Nutritional Composition of Aquatic Species in Laotian Rice Field Ecosystems; Possible Impact of Reduced Biodiversity

By

Mulia Nurhasan

Master's Thesis in International Fisheries Management

(30 stp)

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The picture on the front page shows Laotian fermented fish sauce (*padek)* from Savannakhet province's rice field. Laotians prepare *padek* by mixing gutted fish with rice bran and salt before keeping them in a closed jar to ferment for around 6-12 months. *Padek* is very important in Laotian diets; 37-45 percent of total catch from the rice field fishery was processed and stored, mostly as *padek*. *Padek* contains high contents of nutrients, such as amino acids and minerals required by the Laotians.

Some parts of this master thesis were presented in Cooperation for Biodiversity and Health (COHAB) 2 Conference in Galway, Ireland in February 2008.

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The thesis is dedicated to author's parents, who have been working very hard throughout their lives to send four daughters to school. Last but not least, the author would like to emphasize that the two academic years and the thesis are a manifestation of her belief in Allah the Almighty's first decree to Muhammad, which was; Read, read and read.

Abstract

Nutritional Composition of Aquatic Species in Laotian Rice Field Ecosystems; Possible Impact of Reduced Biodiversity

The population density of Laos PDR has increased from 15 persons per square km in 1985 to 19 persons in 1995 and to 24 persons in 2005. This has threatened food security, which in Laos PDR is generally synonymous with rice availability. Rice production in Laos rose by 70 percent from 1990 to 2004. Evidence from Vietnam, Malaysia and Central Thailand has shown that the rise of rice production steadily decreases the population of aquatic animals in rice field ecosystems, as a result of higher applications of fertilizers and pesticides. In Laos PDR, the use of fertilizers and pesticides is still minimal, but said to be increasing.

The role of aquatic rice field species in rural Laotian diets has been underestimated. Almost 200 species are consumed across the country. These aquatic animals potentially supply most of the vitamins A and B, calcium, iron, sulphur, essential fatty acids and amino acids that are needed by the villagers. However, national and regional food composition data bases contain very limited info on nutritional composition of these species. These aquatic animals are not consumed in large quantities elsewhere.

Field sampling was undertaken in Champasak and Savannakhet provinces for nine species of aquatic animals, including fish, amphibians, crustaceans, molluscs and insects. Additionally four samples of fermented fish sauce were included. The samples were transferred to the University of Tromso for detailed analysis.

The objective of the study was to gain knowledge on the nutritional composition of the most significant species included in the diet in Laos areas. As other studies have made estimates of the quantities consumed, the results of this work enable an assessment for the nutritional contribution of aquatic animals to the diet. The work also discusses the impact on nutrition of a possible decrease in the available species biodiversity.

This study documented that the aquatic animals that are consumed daily contained high amounts of protein, amino acids, calcium, iron and zinc. On the contrary, they contained a low amount of fat, fatty acids, and vitamin A. Amino acids, iron and zinc are important nutrients for the Laotians. As the food supply of rural households in rice farming areas of Laos is critically dependent on the environment, the sustainable existence of the rice-based aquatic animals is crucial factor to the nutritional status of the Laotians.

The objective of biodiversity conservation overlaps local and nutritional needs and should be emphasised in development of national nutrition policies, as well as agricultural development policies and fisheries management practices. Fishery managers and policy makers should integrate pro-poor community-based biodiversity conservation into their ongoing development programmes.

Content

List of Tables

Abbreviations

AI	Adequate Intake
Ca	Calcium
CFCD	China Food Composition Database
CBD	Convention of Biodiversity
CH	Champasak
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
DM	Dry Material
DRI	Dietary Reference Intakes
FAO	Food and Agriculture Organization of the UN
Fe	Iron
FWAE.	Fresh Weight Animal Equivalent
IOM	Institute of Medicine (National Academies and Health Canada)
IZiNCG	International Zinc Nutrition Consultative Group
MA	Millennium Ecosystem Assessment
MUFA	Mono Unsaturated Fatty Acid
NPN	Non Protein Nitrogen
PUFA	Poly Unsaturated Fatty Acid
PDR	People's Democratic Republic
RDA	Recommended Dietary Allowance
SFA	Saturated Fatty Acid
SV	Savannakhet
OAA	Other Aquatic Animals
WM	Wet Material
Zn	Zinc

1. Introduction

The population density of People's Democratic Republic of Laos (Laos PDR) has increased at a rate of 2.5 percent per annum; from 4.25 million in 1990 to 6.4 million in 2006. Laos has had to feed almost 50 percent more people in the last 16 years (SDC, 2007). Food security is often equated with increases in rice production (Krahn, 2005). Thus from 1990 to 2004 rice production increased by 70 percent (NSC, 2005).

The concentration on rice production helps to satisfy dietary energy requirements but the higher fertilizer and pesticide applications necessary to achieve it threaten the biodiversity of the ecosystem (James, 2006). Evidence shows that in Vietnam, Malaysia and Central Thailand the rise of rice production steadily decreased the population of aquatic animals in rice field ecosystems as a result of higher applications of fertilizers and pesticides (Shams, 2007).

In Laos PDR, the use of fertilizers and pesticides is still minimal, but is said to be increasing (Meusch et al., 2003). In addition, there were more fish to catch in the rice field. But increased fishing pressure, conversion of many wetlands into agricultural land and the intensification of rice production has caused a decline of the rice field fishery in many areas (Halwart et al., 2006).

The food supply of rural households in the rice farming areas of the Mekong Basin, including Laos, is critically dependent on the environment. The aquatic ecosystem provides extensive biodiversity and the traditional food habits of Laos include consumption of many species of fish, crustaceans, molluscs, amphibians, reptiles, insects and aquatic plants; almost 200 species are consumed. It is a characteristic of most areas that, as a result of very low cash incomes the ecosystem must therefore provide the dietary energy supplies as well as the protein, lipid and micronutrients (James, 2006).

A Food and Agriculture Organization of the UN (FAO) survey on importance of fish and other aquatic animals (OAA) in rice-based ecosystems reported the species caught and consumed. Nonetheless, reliable information from published sources on the nutritional composition of these resources is scarce (James, 2006). Thus to be able to evaluate the significance of the aquatic species in the diet, and the possible impact of reduced rice field biodiversity there was a need to analyze the content of the nutrients.

The objective of the study was to gain knowledge on the nutritional composition of the most significant species in the diet in Laotian areas. As other studies have made estimates of the quantities consumed, the result of this will enable an assessment of the nutritional contribution of fish and OAA to the diet. The impact on nutrition of a possible decrease in the available species will also be discussed.

2. General background

2.1. Laos' Demographic Problems

Laos is a land locked country bordered by Thailand, China, Cambodia and Vietnam. Geographically, it is located in 17 58 N, 102 36 E and consists of 16 provinces. Total area is 236, 800 sq km; 230,800 sq km land and 6,000 sq km aquatic area. However, only 4.01 percent of the total land is arable. In March 2005, Laos had a total population of 5.62 million people. The population density has increased from 15 persons per square km in 1985 to 19 persons in 1995 and to 24 persons in 2005 (Ahrens, 2007). With this rapidly growing population, Laos requires great increases in food supply.

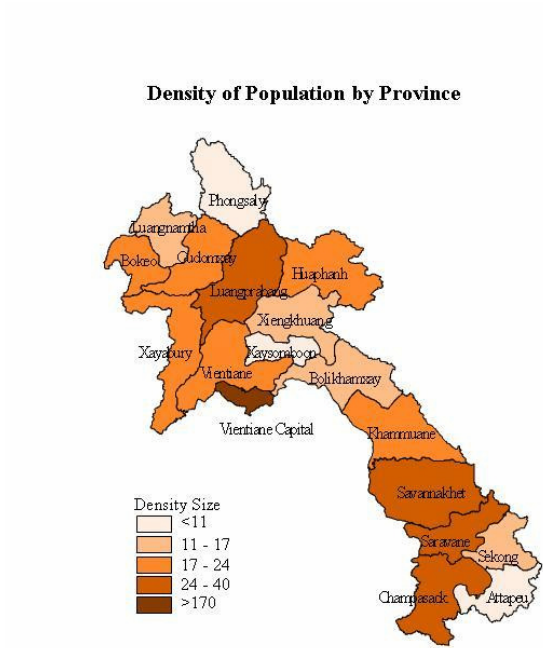

Figure 1. Map of Laos PDR Population Density Size (person per square km) (NSC, 2005)

Food security in Laos is generally synonymous with rice availability (Krahn, 2005). Thus while trying to cope with feeding an additional 2.15 million people within the last 16 years the government has increased rice production by 70 percent, from 1.5 to 2.5 million tons (SDC, 2007).

2.2. From the Ecosystem Service Perspective

The biodiversity in rice field ecosystems forms a network, a way in which one element depends on the others (Halwart et al., 2006).

According to the Convention on Biological Diversity - Rio de Janeiro, Brazil 1992 - biological diversity is defined as; "*the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.*" (CBD, 1992, p.146).

Unfortunately, although aquatic organisms play an important role in the overall biodiversity, not enough information is known on the service provided by the ecosystem (Halwart et al., 2006). Ecosystem Services, according to Millennium Ecosystem Assessment is defined as; *"the benefits people derive from ecosystems"* (MA, 2005).

Understanding the ecosystem service of fish and OAA in rice field ecosystems is essential for management of these ecosystems. Policy makers need to be better informed about the economic and the nutritive value of rice field aquatic organisms. As Lopez, et al (2007) suggested the anthropocentric (utilitarian) factor is more important in determining the human attitudes towards species than the willingness to pay to support biodiversity conservation.

2.3. FAO Surveys on the Importance of Fish and Other Aquatic Animals (OAA) in Rice-Based Ecosystem

An FAO publication has shown that in China, Vietnam, Cambodia and Laos PDR, rice fields are much more than a source of rice (Balzer et al., 2006). This and other studies list 145 species of fish, 15 species of mollusc, 13 species of reptiles, 11 species of amphibians, 11 species of insect and 37 species of plants, caught directly or collected from the rice fields, were consumed daily by the local community (Halwart and Bartley, 2005). Together with other wild animals, fish and invertebrates they were the source of animal protein in rural households (Krahn, 2005).

The role of rice-based ecosystems in Laotian diets has been studied by an FAO survey on the importance of fish and OAA in rice-based ecosystems (Garaway, 2008). The survey was carried out in three provinces each representing different topographical and agro-ecological zones within Laos PDR (1. Champasak: lowland flood plain environment with abundant aquatic resources; 2. Savannakhet: lowland floodplain with areas of abundant aquatic resources and other areas, which are more upland with subsequently lower availability of natural aquatic resources; 3. Xieng Khouang: upland environment).

The survey was conducted at monthly intervals in 240 households of 12 districts from the 3 provinces in two main data periods; dry and wet seasons. The principal objective of the survey was to assess the catch of aquatic biodiversity from rice-based ecosystems and its contribution to the consumption of households in the study. Importance of a species or habitat was measured by the quantity of species consumed or frequency of species found in the days monitored during survey.

Rice fields are reported to be the most important habitat of the species caught during the survey in the rainy season. Champasak Province was discovered to be the most aquatic-resource rich Province- catching approximately double that of Savannakhet (Garaway, 2008).

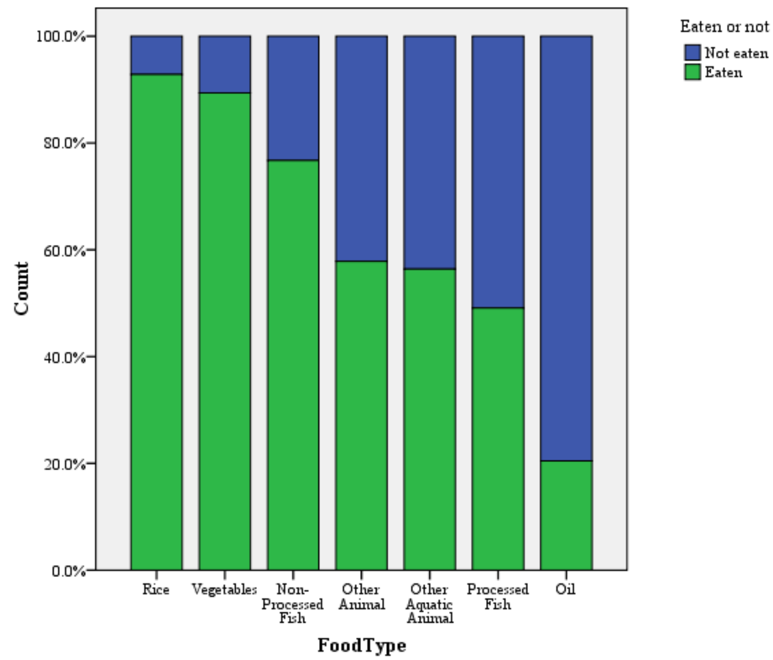

Figure 2. Percentage of days selected foodstuffs were eaten over 6 days in the rainy season (Garaway, 2008).

The survey also reported that 46 fish species, three amphibians, six molluscs, and three reptiles were consumed by the Laotians on a daily basis. Non-preserved (not fermented) fish was the most frequently eaten source of animal protein, being consumed on just fewer than 80 percent of the days monitored. Rice and vegetables are the types of food most frequently consumed during the days monitored (Garaway, in 2008).

2.4. The Role of Rice-Based Ecosystem in Laotian Diets

Average consumption of fish per adult equivalent unit ranges from 114g per day in Champasak to 73g per day in Savannakhet. Assuming all of the stored and processed fish was consumed as fermented fish sauce (*Padek*), Champasak consumes 236g per day and Savannakhet consumes 110g per day. Amphibians were consumed at 94g per day in Champasak and 35g per day in Savannakhet. A similar picture emerges for molluscs with Champasak again consuming significantly more (36g per day) than Savannakhet (18g per day) (Garaway, 2008).

Out of all non-processed fresh fish consumed during the days monitored, most of the fish were prepared as soup (31.72 percent of days monitored), followed by grilled fish (22.89 percent of the days monitored) and the Lao dish *lab,* which is meat of some form minced with chillies and mint (12.21 percent of days monitored). Fried fish is not (yet) common. Preserved fish prepared as Laotian fermented fish sauce (*Padek*), was consumed on 94.48 percent of days monitored, demonstrating the importance of this type of food. The OAA mostly consumed were big frogs (37.84 percent of days), followed by snails (20.78 percent of days), small frogs (9.70 percent of days), insects (8.66 percent of days), crabs (8.05 percent of days) and tadpoles (8.48 percent days) (Garaway, 2008).

2.5. Nutritional Composition of Rice Field Aquatic Animal

As noted by James (2006), reliable information from published sources on nutritional composition of aquatic animal resources from rice fields is scarce since they are not common in the food baskets of most other countries. As a result they have not been much investigated. However, available data from similar species in the surrounding area has been compiled below.

James (2006) included a review of regional and national food composition tables, as well as published data. The selected compilation of the data in table 1 shows great variation between maximum and minimum values.

Table 1. Summary of compositional data per 100 gram fresh weight basis. FAO/WHO, 2002; FAO/WHO/UNU, 1985, 2004 in James (2006).

Fish and OAA	Protein (g)		Fat (g)		Ca (mg)		Fe (mg)		Vitamin A (µg)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Fish	9.7	22.7	0.8	8	17	1751	0.6	9.2	5	1800
Crustacean	10.7	21.2	0.9	3.3	75	5000	0.6	7.5	0	133
Mollusc	7	20.2	0.3	1.4	16	2500	7	26.6	0	243
Frogs	15.1	20.5	0.2	2	19	1293	0.7	3.8	Low	
Insect	3.5	26.2	1.4	8.3	6	120	1.8	30	N/D	
Recommended intake, adults, per day	0.79g/kg body weight or 10-15 of energy		Min 15% of energy		600-1000 mg		5-24 mg		450-600µg	

In 2002, Peking University Medical Press published an electronic database of China Food Composition (CFCD), including the following samples on the list;

Table 2. Some Chinese freshwater species nutritional composition according to CFCD 2002.

Animal Name	Proximate composition of Chinese freshwater species (g/100 g)				
	Edible	Moisture	Protein	Fat	Ash
Rice field swamp eel, freshwater	67	78	18	1.4	1.4
Whiskered catfish, freshwater	50	72.6	15.4	8	0.9
Snake head, freshwater	57	78.7	18.5	1.2	1.6
Mitten crab, freshwater	42	75.8	17.5	2.6	1.8
Rice-paddy field snail, globular, freshwater	26	82	11	0.2	3.2
Frog	37	79.4	20.5	1.2	1
Yuqiyou, fermented fishes and shrimps with salt	100	65.4	11.2	0.2	23.3

2.5.1. Fat and Fatty Acid Composition

In a study by Krahn (2005), snails, crabs and shrimps were reported to be very low in fat content (1.05-1.67 gram per 100 gram), while fish showed a fair amount (4.5 gram per 100 gram) compared with beef (4.3 gram per 100 gram). The intake of polyunsaturated fatty acid (PUFA) derives mainly from aquatic and insect species such as fish, frogs, arthropods and caterpillars (Krahn, 2005).

Table 3. Share of unsaturated fatty acids in total fat of selected foods of Katu tribe in up land southern Laos PDR with Total Fat > 1% (in percent) (Krahn, 2005).

Fatty Acid	Chain	Toad	Crab	Deer	Padek	Cater-pillar	Fish
Myristoleic acid	C14:1	0.2	0.3	1.2	0.1	0.8	2.4
Palmitoleic acid	C16:1	3	2.2	3.7	10.5	7.2	11.6
Oleic acid	C18:1 (cis)	37.2	36.8	23.7	30.5	23	28.5
Linoleic acid	C18:2	13.6	12	1.4	11.1	12.2	0
Gamma linolenic acid	C18:3, n-6	1.5	1.5	0.3	0	0.7	6.6
Linolenic acid	C18:3, n-3	0.2	2.8	0.4	2.6	6	0.2
Eicosatrienoic acid	C20:3, n-6	0.9	0.5	0	1.1	0	0.3
Eicosatrienoic acid	C20:3, n-3	2.1	6.5	0	0.5	5.4	0.8
Eicosapentaenoic acid	C20:5	0.4	2.7	0	1.9	4.3	0
Docosahexaenoic acid	C22:6	0.9	0.1	0	3.7	0	0.1
% of total fat		60	65.4	30.7	62	59.6	50.5

2.5.2. Protein and Amino Acid

In the Chinese food composition database 2002, the protein content of frog (20.5 percent), rice field eel (18 percent) were mentioned to be higher than chicken egg yolk (15.2 percent) (CFCD, 2002).

Table 4. Amino acid content in some of Chinese freshwater species according to CFCD 2002.

Amino acid	Rice field swamp eel (mg/100g)	Whiskered catfish, freshwater (mg/100g)	Snake head, freshwater (mg/100g)	Mitten Freshwater Crab (mg/100g)	Rice-paddy field snail (mg/100g)	Frog (mg/100g)
Isoleucine	769	698	894	676	374	1078
Leucine	1322	1274	1451	1184	764	1947
Lysine	1471	1532	1670	1101	692	2093
SAA	733	430	742	610	335	717
Methionine	476	430	556	351	125	566
Cystein	257		186	259	210	151
Phenolalanine	803	631	718	563	390	951
Tyrosine	604	471	546	626	353	855
AAA	1407	1102	1264	1189	743	1806
Threonine	771	772	804	733	482	1029
Triptophane	250		174	244	131	116
Valine	844	737	945	820	473	1056
Arginine	1300	1306	1168	1428	782	1636
Histidine	409	584	410	451	185	624
Alanine	1128	920	1120	922	626	1418
Aspartic acid	1638	1496	1822	1474	970	1822
Glutamine	2676	1755	2880	2390	1554	2981
Glysine	1231	1039	1073	938	532	1161
Proline	785	641	758	792	318	804
Serine	696	641	686	648	416	958
Water (g/100g)	78	72.6	78.7	75.8	82	79.4
Protein (g/100g)	18	15.4	18.5	17.5	11	20.5

2.5.3. Vitamin A, Calcium, Iron and Zinc

According to Krahn (2005), the Katu tribe of upland southern Laos obtain their Vitamin A from tadpoles (745 microgram per 100 gram), freshwater fish (319 microgram per 100 gram), and quail (490 microgram per 100 gram).

Small aquatic animals such as fish, frogs, toads, water lizards, shrimps, and crabs are important calcium sources, especially when they are consumed with their bones and carapaces. Crab contains exceptionally high calcium (5266 milligram per 100 gram), followed by small fish (1327 milligram per 100g), and fermented fish sauce (3752 milligram per 100 gram). When wild animals including the bony fraction is prepared and mashed in the traditional *tcheruak* (Katu dishes) the calcium content of the dish can be very high (Krahn, 2005).

Table 5. Vitamin A and mineral content in some of Chinese freshwater species (CFCD, 2002).

Food Name	Vitamin. A (µg RE/100g)	Ca (mg/100g)	P (mg/100g)	Fe (mg/100g)	Zn (mg/100g)
Rice field swamp eel, freshwater	50	42	206	2.5	1.97
Whiskered catfish, freshwater		42	195	2.1	0.53
Snake head, freshwater	26	152	232	0.7	0.8
Mitten crab, freshwater	389	126	182	2.9	3.68
Rice-paddy field snail, globular, freshwater		1030	93	19.7	2.71
Frog	7	127	100	1.5	1.15
Fermented fishes and shrimps with salt, *yuqiyou*		24	6	3	0.3

In a study on vitamin A content in small indigenous fish mola (*Amblypharyngodon mola*) and rui (*Labeo rohita*) in Bangladesh Roos et al., (2002) found that 90 percent of vitamin A was found in the eyes and viscera of mola. In rui vitamin A was also found mainly in the eyes, although the amount was far less than in mola.

Table 6. Distribution of vitamin A in parts of Bangladeshi fish; mola and rui, adapted from Roos et al., (2002).

Parts of fish[a]	Vitamin A content in parts (RE/100g)	Weight of parts[b] [g (% of total)]	Location of vitamin A in whole fish[b] [RE/10g (%)]
Mola (*Amblypharyngodon mola*)			
Body tissue, posterior	55	0.68 (23)	12 (1)
Body tissue, anterior (Without eyes)	350	1.88 (49)	170 (7)
Viscera	3620	1.00 (26)	950 (39)
Eyes	62,180	0.08 (2)	1300 (53)
Total, whole raw fish (sum of parts)		3.82 (100)	2430 (100)
Rui (Labeo rohita)			
Body tissue	13	284 (90)	12 (34)
Viscera	180	30 (9)	17 (48)
Eyes	920	2 (1)	6 (17)
Total, whole raw fish (sum of parts)		316 (100)	35 (1000)

[a]For mola, 10 fish were divided in parts and pooled before analysis. For rui, a single fish was divided and analyzed in parts.
[b]For mola, the values are the means of 10 fish, pooled before weighing and analysis

2.6. Dietary Reference Intakes (DRIs)

To be able to compare the adequacy of the evaluated nutrients to the consumer, the Dietary Reference Intakes (DRIs) compiled by the Panel on Macro Nutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Interpretation and Uses of Dietary Reference Intake, Standing Committee on the Scientific Evaluation of Dietary Reference Intake of the Food and Nutrition Board Institute of Medicine, the National Academies and Health Canada in 2005 was used as reference (IOM, 2005).

DRI consists of reference values including: Recommended Dietary Allowance (RDA) and Adequate Intake (AI). RDA defined as; *the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent of) healthy individuals in a particular life stage and gender group.* While AI is defined as; *the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently* *healthy people that are assumed to be adequate—used when a RDA cannot be determined* (IOM, 2005).

The table below presents the information on the recommended intake for individual on nutrients studied in this thesis. The values printed in bold are AI while the rest are RDA.

Table 7. Dietary reference intake adapted from IOM (2005).

Life Stage Group	Vit. A (µg/d)	Ca (mg/d)	Fe (mg/d)	Zinc (mg/d)	Fat (g/d)	Linoleic acid (g/d)	α-Linolenic acid (g/d)	Protein[a] (g/d)	Water[b] (L/d)
Infants									
0-6 mo	400	210	0.27*	2*	31*	4.4	0.5	9.1*	0.7
7-12 mo	500	270	**11**	**3**	30*	4.6	0.5	**11.0**	0.8
Children									
1-3 y	**300**	500	**7**	**3**	**ND**	7	0.7	**13**	1.3
4-8 y	**400**	800	**10**	**5**	**ND**	10	0.9	**19**	1.7
Males									
9-13 y	**600**	1300	**8**	**8**	**ND**	12	1.2	**34**	2.4
14-18 y	**900**	1300	**11**	**11**	**ND**	16	1.6	**52**	3.3
19-30 y	**900**	1000	**8**	**11**	**ND**	17	1.6	**56**	3.7
31-50 y	**900**	1000	**8**	**11**	**ND**	17	1.6	**56**	3.7
51-70 y	**900**	1200	**8**	**11**	**ND**	14	1.6	**56**	3.7
>70 y	**900**	1200	**8**	**11**	**ND**	14	1.6	**56**	3.7
Females									
9-13 y	**600**	1300	**8**	**8**	**ND**	10	1.0	**34**	2.1
14-18 y	**700**	1300	**15**	**9**	**ND**	11	1.1	**46**	2.3
19-30 y	**700**	1000	**18**	**8**	**ND**	12	1.1	**46**	2.7
31-50 y	**700**	1000	**18**	**8**	**ND**	12	1.1	**46**	2.7
51-70 y	**700**	1200	**8**	**8**	**ND**	11	1.1	**46**	2.7
>70 y	**700**	1200	**8**	**8**	**ND**	11	1.1	**46**	2.7
Pregnancy									
14-18 y	**750**	1300	**27**	**12**	**ND**	13	1.4	**71**	3.0
19-30 y	**770**	1000	**27**	**11**	**ND**	13	1.4	**71**	3.0
31-50 y	**770**	1000	**27**	**11**	**ND**	13	1.4	**71**	3.0
Lactation									
14-18 y	**1200**	1300	**10**	**13**	**ND**	13	1.3	**71**	3.8
19-30 y	**1300**	1000	**9**	**12**	**ND**	13	1.3	**71**	3.8
31-50 y	**1300**	1000	**9**	**12**	**ND**	13	1.3	**71**	3.8

a. Based on g protein per kg of body weight for the reference body weight, e.g., for adults 0.8 g per kg body weight for the reference body weight.
b. Total water includes all water contained in food, beverages and drinking water

3. Materials and Methods

3.1. Materials

The work presented here used materials (samples) of aquatic animals taken from Laotian rice field ecosystem. The sampling plan was designed according to Food Composition Data guideline (Greenfield and Southgate, 2003) with flexible adaptation.

Sampling of these animals was done from 21-30 August 2007 in two provinces; Champasak and Savannakhet. At least one village was visited in four districts of each Province. Samples were taken directly from villagers as well as traditional market. Samples species and locations were selected based on available data (per July 2007) from a preliminary report of an FAO project survey on *Aquatic Biodiversity in Rice-based Ecosystems for Lao PDR* (Garaway, 2007). The list of most caught aquatic animals in the dry season of the Champasak and Savannakhet from the report was compared against the list of most caught aquatic animals in the rainy seasons of Xieng Khouang and Houa Phan (Choulamany, 2005). The final list was used as a flexible reference and the selected species were those actually available during the sampling period. Samples were obtained directly from villagers and traditional markets and captured from the surrounding rice fields or natural ponds.

Fish and frog samples were gutted on site. Snail samples were shelled on site. Except cricket, all samples were washed with mineral water and dried on site. Subsequently, all samples were shrink-wrapped, and packed inside double zip lock plastic bags before transported. Transportation from sampling location to temporary storage was done using ice boxes with crushed ice 1:1. Samples were temporarily kept in a freezer with temperature varying from -15 to -40 ^{o}C depending on best possible condition in the field. Shipment to final destination the University of Tromso, in Tromso, Norway was executed on 3 September 2007 by means of air cargo. During transport, the samples were tightly bundled inside ice boxes with dry ice (60 percent of total fish weight). Upon arrival in University of Tromso, Norway, samples were immediately kept at -20 ^{o}C until analysis (approx. four months).

This research is a 30 student credit (one semester) thesis project, therefore it was not possible to analyze all samples taken. Based on the importance and adequacy of the samples, nine species of fish and OAA plus four different groups of fermented fish sauce were selected to be analyzed for nutritional composition. They are; swamp Eel (*Monopterus albus*), snakehead murrel (*Chana striata*), walking catfish (*Clarias batrachus*), Chinese edible frog (*Haplobatrachus rugulosus*), freshwater crab (*Potamon sp*), small apple snail (*Cipangopaludina chinensis*), golden apple snail (species unidentified, the local name is *Hoy pak kuang*), big apple snail (*Pila sp*), cricket (species not yet identified, the local name is *Chie lor*) and fermented fish sauce from Champasak and Savannakhet, each consisted of home made and market sold fermented fish sauce.

Each of the thirteen samples consisted of three sub samples, except for swamp eel (two sub samples due to limited time as eel skin is difficult to homogenize). Each sub sample shows different sample pooling, which may come from different rice fields, traders or composites. Selection of sub samples within species was based on the most possible similarity of age, size and location.

Types of household preparation for each sample were chosen based on knowledge collected from literature and interview with the villagers and researchers involved in the related field. The samples were prepared according to the most common method of preparation for consumption. The selected species, their origin and their method of preparation are listed below:

1. Swamp Eel (*Monopterus albus*) (Savannakhet); gutted and beheaded.
2. Snakehead murrel (*Chana striata*) (Champasak); fillet with skin.
3. Walking catfish (*Clarias batrachus*) (Savannakhet); fillet with skin.
4. Chinese edible frog (*Haplobatrachus rugulosus*) (Champasak); gutted.

Prepared parts of the species listed above were homogenized with a household blender while frozen.

5. Freshwater crab (*Potamon sp*) (Champasak); dorsal and ventral carapace peeled.
6. Cricket (species not yet identified, local name is *Chie lor*) (Champasak); gutted, wings and tail discarded.

Prepared parts of species listed above were homogenized using a household blender.

7. Small apple Snail (*Cipangopaludina chinensis*) (Savannakhet); shelled.
8. Golden apple Snail (Species not yet identified, local name is *Hoy pak Kuang*) (Champasak); shelled
9. Big apple Snail (*Pila sp*) (Champasak and Savannakhet); shelled. .

Prepared parts of species listed above were washed using distilled water until all possible mud and remaining shells were removed. Subsequently, samples were homogenized using a household blender.

10. Fermented fish sauce (local name is *Padek*) (Champasak households)
11. *Padek* (Champasak markets)
12. *Padek* from (Svannakhet households)
13. *Padek* from (Savannakhet markets)

All samples listed above were filtered with traditional bamboo filter.

Detail of sub samples origins are listed in annex 1, picture representing each sample is in annex 2, detail of local names and types of local preparation is listed in annex 3, details for types of Laotian cuisine corresponding to types of preparation is listed in annex 4.

3.2. Methods

The laboratory analysis includes; protein, fat, water, ash, free and total amino acids, fatty acids, vitamin A, Ca, Fe and Zn. Except for mineral composition (Ca, Fe and Zn), all the analysis was done in the Laboratory of the Institute of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromso Norway. While Ca, Fe and Zn analysis was done by the National Institute of Nutrition and Seafood Research, Bergen, Norway.

3.2.1. Protein

Protein content was determined using the Kjeldahl method, AOAC 981.10 (AOAC, 1995). In short: One gram of sample, one Kjeltec catalyst tablet and 10 mL H_2SO_4 was put into a Kjeldahl tube and digested for two hours at 420 ^{o}C. The product was then made basic with 30 percent (w/v) NaOH, before distillation into 0.1 M HCl and titration against 0.25 M NaOH. The factor used to convert nitrogen into crude protein was 6.25

3.2.2. Moisture

Moisture content was determined with a modified version of the AOAC 925.04 (AOAC, 1995). Ten grams of sample was dried at 105 ^{o}C for 24 hours. Water content of the samples was determined gravimetrically.

3.2.3. Fat

Fat content was determined by the AOCS method Ba 3-38 (Gunstone, 1989) using a Soxhlet extractor (Behrotest, Behr Labor Technik GmbH, Dusseldorf, Germany). The water free sample was put into a pre-weight Soxhlet tube and petroleum ether was recycled through the sample for two hours. Remaining ether was evaporated and the sample was dried at 105 ^{o}C overnight. Fat content was then determined gravimetrically.

3.2.4. Ash

Ash content was analyzed using a modified version of AOAC 938.08 (AOAC, 1995). The water and fat free sample was combusted at 500 ^{o}C for 12 hours and ash content was determined gravimetrically.

3.2.5. Amino Acid

Samples for determining free amino acid were prepared as described in (Mierke-Klemeyer, in pres). In short: One gram of sample was mixed with 9 mL distilled H_2O and 1 mL of 20 mmol/L norleucine with an Ultra Turrax T25 basic (IKA Werke GmbH, Staufen, Germany) for 15 seconds before adding 1 mL 35 percent suphosalicylic acid and homogenizing for another 15 seconds. Norleucine served as an internal standard. The suspension was centrifuged at 14000 x g at 4 ^{o}C for 5 minutes. An aliquot of the supernatant was diluted 1:5 with a lithium citrate buffer, pH 2.2 and submitted to amino acid analysis.

For determination of total amino acids, the three sub samples of each species were pooled. 1 gram of the pooled sample was mixed with 1 mL of 20 Mm norleucine, 4 mL distilled water and 6 mL of concentrated HCl. Hydrolysis was performed 110 ^{o}C for 24 hours (Meeren et al., 2008) and the sample was diluted to a suitable concentration in alithium citrate buffer, Ph 2.2 and submitted to amino acid analysis.

All amino acid samples were analysed by a Biochrom 30 amino acid analyser (Biochrom Co, Cambridge, UK). The signal was analysed by Chromeleon software (Dionex, Sunnyvale, CA, USA) and compared to A9906 physiological amino acid standard (Sigma chemical Co, St. Louis, MO, USA).

3.2.6. Fatty Acid

The fat to be used for FFA determination was extracted according to Folch et al., (1957). Briefly; one gram of sample was mixed with 20 mL of chloroform:methanol (2:1, v/v). 2 mg of heptadecanoid acid (C17:0) was added as an internal standard. After filtration, 4 mL of 0.9 percent (w/v) was added and the mixture was centrifuged at 4 oC and 2000 rpm for 10 min. The upper phase (water) was removed and the lower chloroform phase was evaporated using a rotary evaporator (Heidolph Laborota 4000 + Buchi Vacuum Controller B-721). The remaining fat was redissolved in 5 mL of heptane and dried under N2. Fat content was determined gravimetrically. Prior to methylation the extracted fat was redissolved to a concentration of 10 mg/ml in chloroform: methanol (2:1, v/v). The samples were methylated according to (Stoffel et al., 1959) with minor adjustments. 0.1 mL of this solution was mixed with 0.9 mL of chloroform and 2 mL of 2 percent methanol in H2SO4 and boiled for one hour. The fat was then extracted by adding equal amounts of heptane and 5 percent (w/v) NaCl. The upper heptane phase was transferred into anew tube and evaporated under N2. The fat was redissolved in 0.1 mL heptane and transferred into GC vials.

Gas chromatography was performed using an Agilent 6890N equipped with a 7683 B auto injector and a flame ionization detector (FID) with He as the gas carrier. The column used was a Varian CP7419 capillary column (50 m x 250 µm x 0.25 µm nominal).

Injector and detector temperatures were 240 ^{o}C and 250 ^{o}C respectively. A predefined temperature programme was used to ensure the best possible separation of the fatty acids (50 ^{o}C for two minutes, then 10 ^{o}C per min to 150 ^{o}C followed by 2 ^{o}C per min to 205 ^{o}C and finally 15 ^{o}C per min until 255 ^{o}C and stabilization for 10 minutes). The fatty acids were identified by the samples 1895, 1893 and 1891 fatty acid standards from sigma (Sigma Chemicals Co, St. Loius, MO, USA).

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3.2.7. Vitamin A

0.5 grams (exact amount recorded) of homogenized sample were put in a 50 mL centrifuge tube and 2 mL of 2 percent ethanolic pyrogallol (antioxidant) and 2 mL of 10 percent ethanolic potassium hydroxide were added. The tube was shaken vigorously and incubated in the dark for 18 hours. A volume of 1 mL of the sample was transferred to another tube after shaking, where 2 mL petroleum ether and 1.5 mL of Milli-Q water were added and mixed. After phase separation, the organic layer was transferred to another tube (wrapped in aluminium foil). The extraction step was repeated twice with heptane and the extracted organic phases were pooled and dried under nitrogen. Prior to HPLC analyses the sample was redissolved in 500 µl methanol and filtered through a 0.45µm PTFE syringe filter. Retinol standards at two concentrations were extracted with the same procedure as the samples to determine how much of the vitamin that was lost in the extraction. Mean recovery for retinol was 69.2 percent.

Quantitative HPLC analyses were performed on a Waters 2695 Separations Module equipped with a Waters 2996 photodiode array detector (Waters Corporation, MA, USA), using a XTerra reverse phase C-18 column (Waters, 150 × 3.9 mm id, 4µm). Vitamin A was eluted with 85 percent MeOH (0.01 percent trifluoroacetic acid). The flow-rate was maintained at 1 ml min^{-1} and the UV absorption was measured at 325 nm. Concentration of vitamin A in the samples was calculated with a 5-point calibration curve with external standard in the range 500 – 5 ng retinol (Kuhnlein et al., 2006).

3.2.8. Ca, Fe and Zn

Equal amount of all sub samples from each species were pooled and freeze dried. The Ca, Fe and Zn analyses used this freeze dried pooled samples. Prior to the determination of the elements, homogenized samples were submitted to microwave-assisted wet digestion using 2.0 mL HNO_3 (ultra pure quality) and 0,5 mL H_2O_2, in an Ethos Pro microwave system (Milestone, Holger Teknologi, Oslo, Norway). Flame Atomic Absorption Spectrometry (Perkin-Elmer 3300 AAS, Norwalk, CT) with hollow cathode lamp (HCL, Perkin-Elmer, Norwalk, CT) was used for the determination of iron, zinc and calcium. The elemental analyses are all accredited by the Norwegian Metrology and Accreditation Service. The certified reference material NIST Oyster Tissue (National Institute for Standards and Technology, Gaithersburg, MD, USA) was used for quality assurance of the determination of the elements studied (Julshamn et al., 1998; Jorhem and Engman, 2000).

4. Results and Discussion

The results on contents of nutrients composition presented here are three sub samples (n=3) for each item except eel (n=2). The consumption data were adapted from Garaway (2008), based on fresh weight animal equivalent (FWAE) and adult equivalent units (AEU)[1]. Thus it is important to note that FWAE provides only a rough estimation of the actual consumption as it may overestimate the consumed amount of items (e.g; FWAE includes shells for mollusc species). Garaway (2008) grouped consumption data as fish, mollusc and crustacean (one group), amphibian and fermented fish (*padek*). There is no consumption data for insect to represent intake of nutrients from cricket. Reference for required intake of nutrients were adapted from IOM (2005) using the category of the normal adult consumer requiring the highest intake of each nutrient.

4.1. Proximate Composition

The data on moisture, protein, fat and ash content of the 13 analyzed samples, expressed as grams (g) per 100 g edible portion, are presented in Table 8. The fat content of the analyzed samples were generally very low, only cricket and walking catfish had fat contents higher than 1 g/100 g (4.6±1.9 g/100 g and 3.0±0.9 g/100 g). In general, the proximate analysis results are within the range of the published literature values from James (2006) presented in table 1 and slightly different from data published by CFCD 2002 presented in table 2. CFCD 2002, however, is a nutrient composition database of Chinese common food in the whole country, not specifically related to Laotian diets in Mekong area.

The high ash contents in swamp eel, crab and Chinese edible frog, can be explained by the preparation method that included the bones and carapaces (Krahn, 2005). The ash content was also high in snails. This maybe correlated with the ability of snails to ingest soil which is rich in minerals (Beeby and Richmond, 2002). Ash contents in fermented

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[1] AEU was used to convert people of different ages and sexes to standard consumption units. Garaway (2008) assumed that adults between 18 and 59 were distributed equally between sexes.

fish sauces were predictably salt which was added in the preservation process (approx. 5-20 percent of total fish). Total of the proximate composition in all fermented fish are far less than 100 percent, indicating the amount of rice bran utilized in making *padek* (approx. 5-15 percent) which contributed to the carbohydrate content. This study, however did not measure carbohydrate content. In the following sub chapter, more details of the data on water and protein are presented.

Table 8. Proximate composition in analyzed fish, OAA and fermented fish[1] (per 100 g)

English name	Moisture (g)	Fat (g)	Ash (g)	Protein (g)
Fishes				
Swamp eel	77.9 ± 0.8	0.8 ± 0.4	2.4 ± 0.0	19.7 ± 0.8
Walking catfish	76.8 ± 1.4	3.0 ± 0.9	1.4 ± 0.0	19.0 ± 1.0
Snakehead murrel	80.4 ± 0.5	0.4 ± 0.1	1.3 ± 0.0	18.6 ± 0.5
Crustacean				
Freshwater crab	67.5 ± 4.8	0.4 ± 0.0	17.4 ± 3.3	16.3 ± 0.6
Mollusc				
Small apple snail	77.9 ± 2.2	0.4 ± 0.1	4.0 ± 0.5	13.0 ± 0.9
Golden apple snail	83.3 ± 0.9	0.1 ± 0.0	3.0 ± 0.3	11.6 ± 0.5
Big apple snail	82.1 ± 0.7	0.4 ± 0.0	3.4 ± 0.8	11.8 ± 0.8
Amphibian				
Chinese Edible Frog	78.3 ± 0.5	0.8 ± 0.3	3.9 ± 0.4	17.3 ± 0.9
Insect				
Cricket	64.8 ± 5.5	4.6 ± 1.9	1.6 ± 0.3	25.1 ± 3.3
Fermented fish (Padek)				
Champasak Market	76.4 ± 1.8	0.5 ± 0.1	5.8 ± 0.7	3.3 ± 0.4
Champasak Village	73.5 ± 4.2	0.4 ± 0.1	8.1 ± 1.4	7.8 ± 1.6
Savanakhet Market	77.1 ± 0.2	0.6 ± 0.3	5.7 ± 0.6	4.6 ± 0.8
Savanakhet Village	73.8 ± 5.2	0.7 ± 0.1	10.0 ± 2.5	6.8 ± 0.8

[1] Mean ± S.D. of 3 sub samples (n=3) for all samples, except for swamp eel, n=2

4.1.1. Moisture Content

Variation in water content was observed within the fish and OAA. Cricket had the lowest moisture content (64.8 ± 5.5 g/100 g) and golden apple snail had the highest moisture content (83.3 ± 0.9 g/100 g).

When the water content was compared to the data from Chinese freshwater species from CFCD 2002 (Table 2), no significant difference were recorded for most species. . Most similar value was measured from rice field swamp eel where the moisture content was 77.9 g/100 g, while CFCD 2002 have reported moisture content for the same common name at 78 g/100 g. The highest discrepancy between our results and the CFCD 2002 was recorded for freshwater crab, which in CFCD was measured at 75.8 g/100 g while in analyzed samples was 67.5 ± 4.8 g/100 g. This difference could be due to differences in preparation of the samples. The CFCD does not indicate which parts of the crab were included in edible portion. In this analysis, the edible portion only excluded the dorsal and ventral carapace. However, the database stated that edible portion sample is not fixed and can vary widely according to differences in transportation, storage, and processing of the food item. Therefore, wherever there is a discrepancy between the value for edible portion shown in the tables and that judged to be reasonable in actual consumption, the value of edible portion can be varied at the reader's interpretation (CFCD, 2002).

Moisture content of fermented fish (*padek*) was measured using the same method AOAC 925.04 (AOAC, 1995), except that samples were weighed periodically until they reached stable weight. This modification was done as a consequence of difficulty when measuring the weight of *padek* after 24 hours dried at 105 ^{o}C oven, some of the samples remained liquid. *Padek* was made of fish mixed with rice bran and salt. The amount of salt varies approx. from 5 to 25 percent of total fish weight. Salt increases the boiling point of water and decreases freezing point, thus *padek* was more difficult to dry.

4.1.2. Protein Content

Meusch et al., (2003) wrote that aquatic resources make up the majority of animal protein consumed in terms of frequency and quantity in Laos PDR. This is in accordance with the findings of Garaway (2008) which presented in figure 2. To further understand the significance of fish, OAA and fermented fish sauce for Laotian protein intakes in Champasak and Savannakhet, the consumption of proteins was calculated from the consumption data presented by Garaway (2008) and listed in Table 9.

Table 9. Protein composition and adequacy of protein RDA of the analyzed fish, OAA and fermented fish[1]

English name	Mean protein (g/100 g)	Samples consumption[2]		Protein consumption		Adequacy of DRI[3]	
		Champasak (g/day)	Savannakhet (g/day)	Champasak (g/day)	Savannakhet (g/day)	Champasak (%)	Savannakhet (%)
Fishes							
Swamp eel	19.7	114.0	73.0	22.5	14.4	40.2	25.7
Walking catfish	19.0	114.0	73.0	21.7	13.9	38.7	24.8
Snakehead murrel	18.6	114.0	73.0	21.2	13.6	37.8	24.2
Mollusc and Crustacean[4]							
Freshwater crab	12.0	36.0	18.0	4.3	2.2	7.7	3.9
Small apple snail	13.0	36.0	18.0	4.7	2.3	8.3	4.2
Golden apple snail	11.6	36.0	18.0	4.2	2.1	7.5	3.7
Big apple snail	11.8	36.0	18.0	4.2	2.1	7.6	3.8
Amphibian							
Chinese Edible Frog	17.3	94.0	35.0	16.2	6.0	29.0	10.8
Fermented fish (Padek)							
Champasak Market	3.3	236.0	110.0	7.8	3.7	14.0	
Champasak Village	7.8	236.0	110.0	18.4	8.6	32.9	
Savanakhet Market	4.6	236.0	110.0	10.8	5.0		9.0
Savanakhet Village	6.8	236.0	110.0	16.0	7.5		13.3

[1] n=3 for all samples, except for swamp eel, n=2
[2]Consumption data is adapted from Garaway (2008)
[3]DRI Protein >19 years old male (56 g/day)
[4]Consumption data of crustacean and mollusc are grouped into one as in Garaway (2008)

Data from Garaway (2008) indicated that fish, mollusc, crustacean, amphibian and fermented fish were consumed daily both in Champasak and Savannakhet. In 9, these are used to calculate the share in DRI of protein acquired from each of the analyzed items. Table 10 represent an estimation of the minimum and maximum percentage of DRI coverage from the average daily consumption of fish, OAA and fermented fish.

Table 10.Coverage of protein RDA[1] from consumption of fish, OAA and fermented fish per day in two provinces (%)

Food groups	Champasak		Savannakhet	
	min	max	min	max
Fish	37.8	40.2	24.2	25.7
Mollusc and crustacean	7.5	8.3	3.7	4.2
Amphibian	29.0	29.0	10.8	10.8
Fermented fish	14.0	32.9	9.0	13.3
Total	88.3	110.4	47.7	54.0

[1]DRI Protein >19 years old male (56 g/day) adapted from IOM (2005).

If the assumption is made that the villagers in each province consume the lowest protein content items in each food group within a day (snakehead murrel for fish, golden apple snail for mollusc and crustacean, Chinese edible frog for amphibian, cricket for insect and fermented fish from the market). If this is the case, then the people in Champasak province could still cover up to 88.3 percent of their required protein intake (56 g/day). Whilst, the villagers in Savannakhet province who consume less fish, OAA and *padek* covered only 47.7 percent of the required protein intake. On the contrary, if people in each province consume samples from each food group with the highest protein (swamp eel for fish, small apple snail for mollusc and crustacean, Chinese edible frog for amphibian and homemade fermented fish), Champasak province would cover more than the required protein intake (110.4%) whilst Savannakhet province would only cover 54 percent of the RDA.

Homemade *Padek* from both provinces contains higher levels of protein than the market samples. This difference is expressed in the minimum and maximum value of protein adequacy to the RDA required. Compared to *yuqiyou* (Chinese fermented fish and shrimp with salt, Table 2), *padek* contain less protein (<11.2 g/100g). However, moisture content of *yuqiyou* is also lower, thus it is thicker.

Non Protein Nitrogen (NPN) was not analyzed in this study. However when an NPN factor is included, the percentage of protein intake derived from fish will decrease by around 7-12% (Puwastien et al., 1999).

4.2. Amino Acid Composition

Amino acids are subunits forming long chains of protein macromolecules. In the protein molecule, the amino acids are joined together by peptide bonds, which result from the elimination of water between the carboxyl group of one amino acid and the alpha amino group of the next in the chain. Total protein availability in Asian developing countries is almost half that of developed countries. As a consequence, Asian developing countries experience lower amino acid intakes (FAO, 2008). In Laos, rice-based aquatic animals were mentioned as having the potential to supply adequate amounts of required nutrients, including amino acids (Halwart, 2003; Halwart and Bartley, 2005).

Table 11 presented the amino acid composition of the analyzed aquatic animals in milligram per grams protein. Compared to milk, most indispensable amino acids from the samples are lower, but in the vicinity of the referred value. Compared to the CFCD 2002 (table 4), most of the results are similar with low variance. However frogs in the CFCD 2002 contained significantly higher amounts of the indispensable amino acids. The relative proportion of the amino acids nonetheless is similar. This could be due to species and location differences.

Lysine in the three analyzed fish is higher than in milk as adapted from IOM (2005). Zuraini et al., (2006) reported that glutamic acid, aspartic acid and lysine are abundant in Malaysian freshwater snakehead fish, which corresponds to the result of this work. Compared to lysine in marine fish, swamp eel (1557 mg/100g samples), snakehead murrel (1683.1 mg/100g) and walking catfish (1636.8 mg/100g) in this study, the content of lysine in the fresh muscles, is slightly higher than the lysine in raw fillet of rainbow trout (1409.0 ± 21.31 mg/100g; Unusan 2007).

None of the samples analyzed contain tryptophane. This result is similar to findings from other researches (Ijong and Ohta, 1995; Adeyeye and Afolabi, 2003; Verkerk et al., 2006; Zuraini et al., 2005). According to Sanni et al., (2002), tryptophane is presumed to be destroyed during sample hydrolysis.

Table 11. Amino acid composition of analyzed samples (mg/g protein)[1,3]

Samples	mg/ gram protein									Champasak *padek*		Savannakhet *padek*		Milk[2]
	Swamp eel	Walking catfish	Snake head murrel	fresh water crab	Small apple snail	golden apple snail	Big apple snail	Chinese edible frog	cricket	village	market	market	village	
Indispensible														
Histidine	21.2	19.9	**21.2**	18.9	18.7	15.5	10.7	**22.3**	**21.4**	12.0	6.2	22.1	16.7	28.0
Isoleucine	**43.8**	**46.5**	**46.5**	35.2	40.4	39.3	32.2	41.2	37.5	27.0	42.3	37.5	45.8	60.0
Leucine	70.8	79.1	**80.3**	57.7	76.4	77.0	63.0	68.0	68.4	46.9	**80.0**	69.7	**81.2**	98.0
Lysine	**78.9**	**88.6**	**88.1**	49.1	59.6	55.3	44.9	74.3	46.8	48.7	28.2	78.6	70.2	79.0
Methionine	**23.4**	**21.2**	**26.4**	12.5	16.2	12.6	11.3	16.7	11.0	7.3	10.1	15.7	16.1	34.0[2]
Phenylalanine	**37.2**	**40.7**	**40.3**	36.0	37.1	33.9	27.3	35.5	26.5	17.3	29.6	30.6	28.5	96.0[2]
Threonine	42.5	**44.1**	**43.1**	36.7	**45.8**	41.6	36.1	38.9	34.2	18.6	10.8	28.9	35.9	45.0
Valine	45.1	49.0	48.1	41.3	45.5	44.1	38.0	43.3	**59.5**	32.7	**52.7**	47.4	**52.8**	67.0
Dispensible														
Alanine	67.3	63.0	61.3	59.2	54.3	57.7	48.0	61.5	96.8	48.0	108.3	63.3	72.9	
Aspartic acid	91.1	100.6	98.6	75.5	100.7	96.1	79.3	88.4	67.1	60.7	40.3	90.7	87.9	
Serine	37.4	35.0	35.5	29.4	42.1	42.2	37.6	38.7	33.0	15.9	9.6	20.0	29.0	
Conditionally indispensable														
Arginine	63.9	59.9	60.5	61.1	72.5	76.8	67.9	62.1	44.5	11.3	8.3	20.7	17.2	
Glutamine	153.7	155.9	158.4	114.9	151.2	157.1	134.3	148.4	101.4	94.0	92.1	180.3	147.5	
Glycine	87.8	67.1	57.8	53.1	62.7	70.2	71.7	85.1	53.0	31.9	17.8	38.6	42.2	
Proline	56.5	42.0	36.6	39.4	46.3	49.5	43.9	57.6	57.5	22.4	16.6	25.1	28.6	
Tyrosine	22.7	24.0	27.2	21.7	22.6	21.5	17.2	20.1	42.3	6.4	6.8	15.9	8.3	96.0[2]
Nonessential														
Phosposerine	6.8	3.4			11.1	5.1		3.3		6.4	16.1	25.5	10.1	
Taurine	4.5	7.7	12.3	1.1	1.0	0.3	0.5	0.2	0.6	10.9	18.1	19.0	15.7	
Total	943.3	936.6	929.9	741.7	892.1	890.4	763.4	902.1	800.9	501.1	559.7	785.1	780.8	603.09

[1]Analysis were done to pooled sub samples, n=3 for all samples, except for swamp eel; n=2

[2]Data taken from IOM (2005), whereas methionin and cystein together at 34 mg/g protein, phenylalanine and tyrosine together at 96 mg/g protein

[3]Results <1 mg/g protein are excluded; Bold numbers given to the three highest identified amino acid in each samples analyzed

Table 12. Estimated intake of Indispensable amino acid from analyzed samples (mg/day) in Champasak (CH) and Savannakhet (SV)

English name	Estimated consumption of indispensable amino acid from analyzed samples (mg/day)[1]															
	Histidine		Isoleucine		Leucine		Lysine		Methionine		Phenylalanine		Threonine		Valine	
	CH	SV	CH	SV	CH	SV	CH	SV	CH	SV	CH	SV	CH	SV	CH	SV
Fishes																
Swamp eel	476.5	**305.1**	985.9	631.3	1592.8	1019.9	1775.0	1136.6	**526.1**	**336.9**	837.3	536.2	955.4	611.8	1014.1	649.4
Walking catfish	431.5	276.3	1006.9	**644.7**	1713.8	**1097**.5	1918.8	**1228.7**	458.9	293.9	882.2	**564.9**	955.0	611.6	1061.9	**680.0**
Snakehead murrel	449.9	288.1	985.5	631.0	1701.6	1089.6	1865.9	1194.8	559.2		853.0	77.9	912.2	79.5	1018.3	652.1
Mollusc and Crustacean[2]																
Freshwater crab	81.9	40.9	152.4	76.2	250.2	125.1	212.8	106.4	54.1	27.1	155.9	86.4	158.9	106.8	178.8	89.4
Small apple snail	87.2	43.6	188.4	94.2	356.1	178.1	277.9	139.0	75.7	37.9	172.9	70.9	213.6	86.8	212.0	106.0
Golden apple snail	64.9	32.5	164.3	82.1	321.7	160.8	230.9	115.4	52.7	26.4	141.7	57.7	173.6	76.3	184.2	92.1
Big apple snail	45.3	22.7	136.3	68.2	266.6	133.3	190.1	95.1	47.8	23.9	115.5	214.5	152.6		161.1	80.5
Amphibian																
Chinese Edible Frog	361.7	134.7	669.7	249.3	1104.7	411.3	1206.8	449.3	271.7	101.2	576.0		632.5	**235.5**	702.9	261.7
Fermented fish (Padek)																
Village	**711.3**	84.2	**1594.5**	231.0	**2771.3**	409.1	**2879.6**	353.6	430.2	81.3	**1021.3**	143.8	**1101.9**	180.7	**1933.1**	265.8
Market	406.7	46.3	689.8	315.3	1283.7	596.6	1446.6	210.3	288.6	75.4	563.5	220.8	532.5	80.6	872.0	392.8
RDA (mg/day)[3]	868.0		1178.0		2604.0		2356.0		1178.0		2046.0		1240.0		248.0	

[1]Consumption data is adapted from Garaway (2008)

[2]Consumption data of crustacean and mollusc are grouped into one, no available data for cricket consumption (Garaway, 2008)

[3]RDA Protein >19 years old male/female adapted from IOM (2005) with methionine originally summed up with cysteine and phenylalanine with tyrosine

The essential amino acid lysine is lacking in many areas of the world where diets are heavily based on cereals (FAO, 2008). Standard tables of amino acid composition show that the values of lysine in cereals to range from 26 to 38 mg/g protein, whereas the values of lysine in animal food range from 70 to 100 mg lysine/g protein (Pellett in FAO, 2008).

FWAE in Garaway (2008) is used to estimate the daily individual amino acid intakes in each of the provinces. But it was not possible to calculate the consumption of cricket as there is no consumption data. Table 12 expressed the intake of amino acids from the consumption of food items in Champasak and Savannakhet. If the assumption is made that the villagers consume swamp eel, big apple snail, Chinese edible frog and market *padek* from each food group, consequently the villagers in Champasak will have lysine intake twice of RDA level while villagers in Savannakhet can cover up to 80 percent of intake required by RDA (2356 mg/day with average body weight 62 kg). On the contrary, if it is assumed that the villagers consume walking catfish, small apple snail, Chinese edible frog and home made padek from each food group, the villagers in Champasak will have lysine intake up to 2.7 times than RDA level whilst villagers in Savannakhet will cover 90 percent only of RDA level for lysine.

It is important to remember that this will not give a full picture of the diets. Fish and aquatic animals are eaten together with rice and vegetables, whilst the *padek* is often eaten as a condiment or sauce. It is surprising that maximum amino acid intake were offered by homemade *padek*. Although the content of amino acids is slightly lower than in the market *padek*, the protein content is higher thus giving higher intakes of amino acids. Please note that *padek* was the most consumed item among other food groups, thus the contribution is also very high.

Table 13. Amino Acid Composition of Analyzed Laotian Fermented fish compared to Laotian fish sauce (Park et al., 2001)[1]

Amino acid	Fermented fish (*padek*) mg amino acid/100 gram sample[2]				Laotian fish sauce (mg/100 mL)
	champasak village	Champasak market	Savannakhet village	Savannakhet market	
Indispensible					
Histidine	93.8	73.3	113.4	28.4	123 ± 17
Isoleucine	210.3	124.4	310.8	193.6	72 ± 22
Leucine	365.5	231.5	550.5	366.4	143 ± 42
Lysine	379.8	260.9	475.9	129.1	123 ± 17
Methionine	56.7	52.0	109.4	46.3	32 ± 5
Phenylalanine	134.7	101.6	193.5	135.6	36 ± 6
Threonine	145.3	96.0	243.2	49.5	28 ± 13
Valine	255.0	157.3	357.7	241.2	32 ± 45
Total	1641.1	1097.2	2354.4	1190.2	589.0
Dispensible					
Alanine	374.7	210.2	494.6	496.1	179 ± 11
Aspartic acid	473.1	301.2	596.1	184.5	54 ± 59
Serine	124.1	66.3	196.8	44.1	24 ± 10
Conditionally indispensible					
Arginine	87.9	68.6	116.5	38.2	7 ± 4
Glutamine	733.0	598.6	1000.4	422.0	31 ± 34
Glycine	248.6	128.1	286.2	81.5	43 ± 2
Proline	174.7	83.4	194.1	76.1	18 ± 2
Tyrosine	49.8	52.9	56.2	31.2	5 ± 7
Nonessential					
Phosposerine	49.7	84.7	68.2	73.5	
Taurine	84.8	63.0	106.7	82.9	37 ± 00
Urea	806.9	430.1	860.2	1062.9	
Orn	95.2	53.6	72.4	52.8	

[1]unit is in mg/mL

[2] 1 gram of *padek* is compared to 1 mL of fish sauce

Garaway (2008) emphasized the significant role of *padek* consumption in Laos. *Padek* also represent a possibility of fish preservation. The food item will this be available in a longer time span. *Padek* was consumed almost every day (>90 percent of surveyed days). Therefore, there is a need to look closer at the benefit people gain from this consumption. In table 13 the amino acids analyzed on *padek* in this work is compared to the Laotian fish sauce analyzed by Park et al., (2001).

Generally, the analyzed *padek* contain higher amounts of the amino acid than the Laotian fish sauce. However, Park et al., (2001) also analyzed fish sauce from other South East

Asian countries and the results varied. Laotian fish sauce was identified as one of the lowest quality fish sauces. As Park et al., (2001) sampled the fish sauce from the market, this work documented that *padek* sampled from markets were inferior to the homemade *padek.*

Unfortunately cricket consumption data is lacking. The amino acid composition of cricket reveals that this may be a good source of amino acids given the fact that protein content of cricket is the highest among all documented samples (25.06 ± 3.26 g/100 g). Studies on insects as an alternative source of protein brings positive messages: that insect contains high quality protein, they are cheap and have a long history of consumption by humans. They are thus considered safe (Wang, 2007; Ramos-Elorduy et al., 1997; Verkerk et al., 2007; Wang et al., 2004).

4.3. Fatty Acid

As shown in table 8, fat contents of the samples are not high. Nonetheless, fatty acid analysis was done in order to understand the quality of the fat. All samples except the snakehead murrel, were submitted to fatty acid analysis. The reason for this was due to laboratory inefficiency. The fat contents are usually lower when extracted with Folch (1975). The results are presented in the table 14.

Results shows all samples generally containing low SFA, MUFA and PUFA with the exception of cricket, followed by walking cat fish and Chinese edible frog. Data from the Chinese edible frog is similar to what has been indicated by Krahn (2005) for toad.

Fatty acid contents values are inserted into consumption data and presented in table 15. All of the species contribute insignificantly to essential fatty acid required AI, which is 17 g/day for n-6 PUFA (young man) and 1.6 g/day for n-3 PUFA (man). Unfortunately, as there is no data available on insect group species; fatty acid intake from cricket consumption can not be measured.

Linoleic (n-6) or α-linolenic acid (n-3) is not synthesized by humans and lack of it will result in adverse clinical symptomps (IOM, 2005). Field cricket found to contain high quality fatty acids and fatty acid analysis to field cricket (*Gryllus testaceus* Walker) total percentage of oleic acid, linolic acidand linolenic acid was 77.51 percent (Wang et al., 2004). In this study, linolenic acid is absent from cricket and the percentage of linoleic acid and oleic acid is 60 percent.

Table 14. Fatty acid content (mg/gr of fish (w/w)) of analyzed items with fat >0.5 g/100 g.

	mean ± S.D.								Fermented fish (*padek*)			
									Champasak		Savannakhet	
Samples	Walking catfish	Swamp eel	Chinese edible frog	Freshwater crab	Small apple snail	Golden apple snail	Big apple snail	Cricket	villager	market	villager	market
C 14:0	0.3 ± 0.1[b]	0.1	0.3 ± 0.3	0.1[a]	0.9 ± 1.0[b]	0.1	0.2	0.5[a]	0.1	0.2	0.1 ± 0.1	0.1
C 16:0	5.2 ± 2.7[b]	0.8 ± 0.3	1.5 ± 0.7	1 ± 0.1	1.1 ± 0.5	1.1 ± 0.3	0.9 ± 0.2	8.3 ± 1	1.1 ± 0.6	1.6 ± 0.1	1.6 ± 0.3	0.9 ± 0.1
C 16:1	0.7 ± 0.2	0.1[a]	0.7 ± 0.8	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2	0.5 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1
C 18:0	1.8 ± 0.6	0.3 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	2.8 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
C18:1, n-9	5.0 ± 2.3	0.4 ± 0.2	1.4 ± 0.8	0.8a	0.6 ± 0.2	1 ± 0.6	0.4	9.3 ± 1.3	1.2 ± 0.9	1.6 ± 0.5	1.6 ± 0.1	0.9 ± 0.1
C18:1, n-7	0.6 ± 0.1[b]	0.1	0.4 ± 0.3	0.6 ± 0.7	0.1	0.4 ± 0.5	0.1[b]		0.1	0.1	0.1	0.1
C 18:2, n-6	2.5 ± 1.8	0.4 ± 0.2	0.6 ± 0.4	0.8 ± 0.2	0.3 ± 0.2	0.5 ± 0.4	0.4 ± 0.1	9.1 ± 0.8	0.5[b]	1.1 ± 0.1[b]	1.2 ± 0.2	0.5
C 18:3, n-3	0.8 ± 0.1[b]											
C 18:4, n-3	0.2 ± 0.1[b]	0.3[a]	0.3[a]		0.2 ± 0.1[b]	0.2[a]	0.2					
C 20:1, n-9		0.1[a]	0.9[a]			0.1[a]						
C 22:1, n-11		0.1[a]	0.2[a]									
C 20:5, n-3	0.3[b]		0.1[a]	0.2[a]	0.1[a]					0.1a	0.1[a]	
C 22:5, n-3			0.1[a]			0.1[a]						0.1
C 22:6, n-3	0.2[b]		0.1±0.1	0.1 ± 0.1	0.1[a]	0.2			0.1 ± 0.1[b]		0.1	
SFA	7.3	1.2	2.3	1.5	2.4	1.6	1.4	11.6	1.5	2.2	2.2	1.3
MUFA	6.3	0.8	3.6	1.5	0.9	1.6	0.7	9.8	1.4	2	1.9	1
PUFA	4	0.7	1.2	1.1	0.7	1	0.6	9.1	0.5	1.2	1.4	0.6
n-6	2.5	0.4	0.6	0.8	0.3	0.5	0.4	9.1	0.5	1.1	1.2	0.5
n-3	1.5	0.29	0.6	0.3	0.4	0.5	0.2	0	0	0	0.2	0.1
EPA	0.3[b]	-	0.1[a]	0.2[a]	0.1[a]	-	-	-	-	0.1a	0.1[a]	-
DHA	0.2[b]	-	0.1±0.1	0.1 ± 0.1	0.1[a]	0.2	-	-	0.1 ± 0.1[b]	-	0.1	-
n-6/n-3	1.7	1.4	1.0	2.7	0.8	1.0	2.0		-	-	6.0	5.0
total FA	17.6	2.7	7.1	4.1	4	4.2	2.7	30.5	3.4	5.4	5.5	2.9

[a]value only found in 1 sub sample from n=3

[b]value only found in 2 sub samples from n=3

Table15. Estimated intake of n-6[1], n-3[2], EPA[3] and DHA[4] from consumption of analyzed items per day (mg/day)

Samples	EFA content (mg/g protein)		Consumption		Estimated EFA intake				Content of EPA and DHA (mg/g samples)		Estimated EPA+DHA intake (mg/day)			
			CH	SV	Champasak		Savannakhet				Champasak		Savannakhet	
	n-3	n-6	g/day	g/day	n3	n6	n3	n6	EPA	DHA	EPA	DHA	EPA	DHA
Fishes														
Walking catfish	1.5	2.5	114	73	171	285	110	182.5	0.2	0.3	22.8	34.2	14.6	21.9
Swamp eel	0.3	0.4	114	73	34.2	45.6	21.9	29.2						
Crustacean and mollusc														
Freshwater crab	0.3	0.8	36	18	10.8	28.8	5.4	14.4	0.2	0.1	7.2	3.6	3.6	1.8
Apple snail 1	0.4	0.3	36	18	14.4	10.8	7.2	5.4	0.1	0.1	3.6	3.6	1.8	1.8
Golden apple snail	0.5	0.5	36	18	18	18	9	9						
Apple snail 2	0.2	0.4	36	18	7.2	14.4	3.6	7.2						
Amphibian														
Chinese Edible Frog	0.6	0.6	94	35	56.4	56.4	21	21	0.1	0.1	9.4	9.4	3.5	0.94
Fermented fish (Padek)														
Champasak Village	0	0.5	236	110	0	118			0	0.1		23.6		
Champasak Market	0	1.1	236	110	0	259.6			0.1	0	23.6			
Savanakhet Village	0.2	1.2	236	110			22	132	0.1	0.1			11.0	11.0
Savanakhet Market	0.1	0.5	236	110			11	55		0				

[1]n-3 is Omega 3 fatty acid
[2]n-6 is Omega 6 fatty acid
[3]EPA is Eicosapentanoic acid
[4]DHA is Docosahexanoid acid

Table 15 presents the estimated consumption data of fatty acids. It can be seen that the contribution of these species to fatty acid requirements is very low. Using AI from IOM (2005), an adult man requires 17 gram of linoleic acid per day and a young boy requires 1.6 gram of α-linolenic acid per day. Table 16 expresses the inadequacy of n-3, n-6, DHA and EPA intake from the calculated consumption data in Champasak. The Champasak province is taken as an example due to its higher consumption per day. Nonetheless, consumption of fish, OAA and fermented fish sauce in this province can provide only 230.8-629.8 mg/day of omega 6 and 97.8-245.4 mg/day of omega 3, which is 4 percent of the required omega 6 and 15 percent for omega 3 (calculated based on the highest possible consumption value).

Table 16. Range of fatty acid intake from consumption of fish, OAA and fermented fish per day in Champasak (mg/day)

Consumption of Fish, OAA and *padek* in Champasak	Omega 6 (mg/day)		Omega 3 (mg/day)	
	min	Max	Min	max
Fish	45.6	285.0	34.2	171
Mollusc and crustacean	10.8	28.8	7.2	18
Amphibian	56.4	56.4	56.4	56.4
Insect				
Fermented fish	118	259.6	0	0
Total	230.8	629.8	97.8	245.4

The same inadequacy for intake in DHA and EPA is indicated by table 14. ISSFAL (2004) recommend the minimum intake of DHA and EPA combined of 500 mg/day, which is well above the values for all items combined expressed in Table 15.

4.4. Macro Minerals (Ca, Zn and Fe) Content and Intake

Calcium (Ca), iron (Fe) and zinc (Zn) contents were measured on pooled samples (n=3). Equal amounts of sub samples were homogenized into one prior to approx. 24 hours of freeze drying. The results are expressed on a dry material (DM) basis and corrected in accordance to the moisture content of the samples after freeze drying. Moisture content analysis was however conducted 8 weeks after freeze drying, while the analysis was completed within 6 weeks after freeze drying. It is assumed that the water content of DM was the water content of the samples when analyzed. In this discussion, the values are in wet material (WM). Data are presented in Table 17.

Table 17. Contents of Calcium, Zinc and Iron in the analyzed fish, OAA and fermented fish[1] (mg/100 g samples)

English name	Ca Content DM	Ca Content WM	Fe Content DM	Fe Content WM	Zn Content DM	Zn Content WM
Fishes						
Swamp eel	2378.38	524.91	4.97	1.10	6.38	1.41
Walking catfish	1286.17	298.91	2.68	0.62	3.86	0.90
Snakehead murrel	362.09	70.97	1.70	0.33	3.30	0.65
*Mollusc and Crustacean**						
Freshwater crab	20948.18	6812.35	31.97	10.40	9.48	3.08
Apple snail 1	5579.40	1232.49	19.31	4.27	36.48	8.06
Golden apple snail	2662.41	444.36	287.54	47.99	41.53	6.93
Apple snail 2	4530.74	811.91	571.74	102.46	64.72	11.60
Amphibian						
Chinese Edible Frog	5787.78	1256.53	4.07	0.88	7.61	1.65
Insect						
Cricket	97.61	34.33	58.57	20.60	24.95	8.77
Fermented fish (Padek)						
Champasak Market	385.85	90.95	5.47	1.29	1.71	0.40
Champasak Village	898.27	235.44	32.47	8.51	3.57	0.94
Savanakhet Market	274.42	62.82	10.43	2.39	1.54	0.35
Savanakhet Village	511.43	133.99	26.12	6.84	1.96	0.51

[1]Equal amount of sub samples were pooled into one and freeze dried
WM (wet material) and DM (dry material)

There are variations in calcium, zinc and iron contents between the analyzed samples and the data presented in the CFCD 2002 (table 5). The variation is large for all calcium contents, less for iron content and least for zinc contents. However, almost all values are within the range of data from James (2006, table 1).

4.4.1. Calcium

Calcium accounts for 1 to 2 percent of adult human body weight. Over 99 percent of total body calcium is found in teeth and bones. The remainder is present in blood, extracellular fluid, muscle, and other tissues, where it plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission, and glandular secretion (IOM, 1997). In this study, calcium contents of samples which preparation included bone fractures or carapaces are very high. The differences of calcium content of fish, freshwater crab and Chinese edible frog are presumed to occur due to different method of preparation which possibly results in lower value in data from CFCD 2002. The high content of calcium in snails exceeds the range of calcium in mollusc in James (2006). This maybe due to remains of cracked shells included during the sample preparation. Mollusc shells are known for their composite structure of aragonite, other calcareous material and protein glue (Cortie et al., 2006). Consequently introduction of mollusc shells may increase the calcium contents of the samples. Laotians consume snails mostly by boiling them prior to extracting the meat with tooth picks or slurp it directly. Thus the calcium content of snails in this study is maybe overestimated by the cracked shells.

Freshwater crab contained very high level of calcium (6812.35 mg/100 g) compared to the other samples, whilst *padek* from the Savanakhet market contained the least amount of calcium (62.82 mg/100 g). However, all samples of *padek* contained significantly higher levels than *yuqiyou*, the fermented fish and shrimp with salt shown in CFCD 2002. Swamp eel was prepared as eaten with its bones, and thus maybe regarded as a good source of calcium compared to the other fishes analyzed. Nevertheless the value is not as high as freshwater crab, which preparation only discarding ventral and dorsal carapace.

In table 18, an estimation of calcium, iron and zinc intake from the consumption of analyzed items in two provinces; Champasak and Savannakhet are presented. Consumption data were adapted from Garaway (2008) based on FWAE.

Table 18. Estimated intake of Ca, Fe and Zn from analyzed items (mg/day) in Champasak (CH) and Savannakhet (SV).

English name	Ca Content (mg/100g)	Fe Content (mg/100g)	Zn Content (mg/100g)	Estimation of Ca Consumption (mg/day)[1]		Estimation of Fe Consumption (mg/day)		Estimation of Zn Consumption (mg/day)	
				CH	SV	CH	SV	CH	SV
Fishes									
Swamp eel	524.9	1.10	1.41	598.4	383.2	1.3	0.8	1.6	1.0
Walking catfish	298.9	0.62	0.90	340.8	218.2	0.7	0.5	1.0	0.7
Snakehead murrel	71.0	0.33	0.65	80.9	51.8	0.4	0.2	0.7	0.5
Mollusc and Crustacean[2]									
Freshwater crab	6812.3	10.40	3.08	2452.4	1226.2	3.7	1.9	1.1	0.6
Small apple snail	1232.5	4.27	8.06	443.7	221.8	1.5	0.8	2.9	1.5
Golden apple snail	444.4	47.99	6.93	160.0	80.0	17.3	8.6	2.5	1.2
Big apple snail	811.9	102.46	11.60	292.3	146.1	36.9	18.4	4.2	2.1
Amphibian									
Chinese Edible Frog	1256.5	0.88	1.65	1181.1	439.8	0.8	0.3	1.6	0.6
Fermented fish (Padek)									
Champasak Market	90.9		0.40	214.6		3.0			
Champasak Village	235.4		0.94	555.6		20.1			
Savanakhet Market		2.39	0.35		69.1		2.6		
Savanakhet Village		6.84	0.51		147.4		7.5	1.0	0.4

[1]Consumption data is adapted from Garaway (2008)
[2]Consumption data of crustacean and mollusc are grouped into one, no available data for cricket consumption (Garaway, 2008)

Assuming that the people consume snakehead murrel, golden apple snail, Chinese edible frog and padek from the market, the calcium intake will be lowest both in Champasak and Savannakhet. However, the minimum intake of calcium in Champasak (1636.6 mg/day) is exceeding AI value from IOM (1300 mg/day). Whilst the minimum intake in Savannakhet (640.7 mg/day) covers only less than 50 percent of AI value for calcium from IOM (2005).

Table 19. Range of Ca, Fe and Zn intake from consumption of fish, OAA and fermented fish in Champasak and Savannakhet[2]

Food groups	Ca consumption (mg/day)				Fe consumption (mg/day)				Zn consumption (mg/day)			
	Champasak		Savannakhet		Champasak		Savannakhet		Champasak		Savannakhet	
	min	max	min	max	min	max	min	max	min	max	min	max
Fish	80.9	598.4	51.8	383.2	0.4	1.3	0.2	0.8	0.7	1.6	0.5	1.0
Mollusc and crustacean[3]	160.0	2452.4	80.0	1226.2	1.5	36.9	0.8	18.4	2.9	4.2	0.6	2.1
Amphibian	1181.1	1181.1	439.8	439.8	0.8	0.8	0.3	0.3	1.6	1.6	0.6	0.6
Fermented fish	214.6	555.6	69.1	147.4	3.0	20.1	2.6	7.5	1.0	2.2	0.4	0.6
Total	1636.6	4787.6	640.7	2196.6	5.8	59.0	3.9	27.1	6.1	9.5	2.0	4.3
AI[1]	1300	1300	1300	1300	18	18	18	18	11	11	11	11

[1]AI of Calcium taken from intake value of 9-18 years old man/woman, AI for Fe taken from intake value of 19-50 years old woman, AI for Zn taken from intake of >14 years old man (IOM, 2005).
[2]Consumption data is adapted from Garaway (2008)
[3]Consumption data of crustacean and mollusc are grouped into one, no available data for cricket consumption (Garaway, 2008)

When assuming that the villagers are consuming swamp eel, freshwater crab, Chinese edible frog and homemade *padek,* the intake of calcium will be maximized. The intake in Champasak province could exceed more than 3 times of the AI level (4787.6 mg/g). Whilst in Champasak would exceed the recommended amount by 50 percent (2196.6 mg/g). Calcium tolerable upper level (UL)[2] was set at 2.5 g/day. This means there is potency that the villagers in Champasak consume excessive intake of calcium. The use of FWAE of course represents the possibility of error here.

Excess calcium intake is suggested to cause drowsiness, extreme lethargy, impaired absorption of iron, zinc and manganese, calcium deposits in tissues throughout body, and may mimic cancer on X-ray pictures (1stholistic.com, 2008). However in Takasugi et al,. (2005) the affect of calcium excess on mineral metabolism partly depending on its form.

[2] UL= the highest level of daily nutrient intake that is likely to pose no risk of adverse health affect to almost all individuals in the general population (IOM, 2005).

4.4.2. Iron

Iron deficiency is the most common nutritional disorder in the world. 90 percent of 4-5 billion people who suffer live in developing countries (Ho, 2005), including Laos PDR (FAO, 2003). Anemia is identified to be more common in the southern part of Laos, among the female, in rural areas, among children younger than 5 years old and old people over 60 years old, in other words among the vulnerable groups. The government has made an attempt to cope with this by supplying iron tablets to pregnant woman. 13.6 percent of them are offered tablets but the figure varies between rural areas and urban areas (FAO, 2003).

Our results show that snails contain the highest level of iron. Iron content in big apple snail and golden apple snail are far higher than the result presented in James (2006, table 1) and the results in CFCD 2002 (table 5). As snails ingest sludge from the surrounding paddy field (Kurihara et al., 1987), washing was a determining process to minimizing mineral contents before the snails were analyzed. Thus there is a possibility that the high mineral content is due to differences in preparation. However it is impossible to expect households wash equally clean due to individual preparation variance.

Table 19 presented estimated iron intake from consumption of items in each food group. If an assumption is made that villagers consume snakehead murrel, small apple snail, Chinese edible frog and padek from the market within each food group, it is estimated that villagers in Champasak can cover up to 32.2 percent of AI value (18 mg/day) whilst villagers in Savannakhet can cover up to 21.6 percent of AI value. If the villagers consume swamp eel, big apple snail, Chinese edible frog and padek from household preparation, the consumption of villagers in Champasak province could cover more than 3 times of the AI whilst Savannakhet could cover more than 1,5 times than AI.

The UL for iron is set at 45 mg/day (IOM, 2005). Villagers in Savannakhet do not have the same excess iron consumption as villagers in Champasak. Nonetheless, as discussed earlier, Laos PDR has an iron deficiency problem, especially in the rural area. The extremely high iron content from big apple snail has disrupted the iron intake calculation.

Iron content of cricket is within the range of James (2006) as well as Studier and Sevick (1992). The later discussed several species of insect as source of iron. DeFoliart (1992) also indicated similar results; insects contain high amount of zinc and iron. Unfortunately, this study could not provide an estimation of the iron intake from insect consumption due to limited data from Garaway (2008). However our data indicated cricket, consumed by the local Laotian, thus can be potential source of iron.

4.4.3. Zinc

Zinc deficiency is not a pronounced malnutrition problem in Laos PDR as iron, iodine and vitamin A. Nonetheless, according to International Zinc Nutrition Consultative Group (IZiNCG), very few surveys has been conducted to determine directly the extent of zinc deficiency. But IZiNCG has estimated zinc deficiency based on combined information regarding the percent of individual at risk of inadequate zinc intake and the prevalence of childhood growth stunting. Laos PDR has a high prevalence of stunting (42.4 percent, IZiNCG, 2008).

Zinc content of the items analyzed was similar to comparable zinc level in CFCD 2002. Big apple snails contained the highest level of zinc (11.6 mg/100 g) and *padek* from Savannakhet market contain the lowest (0.35 mg/100 g). Snails contain high zinc due to its ability to ingest sludge from the surrounding paddy field (Kurihara et al., 1987). The washing procedure in the laboratory as explained in sub chapter of iron, could also influence the determination of zinc content.

Other than snails, cricket also contained high level of zinc. DeFoliart (1992) observed that some insects could be the source of zinc for humans. Among the *padek* group, Champasak home made contained the highest level of zinc. The same samples also contained the highest level of calcium and iron. Both in Champasak and Savannakhet, home made *padek* contained higher amount of zinc.

Assuming that the villagers consume snakehead murrel, freshwater crab, Chinese edible frog and market sold *padek*, the zinc intake in Champasak will be at 6.1 mg/day, which covers half of the AI value for zinc (11 mg/day) and 2 mg/day, which is less than 20 percent of AI value. On the contrary, if assumption is made that the villagers consume swamp eel, big apple snail, Chinese edible frog and home made padek, then the consumption intake will be maximized, which in Champasak will cover 86.4 percent of the AI value but will cover 39.1 percent of AI value only in Savannakhet.

4.5. Vitamin A Content

Vitamin A analysis was initially conducted on one sub sample of each sample to understand the general levels of vitamin A content in the samples. Consequently, analysis of all sub samples continued only for the samples containing detectable amounts of vitamin A in the initial analysis. The analysis showed that only Chinese edible frog contained vitamin A in all three sub samples, while two of home made fermented fish of both Champasak and Savannakhet province containing vitamin A, but in lower concentration (figure 3).

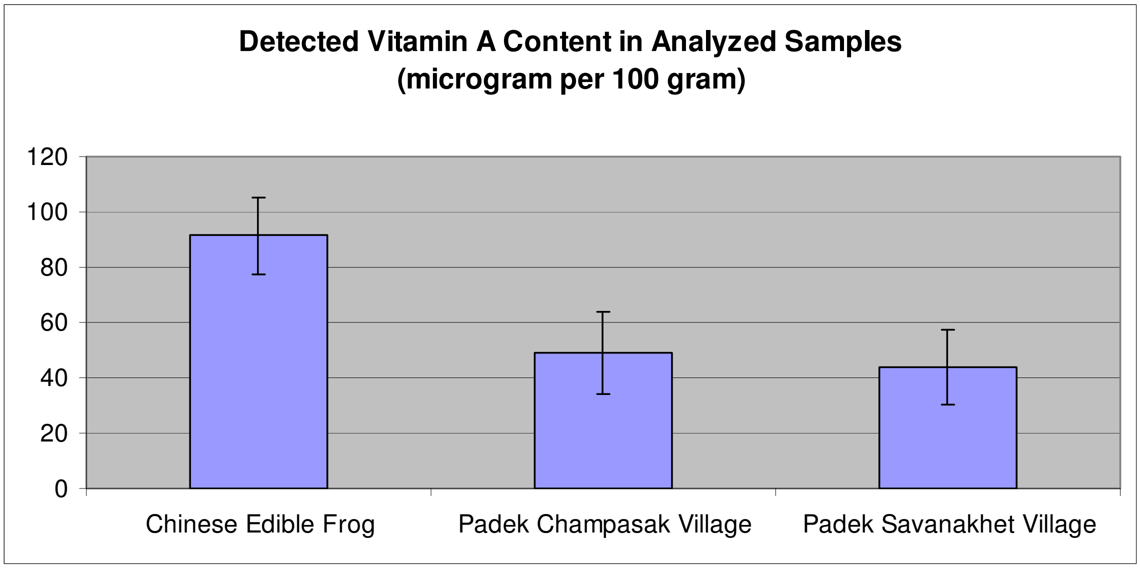

Figure 3. Vitamin A content in analyzed samples (microgram per 100 gram of samples)

Table 5 shows vitamin A content of Chinese freshwater species from CFCD 2002. Vitamin A was detected in freshwater crab (389 µg/100 g of samples), frog (7 µg/100 g of samples), and average snail (26 µg/100 g of samples). The significant difference of vitamin A content in freshwater crab may be due to the fat content that is higher in freshwater crab in the CFCD 2002 (1.2 g/100 g) compared to freshwater crab in this analysis (0.4 g/100 g), as vitamin A is a fat soluble vitamin.

Table 6 shows the data published by Roos et al., (2007) on vitamin A content in small indigenous fishes in Bangladesh. The analyzed fishes, mola and rui, contained most of their vitamin A in eyes and viscera. This probably explains why only these three samples (frog and two types of *padek*) contained detectable vitamin A. In the preparation of frog, the head was included and thus frog eyes were among the material that was analyzed. Fermented fish was prepared including fish heads and eyes. However home fermented fish in both Champasak and Savannakhet contained more fat than the market samples. Viscera, however were discarded in all preparations.

This result correspond to data presented by Krahn’s (2005) which did not suggest fish, aquatic animals and fermented fish to be source of vitamin A. Instead, vegetables were listed as β-Carotene key nutrient.

The Laos PDR National Health Survey in 2000 showed that 3.1 percent of 6 to 59 month-old children were reported suffered from night blindness. Very low intake of fats and oils combined with an inadequate intake of dark green vegetables is the main reason for the prevalence of vitamin A deficiency in Laos (FAO, 2003). However, Krahn’s (2005) research on dietary changes of Southern Laos, did not identify cases of night blindness. Krahn also stated that until now, Laos has been a country with low level of vitamin A deficiency.

5. Conclusion

Rice-based aquatic ecosystems have a high biodiversity and are a rich source of nutrient-dense local food (Halwart, 2006). This study documented that daily consumed aquatic animals contained high amount of protein, amino acid, calcium, iron and zinc. On the contrary, they contained low amount of fat, fatty acid, and vitamin A.

Lysine, which is essential amino acid recognized to be lacking in developing countries is abundant in these food items. Daily consumption of the aquatic animals estimated to cover 80 percent to twice more than the required amount of lysine intake. Iron and zinc deficiencies are also identified among the Laotians. Selected daily food items consumption of these aquatic animals could cover more than the required amount of calcium and iron intake. Zinc required intake, however, can not be fully covered by only consuming these aquatic animals in their recorded daily consumption level and preparation method. Nonetheless, it can cover up to 86.4 percent of RDA/AI.

Method of preparation influences the supply of nutrients; Species prepared with bones or carapace containing higher mineral, samples including eyes part potentially contain higher level of vitamin A. Washing method in snail preparation is presumably determining mineral intake from this food group consumption. Homemade *Padek* has a better nutritional value.

The aquatic animals contained low amounts of fat hence contribute to the low intake of fatty acid and fat soluble nutrient; vitamin A. The analyzed aquatic animals, when prepared and consumed in the same manner as studied here, can not be counted as a good source for these nutrients. Further study on other source of these nutrients is suggested to be conducted.

However, the nutrient intake estimation in this study was based on FWAE estimation of Garaway (2008), and improvements could be made when actual consumption data is provided.

Further study is also suggested on the specific potential contribution of Laotian fermented fish sauce (*padek*) and cricket to amino acid, fatty acid, vitamin A, iron and zinc intake.

From the ecosystem service perspective, this study has revealed the benefits people derive from the rice field ecosystem. As the food supply of rural households in rice farming area of Laos is critically dependent on the environment, the sustainable existence of the rice-based aquatic animals is crucial factor to the nutritional status of the Laotians. The result of this study has provided utilitarian factors to conserve the biodiversity from degradation, which according to Lopez et al., (2007) is more important in determining the human attitudes towards species and the willingness to support biodiversity conservation. The objective of biodiversity conservation overlaps local and nutritional needs and should be emphasised in development of national nutrition policies, as well as agricultural development policies and fisheries management practices. Fishery managers and policy makers should integrate pro-poor community-based biodiversity conservation into their ongoing development programmes.

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Annex 1. List of Species' Origin, Maturity and Length

No	Species/ sample	Sub sample	n	Source	Origin	Approx age/ maturity	Length[1]
1	Swamp Eel (*Monopterus albus*)	Sub sample 1	n.i	Market in Artsaponthong district	Rice field, Kohinkeo village, Champone district, Savannakhet	3-4 months	47-59 cm
		Sub sample 2	n.i	Market in Artsaponthong district	Rice field, Kohinkeo village, Champone district, Savannakhet	3-4 months	47-59 cm
2	Snakehead murrel (*Chana striata*),	Sub sample 1	n.i	Market in Pakse	Rice field, Bahn Song village, Pakse district, Champasak	5 months	approx. 25 cm
		Sub sample 2	n.i	Villager	Rice field, Kied Ngong, Patumphone district, Champasak	4 months	33 cm
		Sub sample 3	n.i	Villager	Rice field, Kied Ngong, Patumphone district, Champasak	4 months	32 cm
3	Walking catfish (*Clarias batrachus*),	Sub sample 1	5	Market in Songkhone	Rice field, Nongkan Nyu village, Songkhone district, Savannakhet	3 months	18-20 cm
		Sub sample 2	7	Market in Songkhone	Rice field, Nongkan Nyu village, Songkhone district, Savannakhet	4 months	18-20 cm
		Sub sample 3	5	Market in Songkhone	Rice field, Nongkan Nyu village, Songkhone district, Savannakhet	5 months	18-20 cm
4	Freshwater crab[2] (*Potamon sp*)	Sub sample 1	12	Villagers in Phontong	Rice field, Muai Phek, Phontong district, Champasak	n.i	5 cm
		Sub sample 2	11	Villagers in Phontong	Rice field, Muai Phek, Phontong district, Champasak	n.i	5 cm
		Sub sample 3	>10	Market in Pakse	Rice field, unknown		3-4.5 cm
5	Small apple snail[3] (*Cipangopaludina chinensis*)	Sub sample 1	>10	Villager in Saipunthong	Rice field, Nong piet village, Champone district, Savannakhet	1 year	1.7-2.5
		Sub sample 2	>10	Villager in Saiphuntong	Rice field, Don Toum village, Saiphuntong district, Savannakhet	1 year	1.0-2.0
		Sub sample 3	>10	Villager in Saiphuntong	Rice field, Don Toum village, Saiphuntong district, Savannakhet	1 year	1.0-2.0

Annex 1. Continued

No	Species/ sample	Sub sample	n	Source	Origin	Approx age/ maturity	Length[1]
6	Golden apple snail[3] (species unidentified, local name is *hoy pak kuang*)	Sub sample 1	>10	Market in Pakse	Rice field and natural pond, Nalao village, Pakse, Champasak	n.i	4.5 cm
		Sub sample 2	>10	Market in Pakse	Rice field and natural pond, Nalao village, Pakse, Champasak	n.i	4.5 cm
		Sub sample 3	>10	Market in Pakse	Rice field and natural pond, Nalao village, Pakse, Champasak	n.i	4.5 cm
7	*Pila sp*[3] (big apple snail)	Sub sample 1	9	Villager	Rice field in Kied Ngong, Patumphone district, Champasak	2 years	n.i
		Sub sample 2	10	Villager	Rice field in Kied Ngong, Patumphone district, Champasak	2 years	n.i
		Sub sample 3	9	Villager	Rice field in Kied Ngong, Patumphone district, Champasak	2 years	n.i
8	Chinese edible frog (*Haplobatrachus rugulosus*),	Sub sample 1	n.i	Market in Pakse	Rice firled, Phatumpone district, Champasak	3 months	15 cm
		Sub sample 2	n.i	Market in Pakse	Rice firled, Phatumpone district, Champasak	4 months	15 cm
		Sub sample 3	n.i	Market in Pakse	Rice field, Phatumpone district, Champasak	5 months	15 cm
9	Cricket (species unidentified, local name is *chie lor*)	Sub sample 1	>10	Market in Pakse	Rice field, Bahn Kang Ko village, Kongsedon district, Champasak	no eggs	4 cm
		Sub sample 2	>10	Market in Pakse	Rice field, Kongsedon district	no eggs	4 cm
		Sub sample 3	>10	Market in Savannakhet	n.i	no eggs	4 cm
10	Fermented fish sauce 1	Sub sample 1	-	Market in Pakse	n.i	5-12 months	Filtered
		Sub sample 2	-	Market in Pakse	n.i	5-12 months	Filtered
		Sub sample 3	-	Market in Pakse	n.i	5-12 months	Filtered

Annex 1. Continued

No	Species/ sample	Sub sample	n	Source	Origin	Approx age/ maturity	Length[1]
11	Fermented fish sauce 2	Sub sample 1	-	Villagers in Patumphone	Households, Patumpone district, Champasak	5-12 months	Filtered
		Sub sample 2	-	Villager in Champasak	Household, Champasak	5-12 months	Filtered
		Sub sample 3	-	Villagers in Patumphone	Households, Patumphone district, Champasak	5-12 months	Filtered
12	Fermented fish sauce 3	Sub sample 1	-	Market in Artsaphonthong	Traders from Champone district, Savannakhet	5-12 months	Filtered
		Sub sample 2	-	Market in Songkhone	Traders from Savannakhet	5-12 months	Filtered
		Sub sample 3	-	Market in Songkhone	Traders from Savannakhet	5-12 months	Filtered
13	Fermented fish sauce 4	Sub sample 1	-	Villager in Savannakhet	Household, Savannakhet	5-12 months	Filtered
		Sub sample 2	-	Villager in Savannakhet	Household, Savannakhet	5-12 months	Filtered
		Sub sample 3	-	Villager in Savannakhet	Household, Savannakhet	5-12 months	Filtered

[1]n.i indicates no information
[2]Lenght measured based on width of the ventral carapace
[3]Lenght measured based on width of the operculum

Annex 2. Representative pictures of the samples

Swamp Eel (*Monopterus albus*)

Snakehead murrel (*Chana striata*)

Walking catfish (*Clarias batrachus*)

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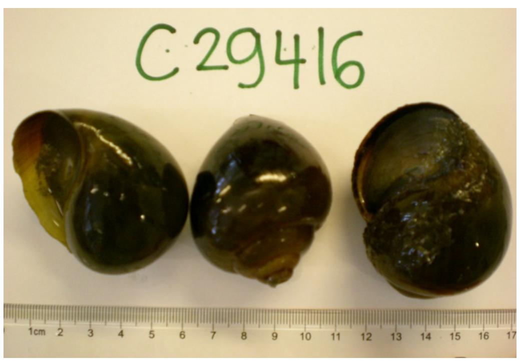
Chinese edible frog (*Haplobatrachus rugulosus*)

Freshwater crab (*Potamon sp*)

Small apple Snail
(*Cipangopaludina chinensis*)

Golden apple Snail (Species not yet identified,
Local name is *Hoy pak kuang*)



Big apple Snail (*Pila sp*)

Cricket (species not yet identified,
local local name is *Chie lor*)

Fermented fish sauce (local name is *Padek*)

Annex 3. General types of Traditional Preparation of Selected Species

No	Species	General preparation types	General edible parts	General discarded parts	Note
1	*Monopterus albus,* local name; *Pa ian* (Swamp eel)	*Om, Poun, Ping, Kheng, Ow, Tom, Cheun, Mouk*	Meat, bones (all but gut), bones, skin, sometimes gut	Gut, head	Some Laotians eat eel as a whole, with the gut and head
2	*Chana striata*, local name; *Pa kor* (Sneakhead Murrel)	*Ping, Kheng, Pon, Cheun, Ow', Padek, Tom, Koy, Mouk,Cheao*	Meat, meat in the head, skin, sometimes gut	Scales, gut, bones	
3	*Clarias batrachus,* local name; *Pa duk* (Walking catfish)	*Ping, Pon, Mouk, Koy, Ow', Jeun, Kheng, Laab, Cheao*	Meat, meat in the head, skin and bones sometimes	Gut, head, bone sometimes	As *Jeun*, fish eaten with the bones, head and skin. As tom and *Laab* only the meat. As *Kheng* eaten with the bones
4	*Haplobatrachus rugulosus*, local name; *Kop na* (Chinese Edible Frogs)	*Ping, Om, Ow', Kheng, Jeun, Tom, Mouk, Yaang*	Meat, head, skin, legs, bones	Gut	Very old frogs that skin very thick and wrinkled normally eaten without skin
6	*Potamon sp,* local name; *Pou* (Freshwater crab)	*Cheun, Chie, Kheng, Poun, Cheo, Laab, Pudong*	All except dorsal and ventral carapace	carapace	few Laotians also discard the legs
7	*Cipangopaludina chinensis*, local name; *Hoy choub* (Small apple Snail)	*Tom, Kheng, Koy, Om, Ow'*	Meat	Shell	Few Laotians also discard the teeth of the snail
8	Species not yet identified, local name; *Hoy pa kuang* (Golden apple snail)	*Tom, Kheng, Koy, Om, Ow'*	Meat	Shells	Some few people also discard the teeth of the snail

Annex 3. Continued

No	Species	General preparation types	General edible parts	General discarded parts	Note
9	*Pila sp*, local name; *Hoy khong* (Big apple snail)	*Cheun, Koy, Ow', Tom*	Meat	Shells	Some few people also discard the teeth of the snail
10	Species not yet identified, local name; *Chie lor* (Cricket)	*Cheun*	Head, meat, carapace	Wings, feet (half part of the leg), gut, tail	Author did not discard the feet
11	Fermented fish sauce, local name; *Padek*	Fermented	Filtered juice	Bones, skull, non dissolved and non filtered part	Sometimes the remaining *padek* after first filer mixed again with water and kept again for sometimes for next fermentation process

Annex 4. Common Methods of Traditional Preparation

Chie

Species normally prepared as *chie*: crab

Thrown into boiling water for 2-3 minutes

After cooking, discard everything except the meat

Ping

Species normally prepared as *ping*: Fishes, frogs

Preparation:

Fish/frogs gutted or for crab - whole

Big size fishes also Scalled

Put on a wooden stick (like barbeque)

Add salt

Grill until well cooked

For crab, abdomen carapace taken away

Eat with rice

Ow'

Species normally prepared as *ow'*: fishes, frogs

Preparation:

Fish gutted

Chopped

Compile salt, fermented fish sauce, seasonings, lemon crush, vegetables (*Ipomia*)

Boil together with the fish in water for 10-20 minutes

Ready for consumption

Om

Species normally prepared as *om*: Fishes, frogs

Preparation:

Fish gutted (some like it with gut),

Chopped

Compile salt, fermented fish sauce, seasonings, lemon crush, vegetables (*Ipomia*)

Boil together with the fish in water for 10-20 minutes

Consume as soup

Snails eaten as *om* prepared with the shells but when eaten, the meat is sucked out by mouth. Some like it better to take out the meat after boiling with water first and then put back in the water with other ingredients.

Ow' and o*m* are different in the amount of water used and served. o*w'* contains more water than o*m*.

<u>Tom</u>

Species normally prepared as *tom*: fishes, snails, frogs

Preparation:

Fish gutted

Chopped

Put species in boiled water

Add salt and vegetables

Eat only the soft part

<u>Kheng</u>

Species normally prepared as *kheng*: snails, fishes

Preparation:

Gutted

Chop

Put into boiling water

Add herbs and spices (garlic, chilli, onion, et)

Add *padek*

Put in vegetables

Add MSG, fish sauce, etc

Boil until well cooked

Edible parts are only soft parts, however some like to consume the whole.

Snails eaten as *kheng* prepared with the shells but when eaten, the meat is sucked out by mouth.

<u>*Poun*</u>

Species normally prepared as *poun*: Crab, fishes

Preparation:

Species cooked first either as Chie or boiled like *jeun* with tamarind and salt.

Remove crab legs and abdomen carapace. Some people prefer to only have the meat

Mince with ingredients (chilli, salt, garlic, fish sauce, vegetables, and the water from boiling the species)

Ready for consumption

<u>*Padek*</u>

Local name of fishes normally used for *padek* preparation: *pa siu*, *pa pak*,

Preparation:

Fishes from one species only or mixed, and washed

Gutted for medium and big fishes, small fishes are not gutted

Salted

Keep for one day or after it smells specific

Add rice bran and mix

Put in a jar

Close the jar and keep it for at least three months

On average, the interviewees said they start to consume *padek* after six months fermentation. Laotians have different way of eating *padek*. Some consume with the fish or only the water that they already filtered. But most of them eat *padek* with the small parts of fish that loosen after fermentation.

<u>Cheao</u>

Species normally prepared as *cheao*: fishes

Preparation:

Boil the fish until well cooked

Take out the meat

Mince the meat

Mixed with garlic and chilli already grilled on fire

Add a little bit of water, MSG and salt

Ready for consumption (normally only the meat, but sometimes with bone when they are soft enough)

<u>Cheun</u>

Species normally prepared as *cheun*: crab, fishes and frogs

Preparation:

Take out the hard parts (dorsal and ventral part for crab, scales for fishes)

Mix with salt and seasoning

Fry in the oil, well cooked and crispy

Details of preparation depend on the species, individual preference and the animals' condition (size, maturity, etc).

<u>Mouk</u>

Species normally prepared as *mouk*: fishes

Preparation:

Scaled

Gutted

Chopped

Put in banana leaves

Add salt, chilli, lemon crush, mixed all together

Steam

Ready for consumption

<u>Koy</u>

Species normally prepared as *koy*: snails

Preparation:

Boil the snail until soft (so it can easily taken out of the shell)

Take out the meat with a tooth pick

Mixed with the following: *padek*, salt, ginger, fish sauce, seasoning, lemon crush, rice, chilli, onion, mint and herbs

Ready for consumption

<u>Laab</u>

Species normally prepared as *laab*: fishes

Preparation:

Gutted

Scaled

Mince the rest

Boil in water with *padek*

Add ingredients (chilli, onion, etc)

Or, instead of boil,

Fry without oil

Ready for consumption

<u>Yaang</u>

Species normally prepared as *laab*: frog

Preparation:

Gutted

Dry under the sun for one day

Keep in an open basket

Fry before consumption

Pudong

Species normally prepared as *pudong*: crab

Preparation:

Cook in boiling water

Add salt

Take out and cool

Put the cold crab into salted cold water

Keep it for five days

Ready for consumption

Annex 5. Representative Pictures from the Field

Laotian Rice Field Ecosystem

Laotian Traditional Dish Serving

Process of cleaning the snails by Laotian Women