CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Determination of the total fat content and fatty acid profile of selected Cambodian smoked fish

MASTER'S THESIS

Prague 2019

Author: Bc. Kateřina Holmanová

Chief supervisor: doc. Ing. Jan Banout, Ph.D.

Second (specialist) supervisor: Ing. Adéla Fraňková, Ph.D.

Declaration

I, Kateřina Holmanová, hereby declare that I have done this thesis entitled "Determination of the total fat content and fatty acid profile of selected Cambodian smoked fish" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 25th April 2019

....

Kateřina Holmanová

Acknowledgements

I would like to use this opportunity to thank my supervisor doc. Ing. Jan Banout, Ph.D for his willingness, patience and helpful comments during our consultations. Furthermore I would like to acknowledge my consultant Ing. Adéla Fraňková, Ph.D. for her guidance and professional assistance in the laboratory, advices and cooperation in research.

Last but not least, I express my thanks to my family and Dobos Botond who have been supporting me for the whole time. Special thanks go to my classmates, namely Sabina Kožichová, David Murcia Higuera and Osama Darwish, for making my studies unforgettable.

Abstract

Fish is one of the main source of food for rural Cambodian population. Next to rice, it is a staple food that provides dominant part of protein intake, as well as essential fatty acids and micronutrients that contribute to better nutrition and overall health state of Cambodians. However, Cambodia still faces severe malnutrition. Fat content and fatty acid profile of 12 smoked fish species, 31 samples in total, which were collected from small-scale smoked fish producers located in 5 provinces (Kampong Chhnang, Battambang, Siam Reap, and Kampong Cham) around Tonlé Sap Lake in Cambodia. Fat content was determined by using semi-automatic Soxhlet extractor. Extracted fat was derivatizated to obtain fatty acid methyl esters, which were determined on GC-MS and GC-FID. Interval of fat content was ranging from 3.28% to 47.43%. The highest mean values were found for *R. hobelmani* and the lowest for *C. laciniata*. Few species showed significant differences at 5% level within one species, which is explained by differences in diet, age or seasonal variation of collected samples. Next to other factors, especially diet composition, has impact on fatty acid profile. The predominant fatty acids found in tested fat samples of smoked fish were oleic acid, palmitic acid, stearic acid, linoleic acid, and n-3 PUFAs (EPA and DHA). The highest value of DHA (13.67%) as well as EPA (12.86%) showed *C. laciniata*, which is significantly higher than other tested samples. The biggest proportion of unsaturated fatty acid showed Cambodian commercially important fish species *C. striata* and *C. macrocephalus.* The results showed strong potential of small fish species, *C. laciniata* especially, and their products in human nutrition term as source of fat rich on n-3 FA. However, further analysis need to be done to confirm these findings.

Keywords: smoked fish, fat content, fatty acid profile, PUFA, DHA, Cambodia.

List of Tables

List of Figures

List of abbreviation

ADB	Asian Development Bank
ALA	α-linolenic acid
CHD	coronary heart disease
CVD	cardiovascular disease
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FA	fatty acid
FID	flame ionisation detector
GC	gas chromatography
HDL	high-density lipoprotein
LA	linoleic acid
LDL	low-density lipoprotein
MS	mass spectrometry
MUFA	monounsaturated fatty acid
NIS	National Institute of Statistics
NIST	National Institute of Standards and Technology
OAAs	other aquatic animals
PUFA	polyunsaturated fatty acid
RAE	retinol activity equivalent
SD	standard deviation
SFA	saturated fatty acid
TG	triacylglycerol
WHO	World Health Organization

Preface

Fish plays an important socio-economic and nutritional role for Cambodian population. Large Mekong water system along with Tonlé Sap Lake cover significant part of the country. Big water areas of rivers, lakes, floodplains, canals and rice fields provide abundant fish and other aquatic animals sources that are easy accessible for all population regardless economic situation. This gives occupation to millions of Cambodians (FAO 2015), and furthermore contributes greatly to food and economic security. On the other hand, fishing and all related activities of fish industry represent fragile system highly dependent on one commodity. Small changes in system can especially affect many people from rural areas.

Fish is one of the main source of food for rural Cambodian population. Next to rice, it is a staple food that provides dominant part of protein intake (Hortle 2007) as well as essential fatty acids and micronutrients that all together contribute to well-being of population and combats micronutrient deficiencies. Despite the fact, Cambodians are one of the biggest fish consumers in the world (Baran 2010), the country faces high percentage of malnutrition. There are several factors of this situation that are related to availability and affordability of nutritious food stuffs, deficiency of food diversity, sufficient awareness about nutritional values of food stuffs and relation of food and health state (Vilain et al 2016).

Fish and mainly marine oily species are rich source of omega-3 polyunsaturated fatty acids that have positive effect in preventing coronary heart disease, proper growth of children and enhancing the immune system (McKeown 2006).

Cambodian water sources are home of hundreds of fish species. However, only small proportion of the number is marketed in local trade or exported to neighbouring countries. It is manly talked about abundant and larger freshwater fish species (Vann et al. 2006). Chemical composition and nutritional values of these species were investigated by several studies (Bano 1977; Roos et al. 2007a; Roos et al. 2007b; Susilowati et al. 2016) but there are still many small indigenous fish species commonly consumed manly by rural population, which should be investigated. For many of these small fish species and their products, there is lack of information from scientific sources about chemical composition and nutritional values.

1 Introduction

1.1 Fish Production and Consumption in Cambodia

Fishing and aquaculture activities are essential part of many Cambodian households. The last census in 2013 showed that 525,952 households (from total number of 2.6 million respondent households) are engaged in fishing and aquaculture activities when 11% is a share for only fishing households (NIS 2015). However, the number slightly increased between November to December due to extensive floods, which occur every year during rainy season on Mekong River, when massive amount of water pushes water flow of tributary called Tonlé Sap River back to a lake of the same name. It results in 4-6 times rise of Tonlé Sap Lake volume which takes 6% of total Cambodian area during rainy season. Water floods large areas around Tonlé Sap River and Lake and so regular agriculture activities are not possible in the flooded area. Total area of Mekong wetlands in Cambodia is 35,000 km^2 (Lacousiere et al. 1998). These are reasons of turn from agricultural to fishing households to secure food and economic stability. 90% of all agricultural engaged households reported that fishing is for their personal consumption (NIS 2015). Large fishes, which are caught in local sources or they are products of aquaculture, are mainly sold on the market.

Mekong River is considered to be the biggest fishery in the world with 2.1 million tonnes of fish per year. Cambodia contributes to the number with 33% of Mekong fish catch (Baran et al. 2014). Production per year of all fisheries, counting together fish and other aquatic animals (OAAs), is estimated to be 745,065 tonnes (FAO 2015). The Ministry of Agriculture, Forestry and Fisheries (2015) reported about 4% decrease of freshwater fish captures between 2012 (505,005 tonnes) and 2014. On the other hand, relatively small coastal zone (435 km) on south-west of the country is place for marine fisheries that showed 17% growth of captures from 99,000 tonnes in 2012 to 120,250 tonnes in 2014. Development of marine aquaculture can be promising alternative for intensive fishing with lower impact on fragile marine ecosystem (FAO 2015). The highest rise and potential can be seen in freshwater aquaculture production, which reached 120,055 tonnes in 2014. Cambodia have many water resources that creates numerous of small lakes, which can be used for aquaculture purposes. Just freshwater pond system has almost 40,000 ponds and floating cage system include 4,224 cages (FAO 2015). High aquaculture production is result of recent spread system throughout of country, even in upland areas. The main cultured species are *Pangasius* spp., *Channa micropeltes, Clarias batrachus, Barbonymus gonionotus* and *Oreochromis niloticus* (Richardson & Suvedi 2018). Increasing demand of fish due to population growth, overfishing in water sources, usage of inappropriate fishing methods, illegal marine fishing, building dams on Mekong River and climate changes have huge impact on ecosystems that can lead to decline of fish population and its diversity and thus food insecurity. Millions of people are dependent on freshwater fisheries of Mekong Basin floodplains and rivers. About 2 millions of people are employed in fishing industry of Tonlé Sap Lake in capture, culture, processing, market and transportation (FAO 2015).

Fish and OAAs are consumed in Cambodia on a daily basis. They are not only the main source of protein but also other essential elements such as fat, minerals (calcium, iodine, iron and zinc) and vitamins, especially vitamin A. A study of Chamnan et al. (2009) mentions that medium and large size fish species are mainly consumed by the rich because they are less bony and tastier compared to small size fish species which can afford poor people because of low price. However, small fish has generally higher nutritional value, and thus it can be of special relevance for poor population. These people are dependent on fish from common-pool resources that are either caught by household members or bought on local markets (Chamnan et al. 2009). The most common Cambodian dish is soup where fish is important ingredient. If there is dry season, common-pool resources decrease and instead of fresh fish, there are consumed mainly dried, smoked and fermented fish (Karim 2006). Around 10-24 kg of fish products per person are consumed every year in 8 provinces around Tonlé Sap Lake (Ahmed et al. 1998). The most known fermented fish products of Cambodia are fish paste (prahok), shrimp paste (kapi) and fish sauce (toeuk trey). Prahok is usually made from *Channa striata* and other small fishes which are first cleaned, eviscerated and without head, then crushed, salted. Fermentation takes place in clay pots with bamboo lid. Other widely used processing method is sun-drying and smoking. These methods can carry several health risks due to contamination by microorganisms before processing, incomplete processing method, contamination by bacteria, moulds, and insects and other animals during processing or wrong packaging and storage method (FAO 2005). Smoked fish can be source of hazardous microorganisms as well as polycyclic aromatic hydrocarbons (PAHs) and PAHs derivatives that can be carcinogenic for human. They can be formed during incomplete combustion of wood during smoking of fish (Mihalca et al. 2011).

1.1.1 Cambodian Fish Species Used for Smoking

In Cambodia, there can be found more than 500 fish species of the 1,200 indigenous species for Mekong Basin (Rainboth 1996). Cambodia also features 468 marine species and 26 species that can live in both environments. Following fish species were found as commonly sold on market or processed in many traditional Cambodian or modern methods. These fish species are smoked by small-scale producers in provinces around Tonlé Sap Lake (Slámová et al. 2017), which is expressed as a percentage of occurrence in Figure 1. According to 31 respondents of Slámová et al. (2017) survey, *Clarias macrocephalus* is the most used fish species for smoking with 84% of occurrence. 45% of respondents answered that they also use *Clarias batrachus* and *Henicorhynchus siamensis*. The rest of mentioned fish species occurred in list of used fish species in less than 30% occurrence.

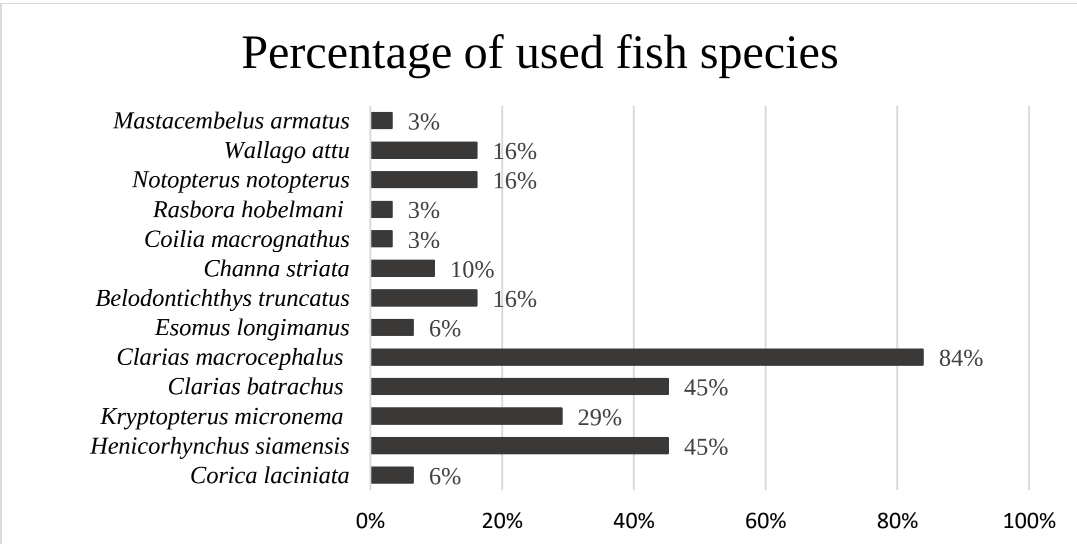

Figure 1. Occurrence of fish species by small scale smoked fish producers (%), (31 respondents).

Source: Slámová et al. 2017

Wallago attu

Figure 2. *Wallago attu*

Source: Warren 1995

Scientific name: *Wallago attu* (Bloch & Schneider 1801)

Family: Siluridae

Local name/English name: Trey sanday/ freshwater shark

Wallago attu is freshwater fish that can naturally occur in brackish water. This fish can be found around all Cambodia, except highland streams, mainly in large rivers of lower Mekong floodplains, or large lakes and tanks. It easily adapts to impoundments. It can reach 200 cm length but commonly it has about 80 cm. Typical for *W. attu* is broad head with depressed snout, caudal fin plainly forked, large mouth that it extending back as far as eye. Its eyes have a free orbital margin. The fish teeth in jaw are set in wide bands. This allows them to feed on smaller fish, crustaceans, and molluscs. It is known to be excellent game fish. It is usually taken by gill-nets and hooks and then sold on market fresh or in ice exported to Thailand (Rainboth 1996).

Channa striata

Figure 3. *Channa striata*

Source: Reyes 2008

Scientific name: *Channa striata* (Bloch 1795)

Family: Channidae

Local name/English name: Trey raws/striped snakehead or Chevron snakehead (FAO name)

Striped snakehead is commercially very important fish species in South and Southeast Asia. Because of its almost boneless firm white flesh and heavy dark skin, it is probably one of the most consumed fish in Thailand, Indochina and Malaysia. Striped snakehead mainly occurs in sluggish, muddy, standing water of rivers, streams, ponds and swamps. During dry season it survives in muddy bottoms of lakes, swamps and canals where it stays as long as skin and air-breathing apparatus is moist. As name indicates, striped snakehead has dark, mottled stripes in black and ochre combination on top and sides of its sub-cylindrical body. Due to large, broad, flatted head, all genus is now called snakehead. Its deeply-gapping mouth is full of small teeth which are used for catching fish, frog, snake, insects or earthworm. This fish is caught with gill-nets, traps or bailed hooks. It is commonly sold on markets fresh or alive as well as processed by smoking or fermentation in prahok (Rainboth 1996).

Clarias macrocephalus

Figure 4. *Clarias macrocephalus*

Source: Tran n.d.

Scientific name: *Clarias macrocephalus* (Günther 1864)

Family: Clariidae

Local/English name: Trey andaing toun/ broadhead catfish or bighead catfish

Broadhead catfish is usually found in rivers, lowland streams, ponds, swamps, rice paddies and pools after floods. It can reach length up to 120 cm. All species of *Clarias* genus are freshwater fishes and furthermore they are type of air-breathing catfish. That means the fish can breathe either under water, so oxygen from water, or above water, and thus oxygen from air. This gained advantage can be useful during dry season when water resource is dried out and the fish needs to get back to water. For this purposes, *Clarias* developed air-breathing organs next to regular gills. This special organ is only used when *Clarias* does not have enough water for breathing (Frimodt 1995). Ability of breathing air can be from several hours to few days as long as fish skin is moist. This advantage is useful for selling fish alive. Other typical characteristics for this family is slender body with long dorsal and anal fins that gives them similar appearance like eels have. Broad mouth with four pairs of sensory barbels is placed on depressed, bony head. *Clarias* spp. occurs in water resources from Africa to East, Southeast and South Asia, and it has been introduced to other parts of the world. 4 species of genus *Clarias* likely to occur in Cambodia (Rainboth 1996). It is very often mistaken with female of walking catfish (*Clarias batrachus*), although broadhead catfish is considered to be better in taste and more nutritious. Broadhead catfish is omnivorous that eats smaller fish, molluscs, and other invertebrates and aquatic weeds. Introduction of the species outside its habitat was found as invasive with ecological impact. Due to air-breathing ability it is usually sold in market alive (Rainboth 1996).

Belodontichthys truncatus

Figure 5. *Belodontichthys truncatus*

Source: Thach n.d.

Scientific name: *Belodontichthys truncatus* (Kottelat & Ng 1999)

Family: Siluridae

Local/English name: twisted-jaw catfish

The twisted-jaw catfish is freshwater fish that belongs to endemic species of the Mekong Basin. The fish can reach 60 cm length. It has strongly upturned head with mouth that is in angle of 60° above horizontal. Natural habitat of the fish is in deeper parts of large rivers. It usually eats smaller fish close to water surface. The fish can be caught by hook-and-line as a game fish, or usually by gill-nets and cast-nets (Rainboth 1996). There can be found two other species of the genus *Belodontichthys*, comprising *B. dinema* (Bleeker 1851) and *B. truncatus*. The natural occurrence of the first mentioned species is in central and southern Thailand, Malaysia, Sumatra and Borneo, while the second species occur in northeast Thailand, Laos, Cambodia and Vietnam. In Cambodia, *Belodontichthys truncatus* is main item for caught, stored in ice and exported to Thailand (Kottelat & Ng 1999). For local consumption, the fish is marketed fresh, dried or salted (Rainboth 1996).

***Henicorhynchus* siamensis**

Figure 6. *Henicorhynchus siamensis*

Source: Warren 1996a

Scientific name: *Henicorhynchus siamensis* (Sauvage 1881)
Family: Cyprinidae
Local name/English name: Trey riel tob/siamese mud carp

Siamese mud carp is freshwater fish that can be found in Mekong and Chao Pryah basins. Cambodian name for siamese mud carp consists of local currency (riel) which shows importance of this fish for annual fishery on the Tonlé Sap Lake. It naturally occurs in large and small rivers where it lives in mid-water to bottom in great shoal. During rainy season, siamese mud carp migrates out to floodplains and comes back when water returns to river. Main signs of *Henicorhynchu* genus are 8 branched dorsal-fin rays, lower lip thin and tightly attached to lower jaw. Typical for Siamese mud carp is plain silvery body, very tiny maxillary barbel and dorsal fin with dark distal margin. Siamese mud carp feeds on algae, periphyton and phytoplankton and it can grow up to 20 cm length. This fish is used for prahok production.

Kryptopterus micronema

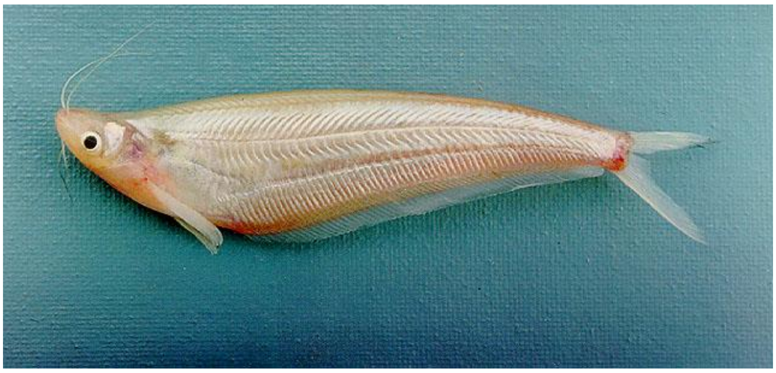

Figure 7. *Kryptopterus micronema*

Source: Warren 1996b

Scientific name: *Kryptopterus micronema* (Bleeker 1846)

Synonym: *Phalacronotus micronemus*

Family: Siluridae

Local/English name: Pa nang khao

Kryptopterus micronema is freshwater fish species native to Mekong River. It is spread in watercourses, such as rivers, lakes and streams, from Thailand to Indonesia. It well adapts to impoundments. Its maximum length is up to 50 cm. Recognition signs for genus *Kryptopterus* are: subcutaneous eye, orbital rim continuous with skin covering eye, mouths is short that is not extending eye, dorsal fin is present or not, maxillary barbel that extends past gill opening, dark spot at caudal-fin base, vomerine teeth in smoothly curved band. Usual food for *K. micronema* is shrimp and pelagic fish. It is mostly consumed smoked or as tradition fish paste called prahok (Rainboth 1996).

Notopterus notopterus

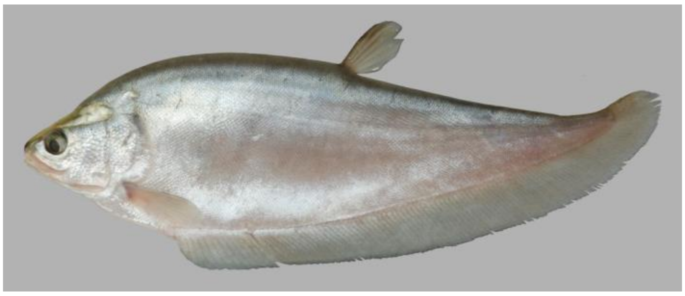

Figure 8. *Notopterus notopterus*

Source: Tran n.d.

Scientific name: *Notopterus notopterus* (Pallas 1769)

Family: Notopteridae

Local name/English name: bronze featherback

N. notopterus naturally occurs in fresh waters of South and Southeast Asia. During rainy season, adults stay in standing waters of lake, pond, river, and canal. The species can be recognized according to brown colouring of adults and slightly concave dorsal head profile. Normally occurring in length of 25 cm. It is fed on insects or fish. The most active is at night and around twilight. Usually it is found fresh or dried (Rainboth 1996).

Corica laciniata

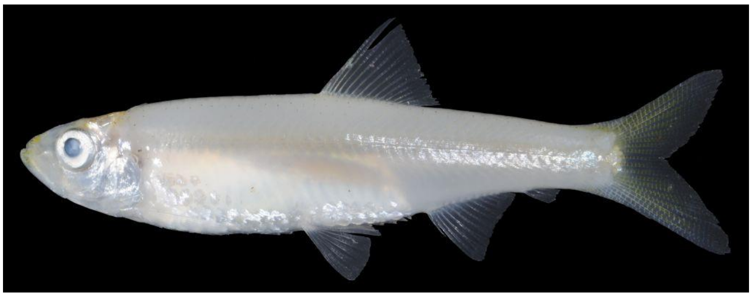

Figure 9. *Corica laciniata*

Source: Tran n.d.

Scientific name: *Corica laciniata* (Fowler 1935)

Family: Clupeidae

Local name/English name: bangkok river sprat

Corica laciniata is freshwater fish that is commonly found tidal zones of large rivers and in floodplains around rivers of Southeast Asia (Whitehead et al. 1988). The fish has moderately elongated body that can have maximal length up to 7 cm. Teeth in jaws are tiny or absent. It is mostly used for prahok and fish sauce production (Rainboth 1996).

Mastacembelus armatus

Figure 10. *Mastacembelus armatus*

Source: Aquatropic 2017

Scientific name: *Mastacembelus armatus* (Lacepède 1800)

Family: Mastacembelidae

Local name/English name: zig-zag eel

Mastacembelus armatus lives along bottom of highland streams or lowland wetlands and occasionally in reservoirs from South to Southeast Asia. It has elongated snake-like body shape that can have up to 80 cm. Its caudal fin is connected to anal and dorsal fins. Body colour is usually dull brown and belly is lighter brown. Its name is according to 1-3 zig-zag lines that gives it specific pattern. The species is active during night and it feeds on benthic insect larvae and worms. It can be usually bought fresh or trade for an aquarium (Rainboth 1996).

Coilia macrognathus

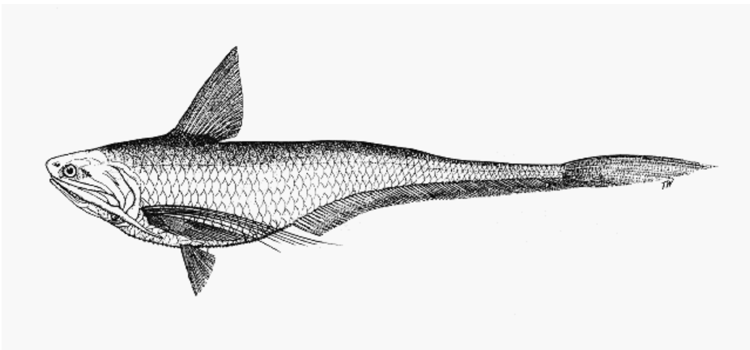

Figure 11. *Coilia macrognathus*

Source: FAO 1988

Scientific name: *Coilia macrognathus* (Bleeker 1852)

Family: Engraulidae

Local name/English name: Trey chonluanh moan/longjaw grenadier anchovy

Coilia macrognathusis freshwater fish that can be found in estuaries or tidal areas of rivers. It is commonly found in Mekong Delta but with less occurrence it is also in Tonlé Sap River and Tonlé Sap Lake. The species usually grows to 20 cm. Typical sign for genus Coilia is elongated caudal fin (Rainboth 1996).

Esomus longimanus

Figure 12. *Esomus longimanus*

Source: Thach n.d.

Scientific name: *Esomus longimanus* (Lunel 1881)

Family: Cyprinidae

Local name/English name: Trey changwa phlieng/mekong flying barb

Esomus longimanus lives in ditches, canals, ponds and it can be also find in areas, where grow submerged aquatic plants. Naturally occurring in Malaysian peninsula, Makong River from Thailand to Cambodia, found also in Tonlé Sap Lake. It is small size species that grows up to 8 cm. Rostral pair of barbels extend well beyond eye and second pair of barbels reach past pelvic fin. It has a dark line starting at a head until a caudal-fin base. It usually feeds on zooplankton and insects. The fish is used for prahok production or it is trade fresh on market (Rainboth 1996).

Rasbora hobelmani

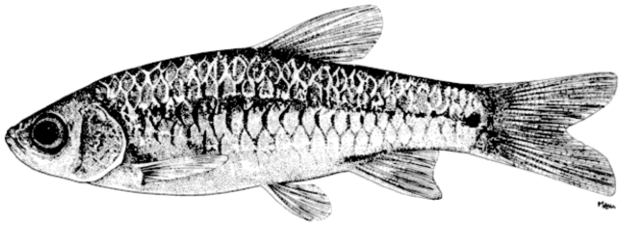

Figure 13. *Rasbora hobelmani*

Source: Rainboth 1996

Scientific name: *Rasbora hobelmani* (Kottelat 1984)

Family: Cyprinidae

Local name/English name: Trey changwa/kottelat rasbora

Freshwater fish *Rasbora hobelmani* can be found from mid-water to surface of small streams and ponds in area from Myanmar to Cambodia. It grows up to 6 cm and it has a dark lateral stripe which ends in a dark spot. It feeds on exogenous insects. The fish is not often seen on markets but may be possibly sold for aquarium trade (Rainboth 1996).

1.2 Fish in Human Nutrition in Cambodia

Food is essential part of Cambodian people life and it poses more than two-thirds of their expenditures (FAO 2014). Total daily food consumption of Cambodian individual is estimated at 955 g in average, where rice comprise the largest and important part of diet. Rice itself or its products are base of many Cambodian dishes so it makes the most consumed starchy food in the country with 302 g per person on daily basis (Morgensen 2001) and the second biggest protein source with 35% after fish. A whole fish is considered as nutrient rich food. So it is no wonder that Cambodians are on the first place in fish consumption in the world (Baran 2010) which gives them 37% of total protein intake and 76% of animal protein intake. Fish protein is mainly consumed from freshwater fish (64%) and marine fish (26%), the rest 10% consists of OAAs and aquaculture (IFReDI 2013). Fish protein is easily digestible and high-quality source that contains all essential amino acids. The importance of essential amino acids is that human body cannot make them on its own and so people need to get them from food. Usual proportion of protein in fish is between 15% and 20% but there are fish species that can have more or less protein than the interval delimits (FAO 2001). As protein is essential for body growth, recommended intake for man is 64 g/day, woman 46 g/day, child 20 g/day (WHO 2007). Fish and fish products contribute to total dietary energy supply (2,411kcal/person/year) with 74 kcal/person/year (FAO 2014).

Fish can be supply of important micronutrients such as vitamins and minerals. These are essential nutrients for human body, because it cannot produce them for the most part, so we need to obtain them from food. Minerals and vitamins are needed in lower amount for human body compared to macronutrients.

1.2.1 Vitamins

Vitamins are organic compounds that have origin from plants or animals with sensitivity on high heat, air and acidy. Various fish processing methods, such as sun drying, oven drying or fermentation, nearly almost or in large quantities destroy vitamin A content. However, vitamin content does not have to be notably reduced in case of gentle processing or preservation practices with low time of storage (FAO 2001). Vitamins can be divided into two groups according to solubility. There are fat soluble vitamins, such as vitamins A, D, E and K, and water soluble vitamins, such as vitamins B and C. Distribution and amount of all vitamins in fish can differ from species to species as well as between individuals. High percentage of fat soluble vitamins can be found mainly in parts which are not often consumed, such as head, liver or gut. But these vitamins can be also found in a flesh. For example, a cod has almost all the vitamin A and D concentrated in a liver, in contrast, an eel has these vitamins presented in flesh. Water soluble vitamins are more likely distributed uniformly in liver, gut, head and roe as well as flesh. When human body do not get sufficient amount of vitamins, it can lead to deficiency diseases, such as rickets (vitamin D deficiency), anaemia (vitamin B12 deficiency) or impaired eyesight (vitamin A deficiency) (National Research Council 1993).

Vitamin A

Vitamin A is an unsaturated organic compound that is important mainly for development of good vision and immune system. Its deficiency can cause worsening of vision until night blindness, diarrhoea, or measles. There are 8.4% of pregnant women and 6.8% lactating women suffering night blindness caused by vitamin A deficiency in Cambodia

(NIS 2011). Only 19% of population absorbs recommended vitamin A intake (IFReDI 2013). Even small amount of fish added to diet can improve nutritional aspect of diet and enhance physical growth and cognitive development of children, furthermore, vitamin A from fish is easier to absorb than from plant food. The main source of vitamin A in Cambodian diet is fish and some of fish species have even double amount to that of vegetables such as carrot or spinach (Vilain et al. 2016). Vitamin A content differs among species and distribution in fish body. In two Cambodian fish species, *Parachela siamensis and Rasbora tornier*, it was found very high presence of vitamin A with concentration over 1500 RAE/100 g of raw, whole fish (Roos et al. 2007a). Deficiencies in vitamin A, zinc, iron and other micronutrients are responsible for a significant proportion of malaria morbidity and mortality. Thus, nutrition programs are recommended to be involved in malaria intervention programs (McLaren & Frigg 2001).

1.2.2 Minerals

On the other hand, minerals, which belong to micronutrients as well, are inorganic compounds that cannot be break down but their presence in fish can be reduced by head, bones or viscera removal before cooking or processing, even though these body parts are rich in micronutrients, in particular zinc, iron and calcium (Vilain et al. 2016). Reasons of their removal are following: these body parts are more sensitive for microbiological contamination, give to food unfavourable appearance or odour. However, study of Roos et al. (2007a) in Cambodia showed that fish species *Esomus longimanus*, which was cleaned by tradition method, has high iron content (45.10 mg/ 100 g) even in its edible parts. Thus, only one portion of local fish soup with rice prepared from *E. longimanus* gives 45% of daily iron requirement for pregnant woman. This fish was also found as very high source of zinc (20.3 mg/ 100 g of edible parts) and good source of calcium (350 mg/100 g of edible parts). Small fish species, which have low market value and they are commonly consumed by poor people in Cambodia, are rich source of minerals with comparison to other animal sources (Roos et al. 2007a). Minerals are important for function of human body in many reasons. Iron transport oxygen to all parts of body, calcium is necessary for strong bones and normal functioning of muscles and nervous system, zinc is needed for most of body processes, in growth and good immune system. Their deficiencies can cause many problems, such as, rickets or osteomalacia (calcium deficiency), anaemia (iron deficiency), poor growth and skin problems (zinc deficiency) (Morgensen 2001).

1.2.3 Malnutrition causes in Cambodia

In 2014 in Cambodia, there was 14% of population living under poverty line (ADB 2018) from total population around 16 million (World Bank 2017). The number is still decreasing (from 48% to 14% between 2007 and 2014), however, there are still around 4.5 million of people living on a border of poverty (USAID 2018). Poverty related problem is undernourishment, which suffers 18.5% of Cambodian population (FAOSTAT 2017). Main factors playing important role in malnutrition, and its vicious circle, is maternal undernourishment, high level of stunting and underweight, and anaemia which mainly causes poor food quality. Pregnant women and young children are the most sensitive for lack of nutrients. This can lead to higher probability of low birth weight baby that can be followed by malnutrition which is then related to child stunting (32.4% in 2014) and wasting (9.8% in 2014) under 5 years of age (ADB 2018).

Despite the fact that Cambodia belongs among countries with the biggest fish production in the world and have abundant sources of fish, many households face serious food insecurity and poor nutritional status. There can be several causes of the scarcity and malnutrition. For rural households, fish is more likely to be sold or exchanged for staple food which is cheaper and providing higher energy supply (Kawarazuka 2010), while rice comprises 63% of energy supply from total 68% of all cereals. Evidence of low price for staple food in Cambodian household is that, only 16% of expenditures are for cereal food, while families usually spend 70% of income for food in total (FAO 2014). Quantity of food is in this case prioritized to its quality, which would be higher intake of meat, fruits and vegetables (Kawarazuka 2010).

Poor health state has direct connection to low nutritional status. Parasites, such as hookworms, roundworms and whipworms cause iron loss and thus decrease of vitamin A absorption that can escalate into anaemia (WHO 2011). When infection of parasites is combined with low quality of drinking water, all these factors lead to diarrhoea and low immune system (FAO/WHO 2004).

Another cause of deficiencies, such as calcium, iron and zinc, can be phytate, which is presented only in plants and in high concentration in cereals. In form of phytic acid, it has strong affinity for binding minerals and trace elements and inhibit their intestinal absorption. On the other hand, with balanced diet and adequate mineral intake phytic acid can be beneficial to human health because of its anticancer, antioxidative and anticalcification activities (Schlemmer et al. 2009).

Very important for well-balanced diet and human health is nutritional education, which is usually missing in developing countries. Furthermore, we can encounter with mistaken beliefs and those eating habits that can result in loss of nutrition diversity. High-risk categories are mainly pregnant women, infants, growing children and elderly (Morgensen 2001).

1.3 Fats

Fats, together with proteins and carbohydrates, are part of macronutrients that are important part of human nutrition. Fats are the most discussed class belonging to lipids which are generally defined as biomolecules that have in common following properties, solubility in nonpolar solvents and insolubility in water. Fats and oils are substances of plant or animal origin that are non-volatile, greasy and oily on touch. They are hydrocarbon molecules which means that they are primary built by carbon and hydrogen atoms. Fats and oils are also called as triacylglycerol (TG) because they are consist of three fatty acids joined to glycerol, a trihydroxy alcohol, as it can be seen in Figure 15. In case that to all three OH groups of glycerol are joined three same fatty acids (FA) it is resulting into simple TG (McMurry 2004; Gurr et al. 2008; Patel 2018). However, natural oils and fats are typically built of, so called, mixed TGs, which are consist of two to three different FA. TGs, which are solid in room temperature (25 °C), are called fats, on the other hand, oils are TGs that are liquid in room temperature. Due to this fact, TGs gained from animal sources we usually call fats and TGs from plant sources, are commonly called oils. Pure fats and oils can be described as tasteless, odourless and colourless. However, animal fats and vegetable oil are more known according to their specific sensory characteristics. It is cause by foreign lipid soluble substances that were absorbed by them (Gurr et al. 2008).

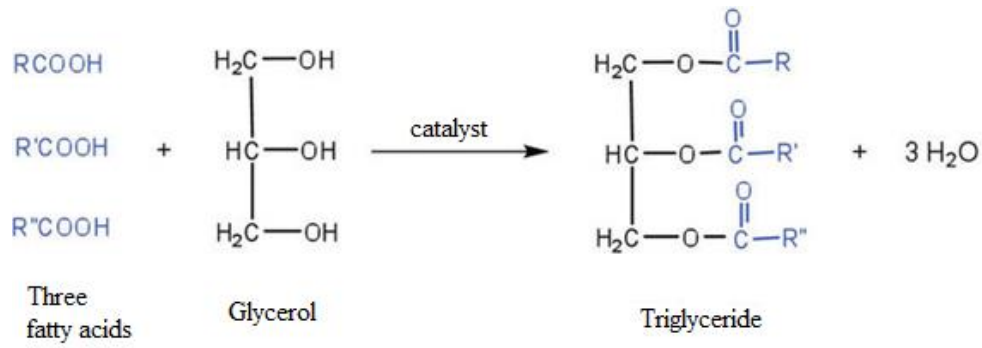

Figure 14. Three fatty acids reaction with glycerol and creation of triacylglycerol and 3 atoms of water

Source: McMurry 2004

Fats can be sorted into categories according to number of double bonding in between carbons in the aliphatic chain. If there is no double bonded carbon we call fat saturated and if there is one or more double bonds, fat is called unsaturated. Furthermore, unsaturated fats are divided into monounsaturated and polyunsaturated. Unsaturated fats are sorted by *cis-trans* isomerism that tells us configuration of carbons in double bonding. *Cis*-FAs occur the most commonly in nature, while *trans*-FAs are rare in nature (McMurry 2004). Nevertheless, *trans*-FAs are results of vegetable oil hydrogenation, often used for margarine or cooking fats production. This process shows Figure 16, when molecular hydrogen reacts with double bond between carbons. Hydrogenated oils get desired properties (soft and solid in room temperature) and other ingredients, colour agents, flavour agents and vitamins A and D are added. Many consumers have switched from butter to margarine in believe of saturated fats and cholesterol intake avoidance but several studies have proved that *trans*-FAs increased cholesterol level and incidence of coronary heart disease (Hu et al. 1997; Zhang et al. 2018).

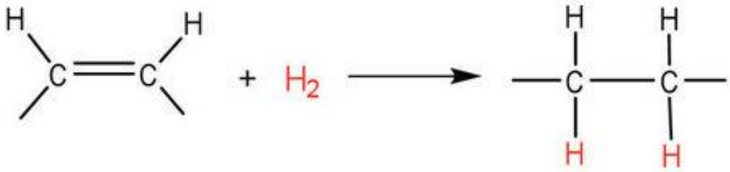

Figure 15. Catalytic hydrogenation reaction

Source: Chemistry Libretexts 2019

Oxidation and hydrolysis are other reactions commonly occurring to fats and oils that cause them to turn rancid that gives it specific unpleasant odour. To avoid oxidation thus rancidity of fats, very small amount of antioxidants are added during oil processing. One of fat physical property is poor heat and electricity conductivity and because of that, it is great insulator (Hernandez & Kamal-Eldin 2013). Fat cells are stored in adipose tissue that insulates body and prevent heat loss. Stored fat serves also as protection of vital organs from heat escape and sudden movements. Fat has other significant functions in human body (Patel, 2018). Fat soluble vitamins A, E, D, and K require daily adequate portion of fat to be dissolved in it and then absorbed. In case of insufficient fat intake in diet, essential vitamin level can drop, ending in vitamin deficiency and its related diseases. Fat serves as the biggest energy source in human body. 1 g of fat contain 9 calories (Patel 2018). Carbohydrates work as the main body fuel that is turned into fat when it is excessively consumed. During exercise, body first use calories from carbohydrates, which have been eaten, then it start spending saved up calories from fat to keep muscles going. On the other hand, fats can be same harmful as they are important. In 2016, more than 1.9 billion of world adults were overweight and 650 million of them were obese (WHO 2018). One of the main cause of overweight and obesity is energy imbalance between consumed and used calories. High popularity of fatty foodstuffs and nutritionally poor diet combined with inactivity lifestyle are problems of developed part of world. Total elimination of fat or low-fat diets are solutions that can possibly cause health risk to individual that choose these practices for losing weight. WHO (2018) suggests reduction of consumption foodstuffs full of *trans*-fats and saturated fats and replace them by food rich in monounsaturated, polyunsaturated fats and omega-3 and omega-6 fatty acids. FAO (2010) recommends minimum amount 15% of energy from fats to ensure adequate total energy, essential fatty acids, and fat soluble vitamins intake for adult. Suggested amount of energy intake for women from developing countries is at least 20% due to the higher prevalence of malnutrition. Maximum energy intake from fat for adult is around 30-35% to prevent excessive consumption and its related health risk. The most discussed disease and the world leading cause of death is cardiovascular disease (CVD), which includes, for example, coronary artery disease, stroke, heart failure, and many others. Among risk factors causing CVD belong obesity, high blood pressure and high blood cholesterol (hypercholesterolemia) (Keys et al. 1957). While talking about cholesterol, low-density lipoprotein (LDL) cholesterol and ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol are the most commonly used indicators for hypercholesterolemia determination. It is considered that combination of following factors: excessive consumption of unhealthy diet and alcohol, sedentary lifestyle and smoking have impact on elevation of cholesterol level in blood. Too high concentration of cholesterol settles on the walls of the blood vessels that can lead to loss of elasticity and get clogged (WHO 2016).

1.3.1 Fatty Acids

FAs are important part of lipids found in animals, plants and microorganisms. Naturally occurring FAs are parts of complex lipid molecules, for example, fats, phospholipids (main components of cellular membrane), or cholesterol esters. The most commonly in nature, they are consist of unbranched chain of an even number of carbon atoms (from 4 to 28), with hydrogen connected along the length of chain and carboxyl group (-COOH) at the end of chain that makes it acid (Gurr et al. 2008; Ridgeway & McLeod 2015). FAs can be categorized according to number of carbons in aliphatic tail into short chain FAs, medium chain FAs, long chain FAs, and very long chain FAs. Other possible sorting of FAs is according to saturation into saturated (SFA) and unsaturated.

Saturated Fatty Acids

SFAs are the simplest from all FAs. They have linear, unbranched chain with single bonding between carbons and carboxyl group at the end. The maximum possible amount of hydrogen atoms is bonded to every carbon. Due to this, SFAs have higher melting points. Table 1 shows the most common SFAs, their trivial names, as well as abbreviation and usual source (Patel 2018). Typical for SFAs are chains of 12-24 carbons long but there are several biochemically important FAs with shorter chain, for instance, butyric (C4:0) and caproic (C6:0) acids well known as FAs found in milk. SFAs of 8 and 10 carbons are found mainly in palm kernel oil or coconut oil. These two oils are significant source of dietary fat in countries of main palm oil producers but also the EU countries and the USA (Index Mundi 2018).

There are different effects of individual SFAs on concentration of lipoprotein cholesterol fraction. Increase of LDL cholesterol cause, for example, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. On the other hand, stearic acid (C18:0) has no effect on LDL cholesterol level. On the grounds of these conclusions, it is recommended to replace SFA (C12-16:0) with PUFAs or MUFAs and thus decrease LDL cholesterol concentration and total/HDL cholesterol ratio (FAO 2010). However, it has been proved that situation around SFA and its role in the causation of coronary artery disease (CAD) is rather minor than association *trans*-fats with CAD (Temple 2018).

Table 1. Commonly occurring saturated fatty acids in food fats and oils

Acid trivial name	Abbreviation	Typical source
butyric	C4:0	dairy fats
caproic	C6:0	dairy fats
caprylic	C8:0	dairy fats, coconut and palm kernel oils
capric	C10:0	dairy fats, coconut and palm kernel oils
lauric	C12:0	coconut oil, palm kernel oil
myristic	C14:0	dairy fats, coconut oils
palmitic	C16:0	most fats and oils
stearic	C18:0	most fats and oils
arachidic	C20:0	peanut oil
behenic	C22:0	peanut oil
lignoceric	C24:0	peanut oil

Source: FAO 2010

Unsaturated Fatty Acids

Unsaturated FAs are specific by one or more double bonds between carbon atoms, which signifies that number of bonded hydrogen atoms is not maximum. Whether FA has only one double bond it is called monounsaturated (MUFA) and whether there are two or more double bonds it is called polyunsaturated (PUFA). Double bonds are further sorted into *cis* or *trans* isomerism, while *cis* configuration means that two atoms of carbon in double bond lie on the same side whereas during trans configuration atoms of carbon lie on the opposite side, as it can be seen in the Figure 17.

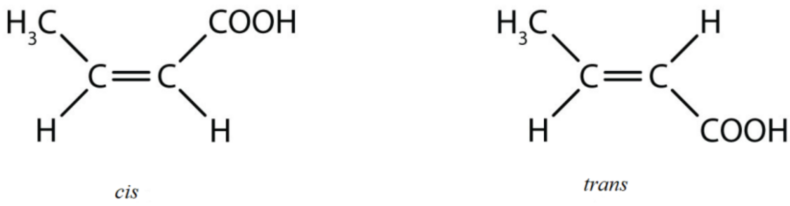

Figure 16. *cis* and *trans* isomerism

Source: Chemistry Libretexts 2019

MUFA

There are up to one hundred naturally occurring MUFA but most of them are very rare compounds. Mediterranean diet is considered as a heathy diet rich on MUFAs that can be commonly found in canola oil, olive oil, red meats, peanut oil, avocados and nuts. The most abundant is oleic acid with 18 carbons and double bond between 9 and 10 carbon. Other often *cis*-MUFAs and their typical sources are shown in Table 2 (FAO 2010). It is highly recommended to integrate MUFA to everyday diets and substitute *trans*-fats with *cis*-configuration MUFA. These steps can prevent risk of CVD because MUFA lower total LDL-cholesterol and maintain HDL-cholesterol level, thus improve TC/HDL cholesterol ratio.

Table 2. Several common *cis*-MUFAs in dietary fats and oils

Trivial name of acid	Delta abbreviation	Typical source
palmitoleic	16:1Δ9c (9c-16:1)	most animal and vegetable oils
oleic	18:1Δ9c (9c-18:1)	all fats and oils
cis-vaccenic	18:1Δ11c (11c-18:1)	most vegetable oils
gadoleic	20:1Δ9c (9c-20:1)	marine oils
gondoic	20:1Δ11c (11c-20:1)	marine oils
erucic	22:1Δ13c (13c-22:1)	mustard seed oil
nervonic	24:1Δ15c (15c-24:1)	marine oils

Source: FAO 2010

PUFA

PUFA with methylene-interrupted double and all *cis*-configuration, which are found commonly in nature, can be sorted out to 12 families. Double bond position ranges from n-1 to n-12, when the most important families in term of human nutrition and health are n-3 and n-6, also known as omega-3 and omega-6 FAs (Gunstone 1999). Examples of these two families are shown in Table 3 and Table 4 with the two essential FA linoleic acid (LA) and α-linolenic acid (ALA) that human body cannot synthetize but they need to be ingested. LA consists of 18 carbons with two double bonds. The first double bond is located between 6^{th} and 7^{th} carbon from the methyl end of the FA chain, hence it belongs to n-6 family. Elongated or enriched on double bonds LA forms series of n-6 PUFAs in Table 3. Ritch sources of LA are most of vegetable oil, especially sunflower and soybean oil. ALA has the same number of carbons as LA but they differ in amount of double bonds (3) and position of the first double bond, which is placed between 3^{rd} and 4^{th} carbon from the methyl end of the FA chain. This characteristics gives the specific name omega-3 (n-3). Same as LA, rich source of ALA are some nuts and vegetable oils, such as flaxseed oil, soybean oil, and canola oil. Other n-3 PUFAs mentioned in Table 4 can be found in fish oils mainly fatty fish species, such as herring, salmon, marcel, sardine, and smelt (Ackman 2007). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important n-3 PUFAs for human nutrition, especially for proper growth of children, positive contribution to brain function and memory, boost processes that decrease blood pressure and blood clotting, which prevents CVD (McKeown 2006). Although human body have the ability to convert ALA to EPA and DHA, the efficiency of conversion is low, especially to DHA. In the view of the fact, it is recommended for man and non-pregnant woman to intake 0.25 g/day of EPA and DHA (FAO 2010).

Table 3. Nutritionally important n-6 PUFAs and their source

Trivial name of acid	N minus abbreviation	Typical source
linoleic	18:2n-6	most vegetable oils
γ-linolenic	18:3n-6	animal tissues, blackcurrant seed oils
dihomo- γ -linolenic	20:3n-6	minor animal tissue
arachidonic	20:4n-6	animal fats, liver and egg lipids, fish

Source: FAO 2010

Table 4. Nutritionally important n-3 PUFAs and their source

Trivial name of acid	N minus abbreviation	Typical source
α-linolenic	18:3n-3	canola oil and soybean oil
stearidonic	18:4n-3	fish oils, hemp oil
eicosapentaenoic	20:5n-3	fish oil; mainly oily fish
docosapentaenoic	22:5n-3	fish oil; mainly oily fish
docosahexaenoic	22:6n-3	fish oil; mainly oily fish

Source: FAO 2010

1.3.2 Fat Content and Fatty Acid Profile of Fish

Fat content and fatty acid profile of fish differ from species to species but there can be found differences even within the same species. Generally speaking, fat content can vary more than water and protein content (Huss 1995). There exists several classifications for determination whether a fish is lean or fatty. According to Bennion (1980), lean fish have less than 5% fat by weight, medium fatty fish have fat content between 5-10%, and fatty fish is considered to be at fat content higher than 10% by weight. Whereas, Ackman (1990) classified fish according to fat content into categories: lean fish (less than 2% of fat), low fat (2-4% of fat), medium fat (4-8% of fat) and high fat (more than 8% of fat). Fat distribution is depended on two kinds of muscles and their proportion in fish species. Fish has, so called, dark and light muscle and according to majority of one of muscle types we sort fish species into white fish or oily (or fatty) fish group. In white fish, such as cod, tilapia, or haddock, the main muscle kind presented is light (or white), while dark (or red) muscle is only distributed in small stripe that is running under lateral line on both side of body. Fat is confined mainly in white fish liver, which works as fat deposit. On the other hand, oily fish species, such as herring, marcel or salmon, have much larger stripe of dark muscle, which contain higher fat content, and several vitamins. Fat cells, which create lipid deposits in oily fish, are usually placed in subcutaneous tissue, belly flap muscle and in muscles that move a fin, head and tail. Some species, which store large amount of fat, may have it saved in belly cavity. Fat can be also found in muscle structure, particularly, with the highest concentration close to myocommata and between light and dark muscles (Johnston 2000). Next to fish physiology, as factor that influence fat content, there are environmental, dietary and seasonal variation factors that have impact on fish fat content and fatty acid profile. One of environmental factor is salinity (Halver

1980). It is proved that marine fish species have lipids richer on n-3 PUFAs than freshwater fish, which have usually higher amount of n-6 PUFAs. Those species that migrates from sea to freshwater or vice versa show significant changes in n-3 and n-6 PUFAs (Ackman 1967). Furthermore, lower temperatures proved to have impact on higher total PUFAs of long chains. Very serious relation was found especially between FA composition and diet of fish and lipid intake. (Halver 1980).

2 Aims of the Thesis

The aim of thesis was to investigate fat content and fatty acids profile of 12 selected Cambodian fish species, which were processed in traditional method of smoking. Obtained data of fat content were statistically compared between species and within same species that were collected from more than one producer. Fatty acid profiles of tested fish samples were compared between species.

3 Materials and Methods

3.1 Samples Collection and Preparation

31 samples of 12 fish species (namely in Table 5) from 19 small-scale smoked fish producers were collected during field study of Slámová et al. (2017) in Cambodia between August and October 2016. Smokehouses of producers were located in villages in wetlands around Tonlé Sap River, Tonlé Sap Lake and Mekong River. Table with all producers by village and province is shown in Table I of Appendix. Obtained fish samples were placed in clean, labelled plastic bags that were then vacuum packed. The samples were transported to Czech University of Life Sciences Prague for further laboratory analyses. The whole fish sample was homogenized in a mortar with addition of about 10 ml of liquid nitrogen. The homogenized samples were stored in amber bottles in refrigerator at temperature below 4 °C.

Table 5. Fish species and number of samples

Fish species	Number of samples
Henicorhynchus siamensis	6
Wallago attu	4
Channa striata	2
Belodontichthys truncatus	5
Clarias macrocephalus	6
Corica laciniata	1
Rasbora hobelmani	2
Notopterus notopterus	1
Kryptopterus micronemus	1
Esomus longimanus	1
Mastacembelus armatus	1
Coilia macrognathus	1

3.2 Soxhlet Extraction

As a method for fat determination, the Soxhlet extraction was used. A principle of the method is transferring the desirable compounds of a solid to a liquid phase using solvent in Soxhlet extraction apparatus.

38 fish samples were analysed with 3 replications. Fine homogenized sample (3-5 g each) was weighted out (with accuracy of 0.0001 g) to thimble and placed to semi-automatic, six-position, Soxhlet extractor VELP Scientifica SER 148 (Italy). Thimble was immersed into approximately 50 ml of petroleum ether (p.a., PENTA, Czech Republic), which was in extraction cup that was previously weighted. Immersion phase of extraction was going for 120 minutes during which extraction cup was being heated up to 110 °C. During washing phase, which took 60 minutes at temperature 110°C, thimble was slid up of extraction cup for dripping of petroleum ether through. The last phase called recovery took 20 minutes, during which petroleum ether was evaporated and recycled. After programme was finished, the extraction cup with fat was dried in oven (Memmert, Germany) to a constant weight. Total amount of fat was obtained from difference of extraction cup after and before extraction.

Total fat content (*w*) was calculated from following equation:

$$w = \frac{m_2 - m_1}{m_0} * 100 \ [\%]$$

Where:

m_0 weight of sample to dry matter content [g]

m_1 weight of dry extraction cup [g]

m_2 weight of extraction cup with extracted fat [g]

3.3 Fatty Acids Analysis

Fatty acids profile of methyl esters in 35 samples was determined by using gas chromatography and mass spectrometry (GC-MS) and gas chromatography with flame ionisation detector (GC-FID).

3.3.1 Derivatization

The fatty acids were esterified by using methanolic sodium hydroxide solution (NaOH, p.a., PENTA, Czech Republic) in the presence of boron trifluoride (BF_3, p.a.,Sigma-Aldrich, Germany) catalyst.

Approximately 50 μl of the extracted fat from sample was placed into 10 ml volumetric flask with addition of 0.4 ml of NaOH (0.5M). The flask was lidded with ground-glass stopper, placed into block heater (Stuart SBH130D, United Kingdom) heated up to 100 °C. After 5 minutes, the flask was cooled down in water bath. Then there was added 0.4 ml of 10% BF_3 in methanol, lidded and heated back up to 100 °C for 5 minutes. After cooling in water bath, 1 ml of extracting solvent, hexane (p.a.,VWR international, France), and 8 ml of saturated sodium chloride (NaCl) solution was added to the flask, which was then gently agitated. Upper phase was separated and transferred to vial that contained 1 mm layer of anhydrous Na_2SO_4 (p.a., PENTA, Czech Republic) to absorb moisture. For gas chromatography analysis, 100 μl of sample and 600 μl of hexane was delivered to vial.

3.3.2 GC-MS and GC-FID

For the identification of fatty acid composition gas chromatograph (Agilent 7890A, USA) with mass detector (Agilent 5975C USA) was used. The GC was equipped with Restek 2560 (USA) biscyanopropyl polysiloxane column (100 m × 250 μm × 0.2 μm). The temperature of the injector was 225 °C and volume of injected sample was 1 μl, split ratio was 1:50. A flow rate of carrier gas (helium) was 1.2 ml/min. A temperature program of the analysis was at initial phase 70 °C hold for 2 minutes, then increased to 225°C (rate 5°C/min) hold 9 minutes, raised up to 240°C (rate 10°C/min) hold for 6.5 minutes. The total run time was 50 minutes. Mass acquisition parameters were set as follows: low mass 40.0, high mass 400.0, MS Source 230 C with maximum 260° C and MS Quad 150 C with maximum 200 C. Fatty acid were identified by comparing their retention times and spectra with the retention times and spectra of available standards (FAME mix, Sigma Aldrich, Czech republic), and by comparing the data with NIST database, version 2.0.

Gas chromatography (Agilent 7890A, USA) with flame ionisation detector (FID) (Agilent 7890B, USA) was used for relative quantification of fatty acids. The chromatographic conditions of the analysis were the same as written above. The FID detector was heated up to 260°C, hydrogen gas flow was 30 ml/min, air flow 400 ml/min and makeup flow was 30 ml/min.

3.4 Dry Matter Content Determination

The water content was determined from the sample weight difference before drying and after drying under defined conditions (105 ± 2 °C for 24 hours). After deducting the water content from 100% the dry matter content was determined.

1-4 g of homogenized sample was weighted out in an aluminium bowl with accuracy of 0.0001 g. The sample dish was placed in an electric oven (Memmert, Germany) at 105°C ± 2 °C to a constant weight of up to 24 hours. The bowl was cooled and weighed.

Dry matter content was calculated by following formulas:

$$w = \frac{m_2 - m_3}{m_2 - m_1} * 100 \ [\%]$$

Where:

m_1	weight of bowl before drying [g]
m_2	weight of bowl with sample before drying [g]
m_3	weight of bowl with sample after drying [g]

Dry matter [%] = 100 – water content (*w*) [%]

3.4 Data analysis

The data obtained from laboratory measurements were firstly processed in Microsoft Office Excel 2013 and then statistically in IBM SPSS Statistics 23 software. For comparison of total fat content in dry matter and individual fatty acids, one-way analysis of variances (ANOVA) with significance level α=0.05 was used and paired test between just two samples. In cases, where null hypothesis was rejected, post-hoc Tukey’s range test with significance level α=0.05 was applied.

4 Results and Discussion

4.1 Fat content

Figure 18 and Table 6 show obtained values from analyses of fat content in dry matter of selected Cambodian freshwater fish species. In this study, fat content ranges from 3.28% (*C. laciniata*) to 47.43% (*R. hobelmani*). There are several classification for definition of lean and fatty fish. However, these classifications cannot be applied on fish samples that lost moisture during processing. Ground of the difference in fat content is ratio between fat (g) and weight of sample (g). Sample of fresh fish contain higher amount of moisture, thus the ratio is lower than in case of smoked fish that during processing loses certain amount of mositure. Due to this reason fat content was calculated in dry matter.

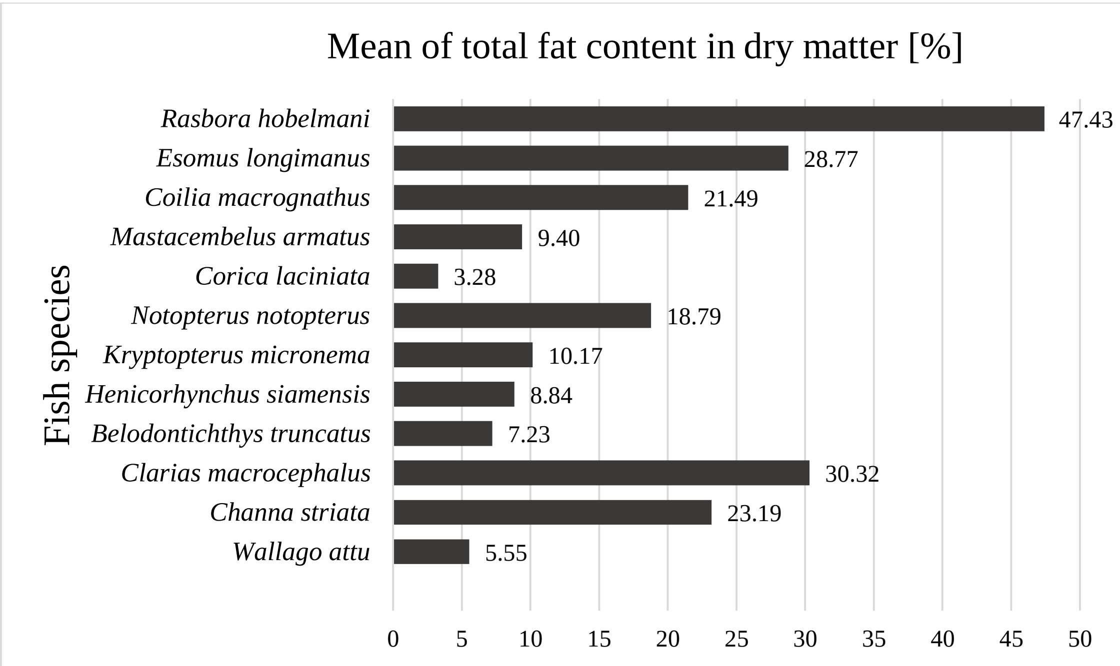

Figure 17. Mean of total fat content in dry matter by fish species (%)

Table 6. Descriptive analysis of fat content in dry matter

Fish species	n	SD	Minimum (%)	Maximum (%)
W. attu	11	±3.27	2.44	10.69
C. striata	6	±2.27	20.26	26.02
C. macrocephalus	17	±6.32	18.03	38.59
B. truncatus	15	±1.71	4.26	9.39
H. siamensis	18	±5.24	1.02	16.47
K. micronema	3	±0.22	9.96	10.39
N. notopterus	3	±0.62	18.18	19.42
C. laciniata	3	±0.14	3.14	3.43
M. armatus	3	±0.08	9.32	9.49
C. macrognathus	3	±0.35	21.11	21.79
E. longimanus	3	±0.63	28.21	29.45
R. hobelmani	6	±3.49	43.59	51.29

n number of replications

Data are not uniform as obvious from standard deviation and minimal and maximal fat content. Table 6 gives detailed data to mean values of fat content in dry matter. Those fish samples, which occurred more than one times, so they have more than 3 replications, show higher standard deviations. The reason of this trend can be mainly because of differences among each species that can vary due to various geographical origin, age, season of catch or different diet (Piggott & Tucker 1990; Rasoarahona et al. 2005). Statistical analysis of variances showed significant difference of fat content of *R. hobelmani* (47.43%) from all other 12 species. The percentage is more interesting with the fact that it is fish species with low body weight and small maximal length (Rainboth 1996). The second highest value of fat showed *C. macrocephalus* (30.32%) that was significantly different from all tested fish species, except *E. longimanus* (28.77%), *C. striata* (23.19%) and *C. macrognathus* (21.49%). *C. macrocephalus* is species analyzed by several studies focused on nutritional vaules (Shirai et al. 2002; Karapanagiotidis et al. 2010). However, their results are comparably lower mainly due to usage of fresh meat that was free of skin, fins and bones. On the other hand, fish used for smoking were only eviscerated, removed from head. Areas beneath skin and around muscles helping fins to move, especieally, are usual lipid deposits (Johnston 2002). Similar case occured with studies investigating nutritional quality of fresh W. attu (Lilabati & Vishwanath 2010; Memon et al. 2010). Study, investigating nutritional values of smoked *Clarias gariepinus* (species of the same genus as *C. macrocephalus*), showed 19.53%±0.79 of fat (Usman 2017), which does not correspond to mean value of fat content of all *C. macrocephalus* in this study. Nevertheless, if we look at data of fat content by producer in Table V in Appendix, a sample from Odombong village shows fat content 20.21% ±2.61, which can be considered as close value compared to the rest values in interval 27.12-36.26%.

Obtained data of the same fish species from more than one producer were statistically compared between each other to find differences among fat content means of repetitive samples of the same fish species. Tables II, III, IV, V, VI and VII give detailed information to mean values of fat content, such as standard deviation, maximal and minimal values and location of smoked fish producer. Mean values of fat content of *W. attu* showed significant difference at $P<0.05$ level among all samples (Table II). An interval of fat content by samples is 2.52-10.39%. Statistical difference at $P<0.05$ level among all samples of same species was also found at case of *H. siamensis* with interval of means 1.10-16.01% (Table VII). In both cases, *W. attu* as well as *H. siamensis*, standard deviation of all mean values have not exceeded more than ±0.61.

4.2 Fatty Acid Profile

Maximum number of FA identified in one sample is 21 plus 6 unidentified which were summed and mentioned in Table VIII. Interval of unidentified FA was in 5.28-0.97%. The FA composition by saturation represent Figure 19 where interval for SFA was 53.66-26.45%, MUFA 45.29-18.51% and PUFA 34.46-11.98%. The species with least proportion of SFA was *C. striata* that had 26.45% SFA and 64.55% of unsaturated FA, while MUFA was 45.29% and PUFA was 19.26%. Study of Jaya-Ram et al. (2018) agrees that *C. striata* has the lowest SFA from tested species, however, in their case it showed 38.4% SFA and 61% unsaturated FA. The second lowest SFA proportion (27.81%), thus high unsaturated FA (42.72% MUFA and 18.14% PUFA), had another commercially important species *C. macrocephalus*. Results corresponded to study of Shirai et al. (2002), which was investigating dietary and seasonal effect on fat composition. The highest SFA proportion (53.66%), thus the lowest unsaturated FA proportion (41.14%) had *B. truncatus*. The highest concentration of PUFA in fat had *C. laciniata* with 34.46%.

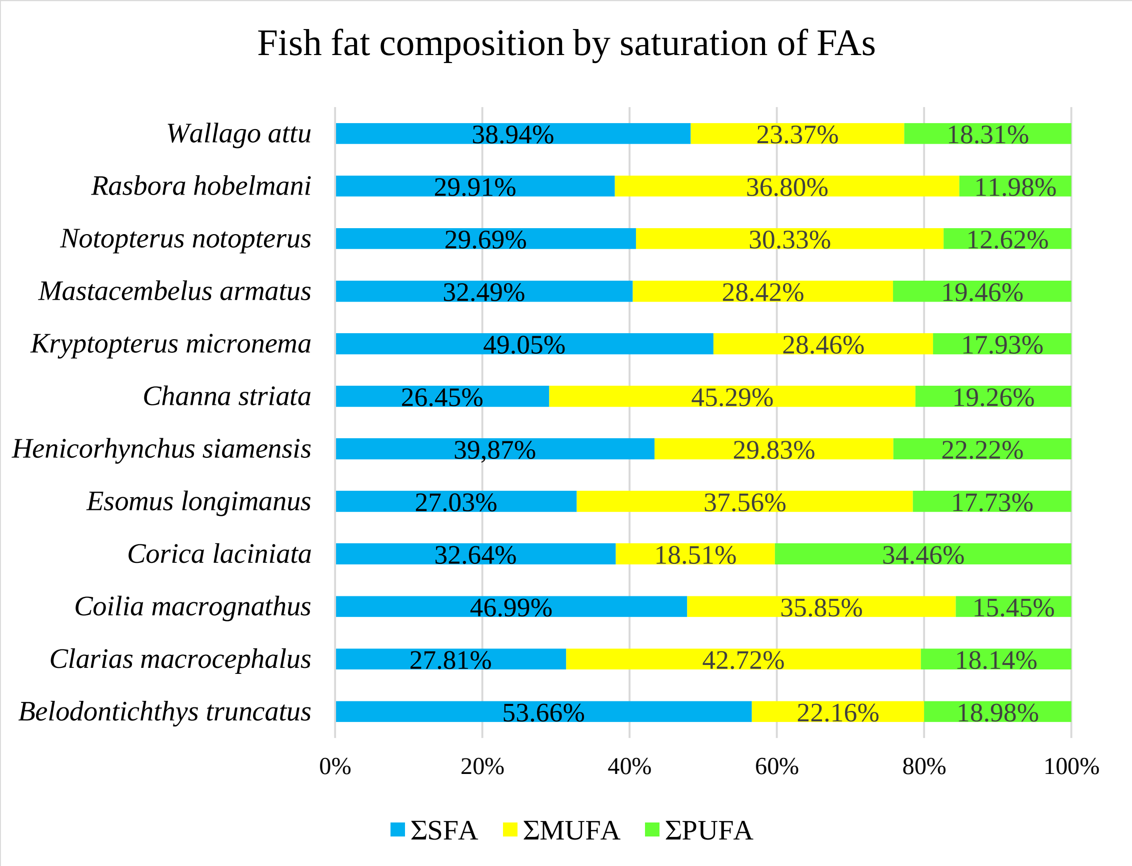

Figure 18. Fish fat composition by saturation of FAs

Figure 19 shows proportional representation of selected FA in fish fat from 12 Cambodian species. Detailed tables with all FA occurring in each fish with percentage of mean values and standard deviations are mentioned in Table VIII in Appendix. FA with the highest mean value was oleic acid (Z9c-18:1; 38.55-5.56%) at species *C. macrocephalus* (38.20%±1.39). The lowest value was for *C. laciniata*. Oleic acid was followed by palmitic acid (16:0; 33.18-22.93%), and then linoleic acid (18:2n-6; 15.55-1.89%) where occurred the same case like at oleic acid. The lowest value belonged to *C. laciniata*, highest value represented by *C. macrocephalus* (12.96%±3.72). Furthermore, the proportion is in agreement with the study of Shirai et al. (2002). *C. striata*, another commercially important species, showed similar percentage (12.49%±0.64), which is higher value than reported by Jaya-Ram et al. (2018). Another FAs, which showed high values after linoleic acid, were DHA (13.67-0.92%) and EPA (12.86-0.24%) where both the highest values belonged to one species *C. laciniata*, which is among rest of the results significantly different. With comparison with other freshwater fish species of this research and Mohanty et al. (2016) and Shirai et al. (2002), none of tested freshwater species has reached up to values of *C. laciniata*. Chemical property investigated of this species was vitamin A concentration by Roos et al. (2007a). The lowest values of DHA and EPA shared *C. macrocephalus*.

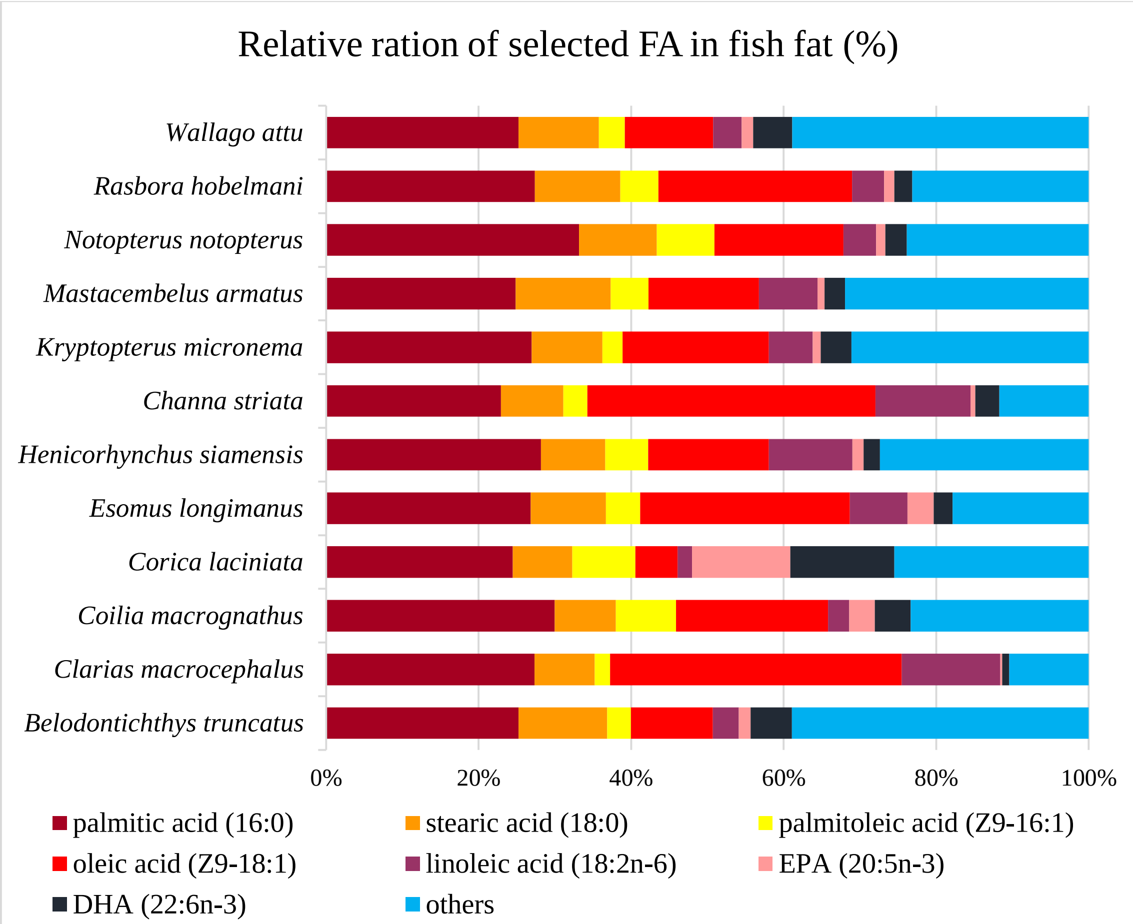

Figure 19. Relative ration of selected FA in fish fat.

The linoleic acid is the major FA from all PUFAs in this research. With α-linolenic acid, it is essential fatty acids for humans. In contrast to LA, ALA was not detected in any of the analysed fish. Low values were found for γ-linolenic acid (18:3n-6) ranging from 1.30% to 0.19%. In Table 7, which shows PUFA/SFA ratio, n-6/n-3 ratio, Σn-3, Σn-6 and DHA/EPA ratio of analysed fish samples, proportion of PUFAs n-3 ranges between 28.68% and 0.83%, PUFAs n-6 ranges between 18.85% and 5.78%. PUFAs n-3 have significant benefit in protection against cardiovascular disease (Moreira et al. 2001). Based on the UK Department of Health, which recommends maximum of 4.0 for n-6/n-3 ratio as ideal proportion, all species, except *C. macrocephalus* (11.84), have not exceeded maximum recommended level, which is set to prevent harmful effect on health (HMSO 1994). The other ratio values ranged from 3.59 to 0.20, where the lowest was for *C. laciniata*. Values in between interval 0.23-0.45 are recommended for PUFA/SFA ratio (HMSO 1994) Interval for PUFA/SFA ratio of Cambodian fish species was from 1.06 for *C. laciniata* to 0.33 for *C. macrognathus*. DHA/EPA ratio was raging from 4.94 for *C. striata* to 0.72 for *E. longimanus*.

It was observed that proportion of each FAs of analysed species varied and in some cases significantly in the $P<0.05$ level. These differences can be explained by various diets (if species are herbivorous, omnivorous or carnivorous), location, water temperature and seasonal variation, which are factors influencing FA composition (Sargent et al. 1995).

Table 7. PUFA/SFA ratio, n-6/n-3 ratio, Σn-3, Σn-6 and DHA/EPA ratio of selected Cambodian fish species

Fish species	PUFA/SFA	n-6/n-3	n-3 (%)	n-6 (%)	DHA/EPA
Belodontichthys truncatus	0.35	1.21	8.57	10.41	3.48
Clarias macrocephalus	0.65	11.84	1.41	16.72	3.77
Coilia macrognathus	0.33	0.74	8.87	6.58	1.41
Corica laciniata	1.06	0.20	28.68	5.78	1.06
Esomus longimanus	0.66	1.68	6.62	11.11	0.72
Henicorhynchus siamensis	0.56	3.59	4.84	17.38	1.48
Channa striata	0.73	3.05	4.75	14.51	4.95
Kryptopterus micronema	0.37	1.80	6.40	11.53	3.87
Mastacembelus armatus	0.60	2.10	6.28	13.17	2.97
Notopterus notopterus	0.43	1.57	4.90	7.71	2.26
Rasbora hobelmani	0.40	1.75	4.36	7.62	1.76
Wallago attu	0.47	1.18	8.42	9.89	3.36

5 Conclusion

Fat content data showed significant differences among several species, moreover, comparison of samples of the same species showed in most of the cases statistical difference as well. The highest (*R. hobelmani*) and the lowest (*C. laciniata*) mean values of the fat content were found in small fish species. From the statistical results, it is possible to conclude that the fat content of each individual sample should be approached separately. However, each fish species showed that the fat content ranged in certain interval that indicates similarity inside species. Reasons of the difference are various, the most reported factors influencing fish fat content are geographical origin, environment, age, seasonal variation and diet.

Analyses of the FA profile showed that commercially important species *C. striata* and *C. macrocephalus* had the highest proportion of unsaturated FA due to the one of the highest values of oleic acid (MUFA) and linoleic acid (PUFA), which were statistically different from other values. Nevertheless, the species had the low EPA and DHA values. High values of essential FA in contrast to *C. macrocephalus* and *C. striata*, *C. laciniata* showed opposite phenomena with the highest EPA and DHA proportions but the lowest oleic and linoleic acid proportions. Nevertheless, sum of PUFAs showed the highest values, which is promising source long chain PUFAs, DHA and EPA especially, in diets of Cambodian population. However, it is needed to carry out more analyses of *C. laciniata* to confirm these significant finding.

The data obtained is expected to provide scientific basis for further investigation of quality and nutrition value in fish species commonly consumed in Cambodia. Small fish species are very often used for production of traditional and indigenous food products, such as prahok and smoked fish that are still commonly consumed. Further investigation of chemical and microbiological properties of these fish species could give quality nutritional values that can be beneficial in attempts to alleviate malnutrition in Cambodia.

6 References

Ackman RG. 1967. Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. Comparative Biochemistry and Physiology 22(3):907-922.

Ackman RG. 1990. Seafood lipids and fatty acids. Food Reviews International 6(4):617-646.

Ackman RG. 2007. Fatty acids in fish and shellfish. In Chow CK, editor, Fatty Acids in Foods and Their Health Implications. CRC Press, London.

Asian Development Bank. 2018. Basic statistics 2018. Available from https://www.adb.org/publications/basic-statistics-2018 (accessed January 2019).

Ahmed M, Navy H, Vuthy L, Tiongco M. 1998. Socioeconomic assessment of freshwater capture fisheries in Cambodia. Mekong River Commission Secretariat, Phnom Penh.

Aquatropic. 2017. The Tire Track Eel (*Mastacembelus armatus*). Available at http://www.aquatropic.com/news/the-tire-track-eel-mastacembelus-armatus (accessed April 2019).

Bano Y. 1977. Seasonal variations in the biochemical composition of *Clarias batrachus* L. Proceedings of the Indian Academy of Sciences Section B, Plant Sciences 85(3):147.

Baran E. 2010. Mekong fisheries and mainstream dams, Fisheries sections in: ICEM 2010, Mekong River Commission Strategic Environmental Assessment of hydropower on the Mekong mainstream. International Centre for Environmental Management, Hanoi.

Baran E, Chheng Phen, Ly Vuthy, Nasielski J, Saray Samadee, Touch Bunthang, Tress, J, Kaing Khim, Tan Sokhom. 2014. Atlas of Cambodia: Maps on Socio-Economic Development and Environment. Save Cambodia's Wildlife, Phnom Penh.

Bennion M. 1980. Introductory foods. New York, USA.

Chamnan C, Thilsted S, Roitana B, Sopha L, Gerpacio RV, Roos N. 2009. The role of fisheries resources in rural Cambodia: combating micronutrient deficiencies in women and children. Department of Fisheries Post-harvest Technologies and Quality control, Fisheries Administration, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh.

Chemistry Libretexts. 2019. Chemical Properties of Alkenes. Available at https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Book%3A_The_Basics_of_GOB_Chemistry_(Ball_et_al.)/13%3A_Unsaturated_and_Aromatic_Hydrocarbons/13.4%3A_Chemical_Properties_of_Alkenes (accessed April 2019).

Chemistry Libretexts. 2019. Cis-Trans Isomers (Geometric Isomers). Available at https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Book%3A_The_Basics_of_GOB_Chemistry_(Ball_et_al.)/13%3A_Unsaturated_and_Aromatic_Hydrocarbons/13.2%3A_Cis-Trans_Isomers_(Geometric_Isomers) (accessed April 2019).

FAO. 2005. Post-harvest changes in fish. Fisheries and Aquaculture Department, FAO, Rome. Available at http://www.fao.org/ (accessed January 2019).

FAO. 2010. Fats and fatty acids in human nutrition. Food and Agriculture Organization, Geneva.

FAO. 2014. Cambodia - Food and Nutrition Security Profiles. FAO, Rome. Available from http://www.fao.org/3/a-at706e.pdf (accessed January 2019).

FAO. 2015. Cambodia. FAO, Rome. Available from http://www.fao.org/cambodia/en/ (accessed January 2019).

FAOSTAT. 2017. Prevalence of undernourishment (%) (3-year average). FAO, Rome. Available from http://www.fao.org/sustainable-development-goals/indicators/211/en/ (accessed January 2019).

FAO/WHO. 2004. Proceedings of expert consultation on human vitamin and mineral requirements in human nutrition. FAO/WHO, Bangkok. Available from https://apps.who.int/ (accessed January 2019).

Frimodt C. 1995. Multilingual illustrated guide to the world's commercial warmwater fish. Fishing News Books, Osney Mead, Oxford.

Gunstone FD. 1999. Fatty acid structure. Pages 1-19 in Gunstone FD, Harwood JL, Padley FB, editors. The Lipid Handbook, Second Edition, Chapman and Hall, London, UK.

Gurr MI, Harwood JL, Frayn KN. 2008. Lipid Biochemistry. GBLHouse, California.

Halver JE. 1980. Fish Feed Technology. Food and Agriculture Organization, Rome.

Hernandez EM, Kamal-Eldin A. 2013. Processing and Nutrition of Fats and Oils. Wiley-Blackwell.

HMSO, UK. 1994. Nutritional aspects of cardiovascular disease (report on health and social subjects No. 46). HMSO, London.

Hortle KG. 2007. Consumption and the yield of fish and other aquatic animals from the Lower Mekong Basin. Vientiane: MRC Technical Paper 16, Mekong River Commission.

Hu FB, Stampfer MJ, Rimm E et al. 1997. Dietary fat intake and the risk of coronary heart disease in women. New England Journal of Medicine 337(21):1491-1499.

Huss HH. 1995. Quality and quality changes in fresh fish. Food and Agriculture Organization, Rome.

IFReDI. 2013. Food and nutrition security vulnerability to mainstream hydropower dam development in Cambodia. Inland Fisheries Research and Development Institute, Fisheries Administration, Phnom Penh.

Index Mundi. 2018. Palm Kernel Oil Food Use Domestic Consumption by Country in 1000 MT. United States Department of Agriculture, USA. Available from www.indexmundi.com (accessed February 2019).

Jaya-Ram A, Fuad F, Zakeyuddin MS, Amir Shah Ruddin Md. Sah. 2018. Muscle Fatty Acid Content in Selected Freshwater Fish from Bukit Merah Reservoir, Perak, Malaysia. Tropical Life Sciences Research 29(2):103-117.

Johnston I. 2000. Fish Physiology: Muscle Development and Growth. Academic Press: UK.

Karapanagiotidis IT, Yakupitiyage A, Little DC, Bell MV, Mente E. 2010. The nutritional value of lipids in various tropical aquatic animals from rice–fish farming systems in northeast Thailand. Journal of Food Composition and Analysis 23:1-8.

Karim M. 2006. The livelihood impacts of fishponds integrated within farming systems in Mymensingh district, Bangladesh. [Ph.D. Thesis]. University of Stirling, Stirling.

Kawarazuka N. 2010. The contribution of fish intake, aquaculture, and small-scale fisheries to improving nutrition: A literature review. The WorldFish Center, Penang.

Keys A, Anderson J, Grande F. 1957. Original Articles: Prediction of Serum-Cholesterol Responses of Man to Changes in Fats in the Diet. The Lancet 270:959-966.

Kottelat M & Ng HH. 1999. *Belodontichthys truncatus*, a new species of silurid catfish from Indochina (Teleostei: Siluridae). Ichthyological Exploration of Freshwaters 10:387–391.

Lacousiere JO, Torell M, Choowaew S. 1998. Inventory and management of wetlands in the Lower Mekong Basin. Mekong River Commission Secretariat, Bangok.

Lilabati H. & Vishwanath W. 2010. Nutritional quality of fresh water catfish (Wallago attu) available in Manipur, India. Food Chemistry 57(2):197-199.

McKeown D. 2006. Fish Consumption – Benefits and risks for women in childbearing years and young children. Medical office of Health, Toronto.

McLaren DS, Frigg M. 2001. Sight and life guidebook on vitamin A in health and disease. Task Force SIGHT AND LIFE, Basel.

McMurry J. 2004. Organic chemistry. Brooks/Cole Publishing Company, California.

Memon NN, Sherazi STH, Talpur FN, Bhanger MI. 2010. Simple Gas Chromatographic Method for the Quantification of Total Cholesterol in Fish Meats. Journal of Aoac International 93(4):1249-1254.

Mihalca GL, Tiţa O, Tiţa M, Mihalca A. 2011. Polycyclic aromatic hydrocarbons (PAHs) in smoked fish from three smoke-houses in Braşov County. Journal of Agroalimentary Processes and Technologies 17: 392-397.

The Ministry of Agriculture, Forestry and Fisheries. 2015. Annual Report 2014 and Actions Plan for 2015. MAFF, Phnom Penh.

Mohanty BP, Ganguly S, Mahanty A, et al. 2016. DHA and EPA Content and Fatty Acid Profile of 39 Food Fishes from India. BioMed Research International 2016:1-14.

Morgensen M. 2001. The importance of fish and other aquatic animals for food and nutrition security in the lower Mekong basin [M.Sc. Thesis]. Royal Veterinary and Agricultural University, Copenhagen.

Moreira AB, Visentainer JV, de Souza NE, Matsushita N. 2001. Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. Journal of Food Composition and Analysis 14: 565-574.

NIS. 2011. Cambodia demographic and health survey. Available from https://dhsprogram.com/pubs/pdf/fr312/fr312.pdf (accessed January 2019).

NIS, Ministry of Planning in collaboration with the Ministry of Agriculture, Forestry and Fisheries National. 2015. Census of Agriculture of the Kingdom of Cambodia 2013.

National Research Council. 1993. Nutrient Requirements of Fish. The National Academies Press, Washington, DC.

Patel VB. 2018. The Molecular Nutrition of Fats. Academic Press, UK.

Piggott GM & Tucker BW. 1990. Effects of technology on nutrition Marcel Dekker, New York.

Rainboth, W. 1996. Fishes of Cambodian Mekong. Species identification field guide for fishery purposes. Food and Agricultural Organization of the United Nations, Rome.

Rasoarahona JRE, Barnathan G, Bianchini JP & Gaydou EM. 2005. Influence of season on the lipid content and fatty acid profiles of three tilapia species (Oreochromis niloticus, O. macrochir and Tilapia rendalli) from Madagascar. Food Chemistry 91:683–694.

Reyes RB. 2008. *Channa striata*. Available from https://www.fishbase.se/photos/PicturesSummary.php?StartRow=1&ID=343&what=species&TotRec=16 (accessed April 2019).

Richardson RB, Suvedi M. 2018. Assessing the Potential for Small-Scale Aquaculture in Cambodia. Environments 5: 76p.

Ridgeway N, McLeod R. 2015. Biochemistry of Lipids, Lipoproteins, and Membranes. Elsevier, New York.

Roos N, Chamnan C, Loeung D, Jakobsen J, Thilsted SH. 2007a. Freshwater fish as a dietary source of vitamin A in Cambodia. Food Chemistry 103: 1104–1111.

Roos N, Thorseng H, Chamnan C, Larsen T, Gondolf U, Bukhave K, Thildted SH. 2007b. Iron content in common Cambodian fish species: Perspectives for dietary iron intake in poor, rural households. Food Chemistry 104(3):1226–1235.

Sargent JR, Bell JG, Bell MV, Henderson RJ, Tocher DR. 1995. Requirements criteria for essential fatty acids. Journal of Applied Ichthyology 11: 183-198.

Schlemmer U, Frølich W, Prieto RM, Grases F. 2009. Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition & Food Research 53(2): 330–375.

Shirai N, Suzuki H, Tokairin S, Ehara H & Wada S. 2002. Dietary and seasonal effects on the dorsal meat lipid composition of Japanese (*Silurus asotus*) and Thai catfish (*Clarias macrocephalus* and hybrid *Clarias macrocephalus* and *Clarias galipinus*). Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology 132(3): 609.

Slámová T, Hubáčková A, Banout J & Fraňková A. 2017. Polycyclic aromatic hydrocarbons in Cambodian smoked fish. Food Additives and Contaminants: Part B Surveillance 10(4): 248–255.

Susilowati R, Sugiyono S & Chasanah E. 2016. Nutritional and Albumin Content of Swamp Fishes from Merauke, Papua, Indonesia. Squalen 3:107.

Temple NJ. 2018. Fat, Sugar, Whole Grains and Heart Disease: 50 Years of Confusion. Nutrients 10(1): 39.

Thach P. n.d. *Belodontichthys truncatus*. Available from https://ffish.asia/?p=none&o=ss&id=69 (accessed April 2019).

Thach P. n.d. *Esomus longimanus*. Available from https://ffish.asia/?p=none&o=ss&id=198 (accessed April 2019).

Tran DD. n.d. *Clarias macrocephalus*. Available from https://ffish.asia/?p=none&o=ss&id=129 (accessed April 2019).

Tran DD. n.d. *Corica laciniata*. Available from https://ffish.asia/?p=none&o=ss&id=147 (accessed April 2019).

Tran DD. n.d. *Notopterus notopterus*. Available from https://ffish.asia/?p=none&o=ss&id=378 (accessed April 2019).

USAID. 2018. Cambodia: Nutrition Profile. Available from https://www.usaid.gov/what-we-do/global-health/nutrition/countries/cambodia-nutrition-profile (accessed January 2019).

Usman IB. 2017. Assessment of the Nutritional Quality of Smoked Catfish (*Clarias Gariepinus*) in Lapai, Niger State, Nigeria. Science World Journal 12(1):18–21.

Vann LS, Baran E, Phen C, Thang TB. 2006. Biological reviews of important cambodian fish species, based on fishbase 2004, Volume 2. WorldFish Center and Inland Fisheries Research and Development Institute, Phnom Penh, Cambodia.

Vilain C, Baran E, Gallego G, Samadee S. 2016. Fish and the Nutrition of Rural Cambodians. Asian Journal of Agriculture and Food Sciences 4: 9p.

Warren T. 1996a. *Henicorhynchus siamensis*. https://www.fishbase.se/photos/PicturesSummary.php?ID=55277&what=species (accessed April 2019).

Warren T. 1996b. *Kryptopterus micronema*. https://www.fishbase.se/photos/PicturesSummary.php?ID=23098&what=species (accessed April 2019).

Warren T. 1995. *Wallago attu*. Available from http://www.fishbase.us/summary/SpeciesSummary.php?genusname=Wallago&speciesname=attu (accessed April 2019).

Whitehead PJP, Nelson GJ, Wongratana T. 1988. FAO species catalogue. Vol.7. Clupeoid fishes of the world. FAO. Fish. Synp. (125)7:305-579.

WHO. 2001. Iron deficiency anemia – assessment, prevention, and control – a guide for programme managers. World Health Organization, Geneva.

WHO. 2007. Protein and amino acid requirements in human nutrition. WHO technical report series 935. World Health Organization, Geneva.

WHO. 2016. Technical package for cardiovascular disease management in primary health care. World Health Organization, Geneva. Available from https

https://www.who.int/cardiovascular_diseases/hearts/Hearts_package.pdf (accessed March 2019).

WHO. 2018. Obesity and Overweight. Available from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed February 2019).

World Bank. 2017. Cambodia. Available from https://data.worldbank.org/ (accessed January 2019).

Zhang Q, Yang Y, Hu M, Li H, Zhong Q, Huang F. 2018. Relationship between plasma *trans*-fatty acid isomer concentrations and self-reported cardiovascular disease risk in US adults. International Journal of Food Sciences & Nutrition 69(8):976.

List of Tables in Appendix

Appendix

Table I. Number of samples by producers, village and province.

Number of samples	Producer	Village	Province
1	1	Kandal	Kampong Chhnang
1	2		
2	3		
2	4	Psear Chhnang	
1	5		
1	6	Chom Karisy	Battambang
1	7	Odombong	
1	8	Norea	
1	9		
1	10		
1	11		
1	12	Takok	
1	13	Psar Kheang	Siam Reap
1	14	Kampong Mkeak	
4	15	Spean Trong	Kampong Cham
3	16		
3	17		
3	18		
2	19		
Total: 31			

Table II. *Wallago attu* total fat content in dry matter (%) and descriptive analysis.

Wallago attu total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
16	Spean trong village	2	5.38	±0.06	5.33	5.42
17	Spean trong village	3	2.52	±0.13	2.44	2.67
18	Spean trong village	3	3.86	±0.15	3.71	4.02
19	Spean trong village	3	10.39	±0.33	10.04	10.69

[abc] Values for each sample with the same superscript letters in the same row are not significantly different at P<0.05; (n) number of replications.

Table III. *Rasbora hobelmani* total fat content in dry matter (%) and descriptive analysis.

Rasbora hobelmani total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
4	Psear Chhnang village	3	44.41	±1.11	43.59	45.67
15	Spean trong village	3	50.44	±1.42	48.80	51.29

* The mean difference is significant at the P<0.05 level; n number of replications.

Table IV. *Channa striata* total fat content in dry matter (%) and descriptive analysis.

Channa striata total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
10	Norea village	3	22.01	±1.52	20.26	22.94
11	Norea village	3	24.36	±2.55	21.43	26.01

* The mean difference is significant at the P<0.05 level; (n) number of replications

Table V. *Clarias macrocephalus* total fat content in dry matter (%) and descriptive analysis.

Clarias macrocephalus total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
6	Chom karisy village	2	29.20^{ade}	±1.21	28.35	30.05
7	Odombong village	3	20.21^{be}	±2.61	18.03	23.10
8	Norea village	3	36.26^{ad}	±2.86	33.07	38.59
9	Norea village	3	32.74^{ade}	±3.83	29.71	37.04
12	Takok village	3	36.04^{ad}	±1.91	33.86	37.41
16	Spean trong village	3	27.12^{abde}	±2.30	25.26	29.69

[abcde] Values for each sample with the same superscript letters in the same row are not significantly different at P<0.05; (n) number of replications.

Table VI. *Belodontichthys truncatus* total fat content in dry matter (%) and descriptive analysis.

Belodontichthys truncatus total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
15	Spean trong village	3	4.36	± 0.10	4.26	4.46
17	Spean trong village	3	8.34[bcd]	± 0.50	7.91	8.88
18	Spean trong village	3	8.02[bc]	± 0.13	7.89	8.15
19	Spean trong village	3	8.90[bd]	± 0.4	8.45	9.39
17	Spean trong village	3	6.52	± 0.13	6.37	6.62

[abcd] Values for each sample with the same superscript letters in the same row are not significantly different at P<0.05; (n) number of replications.

Table VII. *Henicorhynchus siamensis* total fat content in dry matter (%) and descriptive analysis.

Henicorhynchus siamensis total fat content						
Producer	Location	n	Mean (%)	SD	Minimum (%)	Maximum (%)
2	Kandal village	3	10.88	±0.40	10.62	11.33
3	Kandal village	3	3.36	±0.22	3.19	3.61
4	Psear Chhnang village	3	1.10	±0.08	1.02	1.16
5	Psear Chhnang village	3	9.88	±0.16	9.78	10.07
21	Kampong mkeak village	3	11.80	±0.23	11.55	12.00
23	Spean trong village	3	16.01	±0.61	15.31	16.47

[abcdef] Values for each sample with the same superscript letters in the same row are not significantly different at P<0.05; (n) number of replications.

Table VIII. Fatty acids profiles of fish species; mean values (%) with standard deviation.

Fatty acid (%)	*Belodontichthys truncatus*		*Clarias macrocephalus*		*Coilia macrognathus*		*Corica laciniata*		*Esomus longimanus*		*Henicorhynchus siamensis*	
SFA	n=11		n=10		n=2		n=3		n=3		n=13	
13:0	1.45^{a}	±0.34	0.00^{bc}	±0.01	0.05^{bc}	±0.00	0.13^{bc}	±0.16	0.17^{bc}	±0.01	0.38^{bc}	±0.57
14:0	2.83^{abc}	±0.44	2.33^{ab}	±0.89	4.39^{ace}	±0.13	7.53^{de}	±0.04	3.00^{abc}	±0.01	2.84^{abc}	±1.00
15:0	8.12^{ae}	±1.42	0.20^{bcf}	±0.03	0.82^{bcdf}	±0.02	1.34^{bcdf}	±0.15	1.30^{bcdf}	±0.09	2.47^{cdf}	±2.01
16:0	25.24^{abcd}	±1.43	27.34^{abcd}	±2.23	29.97^{abcd}	±0.11	24.48^{abcd}	±0.63	26.82^{abcd}	±1.54	28.15^{abcd}	±3.59
17:0	3.93^{ag}	±0.31	0.24^{bf}	±0.04	1.72^{cde}	±0.01	1.15^{cdf}	±0.04	1.16^{cd}	±0.06	1.94^{ce}	±0.53
18:0	11.64^{a}	±0.93	7.86^{b}	±0.81	8.00^{b}	±0.21	7.79^{b}	±0.16	9.89^{ab}	±0.60	8.46^{b}	±1.71
20:0	0.45^{ac}	±0.04	0.20^{b}	±0.05	0.54^{acd}	±0.02	0.68^{cd}	±0.01	0.43^{ac}	±0.06	0.46^{ac}	±0.12
ΣSFA	53.66		38.17		45.49		43.11		42.78		44.71	
MUFA												
Z9-16:1	3.08^{ac}	±0.37	2.05^{ac}	±1.28	7.91^{bc}	±0.19	8.27^{b}	±0.13	4.47^{abc}	±0.20	5.60^{bc}	±3.41
Z10-17:1	1.77^{ae}	±0.21	0.11^{bd}	±0.02	0.81^{cde}	±0.00	0.40^{bcd}	±0.02	0.48^{bcd}	±0.02	0.61^{cd}	±0.26
Z9-18:1	10.74^{ae}	±1.00	38.20^{b}	±1.75	19.93^{cef}	±0.14	5.56	±0.08	27.49^{df}	±1.19	15.84^{ce}	±3.09
E9-18:1	2.84^{a}	±0.22	1.64^{b}	±0.45	3.34^{a}	±0.04	2.61^{ab}	±0.04	2.57^{ab}	±0.21	2.40^{a}	±0.73
Z9-20:1	3.74^{ab}	±0.91	0.72^{c}	±0.22	3.85^{ab}	±0.09	1.66^{ac}	±0.01	2.55^{abc}	±0.09	5.39^{ab}	±2.72
ΣMUFA	22.16		42.72		35.85		18.51		37.56		29.83	
PUFA												
16:2n-6	0.27^{ab}	±0.06	0.09^{a}	±0.04	0.29^{ab}	±0.00	0.14^{a}	±0.01	0.19^{a}	±0.03	0.77^{b}	±0.65
18:2n-6	3.40^{a}	±0.35	12.96^{b}	±3.72	2.78^{a}	±0.02	1.89^{a}	±0.03	7.56^{b}	±0.21	10.97^{b}	±5.95
18:3n-6	0.22^{ab}	±0.05	1.13	±0.14	0.28^{abc}	±0.01	0.33^{abc}	±0.01	0.36^{abc}	±0.00	0.65^{bc}	±0.32
20:2n-6	0.48^{ab}	±0.03	0.56^{abc}	±0.10	0.25^{ab}	±0.01	0.24^{ab}	±0.00	0.32^{ab}	±0.01	0.53^{abc}	±0.28
20:3n-6	0.59^{aef}	±0.10	1.05^{b}	±0.20	0.27^{cdef}	±0.00	0.24^{cdf}	±0.01	0.58^{acef}	±0.00	0.89^{b}	±0.11
20:4n-6	5.46^{a}	±1.00	0.92^{bc}	±0.14	2.71^{bc}	±0.01	2.94^{bc}	±0.13	2.10^{bc}	±0.07	3.57^{c}	±1.68
20:5n-3	1.56^{a}	±1.22	0.24	±0.15	3.34^{ab}	±3.28	12.86	±0.78	3.45^{b}	±1.26	1.46^{a}	±0.98
22:5n-3	1.58^{ad}	±0.15	0.25^{bc}	±0.05	0.82^{bce}	±0.01	2.15^{adf}	±0.05	0.69^{bce}	±0.00	1.23^{ace}	±0.54

22:6n-3	5.43^{ac}	±0.90	0.92^{be}	±0.34	4.71^{ace}	±0.01	13.67	±0.37	2.49^{cef}	±0.05	2.15^{ef}	±1.05
ΣPUFA	18.98		18.14		15.45		34.46		17.73		22.22	
Unidentified	5.20		0.97		3.21		3.92		1.93		3.23	

[abcdefg] Values for each sample with the same superscript letters in the same row are not significantly different at $P<0.05$; (n) number of replications.

Table VIII continuing. Fatty acids profiles of fish species; mean values (%) with standard deviation.

Fatty acid (%)	*Channa striata*		*Kryptopterus micronema*		*Mastacembelus armatus*		*Notopterus notopterus*		*Rasbora hobelmani*		*Wallago attu*	
SFA	n=4		n=2		n=3		n=2		n=4		n=6	
13:0	0.00^{bc}	±0.00	1.05^{ac}	±0.01	0.19^{bc}	±0.00	0.31^{bc}	±0.00	0.96^{ac}	±0.90	1.62^{a}	±0.37
14:0	2.00^{ab}	±0.04	3.02^{abc}	±0.01	2.60^{abc}	±0.06	5.47^{cde}	±0.03	3.44^{abc}	±0.62	2.83^{abc}	±0.34
15:0	0.40^{bcdf}	±0.05	6.30^{aef}	±0.11	2.84^{bcdef}	±0.08	2.40^{bcdef}	±0.04	3.84^{cdef}	±2.92	8.93^{ae}	±1.23
16:0	22.93^{ade}	±0.93	26.94^{abcd}	±0.21	24.83^{abcd}	±0.25	33.18^{cd}	±0.21	27.39^{abcd}	±0.29	25.21^{abcd}	±0.74
17:0	0.48^{bdf}	±0.01	2.63^{ceg}	±0.03	3.16^{ag}	±0.11	1.99^{cde}	±0.03	1.95^{cde}	±0.75	3.51^{ag}	±0.13
18:0	8.16^{b}	±0.55	9.27^{ab}	±0.12	12.50^{a}	±0.28	10.20^{ab}	±0.16	11.18^{a}	±0.43	10.56^{a}	±0.57
20:0	0.33^{abc}	±0.02	0.39^{abc}	±0.01	0.79^{cd}	±0.03	0.91^{d}	±0.03	0.44^{ac}	±0.01	0.37^{ac}	±0.19
ΣSFA	34.30		49.61		46.93		54.47		49.21		53.04	
MUFA												
Z9-16:1	3.16^{abc}	±0.49	2.67^{abc}	±0.03	4.92^{abc}	±0.16	7.55^{bc}	±0.05	4.99^{abc}	±0.09	3.38^{abc}	±0.60
Z10-17:1	0.39^{bcd}	±0.03	1.29^{ace}	±0.01	1.24^{ce}	±0.05	0.76^{cde}	±0.00	0.94^{ce}	±0.42	1.83^{ae}	±0.24
Z9-18:1	37.77^{b}	±0.60	19.18^{ce}	±0.27	14.50^{ace}	±0.14	16.91^{ce}	±0.09	25.41^{cdf}	±4.36	11.61^{ae}	±0.73
E9-18:1	2.74^{a}	±0.39	2.39^{ab}	±0.03	5.03	±0.10	3.35^{a}	±0.06	2.43^{ab}	±0.47	2.85^{a}	±0.40
Z9-20:1	1.22^{ac}	±0.16	2.94^{abc}	±0.02	2.72^{abc}	±0.05	1.76^{ac}	±0.00	3.04^{abc}	±0.55	3.70^{ab}	±0.31
ΣMUFA	45.29		28.46		28.42		30.33		36.80		23.37	
PUFA												
16:2n-6	0.14^{a}	±0.03	0.19^{a}	±0.02	0.29^{ab}	±0.05	0.33^{ab}	±0.02	0.22^{a}	±0.01	0.19^{a}	±0.10
18:2n-6	12.49^{b}	±0.64	5.75^{ab}	±0.07	7.68^{ab}	±0.11	4.26^{ab}	±0.21	4.20^{a}	±1.78	3.71^{a}	±0.93

18:3n-6	0.54^{abc}	±0.18	0.53^{abc}	±0.01	0.34^{abc}	±0.01	0.25^{abc}	±0.01	0.19^{ab}	±0.05	0.21^{ab}	±0.11
20:2n-6	0.54^{abc}	±0.07	0.62^{abc}	±0.02	0.86^{bcd}	±0.01	1.20^{cd}	±0.02	0.30^{ab}	±0.06	0.36^{ab}	±0.19
20:3n-6	0.31^{cdef}	±0.08	0.68^{aef}	±0.02	0.57^{acef}	±0.01	0.32^{acdef}	±0.01	0.38^{acdef}	±0.10	0.56^{acef}	±0.04
20:4n-6	0.49^{bc}	±0.01	3.75^{ac}	±0.01	3.44^{ac}	±0.04	1.37^{bc}	±0.04	2.32^{bc}	±0.03	4.86^{ac}	±0.62
20:5n-3	0.63^{a}	±0.43	1.04^{ab}	±0.99	0.90^{a}	±0.70	1.24^{ab}	±1.13	1.34^{ab}	±1.06	1.52^{ab}	±0.78
22:5n-3	1.01^{ce}	±0.21	1.32^{acde}	±0.01	2.69^{df}	±0.04	0.87^{abce}	±0.01	0.67^{bce}	±0.05	1.78^{ad}	±0.06
22:6n-3	3.12^{cef}	±0.06	4.03^{acef}	±0.04	2.69^{bcef}	±0.03	2.80^{bcef}	±0.09	2.35^{bef}	±0.10	5.11^{ac}	±1.34
ΣPUFA	19.26		17.93		19.46		12.62		11.98		18.31	
Unidentified	1.15		4.00		5.20		2.58		2.01		5.28	

^{abcdefg} Values for each sample with the same superscript letters in the same row are not significantly different at P<0.05; (n) number of replications