyltransferase1; CPT II= carnitine palmitoyltransferase 2; LACS = long-chain acyl-CoA synthetase; CACT= carnitine acylcarnitine translocase and CoA, coenzyme A. Carnitine enters the cell through active transport by the high affinity carnitine transporter. Long-chain fatty acid is transformed into acyl-CoA after the catalysis of LACS. The carnitine palmitoyltransferase system then transport acyl-CoA from cytoplasm into mitochondrial matrix

for oxidation: CPTI converts acyl-CoAs into acylcarnitines. CACT exchanges acylcarnitine and carnitine between outer and inner membranes of mitochondrial and finally acylcarnitine is converted back into acyl-CoAs for oxidation by CPTII (Adapted from Yu Li et al., 2018).

Nowadays, analyzing vast arrays of metabolites receives much attention in the field of metabolomics that combines strategies to identify and quantify cellular metabolites using sophisticated analytical technologies (Roessner and Bowne, 2018). A technique targeted to nutrient metabolism, is the metabolic profiling of carnitine esters (acylcarnitines) in combination with selected free amino acids. Among other analytical techniques, nuclear magnetic resonance spectrophotometry and tandem mass spectrophotometry are the more frequently reported methods in literature (Bertram and Jakobsen, 2018). Acylcarnitine profiling is frequently used for neonatal screening of metabolic disorders (Antunes et al., 2015). This could be due to its speedy detection system, the capability to analyze for many different compounds in a single analysis, and a minimal need for auxiliary assay reagents (Grüner et al., 2015).

For decades, analyzing of acylcarnitine profile was limited to the field of human medicine to investigate biochemical screening of disorders of fatty acid oxidation and organic acid metabolism in humans (Rinaldo et al., 2008). Recently, the studying of nutrient metabolites via analysis of acylcarnitine profiling using dried blood spot samples has been extending to other animal species including aquatic organisms. Worku et al., (2021) measured seasonal and agro-ecological effects on nutritional status in tropical ranging dairy cows; Brenes-Soto et al., (2019), investigated the real image of nutrient use in anuran (frogs) metabolism; Geda et al., (2017) evaluated metabolic responses to changes in water temperature in carp and tilapia; Dermauw et al,. (2013), evaluated blood and plasma acylcarnitine concentrations in relation to mineral digestibility in zebu cattle and Verbrugghe et al., (2009), studied modulation of glucose and amino acid metabolism in cats. To our knowledge, no results have been reported on nutrient metabolite profiles to explain differences in fish performance in different wild aquatic ecosystems using acylcarnitines and amino acid profiling.

The advantages of using dry blood spot samples for the analysis of acylcarnitines and amino acid profiling have been documented in literature (Grüner et al., 2015). The major highlights are: (i) the volume of blood required is less compared to conventional venipuncture; (ii) the potential risk of bacterial contamination and/or hemolysis with traditional method is minimal with dried blood spot samples, (iii) collection of blood is easy, low-invasive, and economical; (iv) blood spots can be conserved for long periods with almost no deterioration of the analytes.

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CHAPTER 2: Scientific Aims

Fish and fishery products play a pivotal role in food and nutrition security, and its importance is growing worldwide (FAO, 2014; Grafton et al., 2015, FAO, 2018). However, as stated in (Toppe et al., 2017), people have never consumed as much fish nor depended so much on the fisheries sector for their livelihoods. As a matter of fact, micronutrient deficiency is affecting most developing countries (Swaminathan et al., 2013; Joy et al., 2013). This is prevalent in women and children in sub-saharanAfrica and Asia (Micronutrient Initiative, 2009; Kawarazuka and Béné, 2010; Kawarazuka and Béné, 2011; FAO, 2014). In such instance, a diet shift towards diverse fish species could be a sensible remedy. Recently, aquatic food in general, fish in particular, is recognized a sector to be fortified as nutrition-sensitive agriculture (FAO, 2014).

However, in sub-Saharan Africa, specifically in Ethiopia, aquatic sources of food diversification have been overlooked. For instance, despite the country is rich in ichthyofauna (Getahun, 2007; Golubtsov and Darkov, 2008), the nation is accustomed to eat few number of fish species, which is mostly based on consumption of only the fillet (muscle) part. This could have emerged from the nation's lack of knowledge on i) the importance of fish in the human diet, ii) the fish nutritional value of different tissue parts, iii) the nutritive value of overlooked indigenous fish species like *Garra*and *Labeobarbus*. By contrast, it is suggested that a dietary inclusion of varieties of fish and fish products is crucial to increase the intake of important nutrients within the community (Osman et al., 2001; Tacon, and Metian, 2015).

Within Ethiopian fishermen communities, this is an often heard statement: "We don't like to eat *Garra* because of its small size." Acquisition and dissemination of objective insights will be needed to create a change of habits.

Studies have reported that smaller fish species are more nutritious than the larger fish (Kawarazuka and Béné, 2011), since they are consumed whole with bones, heads and viscera. These particular tissues are supposed to sequester more important nutrients and would increase the intakes of valuable micronutrients.

In Ethiopia, both capture and culture fisheries are focusing on a relatively narrow diversity of fish species (Tesfahun, 2019), which likely underutilizes the nation's aquatic resources for food and nutrition provision, because fish species varies considerably in their nutrient composition and density (Bogard et al., 2015). Given the wide diversity of aquatic life, fish nutritive value, especially mineral concentrations could be influenced by various factors, such as species, habitat, climate, and tissue characteristics (metabolic activity and homeostasis) (Bogard et al., 2015; FAO, 2018). Thus, investigating the impact on these factors in nutritive value, essential and non-essential mineral concentrations in different fish species and tissues is paramount in the aquaculture sector development.

The central aim of this thesis was to investigate the differences in environment and species in distribution of nutrient in fish.

Specific objectives of this thesis were:

To analysis the diversity in essential trace elements distribution within the body of various fish species maintained under laboratory conditions.

An exploratory study was conducted to evaluate the importance of species for micromineral (Fe, Zn and Cu) distribution within the fish body. As a model, we used ornamental fish species kept in a commercial aquarium.

To evaluate the impact of species and their edible parts on the nutritive value of fish from the same aquatic environment,

Garra is a small indigenous fish, not yet evaluated for its nutritive value for human consumption in the local community. We were interested to evaluate the nutritive value of whole *Garra* in comparison with commercially known fish, caught from the same environment (Nile tilapia and *Labeobarbus*). It was hypothesized that entire smaller fish species may be a better choice to provide relevant nutrients than larger

fish, especially when only the fillet is consumed. Therefore, the objective was to evaluate the impact of species on proximate composition and mineral concentrations.

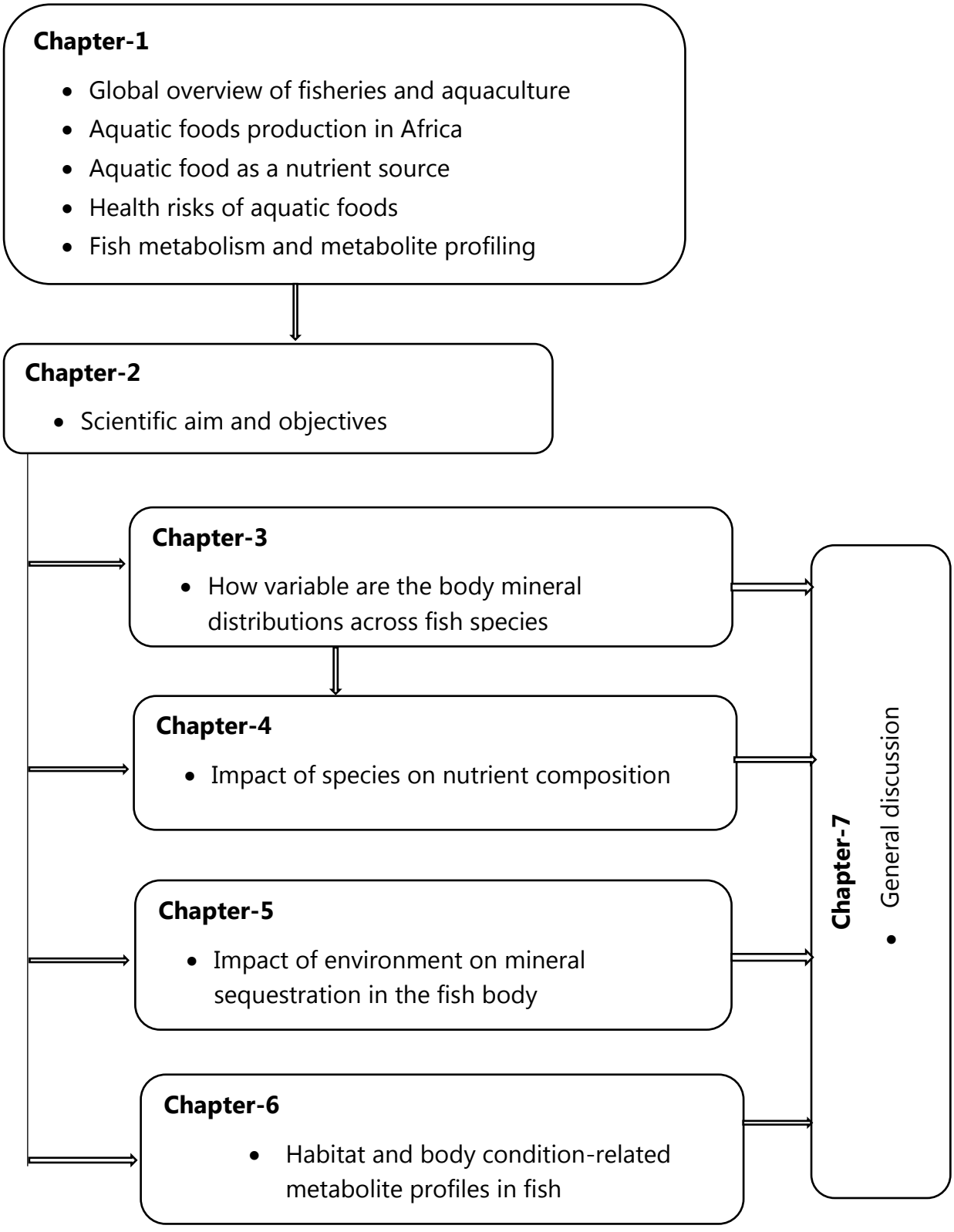
To explore the impact of lake ecosystems on mineral concentrations in tissues of Nile tilapia (*Oreochromis niloticus* L.),

The effect of distinctly different lakes on mineral content of fish was measured to evaluate to which extent the mineral concentrations in fish can be affected by their environment. Nile tilapia was compared from three water bodies, considering that the local community mainly consumed muscle tissue (fillets) and discarded the rest. In addition, the contribution of other fish parts than the fillet in its mineral sequestration was evaluated.

To investigate the nutrient-related metabolite profiles which would explain habitat-dependent differences in body composition and size in fish,

The analysis of circulating concentrations of acylcarnitines and amino acids was aimed to reveal the limiting factors of a specific habitat in energy and nutrient use in fish.

The schematic presentation (helicopter view) of the thesis



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Toppe*,* J., Beveridge*,* M., Graham*,* E. (2017)*. FAO Aquaculture Newsletter*. No. 56*,* pp. 4243. Rom, 2017.

CHAPTER 3: How variable are the body mineral distributions across fish species

Adapted from

Bayissa T.N., Gemeda, G., Du Laing, G., Wakjira, M., G., Janssens, G.P.J. (2020). Diversity in micro mineral distribution within the body of ornamental fish species. *Biological Trace Element Research,* 197, 279–284. https://doi.org/10.1007/s12011-019-01983-1

Abstract

This conceptual study was conducted to evaluate how fish species determine the mineral distribution in their body. Ten different species (n = 3, total = 30) of live ornamental fish were randomly sampled from one big aquarium in a pet store in Belgium. All fish samples were dissected manually for the collection of targeted tissues. The tissue samples were ashed by microwave oven, and the extract was analyzed for copper (Cu), iron (Fe), and Zinc (Zn) by inductively coupled plasma mass spectrometry. Fe was associated with Cu in muscle tissue ($p < 0.05$), but neither of them were associated with Zn in the muscle. However, the three micromineral concentrations were correlated in the heart ($p < 0.05$). Similarly, all of them were correlated in the liver ($p < 0.05$), but none of them showed a significant association in the tail fin. Excess deposition of minerals in heart tissue is a new observation, and it is not known if this is meant as storage or rather the fish heart has a high requirement for microminerals. Storage in the tail fin should be interpreted as a sign of permanent deposition as a tool to dispose of toxic excess. The lack of correlation between the muscular concentrations of Zn on the one hand, and those of Fe and Cu on the other hand, further suggests that fish species distinctly differ in their tissue micromineral accumulation. Although this exploratory study still leaves many questions unanswered, it points to the large diversity in micromineral distribution among fish species and tissues.

3.1 Introduction

In general, a full and comprehensive understanding about micromineral requirements and toxicity in fish is far from complete (Marc, 1999). Identifying the margin between the micronutrient requirement and toxicity is relevant for the aquaculturist. This is because some microminerals are known to have a small margin between requirement and toxicity, leading to situations where a dietary provision of a particular mineral would be near deficiency for one species, but might be toxic for another species (Watanabe et al., 1997).

Nutritional diseases of fish may develop as a result of deficiency (under nutrition), excess (over nutrition), or imbalance (malnutrition) of nutrients present in their food (Stephen et al., 2012; Hixson, 2014). A deficiency in any required nutrient can adversely affect health by impairing metabolic functions and increasing susceptibility to disease (Hixson, 2014). When there is too much food, the excess that is converted to fat and deposited in fish tissues and organs may severely affect physiological functions of the fish (Amar, 2004). Nutrient deficiency should not occur when diets have been formulated and prepared based on the species' requirement. However, some commercially available diets for another species may sometimes be used in the absence of a suitable formulation, resulting in deficiencies (National Research Council, 2011; Hixson, 2014).

As it can be seen from terrestrial species, the body regulates micromineral homeostasis through absorption, storage in and mobilization from tissues, and excretion (Close 2006; Suttle 2010). Copper (Cu), zinc (Zn), and iron (Fe) are examples of essential microminerals that can pose problems with either deficiency or overload across animal species. Their availability in excess inside the cell will change the nature of these elements into toxicity (Marc, 1999; Hixson, 2014). Oxidation-reduction reactions that occur inside the cell play a role in the noxious effect of these elements. Although Cu and Fe attribute to the occurrence of free radicals, Zn acts in the defense against tissue damage.

Historically, keeping ornamental fish is dating back to thousand years for Far East countries and followed by Europe in early seventeenth century. Nowadays, aquarium keeping is among the most popular of hobbies with millions of enthusiasts' worldwide (Earle, 1995; Sales and Janssens, 2003). The production and trade of ornamental fish is a profitable alternative in the aquaculture sector (Ghosh, 2008; Rhyne et al., 2012). Despite the economic importance of this sector, the nutritional information for ornamental fish is scarce; often, few data of the nutritional requirements is available and the dietary requirements of these animals continue to be one of the least explored areas of pet nutrition (Blom and Dabrowski, 2000; Chong et al., 2003; Sales and Janssens ,2003).

Ornamental fishes have traditionally been cultured in multispecies (community aquaria) form in a single aquarium. This may be interesting in terms of hobby and space. However, from a nutritional point of view, it may bear a risk of toxicity for one species and deficiency for another species (Marc, 1999; Suttle, 2010). When ornamental fish are kept in multispecies aquaria, they are fed the same diet in such a setting, hence posing a challenge to feed all species adequately.

In spite of many studies on the nutritional profile of farmed fish, little were performed on ornamental fish (Sales and Geert, 2003). In particular, little is known on the micromineral distribution in different fish species, whereas this study would provide insights in how microminerals are distributed in different tissues and species. The present study mainly targeted on four tissues (heart, muscle, liver, and tail fin) of ornamental fish species as model. Therefore, this conceptual study was conducted to explore the importance of species and tissue parts in determining the distribution of minerals in fish.

3.2 Materials and Methods

3.2.1 Fish Sampling

Ten different species (n = 3, total = 30) of live ornamental fish were randomly sampled from one big aquarium in a pet store in Belgium (Table 3. 1). The fish samples were placed in a double polyethylene bag with sufficient aeration and transported to the nutrition lab at the Faculty of Veterinary Medicine, Ghent University, Belgium. At arrival, they were classified, weighed, measured by total length, and killed by anesthetizing through overdosing of benzocaine (MS 222, 0.8 g/L at pH7.5). Afterwards, all fish samples were dissected manually for the collection of targeted tissues and the tissues were kept frozen at - 20 °C until further analysis.

Table 3. 1. Ornamental fish species used in this study

	Common name	Scientific name	Family name
1	Albino grass carp	*Ctenopharygodon idella*	*Cyprinidae*
2	Golden orfe	*Leuciscus idus*	*Cyprinidae*
3	Golden tench	*Tinca tinca*	*Cyprinidae*
4	Goby	*Gobio gobio*	*Gobidae*
5	Comet tail	*Carassius auratus Var.*	*Cyprinidae*
6	Rudd	*Scardinius erythrophthalmus*	*Cyprinidae*
7	Sarasa comet	*Carassius auratus Var.*	*Cyprinidae*
8	Golden shubunkin	*Carassius auratus* Var.	*Cyprinidae*
9	Sturgeon	*Acipenser sturio*	*Acipenseridae*
10	Blue gill sunfish	*Lepomis macrochirus*	*Centrarchidae*

(For each species three representative samples were taken)

3.2.2 Analytical Procedure

The targeted tissue samples were weighed on a microbalance (Mettler Toledo, AT21 comparator). The samples were homogenized, allowed to react for 12 h in the solvent of 3 mL HNO_3 and 3 mL H_2O_2. Subsequently, samples were ashed by microwave oven

(CEM, MARS6) with the following programme: 10 min at 55 °C and 400 W/10 min at 75 °C and 600 W/40 min at 120 °C and 1200 W. The extract was analyzed for Cu, Fe, and Zn by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, Elan DRC-e). Extracts were diluted 1:10 (v/v) with Ga as internal standard solution. The signal of the sample analyte was always within the standard curve.

3.2.3 Statistical Analyses

All data were checked for homogeneity of variance and normality; the data which were not normally distributed were log-transformed. Pearson correlations were calculated per micro mineral between organs across species. Statistical analysis was performed with SPSS 22.0 for Windows. Significance was accepted at $p < 0.05$.

3.3 Results

The median, minimum, and maximum Fe, Cu, and Zn concentrations across the studied fish showed extreme variations (Table 3. 2). The micromineral concentrations varied with tissue type. The Fe concentration was highest in all tissues except for Zn being higher in tail fin. Zinc concentration was significantly high in the liver and followed by heart, whereas Cu was the mineral with the lowest concentration in every analysed tissue.

Table 3. 2. Tissue concentrations of Fe, Zn and Cu across the ornamental fish species (median, minimum and maximum; based on data pooled per species)

Tissue	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Muscle	13 (5-30)	1.3 (0.4-3.0)	18 (5-48)
Heart	198 (16.5-513)	25 (2-104)	59 (6-220)
Liver	553 (16-21396)	30 (2-350)	51 (4-1118)
Tail fin	33 (13-89)	4 (2-14)	88 (48-223)

Tables 3.3 show variations across species and tissues. In general, muscle tissue showed the lowest concentrations for each of the three microminerals in all species, still with important variation among species. Especially in the liver, the micromineral concentrations varied tremendously, with extremely high values in, for instance, albino grass carp, comet tail, and sarasa. Despite being also a muscle, heart tissue had considerably higher concentrations than body muscle. The tail fin seemed to mainly store Fe and Zn, whereas Cu concentrations were fairly low in that tissue.

Table 3. 3. Mean and standard error (μ± SE, mg/kg) of Fe, Cu and Zn concentrations in the targeted tissues of ornamental fish species (mean

		Sunfish	Sturgeon	Shubunkin	Sarasa	Rudd	Comet tail	Goby	Tench	Orfe	Albino
Muscle	Fe	5.5±0.5	15.0±3.5	15.0±1.0	17.8±3.	12.1±2	17.9±2.5	9.7±1.4	9.7±1.5	9.4 ±1	19.4±6
	Cu	0.4±0.1	1.9±0.5	1.8±0.3	1.4±0.1	1.3±0.1	1.8±0.2	1.0±0.1	1.3±0.1	0.9±0.1	2.3±0.6
	Zn	17±1	25±11	31±4	21±3	22±5	22±2	25±3	7±0.4	7±1	16±7
Heart	Fe	103±7	62±5	148±67	205±14	201±29	181±11	288±24	237±14	178±21	404±73
	Cu	8.6±1.2	8.3±.1	20±9	23±5	14±5	33±4	39±3	30±5	18±2	73±17
	Zn	29±2	39±3	41±18	63±17	64±22	65±5	96±14	86±21	57±13	161±47
Liver	Fe	176±10	221±110	410±279	3837±343	317±255	2786±453	843±103	972±223	99±20	9932 ±5872
	Cu	23±4	37±2	73±57	53±33	33±26	91±15	20±3	40±6	7±2	253±84
	Zn	32±3	44±5	103±68	95±33	222±152	162±43	69±23	83±42	26±4	725±295
Tailfin	Fe	22±6	29±3	37±6	37±6	33±15	40±11	36±2	32±6	30±2	63±13
	Cu	2.4±0.5	4.6±0.6	5.6±1.6	4.2±0.7	9.2±3.4	3.6±1.4	4.4±0.7	4.8±1.0	3.3±1	8.4±1.8
	Zn	148±13	66±5	135±6	110±25	127±49	92±3	101±14	63±8	62±2	92±18

Correlation of the targeted tissues in its micromineral (Fe, Cu, and Zn) deposition levels is shown in Table 3. 4. The level of Fe in liver was significantly correlated with the heart and tail fin ($p < 0.05$). There was no correlation of Fe level in the liver with muscle. Muscle Fe, Cu, and Zn levels were significantly correlated with the tail fin ($p < 0.05$). The level of Zn in the liver was significantly correlated with the heart ($p < 0.05$), but its level in the muscle was negatively correlated with the tail fin. Similarly, the level of Cu in the liver was significantly correlated with the heart ($p < 0.05$).

Table 3. 4. Pearson correlations of Fe, Zn, and Cu concentrations between tissues across ornamental fish

Fe	L	M	H	**Zn**	L	M	H	**Cu**	L	M	H
M	0.54			M	0.09			M	0.49		
H	0.68*	0.19		H	0.72*	-0.34		H	0.57*	0.26	
T	0.77*	0.73*	0.60*	T	0.02	0.70*	-0.42	T	0.31	0.59*	0.31

L=Liver; M= Muscle; H= Heart; T= Tailfin; asterisk indicates a significant correlation at $P < 0.05$

All three microminerals showed correlation between their muscle and tail fin concentrations (Table 3. 5), as well as between their heart and liver concentrations. Remarkably, no correlations were found between body muscle and heart (both being muscles) or between the muscle and liver. Only for Fe, a significant correlation existed between the liver and tail fin concentration. Fe was associated with Cu in the muscle tissue, but neither of them was associated with Zn in the muscle. However, the three microminerals were correlated in the heart and in liver. None of them were correlated in the tail fin.

Table 3. 5. Pearson correlations between Fe, Cu, and Zn concentration in tissues across ornamental fish species

			Liver		
Muscle	Fe	Zn		Fe	Zn
Zn	0.22		Zn	0.80*	
Cu	0.95*	0.25	Cu	0.87*	0.79*

Heart			Tailfin		
	Fe	Zn		Fe	Zn
Zn	0.90*		Zn	-0.16	
Cu	0.88*	0.91*	Cu	0.55	-0.07

Asterisk indicates a significant correlation at $P < 0.05$

3.4 Discussion

The remarkably wide range in micromineral concentrations across species found in this study could be due to a number of factors: Kamaruzzman et al., (2010) and Fernandes et al., (2007) reported that element distribution in different tissues of fish depended on the way of exposure to dietary or aqueous exposure, fish age, gender, and physiological condition of the fish. Similarly, mineral requirement of aquatic animals may be related to the lifestyle (swimming behaviour, habitat) of different species (Table 3.3). In this particular study, there was no information on how long the fish had been exposed to the same diet, so that their feeding history may have been different, at least until they arrived in the same aquarium. The scarce knowledge on mineral tissue distribution in fish especially ornamental fish does not allow considering species-specific sequestrations and comparing the present study with others. Therefore, the observed concentrations in particular species are not necessarily typical for that species. Hence, our aim was to explore the importance of fish species and tissue parts for mineral accumulation as baseline information for the following chapters.

As in many other animals, the liver is a typical storage site of microminerals such as Fe, Zn, and Cu, but especially, this organ showed high variation among species. Studies have reported that minerals are mostly accumulated in metabolically active organs (Dural et al., 2006). Studies have confirmed that the liver has a significant function in basic metabolism, (exchangeable) mineral storage, redistribution, and detoxification or transformation (Agah et al., 2009; Malik et al., 2010). Given that the liver plays a crucial role in synthesizing Fe, Cu, and Zn involved factors, it is not surprising they are highly stored in the liver tissue (Westerlund and Andersen, 1998). The concentration of Fe in the liver was by far higher than other targeted tissues; this is probably due to the presence and metabolism of haemoglobin. Specifically, the liver is the primary organ for Fe bioaccumulation, and it has a vast vascular network where blood passes through. Iron released from the breakdown of haemoglobin, as well as excess Fe found in the body, is stored and detoxified in the liver (Buckley, 1982; Schmidt-Nilson, 1991). This

could also be related to the ability of fish to closely regulate internal micromineral concentrations by producing a large number of binding sites following hepatic metallothionein RNA induction and transcription (Cohen et al., 2006).

The excessive deposition of minerals in heart tissue is a new observation, and it is not known if this is meant as storage or rather the fish heart has a high requirement for microminerals. This requires further study to understand the origin of these high concentrations. One route to look into is whether fish sequester these minerals to organs such as the heart under stressful situations. Close (2006) indicated that some minerals, specifically Cu and Zn, are required at a higher level when animals are under stress conditions.

Because fish fins and scales are void of blood vessels, mobilization of microminerals into the bloodstream is not possible; hence, they can be considered a route for excess storage of microminerals. High concentrations of minerals were sinked in the tail fin, especially Zn, since the Zn concentration in the tail fin even exceeded the hepatic concentration. This agrees with the accumulation of Zn in the hair, claws, and feathers in all studied terrestrial species (Buddhachat et al., 2016; Kaushik, 2002).

Storage in the tail fin should be interpreted as a sign of permanent deposition as a tool to dispose of toxic excess. Excretion of this excess may be an alternative, but since fish then would take in these minerals again from the water they excreted in, permanent deposition in non-accessible tissues would be a better strategy. The fact that this occurs quickly with Zn suggests that, in the tested conditions, Zn can be considered closest to toxicity. Reports confirmed that setting the mineral requirements in fish is much more complicated than that of in terrestrial animals; this could be due to the close interaction of fish with the aquatic environment (Lall, 2002; Fernandes et al., 2007; Kamaruzzaman et al., 2010). The variation among the fish species indicates that the sensitivity to micromineral toxicity may differ substantially among fish species. This raises the issue of feeding multispecies aquaria, because the minimum

requirement for a particular micromineral in one species might already be approaching toxicity for another species.

It is clear from the correlations among minerals within a tissue that homeostasis differs for each micromineral and for each tissue. In the tail fin, storage of one micromineral is unrelated to the others, whereas in the heart and liver, they show clear associations between the three microminerals across species. Therefore, the extents to which fish species differ in their assimilation rate seem similar for the three tested microminerals, but their deposition is markedly different.

The lack of correlation between the muscular concentrations of Zn on the one hand, and those of Fe and Cu on the other hand, further suggests that fish species distinctly differ in their micromineral metabolism. Again, this needs careful consideration, not only when feeding multispecies aquaria with ornamental species but also when it warrants investigation in the differences in micromineral requirements and metabolism of species used for production, since "average" dietary micromineral concentrations may considerably deviate from the actual requirements of particular species. This further underlines the importance of differences in requirements and toxicity of minerals across species. Marc et al., (1999) and Suttle, (2010) already suggested that fish species can vary such in their mineral requirements that a community aquarium may be exposed to a risk of toxicity for one species and deficiency for another species.

It is further remarkable that muscle concentrations did not correlate with liver concentrations but with tail fin concentrations instead. A way to explain this is that fish species with a high metabolic use of microminerals (as reflected by muscular concentrations) have no high need for liver storage, but directly deposit excess in non-exchangeable tissues such as the tail fin. Sampling fin material may therefore not be an accurate method to compare micromineral status in fish. Even muscle types (heart and body muscle) seem to have concentrations independent of each other, again raising the question if one particular tissue is able to present the micromineral status of fish. Although the heart is also a muscle, it thus behaves independently from the

other muscle tissues and in contrast to the other muscle tissue does show clear correlations with hepatic concentrations. This may be due to the morphological proximity of the heart and liver, but a physiological link is not easily explained, and will require further investigation. The heart is of relatively small size in fish compared with, for instance, terrestrial species, and therefore, it is less likely that it serves as a storage organ.

The Fe concentration was the only one of the three minerals that showed an association between the liver and tail fin, which again emphasizes that fish species considerably vary in their ways of dealing with microminerals in metabolism. Even if the exact supply of microminerals in the life history of the fish in our study was not known, the data demonstrate that commonly used diets and management condition exert great differences in micromineral concentrations across fish species, up to levels that are commonly considered to warrant caution for toxicity.

Although this exploratory study still leaves many questions unanswered, it points to the large diversity in micromineral tissue distribution among fish species. This is a general principle that has not received much attention and requires further study since conditions used in commercial aquaculture seem to exert substantial differences in storage across species in fish. It also raises the question on how micromineral status can be assessed in fish species, since deposits such as in the tail fin may not be representative for micromineral status in every species.

In conclusion, the micromineral distribution throughout the body differs substantially across fish species, with different strategies for storage of excess microminerals. These data urge for further study on potential under- of overfeeding of minerals to fish and its implications on reproduction, longevity and nutritive value.

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CHAPTER 4: Impact of species and their edible parts on the macronutrient and mineral composition of fish from the same aquatic environment, the Gilgel Gibe Reservoir, Ethiopia

Adapted from

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Abstract

Fish is an important source of easily digestible animal protein and other essential nutrients. It plays a pivotal role in food security and poverty alleviation in developing countries. However, the nations of the global South consume a limited number of fish species. This study aimed to evaluate the macronutrient and mineral composition of whole fish (*Labeobarbus intermedius*, *Garra quadrimaculata*) and fillet (*Oreochromis niloticus*, *Labeobarbus intermedius*). A total of 64 fish samples were collected from Gilgel Gibe reservoir, Ethiopia, and analyzed for its macronutrient and mineral composition. The proximate composition and mineral contents of fillets and whole body samples were determined. The whole fish showed a much higher fat and ash percentage than the fillets ($p<0.05$). The fillets contained a much higher protein concentration than the whole fish ($p<0.05$). The higher Ca:P ratios in whole fish compared to fillet in our study confirms the importance for a healthy human skeletal development, especially in diets where Ca is typically lacking. Whole *Garra* appeared to be containing important trace elements such as zinc and iron, a feature that was not found to the same extent in the whole *Labeobarbus*. These differences may find its origin in the feeding pattern of these fish species in the reservoir. The advantage of benthic species such as *Garra* to enrich the human diet with essential minerals may, however, coincide with the accumulation of toxic heavy metals as a potential result of soil erosion.

4.1 Introduction

Fish is an important source of easily digestible animal protein and other essential nutrients (Roos et al., 2003; Sarma et al., 2013; Béné et al., 2016). Particularly in the global South, the importance of fish and fish products is crucial for the malnourished, immunocompromised, pregnant women, and nursing mothers (Kawarazuka & Béné, 2011). Achieving and maintaining a nutritionally adequate diet is warranted to maintain the mother's health during pregnancy and breastfeeding (Riordan and Mary, 2005; Marangoni et al., 2016). In developing countries, a diet shift towards diverse fish species could be a sensible remedy. Fish and fish products have a key role in food security and poverty alleviation, in both rural and urban areas of developing countries (Grafton et al., 2015).

As an example, the Ethiopian national diet seems not nutritionally balanced, which is particularly prevalent in children, pregnant women, and lactating women (Mekonnen et al., 2005). Several efforts have been made in reducing childhood malnutrition in the country, but the incidence is still high. A report indicated that more than 1.7 million children, pregnant women, and lactating women are in need of supplementary feeding in the country (FAO, 2016). Modification of the infant, pregnant and lactating women diet with highly nutritious food such as fish then becomes highly imperative (Valenzuela,1999). Osman et al., in (2001), stated that dietary inclusion of varieties of fish and fish products is crucial to increase the intake of important minerals like Fe and Zn within the community.

Ethiopia is rich in ichthyofauna, with about 190 fish species in the country (Getahun, 2007; Golubtsov and Darkov, 2008). Among the mentioned figure of fish species, the nation is accustomed to eat only about six species (Nile Tilapia (*Oreochromis niloticus*), Nile perch (*Lates niloticus*), African catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*) and Labeobarbus (*Labeobarbus intermedius*). This low variety in fish consumption emerged from a lack of knowledge on the importance of fish in the diet as well as consumer acceptance.

In fact, due to increasing population numbers and economic growth, the total demand for fish in Ethiopia is growing. As a result, wild fisheries are over-exploited, specific commercially known fish resources reached their natural limits, and thus led to the decline in stocks of fish of targeted species (NADSE, 2009; Asaminew, 2012). However, the demand for fish consumption is increasing only on the specific tissues (fillet) and a limited number of species (Nile tilapia, African catfish, and Nile perch) are frequently consumed. On the contrary, fishes of the cyprinid family get less attention for consumption in the country. Specifically, the genus *Garra* is hardly known for its food purpose.

Even though there is no proven evidence why genus *Garra* is hardly used for food in the country, there are some assumptions such as small size of the fish, absence of scientific reports demonstrating the acceptable macronutrient and mineral composition of the fish for human consumption, and lack of awareness on aquatic resource utilization in the country. Smaller fish species may however be even more nutritious than the larger fish according to Shakuntala et al., (1996); Torben et al., (2000), small fish species that are consumed whole with bones, heads, and viscera play a critical role in micronutrient intakes, as these parts are known to accumulate more micronutrients. Before recommending those fish for human consumption, investigating its macronutrient and mineral composition compared with locally consumed fish species would be critical (**Chapter 3**). Moreover, comparing the macronutrient and mineral composition of fillets and whole fish can provide insights on whether a shift in eating habits towards entire smaller fish species would be beneficial.

In this study, we evaluated the macronutrient and mineral composition of edible parts of the targeted (Nile tilapia), less targeted (*Labeobarbus*), and non-targeted (*Garra*) fish species in the same water body. The latter is an important aspect because it can show how differences in macronutrient and mineral composition can arise independent of geographical origin.

4. 2 Materials and Methods

4.2.1 Description of the Sampling Area

Fish samples were collected from the Gilgel Gibe reservoir. The reservoir is located 283 km south-west of the capital, Addis Ababa, in Oromia Regional State, South-Western Ethiopia near Jimma town, 70 km north-east of Jimma town (Froese, 2006). GPS based evidence shows that the dam is constructed at an altitude of 1640 m above sea level (asl), at geographic coordinates of 7°42′53′′-7°55′580′′N and 37°11′53′′-37°20′33′′E. Its maximum and minimum water levels during wet and dry seasons are 1671 m and 1653 m asl respectively (Froese, 2006). Its depth ranges between 2 m and 35 m with a mean depth of about 17.6 m and with total coverage of 62 km^2. The reservoir is bordered by three districts (woredas); Omo-Nada, Kersa, and Tiro-Afeta. The area has a sub-humid, warm to hot climate, receives between 1300 and 1800 mm of annual rainfall, and has a mean annual temperature of 19.2°C (Wakjira, 2013).

4.2.2 Animal ethics statement

This study was checked and given approval by Jimma University, College of Natural Sciences Research and Ethical Review Board Committee (Jimma, Ethiopia). The committee has followed the standard procedures written on Article 6 (methods of killing) and Article 9 (Animals are taken from the wild), of directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes and certified this study with the letter referenced (Ref. No: RPG/165/2019). This study was carried out in compliance with the ARRIVE guidelines.

4.2.3 Sample Collection and Preparation

Fish sample collections were carried out during the dry season (October, 2018). Two sampling sites were selected from a range of accessibility and existing variations in habitat type (Bore, as riverine site and Deneba, as a lacustrine site) (Figure 4.1). Moreover, the two sites were included to broaden the validity of the differences in fish proximate composition and mineral content. A total of three fish species (*Garra quadrimaculata* (n=16), *Labeobarbus intermedius whole* (n=16*) and Labeobarbus intermedius* fillets (*n=16),* and *Oreochromis niloticus* (n=16) were collected fresh on the boats as soon as they arrived at the selected landing sites Table 4.1. The Fish samples were thoroughly washed with tap water, separately wrapped in a polyethylene bag, placed on ice, and brought to the laboratory of the zoological sciences of Jimma University, Ethiopia.

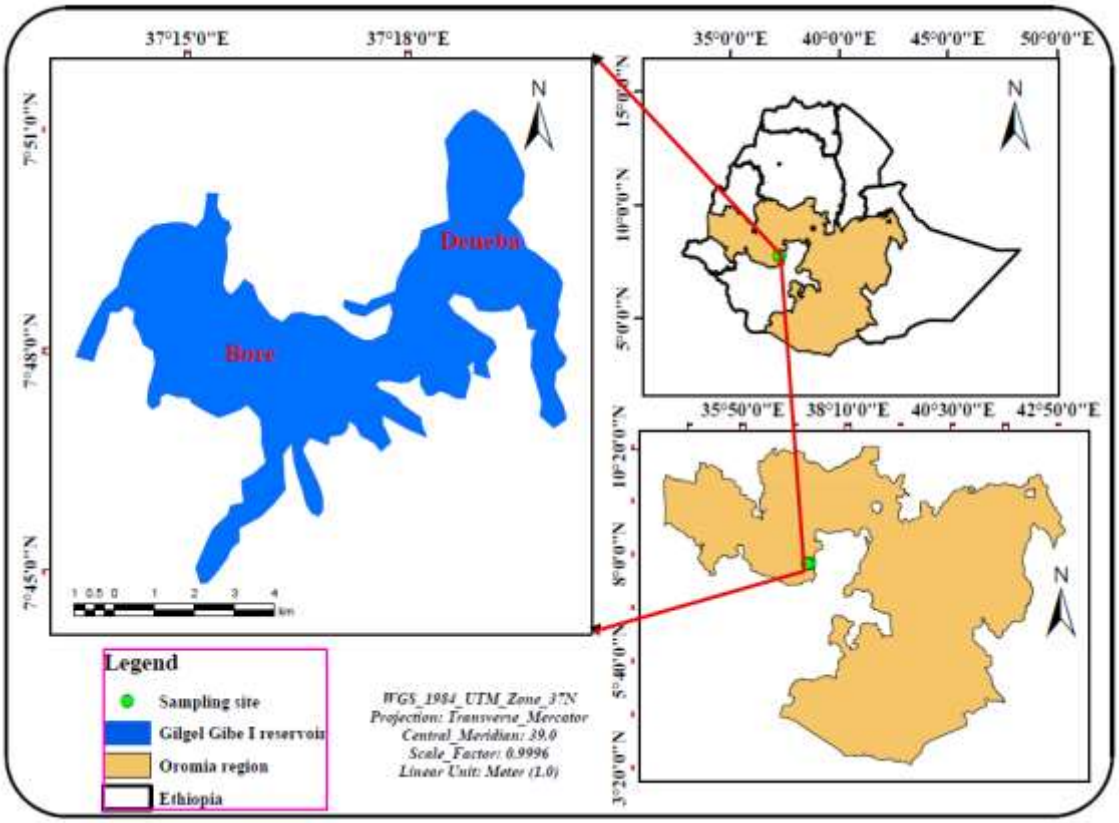


Figure 4.1. Map of the study area (Gilgel Gibe reservoir, Jimma, Ethiopia), 2019

Upon arrival, the total body weight and total body length of all fish were measured to the nearest 0.1 g and 0.01 cm respectively. Because of too small sample amounts of tissues such as muscle to analyses both proximate composition and minerals from individual fish, the fish samples were combined (pooled) into three groups (5, 5, and 6 fish). Each pooled sample was analyzed in triplicate. Samples were analyzed as fillet and/or whole to represent a realistic use of these fish species. *Garra* is small fish and analyzed whole, assumed to be consumed whole. Fillet of Nile tilapia was analyzed, as it is the consumed part, whereas fillet and whole *Labeobarbus* were analyzed assuming that both can be consumed. All materials used in the handling and dissecting of the fish samples were washed in distilled water in advance. Plastic tools (knife and forceps) were used to collect the muscle (fillet) tissues and the samples were stored at −20 °C until further analysis. The whole fish was analyzed with its gut content, to have an idea about the mineral intake of the fish. Finally, all samples were cut into small pieces with a clean stainless steel knife and dried in an oven at 105°C (AOAC, 2000). The dried samples after moisture determination were finely ground for crude protein, crude fat, percentage of ash, and mineral analysis as detailed in the appropriate section.

Table 4.1. Fish species and parts used in this study fish from caught in the Gilgel Gibe Reservoir, Ethiopia

Common name	Scientific name	Local name	Part used	n
Nile tilapia	*Oreochromis niloticus*	Qoroso	Fillet	16
Labeobarbus	*Labeobarbus intermedius*	Bilcha	Fillet	16
Labeobarbus	*Labeobarbus intermedius*	Bilcha	Whole body	16
Garra	*Garra quadrimaculata*	Garbiti	Whole body	16

Figure 4.2. *Garra quadrimaculata* from Gilgel Gibe Reservoir, Ethiopia

Figure 4.3. *Labeobarbus intermedius* from Gilgel Gibe Reservoir, Ethiopia

Figure 4.4. *Oreochromis niloticus* from Gilgel Gibe Reservoir, Ethiopia

4.2.4 Water quality parameter of the reservoir

On site physico-chemical water quality measurements of the reservoir were performed. Day-time dissolved oxygen, water temperature, electrical conductivity, and pH were measured using a multi-probe meter (HQ40d Single-Input Multi-parameter Digital Meter; Hach Company, Loveland, USA). The concentration of nitrate and ammonia were also measured onsite, using a Palin test photometer (Photometer 7500; Tyne and Wear, UK, NE11 0NS) (Table 4. 2).

4.2.5 Proximate analyses

Proximate compositions of fillets (Nile tilapia and *Labeobarbus*) and whole body (*Garra* and *Labeobarbus*) were determined by conventional methods described by the (AOAC, 2000), and data were expressed as g/100g of the fresh sample. The moisture content was determined by oven drying samples at about 105°C until constant weight. For ash content estimation, 3 g of the dried samples were placed in the pre-weighed crucible and heated in a muffle furnace at 550˚C for 4 h. The final weight was subtracted from the initial weight and converted to a percentage, to give an estimate of the ash content.

To obtain crude fat, 3 g of sample was fully extracted by wrapping in a filter paper in a Soxhlet apparatus. Petroleum ether was used as an organic solvent at b.p. 40-60^{o}C and this was done each for 6 h. Crude protein was determined using the automated Kjeldahl apparatus (Velp Scientifica TM UDK 159, F30200150) following the procedure described in ISO 5983-2:2005 (ISO, 2009). The total protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25.

4. 2.6 Mineral Analyses

The whole and filleted fish samples were packed and shipped to Ghent University, Belgium, for the analysis of both micro-and macrominerals. In accordance with the methodology described by AOAC (2000), the samples were weighed on a microbalance (Mettler Toledo, AT21 comparator) and homogenized, allowed to react

for 12 h in the solvent of 3 mL HNO_3 and 3 mL H_2O_2. Subsequently, samples were ashed by microwave oven (CEM, MARS6) with the following program: 10 min at 55 °C and 400 W, followed by 10 min at 75 °C and 600 W, finally /40 min at 120°C and 1200 W. The extracts were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, Elan DRC-e) with a calibration range of 0-50 mg/L.

The limits of detection (LOD) for ICP-MS were calculated as the concentration associated with 3.3 times the standard deviation of the background noise recorded on nine measurements of the procedural blank and given in online resource. Extracts were diluted 1:10 (v/v) with Ga as an internal standard solution. The signal of the sample analyte was always within the standard curve and ISO 11885 international standard method was used to calibrate the instrument. The quantification limit of the analyzed elements was set at 0.005 mg/L for Al, Cr, Cu, Fe, Zn, Mn, 0.01mg/L for Cd, P, 0.05mg/L for Co, Mg, Na, S, Ca, K, and 0.1 for Ni and Pb, 0.2mg/L.

4. 2.7 Risk and Nutritional Assessment

The potential mineral contributions in a 100 g serving of whole fish (Labeobarbus, *Garra*) and fillet (Nile tilapia, *Labeobarbus*) from Gilgel Gibe reservoir to a percentage of the daily value of foods was calculated based on a dry-matter basis of the samples. For the nutritional contribution assessment, the calculated intake of the analyzed essential elements per 100 g serving of whole fish (*Labeobarbus*, *Garra*) and fillet (Nile tilapia, Labeobarbus) were compared to the dietary reference intake (DRI) for children and adults by gender that was set by the Food and Nutrition Board, Institute of Medicine, United States National Academy of Sciences (Monsen, 2000; Trumbo et al., 2001).

4. 2.8 Statistical Analyses

All data were displayed on a scatterplot to check for normality. Effect of the fish product, whole (*Garra*; *Labeobarbus)* fillet *(Labeobarbus* fillet, and Nile tilapia fillet) on the proximate composition and mineral content were evaluated with one-way analysis

of variance (ANOVA) with subsequent post-hoc comparison using Tukey's test. All analyses were done using the statistical package of SPSS version 26.0. The significance was treated at $P < 0.05$ confidence level.

4.3. Results

The physicochemical properties of the water in the two locations are presented in (Table 4. 2). No remarkable variations in water quality parameters were recorded between the sampling sites. The average morphometric feature (weight, length, and ratio) of the three fish species used in the study are presented in (Table 4.3).

Table 4. 2. The physicochemical properties of the water in the Gilgel Gibe Reservoir, Ethiopia

Sites	T(^{0}C)	DO (mg/L)	EC (µS/cm)	pH	NH_3 (mg/L)	NO_3^- (mg/L)
Bore	23.25	6.18	82.05	7.53	0.087	2.08
Denaba	23.34	5.5	77.84	7.54	0.052	3.54

DO= Dissolved oxygen; EC= Electric conductivity; T= Temperature

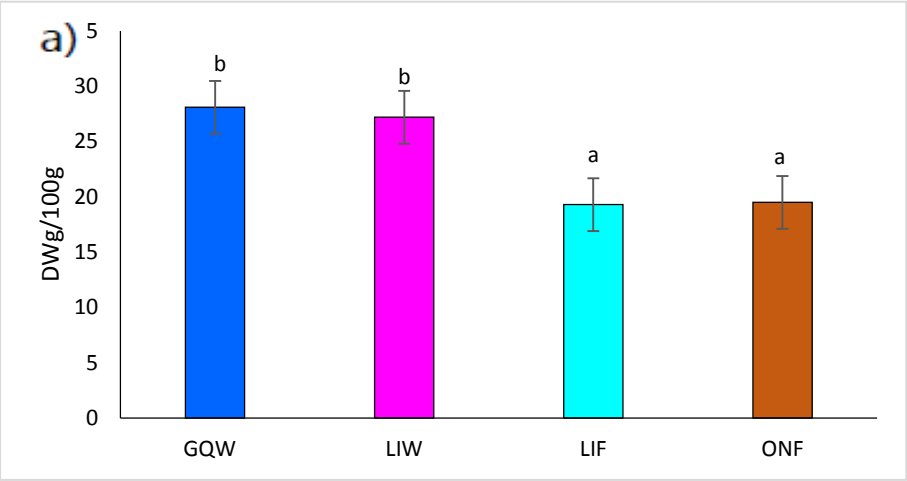
Table 4.3. Morphometric feature of the fishes caught in the Gilgel Gibe Reservoir, Ethiopia

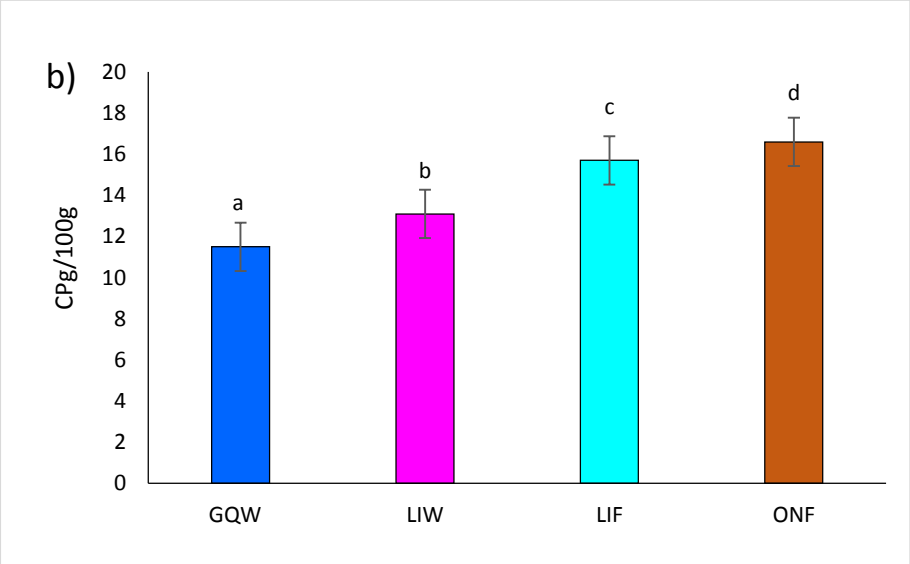
	GQW	LIW	LIF	ONF	p	SEM
Weight (g)	30.4^a	108.4^b	127.6^b	169.3^c	<0.001	7
Length (cm)	14.5^a	$21.6b^c$	23.4^c	19.4^b	<0.001	0.6
Weight:length	2.0^a	4.9^b	5.4^b	8.7^c	<0.001	0.3

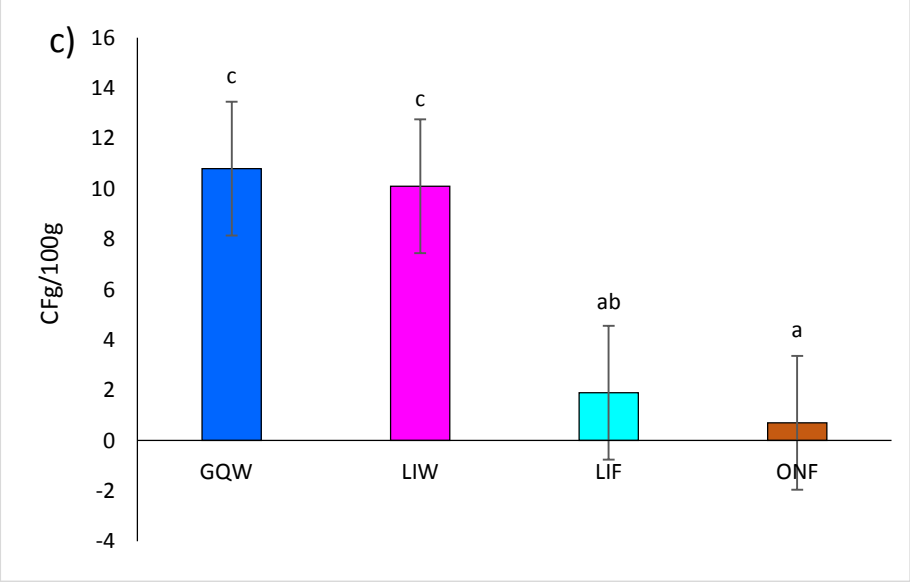
Different superscripts within a row indicate significant differences at p<0.05. GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet; SEM= Standard error of the means

The macronutrient composition and mineral concentrations were significantly varied between the species and tissues. The whole fish showed a significantly higher fat and ash percentage than the fillets, with an even higher ash concentration in the whole

Garra than in the whole *Labeobarbus* (Figure 4.5c, d). The fillets contained a much higher protein concentration than the whole fish (Figure 4. 5b).







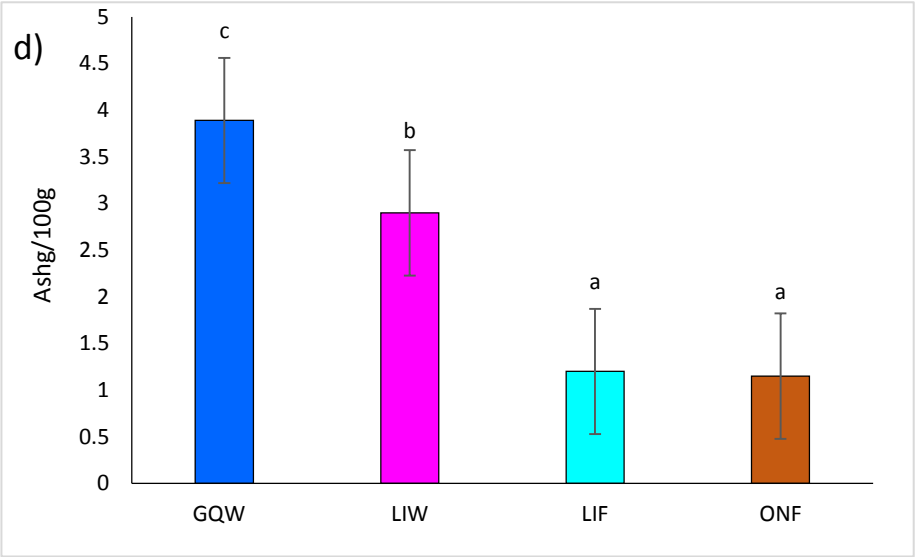


Figure 4. 5 (a-d). Fresh matter proximate composition (g/100 g fresh matter) in whole body and fillet of fish caught in the Gilgel Gibe Reservoir, Ethiopia. a) gram of dry matter per 100g; b) gram of crude protein per 100g; c) gram of crude fat per 100g; d) gram of ash per 100g per 100g DW = dry matter; CP = crude protein; CF = crude Fat; GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet. [a,b,c,d] Mean values with different letters were significantly different (p <0.05).

Within the minerals, Fe and Mn were significantly higher in whole fish compared to fillets, but also typical species differences were observed: Zn, Cu, and especially Mn and Fe were significantly higher in whole *Garra* than in the whole *Labeobarbus*. Even in the fillets, a species difference was found between Nile tilapia and *Labeobarbus*, with Cu being higher in Nile tilapia fillet (Table 4.4).

Table 4. 4. Fresh matter micromineral concentrations (mg/kg fresh matter) in whole body and fillets of fish caught in the Gilgel Gibe Reservoir, Ethiopia

	GQW	LIW	LIF	ONF	*p*	SEM
Zn	95^{b}	39^{a}	14^{a}	14^{a}	<0.001	8
Cu	4.2^{c}	2.2^{a}	0.5^{a}	1.6^{ab}	<0.001	0.3
Fe	1154^{b}	141^{a}	22^{a}	28^{a}	<0.001	115
Mn	55^{b}	11^{a}	1^{a}	1^{a}	<0.001	6

Different superscripts within a row indicate significant differences at p<0.05; GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet; SEM= Standard error of the means

Whereas K was significantly higher in fillets than in whole fish, Ca concentrations were significantly higher in whole fish, with similar P concentrations, implying that whole fish also had much higher Ca:P ratios. Differences in Na and S concentrations were rather mild (Table 4.5). From the evaluated heavy metal elements, Co, Cd, Pb, and Ni were below the detection limit, however, Al was recorded significantly higher (p<0.05) in *Garra* (Table 4.6).

Table 4.5. Fresh matter macromineral concentrations (g/kg fresh matter) in whole body and fillets of fish caught in the Gilgel Gibe Reservoir, Ethiopia

	GQW	LIW	LIF	ONF	*p*	SEM
Mg	2.04^{b}	1.24^{a}	0.93^{a}	1.31^{a}	0.011	0.11
Na	2.1^{b}	1.0^{ab}	0.2^{a}	0.7^{a}	0.001	0.2
S	0.40^{c}	0.22^{b}	0.11^{a}	0.10^{a}	<0.001	0.03
K	7.3^{a}	7.3^{a}	12.0^{b}	16.9^{c}	<0.001	0.8
Ca	35.5^{b}	28.4^{b}	1.6^{a}	2.0^{a}	<0.001	3.5
P	21.8^{c}	19.1^{bc}	7.2^{a}	9.9^{ab}	<0.001	1.6
Ca:P	1.6^{b}	1.4^{b}	0.2^{a}	0.2^{a}	<0.001	0.2

Different superscripts within a row indicate significant differences at *p*<0.05. GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet; SEM= Standard error of the means.

Table 4.6. Fresh matter heavy metal concentrations (mg/100 g fresh matter) in whole body and fillets of fish caught in the Gilgel Gibe Reservoir, Ethiopia

	GQW	LIW	LIF	ONF	*p*	SEM
Al	1020^{b}	76^{a}	8^{a}	9^{a}	<0.001	103
Cd	<0.05	<0.05	<0.05	<0.05	-	-
Cr	1.84^{b}	1.07^{ab}	0.47^{a}	0.72^{a}	<0.001	0.13
Co	<0.1	<0.1	<0.1	<0.1	-	-
Pb	<0.2	<0.2	<0.2	<0.2	-	-
Ni	<0.1	<0.1	<0.1	<0.1	-	-

Different superscripts within a row indicate significant differences at $p<0.05$. GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet; SEM= Standard error of the means

The percentage contribution of the whole fish (*Garra*; *Labeobarbus*) and fillet (Nile tilapia, Labeobarbus) per 100 g serving to the DRI for every mineral is summarized in (Table 4. 7) for children and adults by gender.

Table 4. 7. Contribution (%) of fish from Gilgel Gibe reservoir, Ethiopia to dietary reference intake (DRI) of each element for children and adults by gender (data is compared with USDA standard reference of Nile Tilapia fillet)

Fish	Age	Fe	Zn	Cu	Mn	Mg	Na	K	Ca	P
	Children	1390	119	84	367	136	21	27	532	296
GQW	Female	679	114	47	324	62	14	29	323	247
	Male	1282	86	47	239	50	14	22	323	247
	Children	170	74	44	73	83	10	27	425	259
LIW	Female	83	47	24	65	37	7	29	258	216
	Male	175	35	24	48	30	7	22	258	229
	Children	27	26	10	7	62	2	45	24	98
LIF	Female	13	17	6	6	28	1.3	48	15	82
	Male	24	13	6	4	22	1.3	37	14	86
	Children	34	26	32	7	87	7	43	32	134
ONF	Female	16	17	18	6	39	5	68	20	112
	Male	31	13	18	4	32	5	52	20	119
Reference		31	30	83	16	68	23	64	8	243

GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet. Dietary reference intake (Recommended Daily Allowance) for children and adults by gender that is set by the Food and nutrition board, institute of medicine, United States National Academy of Sciences (Monsen, 2000; Trumbo et al., 2001).

Concentrations of minerals of whole fish (*Garra, Labeobarbus*) and fillet (Nile tilapia, *Labeobarbus*) of this study were also compared with United States Department of Agriculture (USDA) composition data for raw tilapia fillets which are shown in Table 4. 8.

Table 4. 8. Comparison of mineral concentrations (mg/100 g) of fish from Gilgel Gibe reservoir, Ethiopia with USDA compositional data for raw tilapia fillets

Minerals	GQW	LIW	LIF	ONF	USDA composition Data
Mg	204	124	93	131	270
Na	213	103	21	73	520
K	734	732	1202	1688	3020
Ca	3548	2841	160	224	100
P	2182	1914	721	987	1700
Fe	114.5	14.5	2.2	2.8	5.6
Zn	9.5	3.9	1.4	1.4	3.3
Cu	0.42	0.22	0.05	0.16	0.75
Mn	5.5	1.1	0.1	0.1	0.37

GQW = *Garra quadrimaculata* whole; LIW = *Labeobarbus intermedius* whole; LIF = *Labeobarbus intermedius* fillet*; ONF = Oreochromis niloticus* fillet.

4.4 Discussion

The present study shows an overall more balanced proximate composition and mineral content of whole fish versus fillet only. The protein content in the entire fish is markedly lower than in the fillets, but the former can meet the protein requirements for infants or mothers when incorporated in an average diet (Figure 4.5b). Eating just fillets for protein leads to missing out on many nutrients such as fat and minerals that are higher in whole fish. However, protein is essential for growing children, and the present result on the fish fillets confirms the expected high protein concentrations. Although *Garra* showed the least favorable results for protein, it should be placed in perspective, exactly because of the common need for other nutrients in the diet.

Smaller fish species are more nutritious than the larger fish, and according to various reports (Shakuntala et al., 1996; Torben et al., 2000; Roos, 2001), small fish species that are consumed entirely with bones, heads, and viscera play a critical role in micronutrient intakes, as these parts are where most micronutrients are concentrated. Several factors may have affected the distinct differences in nutritive value in the fish products that were tested in our study. The fact that different parts are consumed (in this case the fillet versus the whole fish) plays a prominent role in nutrient intake profiles. Yet, fish species as such seem to affect the nutrient composition. Allometric shifts in tissue proportions can explain that small species differ in nutritive value from large species (Rhiannon et al.,2007; Cleber et al., 2017), but the habitat, feeding habits, size, and age of species will certainly contribute to the observed differences, even when living in the same lake.

Whole *Garra* appears to be high in important trace elements such as Zn and Fe, a feature that was not found to the same extent in the whole *Labeobarbus*. These differences may thus find its origin in the feeding pattern of these fish in the lake; for instance, because of *Garra* being more benthic than pelagic (Zi-ming et al., 2009).

Typically, benthic feeders are more exposed to high mineral concentrations from organisms living in the sediment. *Garra* is indeed described as a benthic feeder (Zi-ming et al., 2009), whereas *Labeobarbus* is rather considered a pelagic feeder (Martin et al., 2008). Nutritive value of fishes differs greatly in the types of food they consume and the differences in nutrient composition between wild and farmed fish of even identical species have been reported (Orban et al., 2003; Kaushik et al., 2006; Hossain, 2011).

Nile tilapia is an omnivore; gut content studies indeed showed diverse food items including phytoplankton, zooplankton, insects, oligochaetes, caridina, and bivalves (Khallaf, 1987; Njiru et al., 2004). The mouth position, ventral (ventro-terminal) of *Garra* is particularly suited to feeding on the bottom of the aquatic ecosystem. Oso et al., 2006, examined the bottom dweller fishes for their gut content and found a high percentage of detritus in their diet. Therefore, the benthic diet of *Garra* is the likely explanation for the higher accumulation of minerals. The distinct differences in micromineral accretion agree with our observation in a study with ornamental fish (Negisho et al.,2020,). The latter study demonstrated that micro mineral tissue accumulation may differ largely between fish species, because the accumulation of microminerals occurred at highly different rates. This implies that the difference between whole *Garra* and whole *Labeobarbus* micromineral profiles may even arise from pure genotypic differences, independent of the actual natural diet.

The higher Fe and Zn concentration in *Garra* is an interesting result as these trace elements are often critically limiting minerals in human nutrition in developing countries. According to the report of (WHO, 2001; Micronutrient Initiative Global Report, 2009), deficiencies of, especially, Fe and Zn are prevalent in human nutrition in developing countries. Among human nutrition deficiencies, the issue of micromineral deficiency is critical due to its occurrence at particular stages of human life (pregnancy, breast-feeding, childhood) which severely affect health and development, leading in some cases to irreversible effects (Kawarazuka & Béné, 2011). In the example of

Ethiopia, infant malnutrition is among the highest in the world, with an estimated 44% of children under the age of five suffering from chronic malnutrition (stunting), and 44% of children suffered from Fe-deficiency anaemia (Sheehy et al., 2019). This also affects physical and cognitive development, pregnancy outcomes, morbidity, and mortality (National Micronutrient Survey, 2013). The inclusion of varieties of fish species such as *Garra* in the human diet may therefore be a useful tool to alleviate these deficiencies.

Similarly, the Ca and P concentrations are by far higher in *Garra* compared with the whole *Labeobarbus*. The proportion of skeleton within the whole body in small species may be the most plausible explanation, but this could also be related to the abundant availability of Ca in sediment and benthic behavior of *Garra* which would help to sufficiently absorb from the detritus matter. According to Love, 1980 cited in (Hassaan et al., 2013), it is generally accepted that fish can absorb Ca from the surrounding water to fulfill part or all of the metabolic Ca requirements. The intake of Ca and P is also higher when small fish are eaten with their bones rather than when the fish bones are discarded, which is obvious from the comparison between whole fish and fillets in this study. A question to be answered is to which extent this skeletal Ca and P is bioavailable, and the effect of cooking on the bioavailability of minerals contained in fishbone is certainly an element for further investigation. The evaluation of the cooking effect on nutrient availability of salmon and mackerel resulted in significant increases in Fe and Ca content (Balladares & Quevedo, 2017). The higher Ca:P ratios in whole fish compared to fillet in our study indicates that the importance of including whole fish in the diet for a healthy human skeletal development, especially in diets where Ca is typically lacking, e.g., in cereal-dominated diets. According to Van paemel et al., (2010), a 1.5 dietary Ca:P ratio is considered ideal for optimum growth of infants and children, which coincides with the whole *Garra* findings.

In contrast to most other minerals, the concentrations of Mg and K were higher in fillets than in whole fish. Differences are expected in mineral sequestration between

the whole body and fillets of fish, this is likely based on the assimilation rates and affinities of the minerals to various compartments (Bevelhimer et al., 1997). Usually, these minerals (Mg and K) are of lesser concern in the human diet because they are typically provided in adequate amounts through vegetal food items. One can easily obtain these minerals; for example, Mg is widely distributed and available in plant and animal foods and even in beverages (Rude et al., 2012).

According to the US Food and Drug Administration (USDA, 2018), food that provides more than 10% of nutrients per serving is considered as a good source of that nutrient. In this study, the mineral contribution to DRI per 100-gram serving was calculated. Whole fish (*Garra, Labeobarbus*) and fillet (Nile tilapia, *Labeobarbus*) from the Gilgel Gibe reservoir in this study could provide more than 10% for several minerals for the children and adults by gender. Whole *Labeobarbus* and *Garra* would provide good sources of minerals. The latter can provide substantial mineral intakes (Ca, P, Zn, Cu, and Mn) for humans. This comparison is made to show that small inclusions of whole *Garra* in the diet may substantially improve the intake of certain minerals. A report indicates that Ethiopian children are suffering from Fe-deficiency anaemia (Sheehy et al., 2019). Moreover, mineral concentrations in whole fish (*Labeobarbus*, Garra) and fillet (Nile tilapia, *Labeobarbus*) in our study showed higher concentrations of minerals compared to USDA composition data of raw Nile tilapia (USDA, 2018). This demonstrates the higher supply of important minerals through whole fish compared with fillets, and this potential should be considered in the human diet.

An important downside for whole *Garra* consumption is the excessive accumulation of Al in *Garra*, which is likely caused by the high inflow of topsoil due to erosion in Gilgel Gibe reservoir (Regassa et al., 2014), and particularly affects *Garra* because it is a benthic feeder. The highest tolerable daily intake of Al for humans according to EFSA recommendations is 0.17 mg/kg of body weight per day (Van paemel et al., 2010). In case Garra would be introduced in aquaculture, this risk can be controlled for, but as

long as *Garra* is consumed from wild-catch, the presence of potentially toxic levels of heavy metals is a concern that requires monitoring.

In conclusion, despite the much higher protein content of fish fillets compared with whole fish, the latter provides a more balanced nutrient profile, with a distinctly higher supply of fat and minerals. The inclusion of particular fish products in the human diet may therefore be chosen in function of existing imbalances. Irrespective of the contrast between the fillet and whole fish, fish species differences are apparent and likely explained by genotype as such and their concomitant natural diet. The case of *Garra* identified that the advantage of benthic species to enrich the human diet with essential minerals may coincide with the accumulation of toxic heavy metals as a potential result of soil erosion.

4.5 Acknowledgements

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CHAPTER 5: Exploring the impact of lake ecosystems on mineral concentrations in tissues of Nile tilapia (*Oreochromis niloticus* L.)

Adapted from

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Abstract

This study evaluates the differences in mineral and toxic trace element concentrations of Nile tilapia (Oreochromis niloticus) tissues from three aquatic ecosystems in Ethiopia Lake Ziway, Lake Langano, and Gilgel Gibe reservoir with a focus on edible (fillet) and discarded (digestive tract, gills, skin, and liver) parts. A total of sixty (n = 60) Nile tilapia samples were collected, comprising twenty (n = 20) fish from each lake, and analyzed by inductively coupled plasma mass spectrometry. All elements varied markedly among tissues and between the lakes. Some differences in element concentrations were attributed to differences in nutrient load in the ecosystems and the function of the tissues. For instance, the calcium concentrations in skin and gill were distinctly higher in fish from calcium-rich Lake Langano. The discarded parts were richer in essential trace elements, showing an opportunity to promote their use in human nutrition to increase the intake of important minerals. However, the accumulation of elements toxic to humans, such as aluminum, should be monitored and, in particular, controlled when rearing these fish in aquaculture.

5.1 Introduction

Food insecurity and malnutrition remain a great problem worldwide, with a prevalent burden on developing countries (IFPRI, 2016; FAO, 2018). Hidden hunger critically affects human health when diet fails to meet nutrient requirements (FAO, 2014). Fish are a source of minerals that is highly favored by consumers in most parts of the world (Abolude and Abdullahi, 2005). Moreover, eating fish provides polyunsaturated fatty acids (PUFAs) that help reduce the risk of cancer and cardiovascular diseases (la Vecchia et al., 2001; Kanakri et al., 2017; Saini and Keum, 2018). Fish can, thus, play a role in fighting malnutrition and the hidden hunger problem (FAO, 2014; Be'ne' et al., 2015). Fish are also generally considered a valuable source of macrominerals, e.g., calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), sulfur (S), potassium (K), and essential microminerals such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) (Porto et al.,2016). However, essential microminerals become toxic upon high-level intake, and fish can accumulate toxic trace elements such as lead (Pb), chromium (Cr), cadmium (Cd), cobalt (Co), and nickel (Ni) (Türkmen, 2009). Nevertheless, mineral distribution in fish tissues is poorly evaluated since studies have mainly focused on the protein and fat composition of fish fillets (Tacon et al., 2013; Khalili and Sampels, 2018).

There are various ways in which fish acquire minerals (Edevaldo et al., 2016): by direct ingestion of suspended particulate matter in the water column in the form of food, by ion exchange of dissolved elements across lipophilic membranes (e.g., the gills), and by adsorption of elements on tissue and membrane surfaces. The elemental distribution in different tissues can be governed by way of dietary and/or aqueous exposure (Edevaldo et al., 2016). Analyzing different fish tissues for mineral distribution can be used to locate mineral sequestration sites, hence providing a basis for advice on the consumption of fish parts.

In natural conditions, the distribution of minerals can vary with the local activity (farming, industrial, or urban activity) that limits the releasing rate of effluents into the nearby aquatic environment. Due to numerous coenzyme functions, minerals play an

important role in human health, growth and development, and disease prevention (Beveridge et al., 2013). Studies on mineral accumulation in fish are essential for understanding the effects associated with the consumption of fish by humans (Farzad et al., 2019). However, the role of fish as a dietary source of minerals is poorly recognized and underevaluated (Allison et al., 2013).

Nile tilapia is a common and well-accepted fish under both capture and culture conditions, especially in tropical and subtropical regions (Asia, Africa, and the Americas) (FAO, 2012). Currently, it is the second most important cultured fish in the world, next to carp species. In Ethiopia, it is widely distributed in lakes, rivers, reservoirs, and swamps and contributes to about 60% of total landings of fish (Akinwumi, 2001; Tesfaye and Wolff, 2014). Moreover, it can consume a wide variety of food items, including phytoplankton, zooplankton detritus, and macrophytes (Canonico et al., 2005; Rivera et al., 2018). The high degree of plasticity and opportunism in their feeding behavior induces variation in nutrient composition among localities; hence, different ecosystems lead to variations in diet composition.

A report demonstrated that the nutrient composition of freshwater fish differs between geographical localities (Zenebe et al., 1998). From an ecological point of view, a fair amount of research was done on how ecosystems affect fish, but the effect on the nutrient composition of fish has been overlooked. It is, however, likely that the accumulation and distribution of beneficial and toxic trace elements in fish will be especially affected by the mineral load in its environment and diet, which, in turn, will reflect soil composition and geological events, such as soil erosion (Nhiwatiwa et al., 2011; Rajeshkumar and Li, 2018). Moreover, there is limited information on the mineral concentration of discarded fish tissues (gill, skin, digestive tract, and liver), but former work has demonstrated that micromineral concentrations can show large concentration differences between tissues (Negisho et al., 2020, **Chapter 3 and Chapter 4**). Because of differences in metabolic activities in each tissue and the

environmental conditions, some tissues, such as the liver, accumulate more toxic trace elements that may harm consumers.

In particular, mineral distribution within the tissues of Nile tilapia is underexplored. Analyzing tissue mineral distribution would help us to understand the physiological role of each tissue and the impact of the ecosystem on mineral and toxic trace element accumulation in the tissues of Nile tilapia. To investigate the impact of environment on the mineral distribution in Nile tilapia tissues, three Ethiopian lakes (Lake Ziway (LZ), Lake Langano (LL), and Gilgel Gibe reservoir (GR) were chosen. Some of the criteria we used to select the three lakes were: geographical location, pollution category, accessibility and ecosystem service provision to the local livelihoods and human activities near the lakes.

The catchment areas of the water sources are different for the three lakes. In recent days, commercial floriculture and water pump irrigation by local farmers have rapidly expanded on the shoreline of Lake Ziway and along its tributary rivers (Tilahun and Ahlgren, 2010). The lake is also under heavy pollution pressure because of expanding urbanization: Batu and Maki are the fast-growing towns near Lake Ziway (Tilahun and Ahlgren, 2010). Compared to other lake basins, Lake Langano experiences only small seasonal water level variations. However, in the surrounding lake, there is an increasing number of resorts and tourists. As a result, untreated effluents are directly discharged into the lake; this could have negative effects (Zenebe, 1998). The water in Gilgel Gibe Reservoir is collected from three agricultural streams (Merewa, Gibe, and Gulufa), three urban streams (Kito, Kochi, and Awetu), and one forest stream. These streams pass through fast-growing urban and intensive agricultural areas. Untreated wastewater, generated by Jimma town inhabitants, is directly discharged into the urban stream, which is the main tributary of the reservoir. Municipal waste discharge, overgrazing, brick preparation, vegetation removal and land conversion to cropland, drainage, and crop cultivation are the major threats from human activities around the tributaries of the reservoir (Ambel et al., 2013).

Based on the above mentioned information, the three lakes were under different degree of pollution pressure, accordingly, Ziway Lake perceived as higher, Gilgel Gibe was intermediate and Langano had a lower degree of pollution pressure. This study aimed to evaluate the effect of varying environment on the mineral distributions in fillet (muscle) and discarded tissue parts (skin, gill, digestive tract and liver) of Nile tilapia from the three lakes. Therefore, we investigate the minerals (Ca, P, S, Mg, Na, K, Fe, Cu, Zn, and Mn) and toxic trace element (Cr, Ni, Pb, Cd, and Co) concentrations of Nile tilapia tissues from these three different environments.

5. 2 Materials and Methods

5.2.1 Description of the Study Area

Fish samples (Nile Tilapia) from LZ, LL, and GR were used in this study, and a summary of limnological information is given in (Table 5.1 and Figure 5.1) (Tilahun and Ahlgren, 2010; Ambelu et al., 2013; Tesfaye and Wolff, 2014). Lake Ziway is located in the central Rift Valley zone of Ethiopia. It has open water and flat swampy margins on all sides except the south and southwest. It fills a depression at an elevation of about 1636 m above sea level. Meki and Ketar Rivers are the main tributaries of the lake. The lake is the shallowest lake in the country and drains into Lake Abiyata via Bulbula River. It is the third-largest freshwater lake in the country. It has a surface area of 434 km^2 and has five islands: Gelila, Debre Sina, Tulu Gudo, Tsedecha, and Fundro. The climate of the lake basin is dry to sub humid. The lowland area surrounding the lake is arid or semiarid, and the highlands are sub dry humid to humid.

Lake Langano is one of the northern Rift Valley lakes of Ethiopia. It is situated at an altitude of 1582 m and has a surface area of 241 km^2 and a mean depth of 17 m. The lake is highly turbid, and the water is usually reddish-brown; it is known by its nickname “Golden Lake”. It is mainly fed by runoff and hot springs. The lake water discharges into Lake Abijata via Hora Kello River. The climate is rainy between June and September, followed by a dry season from November to February.

Gilgel Gibe reservoir is located 283 km southwest of the capital and 70 km northeast of Jimma town. The dam is constructed at an altitude of 1640 m above sea level. Its maximum and minimum water levels during wet and dry seasons are 1671 and 1653 m above sea level, respectively. Its depth ranges between 2 and 35 m, with a mean depth of about 17.6 m, and it covers a total surface area of 62 km^2. It is bordered by three districts (woredas): Omo-Nada, Kersa, and Tiro-Afeta. The area has a subhumid, warm to hot climate and receives between 1300 and 1800 mm of annual rainfall; it has a mean annual temperature of 23.7 °C. In addition to hydropower generation, the reservoir plays an important role for the local community as a fishery resource.

Table 5. 1. Geographic description of the three Ethiopian lakes in this study

Parameters	GR	LZ	LL
Latitude	7°42′53′′-7°55′580′′ N	7°20′54″-8°25′56″N	7°03′0″-7°04″2″N
Longitude	37°11′53′′-37°20′33′′E	38°13′02-9°24′01″ E	38°04′0′′-38°04′9′′E
AL (m) asl	1640	1636	1582
SA (km^2)	62	434	241
CA (km^2)	4200	7025	1600
MD (m)	17.6	2.5	17
DC (km)	283, south-west	165, south	200, south

Reviewed from (Tilahun and Ahlgren, 2010; Ambelu et al., 2013; Tesfaye and Wolff, 2014) GR: Gilgel Gibe reservoir; LZ: Lake Ziway; LL: Lake Langano; CA= catchment area; SA= surface area; DC= distance from the capital; AL= altitude; MD= mean depth

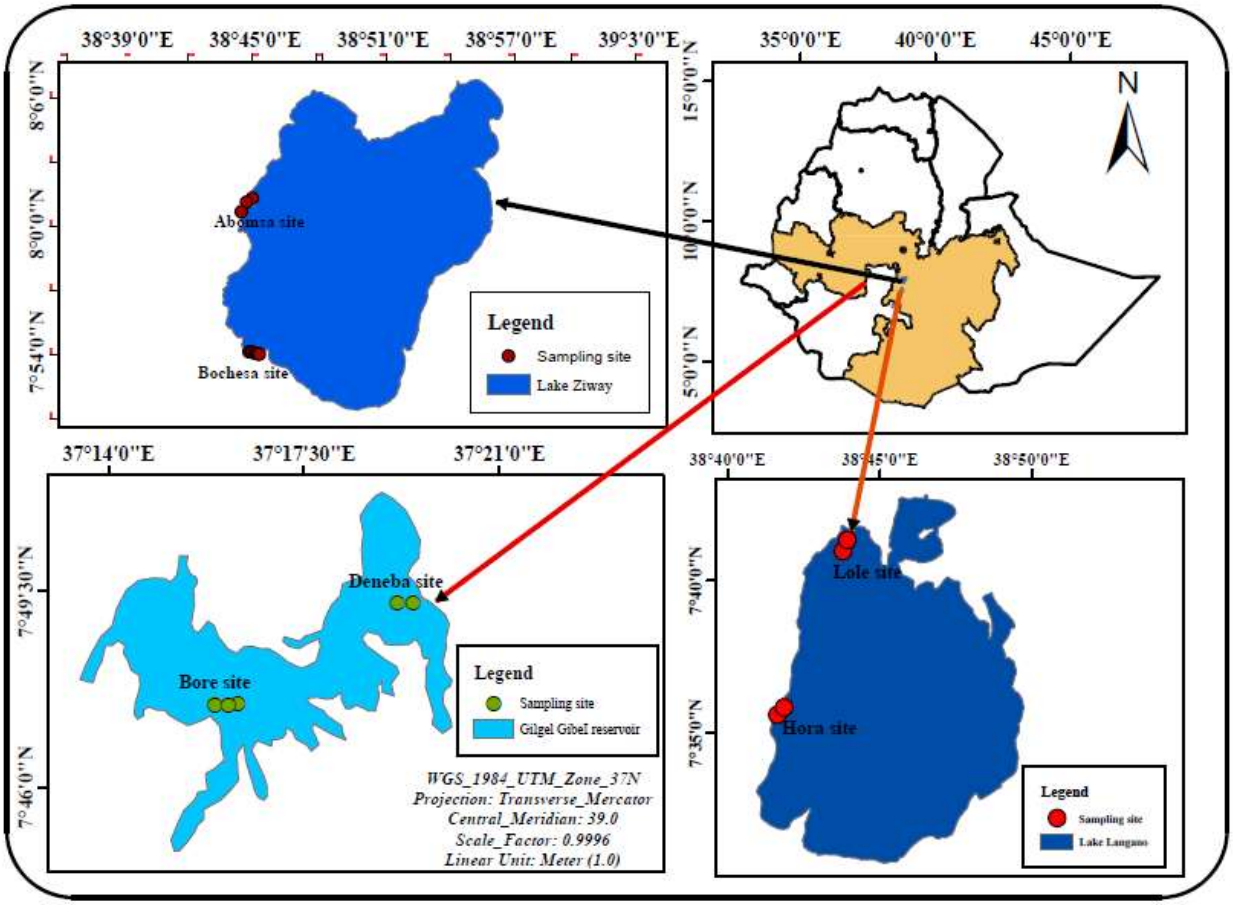

Figure 5.1 Map indicating the three lakes (Ziway, Langano and Gilgel Gibe), 2020

5.2.2 Water Quality Parameters of the Three Lakes

On-site physicochemical water quality measurements of the three lakes were performed. Day-time dissolved oxygen, water temperature, electrical conductivity, and pH were measured using a multi-probe meter (HQ40d Single-Input Multi-parameter Digital Meter; Hach Company, Loveland, CO, USA). In addition, the concentration of nitrate and ammonia were also measured on-site using a Palin test photometer (Photometer 7500; Tyne and Wear, UK, NE11 0NS) (Table 5. 2).

5.2.3 Animal ethics statement

This study was checked and given approval by Jimma University, College of Natural Sciences Research and Ethical Review Board Committee (Jimma, Ethiopia). The committee has followed the standard procedures written on Article 6 (methods of killing) and Article 9 (Animals are taken from the wild), of directive 2010/63/EU of the

European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes and certified this study with the letter referenced (Ref. No: RPG/165/2019). This study was carried out in compliance with the ARRIVE guidelines.

5.2.4 Fish Sample Collection and Preparation

Nile tilapia samples were collected from LZ, LL, and GR during the dry season (October 2018). Nile tilapia was selected based on its relevance for commercial fishing in the country and fish consumption by the local population. The target tissues were chosen based on the edible and discarded parts. A total of sixty (n = 60) fish samples were collected, comprising 20 (n = 20) fish from each lake, caught using standard gillnets. Fish samples were thoroughly washed with tap water, separately wrapped in plastic foil (parafilm), placed on ice, and brought to the laboratory. Fish from Gilgel Gibe Reservoir were transported to Jimma University Zoological Sciences, whereas fish from Lake Ziway and Lake Langano were brought to the Batu Fisheries and Other Living Aquatic Resource Research Center.

Because of the too small sample amounts of tissues such as the liver, the fish samples were combined (pooled) into three groups (7, 7, and 6 fish). Each pooled sample was analyzed in triplicate. All materials used in the handling and dissecting of the fish samples were washed in distilled water in advance. Plastic tools (knife and forceps) were used to collect the target tissues, and the samples were stored at − 20 °C until further analysis.

The digestive tract was analyzed with its contents to have an idea about the mineral intake of the fish. The skin was analyzed with scales, and, in this study, both skin + scales are referred to as “skin”; this is because of an assumption that scales can potentially be a rich source of minerals. The concentrations of macrominerals, microminerals, toxic trace elements, and ash content were determined in all targeted tissues.

5.2.5 Percentage of Ash Determination

Three grams of the dried samples were placed in a pre-weighed crucible. Before placing in the oven, the samples in the crucible cups were charred on hot plates for 30 min to prevent excess smoke production. For ashing, the weighed samples were incinerated in a muffle furnace (550 °C) for 6 h, and the percentage of ash content was calculated (AOAC, 1990)

5.2.6 Mineral Analysis

The fillet and discarded tissue parts of the fish samples were packed and shipped to Ghent University, Belgium, for the analysis of minerals and toxic trace elements. Following the methodology described by Association of Official Analytical Chemists, AOAC (1990), the samples were weighed on a microbalance (Mettler Toledo, AT21 comparator), homogenized, and allowed to react for 12 h in a mixture of 3 mL HNO_3 and 3 mL H_2O_2. Subsequently, the samples were heated in a microwave oven (CEM, MARS6) with the following program: 10 min at 55 °C and 400W, followed by 10 min at 75 °C and 600W, and, finally, 40 min at 120 °C and 1200W.

The extracts were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, Elan DRCe) with a calibration range of 0–50 mg/L. The instrument of detection (LOD) for ICP-MS was calculated as the concentration associated with 3.3 times the standard deviation of the background noise recorded on 9 measurements of the procedural blank and given in online resources. Extracts were diluted 1:10 (v/v) with Ga as an internal standard solution. The signal of the sample analyte was always within the standard curve, and the ISO 11885 international standard method was used to calibrate the instrument. The quantification limits of the analyzed elements were set at 0.005 mg/L for Al, Cr, Cu, Fe, Zn, and Mn, 0.01 mg/L for Cd and P, 0.05 mg/L for Co, Mg, Na, S, Ca, and K, 0.1 mg/L for Ni, and 0.2 mg/L for Pb.

5.2.7 Nutritional Contribution Assessment

Possible mineral contributions in a 100-g serving of Nile tilapia tissues from the three Ethiopian lakes to a percentage of the daily value of foods were simulated based on a dry-matter basis of the samples. For the nutritional contribution assessment, the calculated intake of the analyzed essential elements per 100 g serving of Nile tilapia tissues was compared to the dietary reference intake for women aged between 19 and 30 years as an example that was set by the Food and Nutrition Board, Institute of Medicine, United States National Academy of Sciences (Monsen, 2000; Trumbo, 2001; NAC, 2011).

5.3.8 Statistical Analysis

All data were displayed on a scatterplot to check for normality. The effects of tissues on mineral, heavy metal, and ash concentrations were evaluated with a general linear model repeated measure analysis of variance (ANOVA). One-way analysis of variance (ANOVA) was also used to determine the effect of locality on mineral and toxic trace element concentrations. For both analyses, a subsequent post-hoc comparison using Tukey's test was performed. All analyses were done using the statistical package of SPSS version 26.0. Significance was evaluated at $p < 0.05$ confidence level.

5.3 Results

The physicochemical properties of the three Ethiopian lakes used in this study are presented in Table 5. 2. The two Rift Valley lakes (Ziway and Langano) showed no observed differences except electric conductivity was markedly higher in Lake Langano than in Lake Ziway. However, Gilgel Gibe showed notably lower values for all parameters except for dissolved oxygen in which there was no pronounced difference among the three lakes.

Table 5. 2 Physicochemical water quality parameters of the three Ethiopian lakes in this study.

Lake	T (^{0}C)	DO (mg/L)	EC (µS/cm)	pH	NH_3 (mg/L)	NO_3^- (mg/L)
LZ	26.98	6.31	1345	8.55	0.64	7.97
LL	27.41	6.11	1634	8.73	0.68	9.26
GR	23.29	6.74	456	7.56	0. 07	2.81

(*In situ* measurement); T = Temperature, DO = Dissolved oxygen, EC = electric conductivity; LZ= Lake Ziway; LL= Lake Langano; GR= Gilgel Gibe Reservoir

Across the lakes, the concentrations of macrominerals Na, Mg, and K were significantly higher in the digestive tract compared to other tissues. However, in the three lakes, the concentrations of Ca and P were significantly higher ($p<0.05$) in the skin and gill tissues compared to other tissues. Gills from Lake Langano contained more Ca compared to the other two lakes. There was no significant difference in muscle ash content between the lakes. The ash content in gill and skin was significantly lower in Gilgel Gibe fish compared to the other two lakes. However, significant higher ash content ($p< 0.05$) was observed in the digestive tract and liver from Lake Langano fish compared to the other two lakes (Table 5.3).

Table 5.3. The concentration of ash and macrominerals in Nile tilapia tissues from the three lakes in Ethiopia (g/kg dry matter)

	Tissues	GR	LZ	LL	SEM	P_L	$P_{(L \times T)}$
Ca	Muscle	2.2^{A}	2.3^{A}	2.4^{A}	0.3	0.803	<0.001
	Gill	49^{aC}	66^{abC}	76^{bC}	4	0.007	
	Skin	46^{aC}	58^{aC}	95^{bC}	3	<0.001	
	Digestive tract	13^{B}	17^{B}	9^{B}	3	0.186	
	Liver	3^{A}	2^{A}	5^{A}	2	0.768	
	P_T	<0.001	<0.001	0.005	2	-	
Mg	Muscle	1.4^{A}	0.9^{A}	0.8^{A}	0.1	0.054	<0.001
	Gill	1.2^{bA}	1.5^{cA}	0.7^{aA}	0.1	<0.001	
	Skin	1.2^{bA}	1.1^{bA}	0.5^{aA}	0.1	<0.001	
	Digestive tract	3.9^{bB}	4.8^{bB}	1.8^{a}	0.2	<0.001	
	Liver	1.7^{bA}	1^{aA}	0.6^{aA}	0.1	0.043	
	P_T	0.02	0.001	<0.001	0.1	-	
Na	Muscle	1^{A}	3^{A}	2.3A	0.4	0.059	0.002
	Gill	4.9^{aA}	6.7^{bB}	8.9^{cB}	0.3	0.013	
	Skin	1.3^{aA}	1.1^{aA}	6.1^{bA}	0.9	0.024	
	Digestive tract	26^{C}	12^{C}	25^{D}	4	0.089	
	Liver	12^{B}	13^{C}	11^{C}	2	0.782	
	P_T	<0.001	0.001	0.002	1	-	
Ash	Muscle	59^{A}	63^{A}	69^{A}	5	0.19	0.037
	Gill	153^{aB}	199^{bC}	205^{bC}	10	<0.001	
	Skin	135^{aB}	226^{bD}	234^{bC}	7	<0.001	
	Digestive tract	323^{aC}	371^{bD}	316^{aD}	10	0.001	
	Liver	143^{bB}	121^{aB}	140^{bB}	7	0.01	
	P_T	<0.001	0.003	0.001	8	-	

Table 5.3 **Continued**

	Tissues	GR	LZ	LL	SEM	P_L	$P_{(L*T)}$
	Muscle	0.1^{A}	0.1^{A}	0.2^{A}	0.05	0.238	
	Gill	0.7^{aB}	0.2^{aA}	1.7^{bC}	0.1	0.002	<0.001
S	Skin	0.1^{aA}	0.2^{aA}	1.3^{bC}	0.03	<0.001	
	Digestive tract	1.5^{bC}	0.5^{aB}	0.6^{aB}	0.1	0.003	
	Liver	0.1^{A}	0.4^{B}	0.3^{A}	0.06	0.064	
	P_T	0.01	0.01	0.004	0.07	-	
	Muscle	10^{A}	8^{A}	8^{A}	1	0.258	
P	Gill	27^{aB}	37^{bC}	44^{bC}	2	0.003	<0.001
	Skin	26^{aB}	31^{aC}	50^{bC}	2	<0.001	
	Digestive tract	24^{bB}	9^{aA}	14^{aB}	2	0.018	
	Liver	25^{bB}	15^{aB}	13^{aB}	1	0.003	
	P_T	0.01	0.002	0.001	2	-	
	Muscle	18^{B}	14^{B}	15^{B}	2	0.379	
K	Gill	5^{A}	6^{A}	7^{A}	1	0.132	0.006
	Skin	6^{bA}	3.5^{aA}	4.8^{abA}	0.4	0.013	
	Digestive tract	28^{bC}	15^{aB}	17^{abB}	3	0.047	
	Liver	26^{bC}	17^{aB}	14^{aB}	2	0.008	
	P_T	0.001	<0.001	<0.001	2	-	

^{a,b,c,} Different superscripts in small letter within a row and ^{A,B,C,D,} superscripts in capital letter within a column indicate significant differences at P <0.05; GR= Gilgel Gibe reservoir, LZ= Lake Ziway; LL=Lake Langano; SEM= Standard error of the means; P_T= P value for tissues, P_L= P value for Lakes and P_{T*L}= P-value for the interaction.

The concentration of the evaluated microminerals (Fe, Zn, Cu, and Mn in mg/kg dry matter) significantly varied between the tissues and the lakes (Table 5. 4). A significant higher concentration of Fe was observed in the gill tissue from Lake Ziway and Gilgel Gibe fish than Lake Langano fish. However, Fe concentration in the liver tissue from Gilgel Gibe fish was significantly higher than from the other two lakes ($p<0.05$). There was no significant variation in Zn concentration in digestive tract tissue between the three lakes, but it significantly varied in gill and skin. Mn concentration was significantly higher in the skin and digestive tract from Gilgel Gibe fish ($P<0.05$). Muscle from Lake Langano had significantly higher Cu concentration compared to the other two lakes.

However, the liver samples from the same lake showed lower Zn concentrations than the two other lakes. The measured concentrations of toxic trace elements (Al, Cr, Co, Ni, and Cd in mg/kg dry matter) varied depending on the analyzed tissue and the origin of the sample (Table 5.4). The highest concentrations were found in liver and digestive tract tissues in the three lakes. For instance, the liver and the digestive tract from Gilgel Gibe fish contained significantly higher concentrations of cobalt than the same tissues from the other two lakes, but tissue cobalt concentrations did not significantly vary between lakes. Muscle, gill, and skin contained lower concentrations of Al and Cr than in digestive tract and liver tissues across the three lakes. The concentration of cadmium was below the detection limit in all analyzed tissues and lakes, except in the liver tissue from Lake Ziway. Digestive tract samples from Gilgel Gibe and Ziway contained notably higher concentrations of Al than other tissues, but these were several-fold lower than samples from Lake Langano.

Table 5.4 Concentrations of trace elements in Nile tilapia tissue from the three lakes in Ethiopia mg/kg dry matter)

	Tissues	GR	LZ	LL	SEM	P_L	$P_{(L*T)}$
	Muscle	3	1	14	7	0.432	
	Gill	5	5	3	1	0.223	NS
Mn	Skin	10^{b}	5^{ab}	4^{a}	1	0.036	
	Digestive tract	1.5^{b}	0.4^{a}	0.5^{a}	0.1	0.001	
	Liver	2.6	2.4	1.3	0.4	0.305	
	Muscle	228^{B}	249^{B}	237^{B}	21	0.451	
	Gill	482^{C}	537^{C}	547^{C}	57	0.711	
Fe	Skin	53^{A}	97^{A}	115^{A}	21	0.168	<0.001
	Digestive tract	16196^{bE}	15405^{bE}	5573^{aD}	1389	0.004	
	Liver	2497^{bD}	783^{aD}	621^{aC}	208	0.002	
	P_t	<0.001	0.01	<0.001	339	-	
	Muscle	15^{A}	24^{A}	47^{A}	8	0.084	
	Gill	52^{aB}	76^{bB}	75^{bB}	5	0.025	<0.001
Zn	Skin	55^{aB}	63^{aB}	93^{bB}	7	0.014	
	Digestive tract	178^{C}	124^{C}	141^{C}	14	0.083	
	Liver	178^{C}	95^{C}	128^{C}	22	0.083	
	P_t	0.001	<0.001	<0.001	11	-	
	Muscle	2^{aA}	1.6^{aA}	5^{bA}	0.2	<0.001	
	Gill	2^{A}	3^{A}	6^{A}	2	0.269	0.001
Cu	Skin	1.2^{A}	1.6^{A}	4^{A}	0.9	0.119	
	Digestive tract	30^{B}	37^{B}	29^{B}	7	0.683	
	Liver	1816^{bC}	1099^{bC}	126^{aC}	406	0.049	
	P_t	0.01	0.01	0.03	83	-	
	Muscle	111^{B}	259^{C}	105^{A}	41	0.335	
	Gill	113^{B}	124^{B}	193^{B}	40	0.431	
Al	Skin	29^{A}	64^{A}	216^{B}	74	0.253	<0.001
	Digestive tract	18406^{bD}	23604^{bE}	9411^{aD}	983	0.017	
	Liver	461^{C}	702^{D}	366^{C}	203	0.694	
	P_t	<0.001	0.001	0.004	268	-	

Table 5.4 **continued**

	Tissues	GR	LZ	LL	SEM	P_L	$P_{(L*T)}$
	Muscle	0.80	0.93	0.96	0.08	0.364	
	Gill	1.47	1.88	1.29	0.23	0.251	<0.001
Cr	Skin	2.04^{c}	1.41^{b}	0.75^{a}	0.12	0.001	
	Digestive tract	11.7^{a}	17.3^{b}	12.6^{a}	0.9	0.024	
	Liver	15.6^{b}	2.3^{ab}	1.3^{a}	3.2	0.047	
	P_T	<0.001	0.004	<0.001	1	-	
	Muscle	<0.1	<0.1	<0.1	-	-	
	Gill	<0.1	<0.1	<0.1	-	-	NS
Co	Skin	<0.1	<0.1	<0.1	-	-	
	Digestive tract	<0.1	<0.1	<0.1	-	-	
	Liver	11^{b}	7^{a}	3^{a}	0.5	<0.001	
	Muscle	<0.1	<0.1	<0.1	-	-	
	Gill	<0.1	<0.1	<0.1	-	-	NS
Ni	Skin	<0.1	<0.1	<0.1	-	-	
	Digestive tract	7^{b}	2^{a}	2^{a}	1.4	0.011	
	Liver	5.3^{b}	1.3^{a}	1.3^{a}	1.3	0.001	
	Muscle	<0.01	<0.01	<0.01	-	-	
Cd	Gill	<0.01	<0.01	<0.01	-	-	NS
	Skin	<0.01	<0.01	<0.01	-	-	
	Digestive tract	22	26	8	1.3	0.167	
	Liver	<0.01	0.07	<0.01	-	-	

^{a,b,c,} Different superscripts in small letter within a row and ^{A,B,C,D,E,} superscripts in capital letter within a column indicate significant differences at P <0.05; GR= Gilgel Gibe reservoir, LZ= Lake Ziway; LL=Lake Langano; SEM= Standard error of the means; NS= not significant; P_T= P value for tissues, P_L= P value for lakes and P_{T*L}= P value for the interaction.

The percentage contribution of Nile tilapia tissues from the three Ethiopian lakes per 100g serving to a mineral's dietary recommended intake (DRI) is summarized in Table 5.5 for women aged between 19 and 30, as an example. Gill and skin provided a higher percentage of DRI of Ca and liver provided a higher percentage of DRI for Fe, Na, and Cu than other tissues per 100 g of serving for women.

Table 5.5 Contribution (%) of Nile tilapia tissues from the three Ethiopia Lakes to dietary reference intake (DRI *) for females aged between 19 and 30, as an example (compared with USDA standard reference)

Lake	Tissue	Mg	Na	K	Ca	P	Fe	Zn	Cu	Mn
	M	45	3	69	22	143	126	19	23	17
	G	38	12	19	490	386	267	65	22	28
GR	S	38	3	23	460	371	29	68	13	55
	L	54	30	100	30	357	1387	222	20177	14
	M	29	7	53	23	114	138	30	17	6
LZ	G	48	17	23	660	259	298	96	33	28
	S	35	3	13	580	442	53	78	17	28
	L	32	32	65	20	214	435	118	12211	13
	M	26	6	57	24	114	131	58	55	77
LL	G	22	22	26	760	629	303	93	66	16
	S	16	15	18	950	714	63	116	47	22
	L	19	27	53	50	186	345	160	1400	7
Reference		68	23	64	8	243	31	30	83	16

GR= Gilgel Gibe reservoir, LZ= Lake Ziway, LL= Lake Langano. Ref.:- Reference; *Dietary reference intake (Recommended Daily Allowance) for women aged between 19 and 30 years is set by the Food and nutrition board, institute of medicine, United States National Academy of Sciences (chapter, 4). M= Muscle; G= Gill; S=Skin, L=Liver

5.4 Discussion

Ash is the indication of overall mineral content in the samples (Ayanda et al., 2019). From the ash and individual element concentrations, it is clear that muscle (fillet) is not a rich source of minerals (**chapter, 3 and 4**). Therefore, encouraging the consumption of other tissues, or the entire fish, could substantially increase mineral intake in the human diet.

Higher ash content in the digestive tract likely reflects high mineral concentrations in the diet, potentially including sediment. In several commercially important fish species (Zenebe et al., 1998; Khalili et al., 2018), ash content responded to changes in localities. The sum of all analyzed elements only represents a fraction of the total ash in the digestive tract, suggesting that silicon-containing soil dominates total ash content in the digestive tract samples. The higher ash concentration in LZ digestive tract may, therefore, indicate the eutrophic status of the lake and a more particulate-suspension-feeding strategy of the fish in that lake (Tilahun and Ahlgren, 2010), resulting in a high intake of insoluble, non- absorbable minerals, as can be seen from the lower ash concentration in the liver of LZ fish, as well as the high intestinal Ca: P ratio, typical for calcium-based soil material.

The “bony” structures in gills and skin can explain the higher deposition of Ca and P in those tissues. Both external tissues deposit hydroxyapatite in the vitrodentin of the scales, composed of collagen covered with Ca (Alina and Justyna, 2014). In these tissues, the highest concentrations of these minerals were observed in fish from LL, showing an adaptation of these fish to the high electric conductivity of this lake. Likely the high Na concentrations in gills and skin in the same lake adhere to that principle as well. This means that the environment affects macromineral concentrations in fish tissues.

Lake Langano is indeed a lake with high Na and Ca concentrations in the water (Tadesse, 1999), compared to the two other lakes. In contrast to gills and skin, the low

Ca concentration in the digestive tract of LL fish means that diet items are not necessarily higher in Ca in contrast to the water. However, reports have indicated that the elevated concentration of minerals in gills reflects a higher concentration in the diet (Samar, 2012), which may still hold for some of the microminerals, as will be discussed further.

Most S in the body is found in S-containing amino acids such as methionine and cysteine. The latter is especially needed to make strong protein structures through disulfide bonds. It is, therefore, logical to find the highest concentrations, again, in skin and gills and fairly low concentrations in muscle, despite its high protein content. Here, too, the high S concentration in LL skin and gills corresponds with the apparent need for stronger structures in the gills and skin to resist the high conductivity and mineral load in that lake. The high S concentrations in the digestive tract and especially those from GR are, again, likely to reflect the high S concentrations in the diet items because of the importance of disulfide bonds in the strength of chitin (Montroni et al., 2020), a structure dominating the exoskeleton of zooplankton (Cauchie, 2002).

Further investigation is warranted to identify the origin of the very high intestinal Fe concentration in all fish. Since it coincides with high concentrations of Al, the intake of suspended soil or the accidental intake of particulate matter during suspension feeding may be a plausible explanation. The three lakes are surrounded by Al- and Fe-rich soils (Tilahun and Ahlgren, 2013; Ambelu et al., 2013; Merga et al., 2020), increasingly challenged by soil erosion (Wolka et al., 2015). Although Fe is an essential mineral for animals, extremely high concentrations may become a burden to the fish; although Fe uptake is strongly regulated, hepatic accumulation seems to occur (Anderson, and Shah, 2013; Kondaiah et al., 2019). Fish are known to regulate elevated Fe concentrations in the whole body by controlling the level in blood, subsequently transferring Fe to the liver for storage (Cohen et al., 2001). As LL has more Ca-rich soils, the problem is less apparent than in LZ and GR. The overall picture regarding Fe confirms the report by (Rajkowska and Protasowicki, 2013) that essential minerals can

be found in fish tissues, with the highest concentrations in the liver, gills, kidneys, and spleen and the lowest concentrations in skin and muscle. The liver has a crucial function in basic metabolism, (exchangeable) mineral storage, redistribution, and detoxification or transformation (Agah et al., 2009; Malik et al., 2010).

Iron deficiency causes anemia, and fish are a major source of Fe (Roos et al., 2007). In the present study, the Fe concentration of the liver, digestive tract, and gills of Nile tilapia is several folds higher than in muscle and skin. Therefore, the liver and gills of Nile tilapia may be a good source of iron for human nutrition, depending on dietary needs. Similarly, Cu, Zn, and Mn were also higher in those tissues compared with muscle.

Fish liver and gills accumulate not only essential elements like Fe, Cu, Zn, and Mn, which may also become toxic at too-high uptake levels but also toxic trace elements like Cr, Al, Cd, Ni, and Co (Türkmen et al., 2009). It is, therefore, important to be careful in the consumption of Nile tilapia liver due to the high content of these toxic trace elements. For this reason, only the liver and gills of Nile tilapia that are harvested or cultured under controlled environmental conditions can be considered safe for consumption.

According to the US Food and Drug Administration (USAD, 2018), food that provides more than 10% of nutrients per serving is considered a good source of that nutrient. In this study, mineral contribution to DRI per 100-g serving was calculated (Table 5.5). Nile tilapia tissues from the three Ethiopian lakes of this study can provide more than 10% of minerals (Ca, P, Fe, Zn, Cu, Mg, Mn, Na, K) for women aged between 19 and 30, hence making Nile tilapia tissues (other than muscle) good sources of these minerals. This comparison is made to show that small inclusions of such parts (tissues) in the diet may substantially improve the intake of certain minerals. This possibly indicates that wild tilapia may have higher mineral concentrations than fish in cultured conditions, and this should be considered in the contribution of fish to human mineral intakes. Several factors could contribute to these differences, such as diet,

geographical variations, the environment that the fish were grown in, and the methods that were used to measure the minerals.

The concentration of heavy metals in water influences the content of toxic trace elements in fish tissues (Chale, 2002). In our study, the highest concentrations of these toxic trace elements were recorded in the liver and digestive tract, followed by gills, and the lowest in muscle and skin. This agrees with other reports that the metal accumulation capacity of muscles in fish is lower than that of other tissues such as gills, gut, and liver (Meletem et al., 2007). Cadmium is a nonessential element found in natural waters, with potential toxicity to fish even at low levels (Edevaldo et al., 2016). The detection of Cd only in the liver tissue from LZ could be related to intensive human activities carried out in every corner of that lake. A recent publication reported that the lake is under increasing agricultural and urban pressure and is exhibiting deteriorating trends in several water-quality and ecological parameters; additionally, toxic trace element concentrations of the lake have shown increasing temporal trends (Merga et al., 2020).

Similarly, the higher Al accumulation in the digestive tract from Gilgel Gibe and Ziway fish is likely caused by the high inflow of eroded topsoil due to intensive unprotected farming activities in the surrounding ecosystems. Whereas the other toxic trace elements were still at acceptably low, the high concentrations of Al in the digestive tract may be a concern for human consumption as it exceeds the maximum tolerable intake (Van paemel et al., 2010).

As a conclusion, differences in minerals and toxic trace element concentrations were observed between tissues in Nile tilapia. In addition, these concentrations were substantially affected by the lake where these fish caught from. Some differences could be attributed to the water quality of the lakes, whereas others may refer to the trophic status of the lakes (food web). Compared to fillet (muscle), the typically non-eaten parts were much richer in minerals, leaving an opportunity to fortify human nutrition with essential minerals. However, caution is needed due to the accumulation of some

toxic trace minerals such as Al, likely arising from anthropogenic soil erosion. Moreover, the route by which these toxic trace elements enter into fish tissue should be optimally identified, and responsible bodies should pay attention to public health safety. Quality control for toxic trace elements in water and diet is therefore warranted when culturing these fish.

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CHAPTER 6: Nutrient-related metabolite profiles explain habitat dependent differences in body composition and size in Nile tilapia (*Oreochromis niloticus)* from different Ethiopian lakes

Adapted from

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Abstract

This study investigated how metabolite analysis can explain differences in tissue composition and size in fish from different habitats. We, therefore, studied Nile tilapia from three Ethiopian lakes (Gilgel Gibe, Ziway, and Langano) using dried bloodspot (DBS) analysis of carnitine esters and free amino acids. A total of sixty (N=60) Nile tilapia samples were collected comprising twenty (n=20) fish from each lake. The DBS samples were analyzed for acylcarnitine and free amino acid profiles using quantitative electrospray tandem mass spectrometry. Metabolite ratios were calculated from relevant biochemical pathways that could identify relative changes in nutrient metabolism. Marked differences were observed in Nile tilapia metabolic activity between the lakes. For instance, the lower body weight and body condition of the fish in Lake Langano coincided with several metabolite ratios pointing to a low flow of glucogenic substrate to the citric acid cycle. In the Gilgel Gibe fish, the metabolic markers for lipogenesis and metabolic rate could explain the high fat concentration in several parts of the body. Our results show that nutrition-related blood metabolite ratios are useful to understand the underlying metabolic events leading to the habitat-dependent differences in growth of Nile tilapia, and by extension, other species.

6.1 Introduction

Fish populations are declining worldwide because of several threats (Cury et al., 2011; Pikitch, 2012; FAO, 2018). The causes are anthropogenic factors: habitat destruction, the introduction of new species, pollution, and overfishing (Branch and Steffani, 2004; de greef et al., 2004; Davidson et al., 2014; Landrigan et al., 2018) and are accompanied by climate change (Döll et al., 2015). These threats not only affect the fish production potential but also the nutritive value and metabolic activities of fish. They are altering the aquatic ecosystem by shifting distributions, nutrient load, and phenologies (Whitehead et al., 2009; Knouft and Ficklin et al., 2017), but understanding how fish metabolically respond to these changes remains a challenge (Claireaux and Chabot, 2016).

Reports indicate that fish can perform differently in different ecosystems: even the same fish species in different habitats show variation in proximate composition especially on fat content (Hadjinikolova et al., 2008; Ljubojević et al., 2013). This may be directly linked with the composition of the food web in each habitat (Pyz-Łukasik and Paszkiewicz, 2018). To understand how the food web affects fish growth, there is a need to identify how nutrition is affecting fish metabolism. Environmental conditions have been reported as key factors to affect fish metabolism (Enders and Boisclair, 2016), yet studies on fish metabolism are usually performed under lab conditions, minimizing the complexity that is present in nature (Zheng et al., 2014; Geda et al., 2015; Sabzi et al., 2017).

In such experiments, fish blood analyses have been used as a tool to investigate amino acid and fatty acid catabolism (Geda et al., 2012; Zheng et al., 2014; Geda et al., 2015; Sabzi et al., 2017; Geda et al., 2017). Most of these studies were conducted under controlled parameters like temperature, oxygen, and given a known diet. For instance, in carp, mildly elevated temperature resulted in increased amino acid catabolism (Zheng et al., 2014) and dietary L-carnitine supplementation stimulated lipid

catabolism and increased glycogen and protein deposition in Nile tilapia muscle (Li et al., 2020).

However, fish in their natural ecosystem are experiencing a heterogeneous habitat and complex flow field (Butler et al. 2004; Forster et al. 2012). A natural ecosystem harbours a diversity of factors, making it hard to identify the most limiting factors for fish growth without unravelling the effects on metabolism mechanistically. To our knowledge, no results have been reported on nutrient metabolite profiles to explain differences in fish performance in different wild aquatic ecosystems using acylcarnitines and amino acid profiling. We here used dried bloodspot analysis for nutrient metabolite profiling (carnitine esters and free amino acids) to identify why Nile tilapia differs in body composition and size between three different Ethiopian lakes.

6.2 Material and Methods

6.2.1 Animal ethics statement

This study was checked and given approval by Jimma University, College of Natural Sciences Research and Ethical Review Board Committee (Jimma, Ethiopia). The committee has followed the standard procedures written on Article 6 (methods of killing) and Article 9 (Animals are taken from the wild), of directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes and certified this study with the letter referenced (Ref. No: RPG/165/2019).

6.2.1 Fish sample collection and euthanasia

Nile tilapia samples were collected from three Ethiopian lakes (Gilgel Gibe, Ziway, and Langano) (**Chapter 5,** Figure 5.1 and Table 5.1). For this study, Nile tilapia was selected based on its relevance to commercial fishing in the country and the fish consumption by the local population. A total of sixty (N=60) Nile tilapia samples were collected comprising twenty (n=20) fish from each lake caught using same standard gillnets.

Immediately, the fish were euthanized by pithing the brain (Clark et al., 2011) for blood collection.

Then, the samples were thoroughly washed with tap water to remove any adhering contaminants and then transported using an icebox to the laboratory (samples from Gilgel Gibe reservoir were transported to Jimma University Zoological Sciences whereas samples from Lake Ziway and Langano were transported to Batu Fisheries and Other Living Aquatic Resource Research Center). Upon arrival, all fish were measured for their weight and length to the nearest 0.10 g and 0.01 cm respectively. Afterward, the samples were re-washed thoroughly with potable water, then dissected (using plastic tools) and targeted tissues (muscle, gills, skin, gut, and liver) were collected. Macronutrient composition (dry matter, crude protein, and crude fat content) of all targeted tissues were determined.

6.2.3 Blood sampling and analysis of acylcarnitine

Blood samples were drawn from the caudal vein (Ferguson, 2005) by puncturing with a 1 mL syringe (Becton Dickinson S.A., Madrid, Spain) and a 26-G needle (Becton Dickinson, Drogheda, Ireland) rinsed with heparin (LEO Pharma, Ballerup, Denmark). Blood collection from each fish lasted less than 3 min to avoid cortisol increase (Arends et al., 1999) due to manipulation upon sampling. Consequently, the blood samples were blotted and dried on filter paper (Whatman903^{TM}, Cardiff, CF147YT, UK). The dried blood spot (DBS) samples were shipped to Ghent University, Belgium, for further analyses. Finally, the acylcarnitine and free amino acid profile of the DBS was determined using quantitative electrospray tandem mass spectrometry following (Zytkovicz et al., 2001; Vieira Neto et al., 2012) at the Laboratory for Metabolic Diseases at Ghent University Hospital. Metabolite ratios were calculated from relevant biochemical pathways that could identify relative changes in nutrient metabolism. The metabolite ratios used in this study were as followed; acetyl carnitine : free carnitine (C2:C0); propionyl- : acetyl carnitine (C3:C2); 3-hydroxybutyryl- : acetyl carnitine (3OH-C4:C2); methylmalonyl- : propionyl carnitine (C4DC:C3); methylmalonyl carnitine :

(valine+methionine) (C4DC:val+met); malonyl carnitine : acetyl carnitine (C3-DC:C2); valine : leucine (val: leu) and sum of 3-hydroxy-long-chain fatty acids : sum of long-chain fatty acids (3-OH LCFA:LCFA).

6.2.4 Proximate analysis:

The proximate composition of all targeted tissues of Nile tilapia was determined by conventional methods described by the Association of Official Analytical Chemists (AOAC, 1990) for crude fat) and crude protein.

6.2.4.1 Crude fat determination

Crude fat was analysed using the ether extract method (AOAC, 1990). A 2 g dried sample was inserted into a porous thimble allowing rapid flow petroleum ether. The sample was wrapped in filter paper, placed into the thimble, and covered with glass wool. Anhydrous ether was placed into a weighed boiling flask, which, together with the Soxhlet flask and condenser, was assembled into the Soxhlet apparatus. Crude fat was extracted into a Soxhlet extractor for 6 hours, by the heating solvent in the boiling flask. The boiling flask with extracted fat was dried in an air oven at 100°C for 30 minutes, cooled in a desiccator, and weighed. Finally, the percentage of crude fat was computed following.

6.2.4.2 Crude protein determination

Crude protein was determined by an automatic Kjeldahl analyser method (Pearson 1999), using Velp Scientifica ™ (UDK 159, F30200150). A VELP DK Series Digestion Unit and a VELP UDK 159 automatic digestion and titration system were used subsequently. Every sample flask was prepared by adding 2 catalysts for digestion. This included 1 g of the dried sample, 0.2 g $CuSO_4$, and 7 g of K_2SO_4. For the actual digestion, 20 ml of H_2SO_4 was added. The program was set at 30 minutes at 250°C, 30 minutes at 350°C, and 1 hour at 420°C. Ultimately the samples were cooled down to 50°C.

Then, distillation took place by adding distilled water followed by adding 10 ml and 7 ml of indicators bromocresol green and methyl red respectively. The nitrogen was separated from the digested mixture by steam distilling with the UDK 159 to extract ammonia from the alkaline solution. Sodium hydroxide (35%) was added to raise the pH and convert solid NH_4^+ into gaseous NH_3. The distilled nitrogen was then trapped by adding boric acid.

For the final colorimetric titration, hydrochloric acid was added to react with the ammonia and the indicators. The volume of titrant that was needed to reach the endpoint allows to automatically calculate the amount of nitrogen, expressed as a percentage of nitrogen or display the percentage of protein directly.

6.2.5 Statistical analyses

All data were evaluated for normality. The crude protein and crude fat content of tissues were analysed by repeated measure analysis of variance within tissues as within-subject variable and lake as between-subject variable. One-way analysis of variance was used to analyse the ratios of selected acylcarnitines and free amino acids. For both analyses, a subsequent post-hoc comparison using Tukey's test was performed. All analyses were done using the statistical package of SPSS version 26.0. The significance was treated at $p<0.05$ confidence level.

6.3 Results

6.3.1 Physicochemical properties of the three Lakes

The physicochemical properties of the three Ethiopian lakes used in this study were presented in Chapter 5, Table 5. 2. The two Rift Valley lakes (Ziway and Langano) showed no observed difference except electric conductivity was markedly higher in Lake Langano than in Lake Ziway. However, Gilgel Gibe showed notably lower values for all parameters except for dissolved oxygen in which there was no pronounced difference among the three lakes.

6.3.2 Morphometric feature of Nile tilapia from the three lakes

Morphometric feature of Nile Tilapia caught for this study from the three lakes were presented Table 6.1. Though, fish sample collection method and material used were similar, the caught composition showed distinctive weight length variation. Accordingly, the caught Nile tilapia from Lake Ziway were largest (178 g), followed by Gilgel Gibe reservoir (134 g) and Lake Langano (118 g).

Table 6.1. Morphometric features of Nile tilapia caught from three lakes in Ethiopia

	GR	LZ	LL	*P*	SEM
Weight (g)	134^{b}	178^{c}	118^{a}	<0.001	6
Length (cm)	19.6^{b}	22.4^{c}	17.3^{a}	<0.001	0.3
Weight:Length	7.8^{b}	7.9^{b}	4.7^{a}	0.002	0.2

Different superscripts within a row indicate significant differences at P <0.05; GR=Gilgel Gibe reservoir, LZ= Lake Ziway; LL= Lake Langano and SEM= Standard error of the means

6.3.3 Crude protein and crude fat composition of Nile Tilapia tissues

All tissues except liver showed a significant differences in crude fat concentrations among tissues, but only two tissues showed significant variation in crude protein composition among the lakes (Table 6.2). The source of fish affected the composition of crude fat in several tissues. For most tissues, crude fat concentration was

significantly higher in GR fish and lowest in LL fish. Crude protein concentration was slightly higher in guts from Gilgel Gibe fish and gills from Ziway fish. For the other tissues, there were no significant differences in crude protein concentration between the lakes.

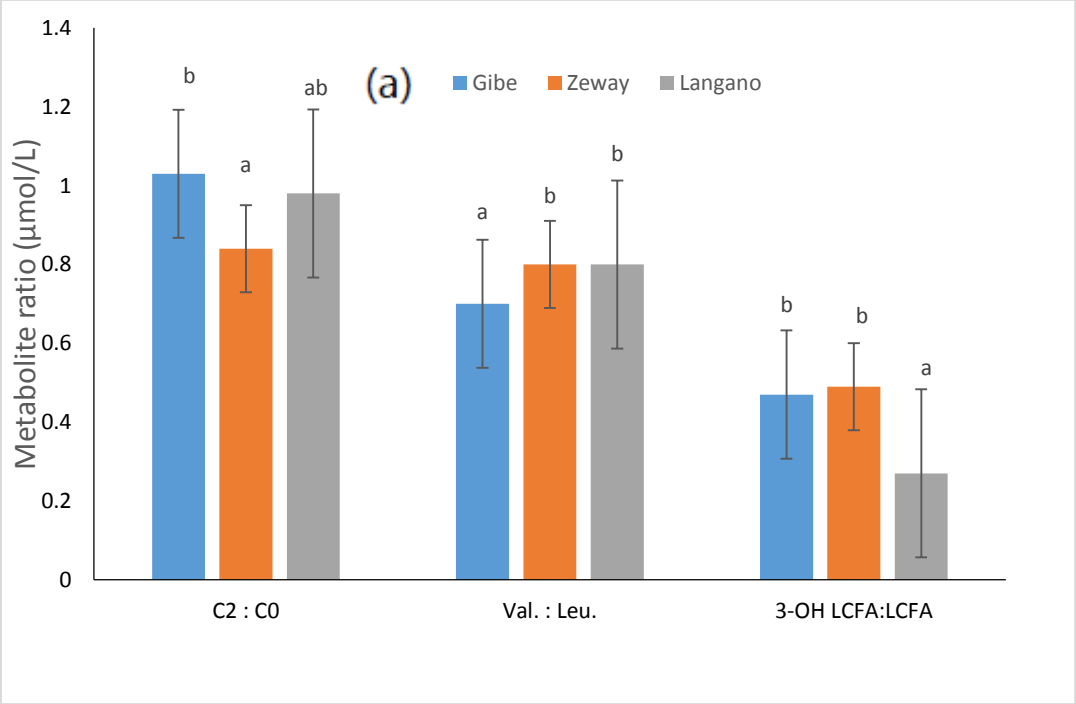
Table 6.2. Crude fat and protein content of Nile tilapia tissues from the three lakes in Ethiopia (g/kg on dry matter basis)

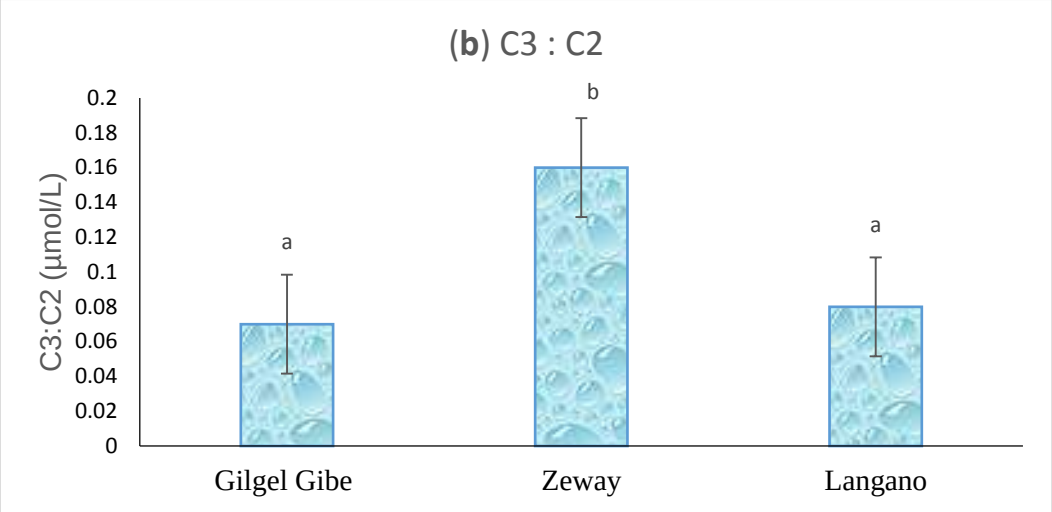
	Tissues	GR	LZ	LL	SEM	P_L	$P_{(L*T)}$
Crude fat	Muscle	34^{bA}	31^{abA}	26^{aA}	2	0.008	<0.001
	Gill	287^{bC}	215^{aC}	219^{aC}	7	<0.001	
	Skin	49^{bA}	46^{bA}	31^{aA}	3	<0.001	
	Gut	179^{bB}	169^{aB}	168^{aB}	5	0.01	
	Liver	165^{B}	166^{B}	171^{B}	4	0.13	
	P_T	<0.001	0.002	<0.001	4	-	
Crude protein	Muscle	856^{D}	851^{C}	844^{C}	8	0.28	0.001
	Gill	473^{abA}	496^{bAB}	445^{aA}	14	0.005	
	Skin	712^{C}	690^{B}	684^{B}	11	0.061	
	Gut	$407^{b\ A}$	399^{abA}	386^{aA}	5	0.004	
	Liver	604^{B}	603^{B}	606^{B}	10	0.08	
	P_T	0.003	0.001	<0.001	9	-	

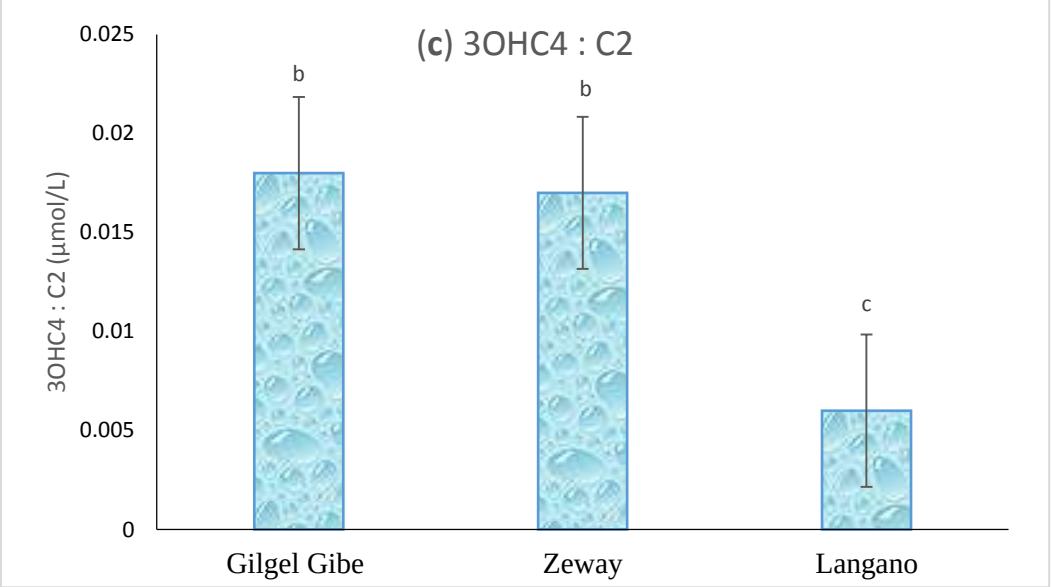
^{a,b,c,} Different superscripts in small letter within a row and ^{A,B,C,D,E,} superscripts in capital letter in column indicate significant differences at P <0.05; GR= Gilgel Gibe reservoir, LZ= Lake Ziway; LL=Lake Langano; SEM= Standard error of the means; P_T= P-value for tissues, P_L= P-value for Lakes and P_{T*L}= P-vale for the interaction

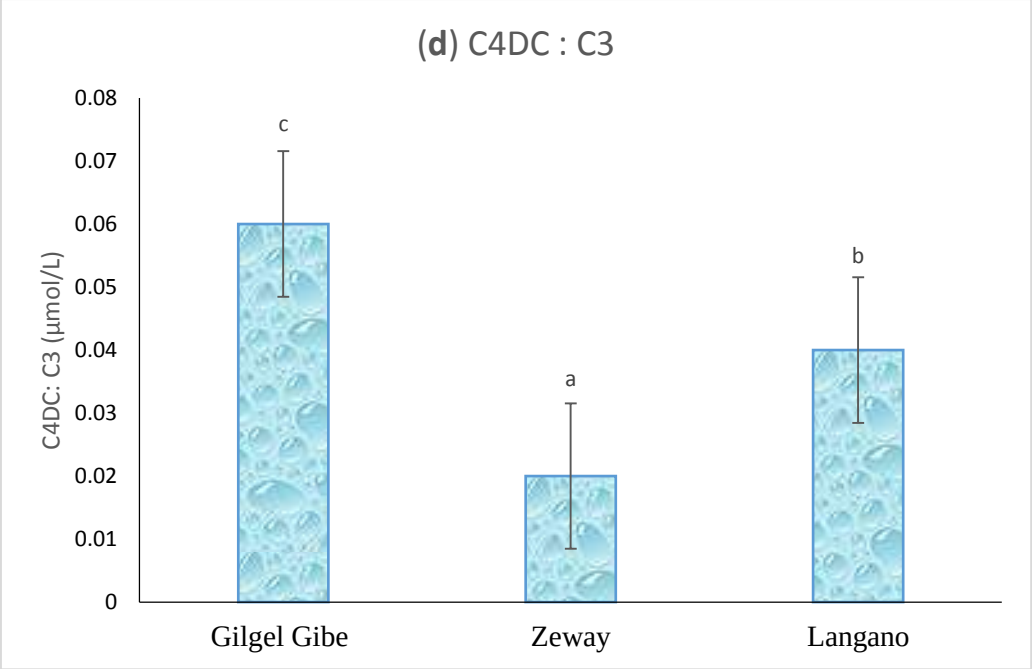
6.3.4 Selected acylcarnitine and free amino acids ratio

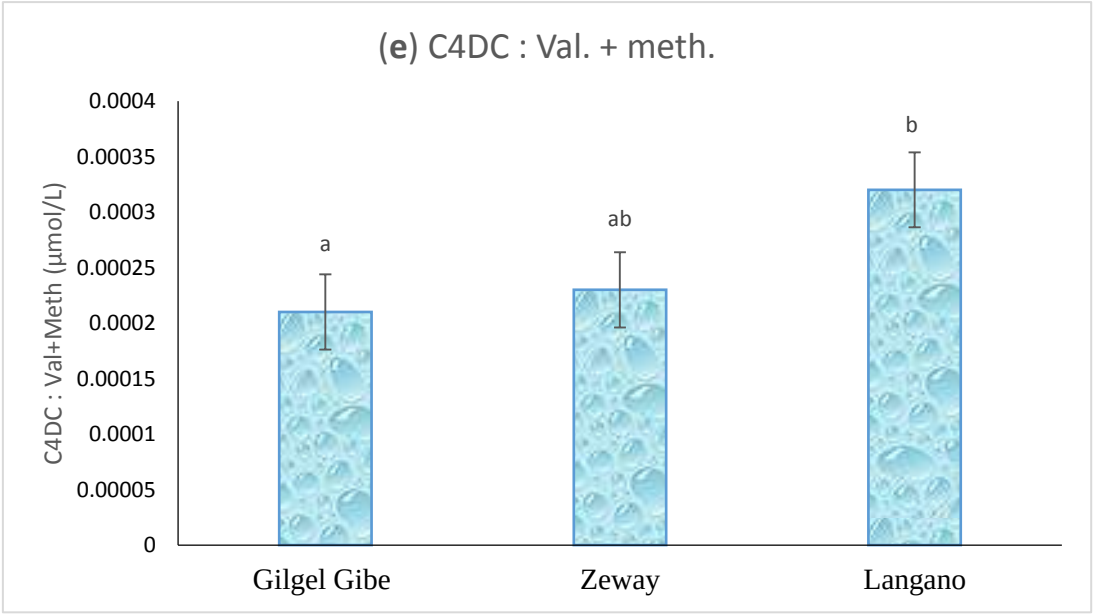
The results of selected acylcarnitine and free amino acids ratio are presented in (Figure 6.1a-f). Significant differences were observed in Nile tilapia metabolic activity among the lakes. The C2:C0 ratio, here used as a marker for metabolic rate, was higher in Nile tilapia from Gilgel Gibe than Ziway, with intermediate values for Langano. The C3:C2 ratio was higher in Nile tilapia from Lake Ziway than the two other lakes. In Langano fish, the 3OHC4:C2 ratio was lower than fish from the other lakes. The C4DC: C3 ratio was highest in Gilgel Gibe, followed by Langano and then Ziway. Yet, the C4DC: val+met ratio was lower in Gilgel Gibe than in Langano, with Ziway as intermediate. Langano fish had a lower C3DC: C2 ratio and a lower 3OH-LCFA: LCFA ratio than fish from the other lakes. The ratio of valine to leucine in Gilgel Gibe fish was significantly lower compared to other lakes.




(b) C3 : C2
C3:C2 (µmol/L)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
a
b
a
Gilgel Gibe
Zeway
Langano


(c) 3OHC4 : C2
3OHC4 : C2 (µmol/L)
0.025
0.02
0.015
0.01
0.005
0
b
b
c
Gilgel Gibe
Zeway
Langano


(d) C4DC : C3
C4DC: C3 (µmol/L)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
c
a
b
Gilgel Gibe
Zeway
Langano


(e) C4DC : Val. + meth.
C4DC : Val+Meth (µmol/L)
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
a
ab
b
Gilgel Gibe
Zeway
Langano

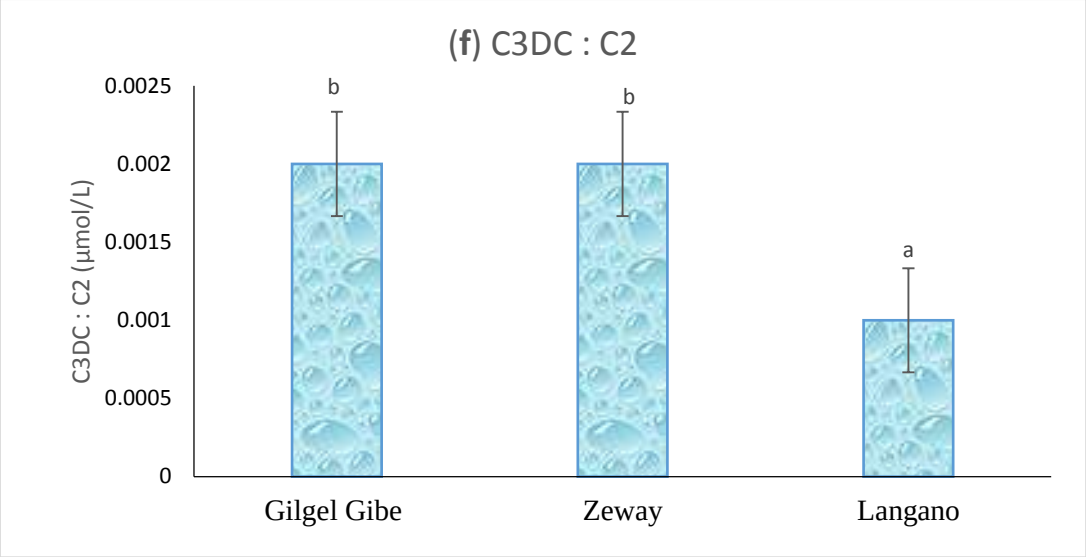


Figure 6.1 (a-f). Ratios of selected acylcarnitines and free amino acids in Nile tilapia blood (n=20) from each Lake. C2:C0= acetyl carnitine : free carnitine; C3:C2 = propionyl : acetyl carnitine; 3OH-C4:C2 = 3-hydroxybutyryl : acetyl carnitine; C4DC:C3 = methylmalonyl: propionyl carnitine; C4DC:val+met = methylmalonyl carnitine : (valine+methionine); C3-DC:C2 = malonyl carnitine : acetyl carnitine; val: leu valine : leucine and 3-OH LCFA:LCFA = sum of 3-hydroxy-long-chain fatty acids : sum of long-chain fatty acids. [a,b,c,]Mean values with different letters across the row of the same legend are significantly different ($P < 0.05$).

6.4 Discussion

Despite using the same fishing technique in the three lakes, a profound difference in fish size and weight were found. Although other factors may have influenced the fish size of the catch in every lake, fishermen confirmed that it was representative of a typical catch in those lakes. Therefore, it can be concluded that the food webs (trophic status of the lakes) and conditions in the lakes exerted different growth patterns. This is as such not a novel finding, but we here demonstrated that targeted metabolite analysis can provide a mechanistic explanation for the observed differences in growth performance, as a first step in identifying the nutritional limitations in a particular habitat. The carnitine ester and free amino acid profile have been used in several species including fish to demonstrate differences in nutrient metabolism, for instance (Geda et al., 2017; Li et al., 2020).

The citric acid cycle, as the most efficient energy-producing pathway in the body, needs substrates for acetyl coenzyme A (CoA) and oxaloacetate to form citric acid. In an aquatic environment, digestible carbohydrates are very limited, meaning that glucose will not be available as the main precursor for oxaloacetate in the citric acid cycle. Instead, to form oxaloacetate, fish will need to use amino acids from dietary protein or – in case of a fair amount of intestinal microbial fermentation – propionic acid. Several metabolite ratios reflecting amino acid and propionic acid use were indeed lowest in Gilgel Gibe fish. First, the low propionyl- to acetylcarnitine ratio (C3:C2) in Gilgel Gibe fish is indicating that more of the available acetyl CoA is not led into the citric acid cycle, but instead will be used for fat synthesis. Although only numerically, the higher ratio of malonyl- to acetylcarnitine is confirming that conclusion, since malonyl CoA is a marker for lipogenesis. Furthermore, prominent glucogenic amino acids as methionine and valine were less converted in methylmalonyl- and succinyl carnitine (witnessed by the C4DC:met+val ratio), leading to less precursor provision for oxaloacetate in the citric acid cycle to link with acetyl CoA (low C4DC: C3 ratio). The final support for the higher fat accretion in Gilgel Gibe

fish, is the lower free amino acid ratio of valine to leucine, because valine is a typical glucogenic amino acid (hence precursor for oxaloacetate), whereas leucine is strictly lipogenic and can only provide acetyl CoA.

It thus appears that Gilgel Gibe harbors environmental conditions that lead to a relatively lower supply of glucogenic substrate in the diet, likely because of the relatively lower availability of protein-rich diet items. When considering the acetylcarnitine to free carnitine ratio (C2:C0) as an indicator of the metabolic activity in fish, the higher ratio in Gilgel Gibe fish compared with larger-sized Lake Ziway fish, suggests that Gilgel Gibe still provides sufficient amounts of food for the fish. The trophic status of the lakes indeed differs substantially: for instance, Lake Ziway is characterized as a eutrophic lake (Tilahun and Ahlgren, 2010; Vijverberg et al., 2014), and mainly dominated by chroococcal cyanobacteria with numerous Microcystis species (Dagne et al., 2008; Engdaw et al., 2013). Most cyanobacteria including Microcystis species are known to have poor nutritional value and less palatable to the zooplankton (rotifers and crustaceans) (Gouni and Sommer 2020), which is the main diet for Nile tilapia (Menezes et al., 2010; Ibrahim et al., 2015). In contrast, Lake Langano is characterized as a mesotrophic lake (Vijverberg et al. 2014), with lower primary productivity. Gilgel Gibe has a mesotrophic status (Ambalu et al., 2013), but it is a reservoir that could have developed a different food web than the two lakes.

The smaller size of the fish from Lake Langano agrees with a lower metabolic activity (lowest C2:C0 ratio). Besides, the ratio of 3-hydroxy forms of long-chain fatty acids to those long-chain fatty acids is lowest in fish from this lake, showing a low potential of lipogenesis that reflects their low body energy reserves. These fish seem to face a challenge to find sufficient food: despite a high proportion of protein catabolized to provide a glucogenic substrate to the citric acid cycle (high C3:C2, high C4DC: val+met), these metabolites do not sufficiently enter the citric acid cycle as witnessed by the low C4DC: C2 ratio.

The ratio of 3-hydroxybutyryl- to acetylcarnitine (3OHC4:C2) indicates the proportion of acetyl CoA that cannot enter the citric acid cycle and is deviated to ketone synthesis, usually due to a relative lack of glucogenic substrate. Despite the lowest body weight and body condition in Langano fish, they had the lowest 3OHC4:C2 ratio, which means that their poorer performance was not due to a relative lack of glucogenic substrate rather than an overall insufficient nutrient supply that reduced their metabolic rate, as witnessed by the low C2:C0 ratio.

This relative lack of glucogenic supply to the citric acid cycle, therefore, does not seem to come from a relative lack of glucogenic substrate but can point to a deficiency of specific micronutrients needed to support the conversion to succinyl CoA, such as magnesium. We indeed found a lower magnesium status in fish from Lake Langano (Bayissa et al., 2021). The present metabolite analysis thus points to further routes of investigation to identify the underlying causes of poor development in fish.

The largest fish were found in Lake Ziway, yet with less body fat. Based on the intermediate values of the C2:C0 ratio, their metabolic rate was not as high as in the smaller Gilgel Gibe fish. This can be explained by a higher growth efficiency: because fat is highly energetic per unit of weight, lean growth comes with a higher food utilization efficiency: less food will be needed for the same amount of growth when that growth is leaner (protein and water) compared with fat deposition. Remarkably, Lake Ziway fish had the lowest C4DC: C2 ratio, suggesting that relatively less protein was needed to support the generation of energy. A possible, but so far not investigated route, is the use of chitin as an energy source. Many aquatic organisms such as crustaceans and insects have an exoskeleton containing chitin (Puvvada et al., 2012; Zhang et al., 2012; Philibert et al., 2017). It has been demonstrated that many fish species secrete endogenous chitinase in their gut (Matsumiya and Mochizuki, 1996; Gutowska et al., 2004; Molinari et al., 2007), but very little information is available on the metabolic fate of chitin that is enzymatically degraded. Chitin mainly consists of N-alpha-acetyl glucosamine units, which after absorption, should split in an acetyl

group that can provide acetyl CoA and glucosamine that can easily convert to glucose. That would mean that fish would still have another glucogenic substrate apart from amino acids and eventually propionate from fermentation, but, as said, this is still uncovered. Yet, it could explain the high lean growth of Lake Ziway fish. The gut content analysis of Nile tilapia from Lake Ziway showed a higher proportion of zooplankton (crustacean and rotifer) and aquatic insects (Ibrahim et al., 2015). Crustaceans and aquatic insects are the main chitin source in the aquatic environment (Cauchie, 2002). Thus, a larger size of Lake Ziway fish, but lower fat content (leaner) compared to smaller size Gilgel Gibe fish is possible, Lake Ziway fish would use chitin as an alternative glucogenic substrate instead of amino acids.

In conclusion, the nutrition-related blood metabolite ratios in our study explained the habitat-dependent differences in the size of Nile tilapia. Therefore, we provide a method to explore the underlying causes of growth (size) and differences in fish.

6.5 Acknowledgments

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Chapter 7: General Discussion

Fish is a valuable source of high-quality macronutrients (protein and fat) and micronutrients (vitamins and minerals). It is often referred to as "rich food for poor people" (Beveridge et al., 2013), yet underused in high-need regions such as in sub-Saharan countries. In this doctoral thesis, the impact of species, environment and different tissues on the macronutrient (protein and fat) composition, and micro- and macromineral distribution in fish have been investigated, while using blood metabolite analysis to explain habitat- and size-dependent differences.

The exploratory study in this thesis demonstrated that species is an important factor determining the distribution of mineral concentrations in the body of fish (**Chapter 3**). From this chapter, it was concluded that these factors should be further investigated in view of species and environment in nutrient concentration in fish from natural environment. If this range in mineral concentrations would occur in production fish as well, this would be great opportunity to optimize the mineral intake consumers. To get these insights, the differences between species in the Ethiopian real-life context, species and their edible parts on nutrient composition (proximate composition and mineral concentrations) of three fish from the same aquatic environment were analyzed (**Chapter 4**). Although the range in mineral concentrations was overall lower, it was still of magnitude that allowed research towards targeting fish species and different tissue parts in mineral concentrations.

In addition to the species effects, **Chapter 5** showed that distinct differences in mineral concentrations were observed in the same species caught from different aquatic ecosystems. This inherently points to a potential to modulate mineral enrichment of fish and fish products through targeted aquaculture. The metabolic profiling in **Chapter 6** is an example of how the impact of changes in environment on fish composition can be explained and modulated.

7.1 Does this study contribute to dietary diversity and to alleviating malnutrition?

Around the world, malnutrition is a serious public health burden. Reducing malnutrition can have a significant contribution in reducing poverty and achieving better health (IFPRI, 2014). Aquatic foods offer ample opportunities to reduce hunger and improve nutrition, alleviate poverty, generate economic growth and ensure better use of natural resources (FAO, 2017). This complies with some of the sustainable development goals (SDG) 1, 2, 3, 8 and 12 which are about no poverty, zero hunger, good health, economic growth and responsible consumption and production, respectively (UN, 2015).

However, an estimation report indicated that 47 million children under five years old are stunted in sub-Saharan Africa (WHO, 2017). Obviously, its consequences include nutritional blindness, poor learning capabilities, poor growth and increased morbidity and mortality rates. In Ethiopia predominantly poor households are often characterized by a lack of dietary diversity. Chronic shortages of micronutrients can pose a severe but often invisible threat to health, especially for women and children (Black et al., 2013).

The energy supply is still remarkably low in micronutrients and fat in the country (Roba, 2016; Sheehy et al., 2019). There is little evidence yet of changes that are usually associated with a nutrition transition (Sheehy et al., 2019), especially to aquatic food production in the country. Currently, the country is suffering from lack of access to enough food and nutrition. A recent nutritional data report showed that 38% of children less than 5 years of age are stunted, 24% are underweight, 10% are wasted and more than 50% are anaemic, along with 18% of men and 23% of women in the 15–49-year age group (Amare et al., 2012; CSA and ICF, 2016). A number of reports indicated that (Abebe, 2008; FAO, 2008; Amare et al., 2012; Kumera et al., 2015; Sheehy et al., 2019), Ethiopia is suffering from micronutrient deficiencies, such as Fe and Zn, these are major public health concerns.

From the above, the dietary gap for energy intake of the nation urges for a higher fat and micronutrients consumption. In this regards, whole small fish proved to be the better candidate, for instance study reported by (Kawarazuka and Be´ne´, 2013) indicated that, small fish are consumed as a whole, they play a critical role in micronutrient intakes since bones, heads, and viscera concentrate most of them. Though there is limited data on the intake of each minerals in Ethiopian diet, study reported for some minerals showed low serum mineral concentrations of nation of the country, indicating that the nation mineral intake is lower than reference dietary intake (Amare et al., 2012; Ayana et al., 2018; Belay et al., 2021).

Ethiopia mainly relies on carbohydrates as sources of energy supply with limited protein (due to lack of excess food) and fat energy sources (Sheehy et al., 2019). Lipids and proteins are the major components in fish body composition (**Chapter 5**) and carbohydrates are usually detected at very low levels (<0.5 %) (Cheung and Mehta, 2015). Apart from an energy source, protein is of course crucial as a supplier of amino acids that serve many functions, including protein synthesis and precursor functions.

The supply of minerals per capita in Ethiopia is low (Sheehy et al., 2019). In such case, fish is an excellent source of valuable micronutrients (vitamins, and minerals). The 50-year trends in micronutrients such as Ca, Fe and Zn supply in Ethiopia between 1961 and 2011 are shown in Figure 7.1. However, the result of this thesis indicated that the concentration of some macrominerals like Ca and P are high in 100 g serving of *Garra* and whole *Labeobarbus* (**Chapter 4**). The higher Ca and P indicates the importance of these species for a healthy human skeletal development. This is crucial for a nation such as Ethiopia where Ca is lacking in the diet (Kumera et al., 2015; Sheehy et al., 2019). For instance, the ratio of Ca and P from *Garra* fulfill the dietary recommendation for optimum growth of infants and children (**Chapter 4**).

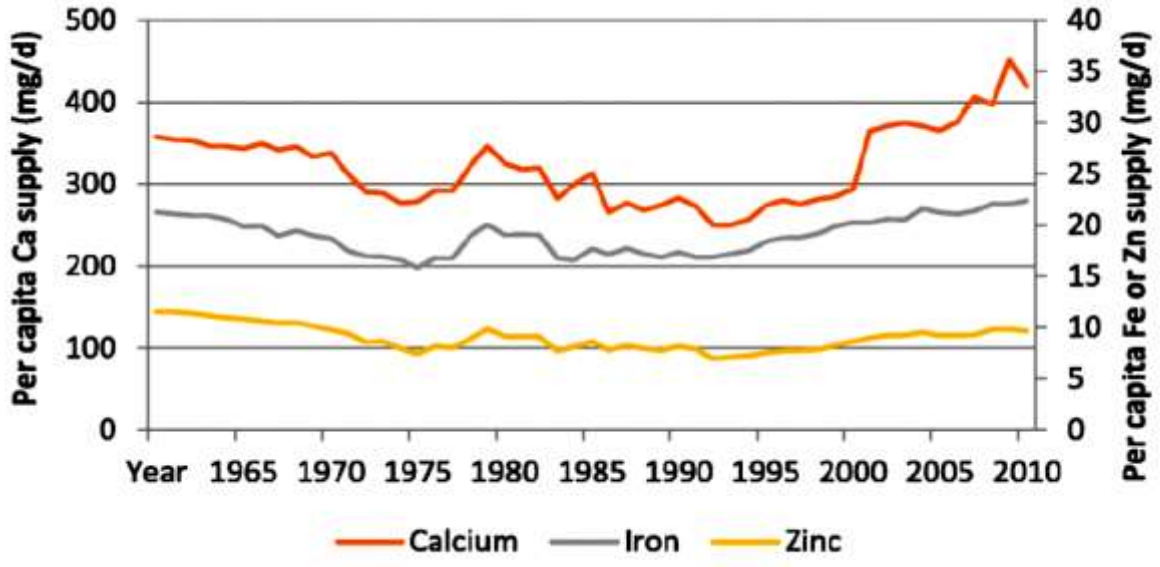

Figure 7.1. Per capita supply of calcium, iron and zinc (mg/d) in Ethiopia between 1961 and 2011 (Sheehy et al, 2019)

This thesis indicated that whole fish (*Garra* and *Labeobarbus*) have more nutritional balance than fillet alone (**Chapter 4**). The fillet parts resulted in high protein with low fat and minerals. Our present result on the fish fillets confirm the expected high protein concentrations. However, the thesis demonstrated that consuming fillets for protein leads to missing out on many nutrients such as fat and minerals that are higher in whole fish.

Similarly, in **Chapter 5 and 6**, nutritional analysis of different tissue parts of Nile tilapia resulted in higher in minerals and fat compared to the fillet part respectively. Moreover, various fish species are known to accumulate essential trace elements in different tissues (**Chapter 3**). In this PhD study, we observed that eating whole fish can have many advantages over eating only fillet to combat malnutrition in the countries like Ethiopia: it still provides enough protein, but also provides more fat and minerals. According to Kawarazuka and Be´ne´ (2013), whole small fish offer more nutritional advantages than big, filleted fish. Some of the benefits are: (i) it can be processed and stored for a long period, (ii) it is more affordable for the poor as they

can be purchased in small quantities, and (iii) they can be more evenly divided among household members.

Generally, small fish such as *Garra* could be a promising fish to consider for consumption in the country. In this regard, there is a good trend in Peru in converting overlooked small fish for human consumption with a local slogan "little fish can provide big health and nutritional benefits". Previously, Peruvian underestimated (considered as a low-value fish) Peruvian anchoveta (*Engraulis ringens*) for human consumption, but the country produces on average of 5-6 million tonnes of this fish and almost all production was destined to industry for fish meal and fish oil production. However, recently Peruvian scholars came to learn that Peruvian anchoveta is a high value fish when it comes to human nutrition. Because of their small size, they can be consumed whole with skin and bone, hence contributing more nutrients to the diet (FAO, 2017).

7.2 Do environment and species impact nutrient distribution in fish?

The main goal of this PhD study was to investigate the nutrient distribution in fish in response to species and environment (**Chapter 3, 4 and 5**). We have shown that mineral intake can differ substantially in response to the environment, meaning that there is a clear path for aquaculture to create "mineral- enriched" fish products. Such fish products could then aim for the ideal mineral profile, with more of the minerals that are lacking in the present human diet, less of their antagonists, and less of the toxic minerals. Moreover, like terrestrial animals, fish require dietary sources of minerals, which they utilize for structural purposes, osmoregulation, and as cofactors in metabolic reactions (Lall, 2002) (Table 7.1). In domestic animals and humans, functions of essential trace elements have been widely established (Close, 2006; Suttle, 2010; López-Alonso, 2012). Relatively little is known about the uptake, function, and biological availability of many trace elements in fish (Halver and Hardy, 2002). Marc, (1999) reported that a comprehensive understanding about micromineral requirements and toxicity in fish is far from complete.

Table 7.1. Essential metalloenzaymes in aquatic animals (each enzyme with respective function after comma) (Lall, 2002)

Trace element	Enzymes	Function
Iron	Succinate dehydrogenase, Cytochromes (a,b,c) Catalase	Aerobic oxidation of carbohydrates, Electron transfer, Protection against H_2O_2
Copper	Cytochrome oxidase, Lysyl oxidase, Ceruloplasnin (ferroxidase), Superoxide dismutase	Terminal oxidase, Lysine oxidation, Iron utilization and copper transport, Dismutation of the super oxidase free radical ($O_2^{\cdot}$)
Zinc	Carbonic anhydrase, Alcohol dehydrogenase, Carboxypeptidase, Polymerase, Collagenase	CO_2 formation, Alcohol metabolism, protein digestion, Hydrolysis of phosphate esters, Synthesis of RNA and DNA chains, Wound healing
Manganese	Pyruvate carboxylase, Superoxide dismutase	Pyruvate metabolism, Dismutation of the superoxide free radical ($O_2^{\cdot}$)
Molybdenum	Xanthine dehydrogenase, sulfite oxidase, Aldehyde oxidase	Purine metabolism, Sulfite Oxidation, Purine metabolism
Selenium	Glutathione peroxidase Type I and III deiodinases	Removal of H_2O_2, Conversion of thyroxine to the active form

Findings in the thesis (**Chapter 4, 5 and 6**) demonstrate that the macronutrient composition and mineral concentrations varied greatly between the species and tissues even within the same natural aquatic ecosystem. Various studies have reported that the regulation of the intake of macronutrients is strongly influenced by organismal traits and states that it includes foraging guilds, species-specific life histories (Clark et al., 2013), and nutritional status (Pompilio et al., 2006).

7.3 The implications of this thesis for the aquaculture sector

The global aquaculture production has shown a continuous growth for a couple decades, but recently there are concerns regarding its sustainability (Ahmed et al., 2019). There are challenges that would hinder the sustainability of aquaculture development, unless a solution is timely forwarded. Some of these challenges are the competition on land with other terrestrial farming activities, fish feed availability and cost, environmental degradation (e.g. mangrove degradation by shrimp farming in coastal areas), pollution (waste generated by aquaculture farming), the use of antibiotics and genetic pollution due to escape of farmed fish (Ottinger et al., 2016; Reid et al., 2020). Due to this fact, organic or ecological aquaculture has been recently recommended. It is aquatic farming (aquaculture) which meets a criterion of less damage to the aquatic environment, use of pollutant-free water, absence of genetically modified organisms, low culture density, maintenance of typical species behavior, use of oxygen to improve animal welfare (not to increase culture density) and feeding from sustainable sources (Ottinger et al., 2016; Ahmed & Thompson, 2019).

In contrast to most countries, aquaculture is still underexploited in Ethiopia, but it has a large, untapped potential to combat against malnutrition and food security. To become less dependent on capture fisheries and produce fish all year around, aquaculture needs to sustainably develop in the country. One of the specific challenges for aquaculture development in Ethiopia is the search for sustainable fish feed resources. Research on different potential fish feed sources have been reported and agricultural by-products can increase the yield opposed to the natural possible yield of the pond in small-scale fish farming. For commercial fish farming these by-products can partly replace the formulated feed and lower feed costs (Hirpo, 2017). Fish carcass remains have all shown great potential as fish feed (Kassahun et al., 2012). In this regard, the digestive tract of Nile tilapia showed a potential for fish feed ingredient to be considered (**Chapter 5**).

The other bottleneck is that the nation depends on limited number of fish species and fillet part. In this regard, the result in this thesis indicated that, if different fish species and tissue parts (**Chapter 4 and 5)** are adapted to be either directly eaten or used as a food ingredient to boost the nutritional value of local diets in the nation, the aquaculture candidate fish would be of several choice and increase the chance for its development in the country. This could also contribute to the country's sustainable aquatic resources utilization and in alleviating the present malnutrition and poverty.

However, there is still a lot to be done in terms of research (aquatic food system); - nation-wide awareness on small and whole fish consumption, and the aquaculture adaptability (domestication) these species (*Garra* and *Labeobarbus)*. Therefore, further study would focus on the feeding habit, growth performance, survival and physiological adaptability of these fish under culture condition. As an alternative, for example, *Garra* can be raised in aquaculture, or which alternative would score best in terms of the production of edible biomass with optimal nutritive value. For instance, if Garra has a far lower biomass production than other species, this may still undermine its potential for aquaculture, despite its interesting nutritive value.

The other way around, our study results (**Chapter 3, 4, 5 and 6**) made clear that fish can vary widely in nutritive value, so that edible biomass production should not be the only criterion in aquaculture but consider how well certain fish products and species can help in beating imbalances in the human diet. The metabolic response to trace elements differs greatly among fish species. The importance of this is reflected by the mention of devastating micronutrient deficiency in the National Nutrition Strategy of Ethiopia. Finding out which species would perform best in which conditions on readily available feed resources will be a major goal in aquaculture research. This can be indicated by the evaluation of various body parts of fish caught in different lakes with variable water conditions (**Chapter 5**) and the blood metabolite profile of the fish (**Chapter 6**).

Still a lot of work is warranted to optimize aquaculture in view of the aims of nutrition-sensitive aquaculture but the work in this thesis also showed the new techniques can help to speed up the process. Metabolite analysis can indicate if fish are thriving well on a certain diet and aquatic environment (**Chapter 6**). Refining these techniques may overcome the need for large-scale long-term growth trials. Monitoring nutrient metabolism in fish may allow faster adjusting of the diet and conditions.

In addition to its relevance for aquaculture, this metabolite profiling seems also valuable to identify suboptimal conditions of free-living fish due to for instance pollution, erosion or other threats to their habitat. Therefore, this approach can also be useful for ecological research, whereas this has not been used to date. Also, the mineral profiling in free-living fish can also indicate whether they are exposed to potential deficiencies or toxic levels. The latter can make people aware that pollution, e.g., with heavy metals, can cause a threat for their own health when consuming contaminated fish from lakes. Reports indicated that excess intake of even essential trace element could have health risk. Aquaculture systems should allow a better control of such contaminants, hence creating a healthier solution for the human diet. For human consumption safety issue, international organizations such FAO, WHO and different countries set the permitted levels of trace elements in fish guidelines (Table 2).

Table 2. Summary of permitted levels of trace elements in fish (mg/kg wet weight) established by different guidelines

Country	References	Cu	Zn	Fe	Mn	Cr	Pb	Cd	Ni
Worldwide	FAO (1983)	30	40-100	–	–	–	0.50	0.05	–
Worldwide	FAO/WHO (1989)	30	50	–	–	1.0	0.50	0.10	–
UK	MAFF (2000)	20	50	–	–	1.0	2.0	0.20	–
Europe	EC (2006)	–	–	–	–	–	0.30	0.05	–
Turkey	TFC (2002)	20	50	–	20	–	1.0	0.1	–
Montenegro	Official Gazette MN (2009)	–	–	–	–	–	0.30	0.05	–
Bulgaria	Anonymous (2004)	10	50	–	-	0.3	0.40	0.05	0.5

Furthermore, in fish nutrition, despite species variation exists, low mineral supply causes deficiency or death and excessive mineral intake causes toxicity or death to the fish (Lall, 2002; Figure 7.2).

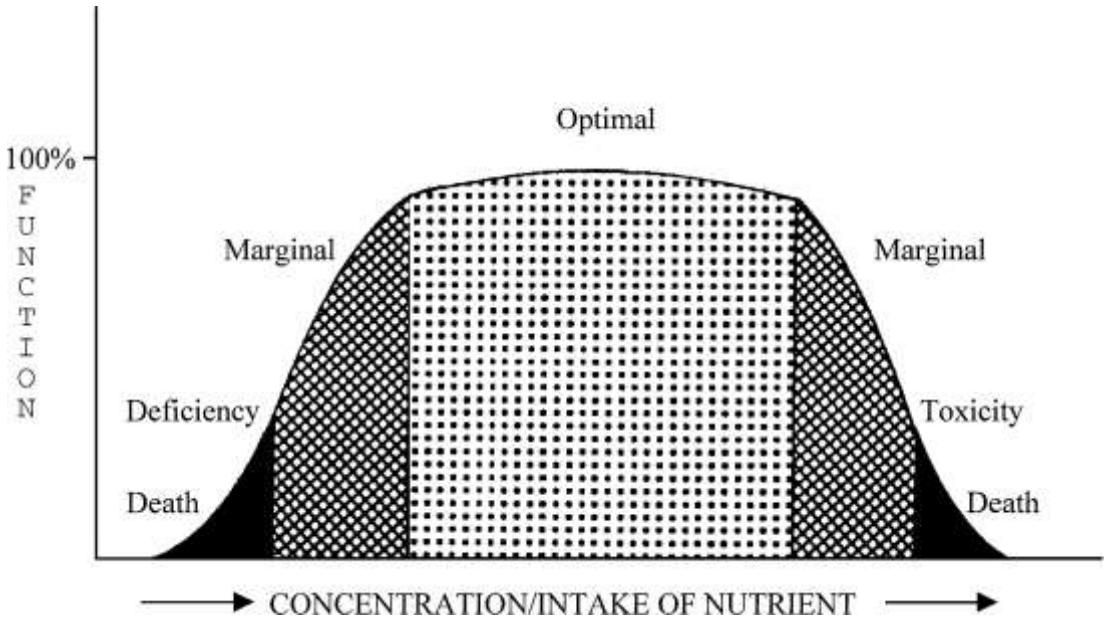

Figure 7.2. Nutrient intake and its impact in fish nutrition (Lall, 2002)

In aquaculture context, it is important to know the role of trace elements in fish metabolism. Understanding its role would help in fish feed formulation, hence the mineral accumulation and macronutrient composition in fish can be affected by type

of feed. This is an indication that not every fish species can thrive with every feed resource. Further studies therefore need to evaluate which combination can lead to the best "gap closure" in the human diet.

Limitation of the study: - This thesis provided several important data which will help to promote aquatic food production and food and nutrition security in the country. However, there are some limitations of the study; (i) the limited sample size in some chapters, which is directly related to logistics and budget (ii) lack of collaborative work with human nutrition researchers; this would enable a better use of the data generated to the human diet integration (iii) evaluation of *Garra* muscle and whole Nile tilapia in chapter 4 would have given a completer picture of the species aspect.

7.4 Future prospects

Despite the aquatic food production, specifically aquaculture, is growing worldwide, it is still embryonic in developing countries such as Ethiopia. This is due to several reasons described above. The studies in this thesis demonstrated the potential of small fish (*Garra*) aquaculture to complement shortages in the human diet in Ethiopia. This study was conducted on fish caught from the wild taking species and environment as factors. Some results came up with higher toxic mineral accumulation for example Al in Garra. Thus, it is crucial to evaluate the potential of *Garra* in aquaculture, taking the following points in to consideration.

- It would help the sector development if future studies focus on pond trials under intensive monitoring on *Garra* and *Labeobarbus*. Such studies should focus on the reproduction potential, feeding habits, mineral intake and accumulation, feed utilization and body proximate composition of these fish species, using blood metabolite profiling to gain mechanistic understanding.
- To make the study a package, a future study should focus on how to handle the post-harvest processes, monitor the safety and health effects, and increase shelf-life (cooking, smoking and sun drying) of small fish. Consequently, studying and providing awareness creation for local communities on (i)

knowledge of the beneficial health effects of consuming small fish (ii) the prioritization of small fish for direct human consumption (iii) improving quality by upgrading post-harvest processing, reduce spoilage and contamination.

- Moreover, a collaborative study with human nutrition researchers can be conducted on the nutrient balancing with whole small fish as a diet ingredient or supplement, especially for children and pregnant women, to tackle the Zn and Fe deficiency and anemia in the country.
- Socio-economic analysis should evaluate how small-scale aquaculture can best be integrated in the existing agricultural practices.
- The consistency of blood metabolite analysis would be further investigated as the technique is a promising approach to monitor nutrient metabolism in fish that may allow faster adjusting of the diet and conditions for fish in the aquaculture sector.

As take-home messages: - In conclusion, this thesis demonstrated that:

- Mineral distributions throughout the body differs substantially between various fish species and body parts.
- Despite the much higher protein content of fish fillets compared with whole fish, the latter provides a more balanced nutrient profile, with a distinctly higher supply of fat and minerals.
- Distinct differences in mineral concentrations were observed between tissues in Nile tilapia and also these concentrations were substantially affected by the ecosystem where these fish were caught from.
- The profiling of blood metabolites can be a valuable tool to speed up and deepen the evaluation of dietary and environmental effects on fish metabolism.

7.5 References

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Summary

Aquatic food production plays a pivotal role in providing food, nutrition, employment, recreation, trade and economic well-being for people throughout the world, for present and future generations. It offers a life-changing opportunity for the hundreds of millions of undernourished people around the world, particularly in low- and middle-income countries. Moreover, aquatic food production is one of the candidate sectors recently foreseen as fortification of nutrient-sensitive agriculture. Aquaculture is continuously increasing worldwide to meet the global market demand for fish and fishery products, driven by the increasing human population and over-exploitation of wild capture fisheries. Though overexploitation of wild capture fisheries has been reported, still it dominates the fish supply in Africa, and aquaculture production in the continent contributes an insignificant percentage. To sustain aquatic food production, there would be a diversifying option of fish species and parts used for human consumption (**Chapter 1**).

People have never consumed as much fish nor depended so much on the aquatic food products for their livelihoods. As a matter of fact, micronutrient deficiency is affecting most developing countries. In such condition, diet shift towards diverse fish species could be a sensible remedy for micronutrient deficient nations. In Ethiopia, both capture and culture fisheries are focusing on a relatively narrow diversity of fish species, which likely underutilizes the nation's aquatic resources for food and nutrition provision, because fish species vary considerably in their nutrient composition and density. Given the wide diversity of aquatic life, fish nutritive value, especially mineral concentrations could be influenced by various factors, such as species, habitat, climate, and tissue characteristics (metabolic activity and homeostasis). Therefore, investigating the impact of these factors in nutritive value, essential and non-essential mineral concentrations in different fish species and tissues is paramount in the aquaculture sector development. The general aim of this PhD thesis thus was to investigate the impact of species, tissues and ecosystem on the nutrient metabolism and nutritive value of fish as part of a nutrition- sensitive agriculture **(Chapter 2)**.

Fish nutrient deficiency should not occur when diets have been formulated and prepared based on the species' requirement. However, some commercially available diets for another species may sometimes be used in the absence of a suitable formulation, resulting in deficiencies. **Chapter 3** compared micromineral (Fe, Zn and Cu) homeostasis across ten ornamental fish species. Ten different species of live ornamental fish were randomly sampled from one big aquarium in a pet store in Belgium. All fish samples were dissected manually for the collection of targeted tissues and analyzed for the above mentioned microminerals. In general, muscle tissue showed the lowest concentrations for each of the three microminerals in all species, still with important variation among species. Fe was associated with Cu in muscle tissue ($p < 0.05$), but neither of them were associated with Zn in the muscle. However, the three micromineral concentrations were correlated in the heart ($p < 0.05$). Similarly, all of them were correlated in the liver ($p < 0.05$), but none of them showed a significant association in the tail fin. Excess deposition of minerals in heart tissue is a new observation, and it is not known if this is meant as storage or rather the fish heart has a high requirement for microminerals. Storage in the tail fin should be interpreted as a sign of permanent deposition as a tool to dispose of toxic excess. The lack of correlation between the muscular concentrations of Zn on the one hand, and those of Fe and Cu on the other hand, further suggests that fish species distinctly differ in their micromineral metabolism. Although this exploratory study still leaves many questions unanswered, it points to the large diversity in micromineral metabolism among fish species.

Consequently, **Chapter 4,** aimed to evaluate the macronutrient and mineral composition of whole fish (*Labeobarbus intermedius*, Garra *quadrimaculata*) and fillet (*Oreochromis niloticus*, *Labeobarbus intermedius*) from the same water body. A total of 64 fish samples were collected from Gilgel Gibe reservoir, Ethiopia, and analyzed for its macronutrient and mineral composition. The proximate composition and mineral contents of fillets and whole body samples were determined. The whole fish showed a much higher fat and ash percentage than the fillets ($p<0.05$). The fillets contained a

much higher protein concentration than the whole fish ($p<0.05$). The higher Ca: P ratios in whole fish compared to fillet in our study confirms the importance for a healthy human skeletal development, especially in diets where Ca is typically lacking. Whole *Garra* appeared to be containing important trace elements such as zinc and iron, a feature that was not found to the same extent in the whole *Labeobarbus*. These differences may find its origin in the feeding pattern of these fish species in the reservoir. The advantage of benthic species such as *Garra* to enrich the human diet with essential minerals may, however, coincide with the accumulation of toxic heavy metals as a potential result of soil erosion.

In natural conditions, the distribution of minerals can vary with the local activity (farming, industrial, or urban activity) that limits the resealing rate of effluents into the nearby aquatic environment. The accumulation and distribution of beneficial and toxic trace elements in fish tissues can be affected by the mineral load in its environment and diet, which, in turn, will be a reflection of soil composition and geological events, such as soil erosion. In **Chapter 5**, a study was aimed to evaluate the differences in mineral and toxic trace element concentrations of Nile tilapia tissues from three aquatic ecosystems in Ethiopia namely, Lake Ziway, Lake Langano, and Gilgel Gibe reservoir with a focus on edible (fillet) and discarded (digestive tract, gills, skin, and liver) parts. From each lake, twenty Nile tilapia samples were collected, dissected for the targeted tissues and analyzed by inductively coupled plasma mass spectrometry. All elements varied markedly among tissues and between the lakes. Some differences in element concentrations were attributed to differences in nutrient load in the ecosystems and the function of the tissues. For instance, the calcium concentrations in skin and gill were distinctly higher in fish from calcium-rich Lake Langano. The discarded parts were richer in essential trace elements, showing an opportunity to promote their use in human nutrition to increase the intake of important minerals. However, the accumulation of elements toxic to humans, such as aluminum, should be monitored and controlled when rearing these fish in aquaculture.

A natural aquatic ecosystem harbors a diversity of factors, making it hard to identify the most limiting factors for fish development and body composition. In **Chapter 6**, study was conducted to explore how metabolite analysis can mechanistically explain differences in tissue composition and size of Nile tilapia from different habitats. Dried blood spot (DBS) samples of Nile tilapia were collected from three Ethiopian lakes (Gilgel Gibe, Ziway, and Langano) and analysed for the carnitine esters and free amino acids. Metabolite ratios were calculated from relevant biochemical pathways that could identify relative changes in nutrient metabolism. Marked differences were observed in Nile tilapia metabolic activity between the lakes. For instance, the lower body weight and body condition of the fish in Lake Langano coincided with several metabolite ratios pointing to a low flow of glucogenic substrate to the citric acid cycle. In the Gilgel Gibe fish, the metabolic markers for lipogenesis and metabolic rate could explain the high fat concentration in several parts of the body. The nutrition-related blood metabolite ratios are useful to understand the underlying metabolic events leading to the habitat-dependent differences in growth of Nile tilapia, and by extension, other species.

In conclusion (**Chapter 7**), this thesis demonstrates the potential for targeted enrichment of the human diet with minerals and other nutrients through fish. Apart from the fish diet and environment, the choice of the fish species and which parts are eaten are available strategies to that end. Whole blood metabolic profiling in fish showed to be a valuable tool to facilitate and speed up the evaluation of these strategies.

Samenvatting

Aquatische voedselproductie speelt een centrale rol in de voorziening van voeding, tewerkstelling, recreatie, handel en economische welstand voor mensen overal ter wereld, voor zowel huidige als toekomstige generaties. Het betekent een unieke opportuniteit voor de honderden miljoenen ondervoede mensen rondom de wereld, vooral in landen met laag en middellaag inkomen. Aquatische voedselproductie behoort bovendien tot de sectoren die gezien worden om aanrijking te genereren binnen het kader van nutriëntsensitieve landbouw. Aquacultuur stijgt wereldwijd continu om de globale marktvraag naar vis en visproducten te volgen, gestuurd door de groeiende menselijke bevolking en de overexploitatie van wildvangstvisserij. Hoewel deze overexploitatie ondertussen bekend is, domineert het nog steeds de visvoorziening in Afrika, en betekent de productie vanuit aquacultuur nog steeds weinig. Om de aquatische voedselproductie te onderhouden, moet er een diversificatie komen van vissoorten en de delen die worden gebruikt voor humane consumptie (Hoofdstuk 1).

Mensen hebben nog nooit eerder zo veel vis gegeten of zo sterk van aquatisch voedingsproducten afgehangen voor hun levensonderhoud. Eigenlijk komen tekorten van micronutriënten voor in de meeste ontwikkelingslanden. In zo'n situatie zou een verschuiving van het dieet naar diverse vissoorten een zinvolle remedie kunnen zijn voor landen met een micronutriëntentekort. In Ethiopië richten zowel de visvangst als de visteelt zich op een relatief nauwe verscheidenheid van vissoorten, hetgeen vermoedelijk de landelijke aquatische voedselbronnen onderbenut omdat vissoorten behoorlijk kunnen verschillen in hun nutriëntensamenstelling en -gehalte. Gegeven de grote diversiteit van aquatische levensvormen, kan de voedingswaarde van vis, en in het bijzonder de mineralenconcentraties, worden beïnvloed door verschillende factoren waaronder species, habitat, klimaat en de eigenschappen van weefsels (metabolische activiteit en homeostase).

Het is daarom belangrijk om de impact van die factoren op de voedingswaarde en de concentraties van essentiële en niet-essentiële mineralen in verschillende vissoorten

en -weefsels te onderzoeken om zo de ontwikkeling van de aquacultuursector te steunen.

Het doel van dit proefschrift is dus om de invloed te onderzoeken van species, weefsel en ecosysteem op het nutriëntenmetabolisme en de voedingswaarde van vissen als deel van een nutriëntenssensitieve landbouw (Hoofdstuk 2).

Nutriëntendeficiënties zouden niet mogen voorkomen bij vissen wanneer de voeders werden geformuleerd en bereid op basis van de behoeften van die soort. Echter, bepaalde commercieel beschikbare voeders voor een bepaalde soort worden soms gebruikt voor een andere soort bij gebrek aan gekende normen, en toch leiden tot deficiënties. Hoofdstuk 3 vergeleek de homeostase van de micromineralen Fe, Zn en Cu tussen tien siervissoorten. Tien verschillende soorten siervissen werden willekeurig verzameld vanuit een groot aquarium in een Belgische winkel voor gezelschapsdieren. Alle visstalen werden manueel ontleed voor het verzamelen van specifieke weefsels en geanalyseerd voor hogervermelde micromineralen. Het spierweefsel toonde de laagste concentraties voor elk van de drie micromineralen bij alle soorten, met toch nog een belangrijke variatie tussen de soorten. Fe toonde een associatie met Cu in spierweefsel maar geen van beide was geassocieerd met Zn in spierweefsel. Nochtans toonden de concentraties van alle drie een correlatie in het hart en ook in de lever maar niet in de staartvin. De hoge concentraties aan mineralen in het hart is een nieuwe waarneming, en het is nog niet gekend of die duidt op stockage of een hoge behoefte voor het hart. De hoge concentratie in de staartvin kan worden opgevat als een teken van permanente afzet als middel om van een toxische overmaat af te geraken. Het gebrek aan correlatie tussen spierconcentraties van Zn aan de ene kant, en die van Fe en Cu aan de andere kant, doen veronderstellen dat vissoorten aanzienlijk verschillen in hun micromineralenmetabolisme. Hoewel deze verkennende studie nog vragen onbeantwoord laat, duidt ze op de grote verscheidenheid in micromineralenmetabolisme onder vissoorten.

Daarom had Hoofdstuk 4 asl doel om de samenstelling van macronutriënten en mineralen te vergelijken tussen gehele vis (*Labeobarbus intermedius*, Garra *quadrimaculata*) en visfilet (*Oreochromis niloticus*, *Labeobarbus intermedius*) uit hetzelfde waterreservoir.

In totaal werden 64 visstalen gecollecteerd uit het Gilgel Gibe reservoir in Ethiopië, en geanalyseerd voor de concentraties aan macronutriënten en mineralen. Die werden bepaald op de filets en de gehele vissen. De gehele vissen toonden een veel hoger gehalte aan vet en as dan de filets. De filts bevatten een hoger eiwitgehalte dan de gehele vis. De hogere Ca:P verhouding in de gehele vis in vergelijking met de filet in onze studie bevestigt het belang voor een gezonde ontwikkeling van het menselijk skelet, in het bijzonder in een dieet met een tekort aan Ca. Gehele *Garra* bleek een belangrijke bron van sporenelementen zoals zink en ijzer, een kenmerk dat niet in dezelfde mate werd gevonden in gehele *Labeobarbus*. Die verschillen kunnen hun oorsprong vinden in het voedingspatroon van deze vissoorten in het reservoir. Het voordeel van benthische soorten zoals *Garra* om het menselijke dieet te versterken met essentiële mineralen kan echter samengaan met een stapeling van toxische zware metalen vanwege bodemerosie.

In natuurlijke omstandigheden kan de verdeling van mineralen variëren naargelang de plaatselijke activiteiten (landbouw, industrie, stad) hetgeen de mate van uitstroom naar nabijgelegen aquatische systemen bepaalt. De stapeling en verspreiding van gewenste en toxische sporenelementen in visweefsels kan worden beïnvloed door de hoeveelheid mineralen in de omgeving en in het dieet, hetgeen op zijn beurt de bodemsamenstelling of geologische gebeurtenissen zoals bodemerosie weerspiegelt. De studie in Hoofdstuk 5 ging de verschillen onderzoeken in de concentraties aan mineralen en toxische metalen in weefsel van Nijltilapia uit drie aquatische ecosystemen in Ethiopië, namelijk het Ziway-meer, het Langano-meer en het Gilgel Gibe-reservoir, met een focus op eetbare (filet) en verwijderde delen (darmstelsel, kieuwen, huid en lever). Van elk meer werden twintig Nijltilapia's verzameld,

gedissecteerd voor specifieke weefsels en geanalyseerd met inductief gekoppelde plasma massaspectrometrie. Alle elementen varieerden aanzienlijk tussen de weefsels en tussen de meren. Sommige verschillen in elementconcentraties konden worden toegewezen aan verschillen in de aanwezigheid van nutriënten in de ecosystemen en de functie van de weefsels. De calciumconcentraties in huid en kieuwen waren bijvoorbeeld opmerkelijk hoger in vissen uit het calciumrijke Langano-meer. De verwijderde delen waren rijker in essentiële sporenelementen, hetgeen duidt op de mogelijkheid om hun gebruik in de menselijke voeding te promoten om de inname van belangrijke mineralen te bevorderen. Wel moet de stapeling van elementen die toxisch zijn voor de mens, zoals aluminium, worden gemonitord en in het bijzonder worden gecontroleerd wanneer die vissen in aquacultuur worden gekweekt.

Een natuurlijk aquatisch ecosysteem bevat diverse factoren, die het identificeren van de meest limiterende factoren voor de ontwikkeling van vissen en hun lichaamssamenstelling bemoeilijken. In Hoofdstuk 6 werd een studie uitgevoerd om te verkennen hoe metabolietenanalyse op een mechanistische manier de verschillen in samenstelling tussen weefsels en de grootte van Nijltilapia kan verklaren in verschillende habitats. Stalen van gedroogde bloeddruppels van Nijltilapia werden verzameld van drie Ethiopische meren (Gilgel Gibe, Ziway en Langano) en geanalyseerd op carnitine-esters en vrije aminozuren. Metabolietverhoudingen werden berekend voor relevante biochemische routes die de relatieve veranderingen in nutriëntenmetabolisme konden identificeren. Opvallende verschillen werden waargenomen in de metabolische activiteit van Nijltilapia uit de verschillende meren. Het lagere lichaamsgewicht en lichaamsconditie van vissen uit het Langano-meer viel bijvoorbeeld samen met verschillende metabolietverhoudingen die duiden op een lage toevoer van glucogeen substraat naar de citroenzuurcyclus. In de vissen uit Gilgel Gibe konden de metabolische merkers voor lipogenese en metabolische snelheid de hoge vetconcentratie in verschillende delen van het lichaam verklaren. De voedingsgerelateerde bloedmetabolietverhoudingen zijn nuttig om de onderliggende

metabolische acties te begrijpen die leiden tot habitat-afhankelijke verschillen in groei van Nijltilapia, en bij uitbreiding, van andere soorten.

Tot slot (Hoofdstuk 7) toont dit proefschrift het potentieel voor een gerichte aanrijking van de menselijke voeding met mineralen en andere nutriënten via vis. Behalve de voeding van de vissen en hun omgeving, behoren de keuze van de vissoort en welke delen worden gegeten tot de mogelijke strategieën om dit na te streven. Het profiel van metabolieten in het bloed van vissen bleek een waardevol middel om de evaluatie van deze strategieën te faciliteren en te versnellen.

Curriculum Vitae

Tokuma Negisho Bayissa was born on August 31th, 1986 in Shambu, West Oromia, Ethiopia. In 2005, he completed preparatory school at Shambu Secondary and Preparatory School. He graduated in bachelor degree in applied biology from Ambo University College in 2008. He joined Oromia agricultural research institute, Batu fish and other aquatic life research center and worked as assistant researcher from 2009 to 2010.

In 2011, he was awarded a VLIR-UOS international course program scholarship and studied Masters of sciences in aquaculture at Faculty of Bioscience Engineering, Ghent University, Belgium. He obtained MSc. in aquaculture with a thesis on impact of climate change on aquatic ecosystems. In 2013, he joined College of Natural Sciences, Jimma University (Ethiopia) as lecturer and promoted to the academic rank of an assistant Professor in 2017. In 2018, he started his doctoral studies at Department of Nutrition, Genetics, and Ethology, Faculty of Veterinary Medicine, Ghent University, Belgium and his studies was funded by VLIR-UOS Networking Program. Additionally, during his PhD studies he supervised several masters' students.

Tokuma is author and co-author of several publications in peer-reviewed scientific journals. He presented his work at national and international conferences in virtual mode. He also virtually attended several conferences.

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