

Potential and Efficiency of Underused Agricultural and Fishery Resources in Laos



Edited by Shinsuke Morioka, Katsumi Hasada

March 2020

**Japan International Research Center for Agricultural Sciences
Tsukuba, Ibaraki, Japan**



Cooperated by

National Agriculture and Forestry Research Institute (NAFRI)

and

National University of Laos (NUoL)

Vientiane, Lao P.D.R.



JIRCAS Working Report No. 90

ISSN 1341-710X

Potential and Efficiency of Underused Agricultural and Fishery Resources in Laos

Edited by

Shinsuke Morioka, Katsumi Hasada

March 2020

**Japan International Research Center for Agricultural Sciences (JIRCAS)
Tsukuba, Ibaraki, Japan**

Cooperated by

**National Agriculture and Forestry Research Institute (NAFRI)
Vientiane, Laos**

and

**National University of Laos (NUoL)
Vientiane, Laos**

Preface

Laos is an inland country in which approximately 70% of population (ca. 6.9 million in 2018) practices agriculture and its related industries. This country has shown remarkable economic development with high GDP growth (6-7%/year) in recent decade, but has remained as a least developed country (LDC) with low GDP/capita/year (ca. 2,500 USD in 2017), placing 133rd in GDP ranking among 190 countries. Although Laos has been self-sufficient in terms of rice production since 2000, an increase in food production is still required due to the high rate of increase in national population (1.45%/year). Moreover, deficient animal protein intake (14g/day/capita) is another concern that might have led to a high undernourished population rate (21%) over the country particularly in rural areas, indicating the great need to increase the animal protein supply.

In the context of the above, the Japan International Research Center for Agricultural Sciences (JIRCAS) has implemented various research activities in partnership with the National Agriculture & Forestry Research Institute (NAFRI) and the National University of Laos (NUoL) since 2016 under JIRCAS's "Value-adding technology" research program comprising four projects, namely, "Multiple use of regional resources in semi-mountainous villages", "Food value chain", "Higher value forestry", and "Aquatic production in tropical areas".

Through our vigorous activities, various technical and informative achievements have been attained, and they have contributed greatly to the sectors of agriculture, forestry and fisheries in Laos. These achievements were disseminated as applicable farming techniques, as useful references for central and local administrators, and as assets for scientific research. To summarize the achievements and share the information among researchers and relevant central and regional stakeholders, and to discuss their practical applications in the future, JIRCAS held a workshop entitled "Technical achievements of the projects and their applications - Potential and efficiency of underused agricultural and fishery resources in Laos –" on October 30, 2019 in Vientiane. Ten (10) research topics relevant to lowland/upland rice production and evaluation, agricultural management in mountainous slope areas by tropical fruits and teak, informative research on nutritional conditions in rural villages, and aquaculture of indigenous fish and shrimps, were provided to workshop participants. This Working Report consists of these representative achievements presented in the above workshop and some supplemental outputs. We do hope that this Working Report can provide useful insights from various practices and contribute to their wider application in Laos.

Finally, we would like to express our gratitude and appreciation to JICA and the Japanese Embassy in Laos as well as various local agencies, for their considerable support to our research activities. To end, I would like to thank all the contributors for making this Working Report possible.

Yukiyo Yamamoto
Program Director
JIRCAS

Contents

Preface	-----	i
Section I: Productivity improvement and value addition of lowland/upland rice		
1: Key agronomic traits for variety selection by farmers in upland rice systems of Lao		
	Hidetoshi Asai, Pheunphit Soisouvanh	
	-----	1
2: Toward efficient farm management with remote sensing technologies in lowland rice fields in Laos		
	Kensuke Kawamura, Hiroshi Ikeura, Naruo Matsumoto, Hidetoshi Asai, Sengthong Phongchanmixay, Phanthasin Khanthavong, Soukasdachanh Souvannasing, Thavone Inthavong	
	-----	15
3: Soil–water conditions and efficient fertilization strategies to increase rice grain yield in rainfed lowland fields in Savannakhet Province		
	Hiroshi Ikeura, Sengthong Pongchanmixay, Amphone Chomxaythong, Bounson Bounyavong, Phanthasin Khanthavong, Thanouphone Khanthavong, Naruo Matsumoto, Kensuke Kawamura, Laeh Homsengchanh, Somphone Inkhamseng	
	-----	23
4: Current status of paddy field agriculture and efficient water use in existing reservoirs for fish aquaculture in a semi-mountainous village in Vientiane Province, Laos		
	Hiroshi Ikeura, Toshihiko Anzai, Amphone Chomxaythong, Khaykeo Keokhampui, Salermphon Phonsangone, Phetyasone Xaypanya, Sengthong Pongchanmixay, Somphone Inkhamseng, Somnuck Soubat, Haruyuki Fujimaki	
	-----	39
Section II: Agricultural management in mountainous slope area		
1: Soil suitability map for planted teak (<i>Tectona grandis</i> L.f.) stand growth in the mountainous area of northern Lao People’s Democratic Republic		
	Akihiro Imaaya, Simone Vongkhamho, Phutthavong Sikhot	
	-----	53
2: Collections and genetic diversity analyses of SSR markers for mango native genetic resources in Lao PDR		

Shinsuke Yamanaka, Tatsushi Ogata, Phaythoune Mounsena, Nouphone
Khamsonphou, Hiroshi Matsuda, Bounneang Douangboupha
----- 65

Section III: Nutritional improvement in rural areas and value addition of fishes

- 1:** *Padaek* distribution and business management in Vientiane capital, Lao PDR
Katsumi Hasada, Phonesanith Phonhnachith, Phonevilay Sinavong,
Manivanh Phimpachanvongsod, Shintaro Kobayashi, Junichiro Marui
----- 71
- 2:** Assessment of food access in Laos from macro and micro perspectives
Katsumi Hasada, Souny Phomdouangsy, Phonevilay Sinavong,
Phonesanith Phonhnachith, Manivanh Phimpachanvongsod
----- 79
- 3:** Assessment of usage and fermentation-related components of *pa daek*, a salt-fermented
freshwater fish paste, for household use in a semi-mountainous village in Laos
Junichiro Marui, Katsumi Hasada,
Souphachay Phoupasouk, Sayvisene Boulom
----- 89
- 4:** Fermentation period-dependent changes of lactic and amino acid concentrations in *pa daek*, a
salt-fermented freshwater fish paste in Laos
Junichiro Marui, Yethao Giavang, Souphachay Phoupasouk,
Yanglao Yiale, Sayvisene Boulom
----- 95
- 5:** Nutritional analysis of freshwater fish and shellfish for improving human nutrition in rural area
in Laos
Kaori Fujita, Philavanh Boutsavath, Xaypunya Mahathilath
----- 101

Section IV: Sustainable aquaculture and fisheries

- 1:** A study on larval rearing of the local freshwater shrimp, *Macrobrachium dolatum*, in Laos: a
potential target species for aquaculture
Tomoyuki Okutsu, Aloun Khounthongbang, Phonenaphet Chanthasone,
Phoutsamone Phommachan, Sayaka Ito
----- 109

- 2:** Importance of resources of small-sized fishes as fundamental components of food resources and fish diversity in Lao PDR

Shinsuke Morioka, Bounsong Vongvichith

----- 117

- 3:** Technical achievements of indigenous fish aquaculture in rice-paddy and pond in rural areas of Lao PDR: summarized results obtained by the JIRCAS-LARReC project

Bounsong Vongvichith, Shinsuke Morioka

----- 127

Section I

Productivity improvement and value addition of lowland/upland rice



Rice planting in mountainous village in Laos



Black-colored upland rice in Laos

Key agronomic traits for variety selection by farmers in upland rice systems of Lao

Hidetoshi Asai¹, Pheunphit Soisouvanh²

¹Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

²Rice Research Center (RRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

In the mountainous areas of Lao P.D.R, where upland rice production under shifting cultivation has been a major system for rice security, farmers have grown diverse varieties according to their indigenous knowledge. However, the policy for forest conservation and the shift to a market economy since the 1990s has dramatically changed the nature of shifting cultivation, which potentially affects the farmers' selection of varieties of upland rice. To identify the key agronomic traits for variety selection by upland rice farmers, we conducted a germplasm collection and an interview survey of rice characteristics and cultivation frequency in a total of 26 villages in the Luang Prabang, Vientiane and Sekong Provinces during the period from 2014 to 2015. Characteristics, such as plant height, tiller number, soil adaptability, and maturity, were recorded as categorical parameters with two to five classes; grain shape (length and width) and grain weight were measured as continuous parameters.

We collected 244 accessions from 26 villages. The number of accessions per village was highest in Luang Prabang Province, which proportionately has the largest area of shifting cultivation in Lao. For each characteristic evaluated with the interview survey, a Pearson's chi-square test was performed to compare the frequency distribution between the primary genotype group ($n=27$), which were the genotypes with the highest cultivation frequency in each village, and the total population ($n=244$). The results showed a significant difference in two characteristics: environmental adaptability and maturity. The frequency distribution of environmental adaptability to poor, moderate and good environments was 29%, 55% and 24% for the total population but 15%, 22% and 63% for the primary genotype group. The frequency distribution of maturity for early, middle and late maturity was 19%, 30% and 51% for the total population but 7%, 11% and 82% for the primary genotype group. These results indicate that upland rice farmers in Lao tend to prefer varieties with late maturity that are adapted to good environments.

Introduction

The Lao P.D.R. (hereafter, Lao) is a landlocked country in Southeast Asia and has the second highest annual rice consumption per capita in the world (approximately 170 kg per capita,

GRiSP, 2013). Traditionally, shifting cultivation has been one of the dominant rice ecosystems in Lao due to the geographic characteristics of the country; mountainous terrain occupies 70% of the total land area. Particularly in the northern region, upland rice production has been a key practice for food security for the resource-poor farmer with 60% of rice producers engaged in shifting cultivation (Steering Committee for the Agricultural Census 2012).

Lao is the one of the countries with the largest genetic diversity of rice (Rao et al. 2002). Due to the erratic rainfall and lack of fertilizer input, the yield is generally low, approximately 1.7 t ha⁻¹ (Committee for Planning and Investment 2006; Ministry of Planning and Investment 2013). To achieve stable rice production under such poor conditions, upland rice farmers utilize their genetic resources according to the local indigenous knowledge, which is based on their long-term experience. In addition, the cultivation of multiple varieties in a single field, which is practiced for convenient management, accelerates natural interbreeding on farms and consequently promotes genetic diversification in upland rice (Rao et al. 2006). There has been growing concern regarding rice diversity conservation in Lao; germplasm surveys have been widely conducted across Lao since the 1990s (Sato 1991; Roder et al. 1996; Rao et al. 2002; Kuroda et al. 2006).

Changes in socioeconomic conditions during the past decade have greatly influenced shifting cultivation in Lao. To control shifting cultivation and conserve forest area, the land allocation policy, which sets a clear boundary for the agricultural zone and the conservation forest zone within a territory of each village, was implemented in 1996. This led to a decrease in arable land and resulted in a shortened fallow period with a reduction from 40 years in 1950 to 2–3 years in 2000 in some areas (Roder 2001; Linqvist et al. 2007). Shortened fallow periods caused soil degradation and decreased yield leading to a rice shortage at the household level (Ducourtieux and Castella 2006; Saito et al. 2006a). However, in 2000's, market economy has rapidly extended even into the rural area and diversified the livelihood of local farmers, resulting in the increasing opportunities for cash crop production and for off-farm works. Such changes also have led the local farmers to secure rice sufficiency at household level not by self-production, but by purchase at market. Overall, the demand on shifting cultivation for rice sufficiency has decreased. At the national level, there has been a gradual decrease in the area involved in shifting cultivation (Hunri et al. 2013).

Understandably, these socioeconomic changes have influenced the farmers' selection of varieties. After implementation of the land allocation policy, farmers initiatively adopted varieties with early maturity and adaptability to poor soil conditions; early maturity made it possible to harvest during the period when rice is least available and poor soil adaptability contributed to stable rice production under the conditions created by short fallow periods (Songyikhangsuthor et al. 2002; Saito et al. 2006a, b). During this period, the participatory variety selection program selected promising traditional varieties with the desirable traits of early maturity and yield superiority under poor soil conditions (Linqvist et al. 2006). However, it is unclear how the current diversification of livelihood and the trend towards decreasing shifting cultivation influence the farmers' selection of rice varieties.

The objective of this study is to investigate the key traits for farmers' selection of varieties

for upland rice production in shifting cultivation ecosystems in Lao. We conducted a survey in 26 villages of three provinces: Luang Prabang (north), Vientiane (central) and Sekong (south). In this survey, we collected information on agronomic traits such as maturity, plant height, lodging resistance, etc., for each variety and identified the primary genotypes that were cultivated most frequently for each village. From the morphological data, we identified which agronomic traits were the key parameters for the selection of these primary genotypes.

Materials and Methods

Study area

We conducted the survey in 26 villages of three provinces: Luang Prabang (north), Vientiane (central) and Sekong (south) (Table 1). The survey villages were located on the main road with good accessibility. Thus, their location has the socioeconomic background such that local livelihoods are easily influenced by the market economy and forest conservation policy.

All the villages surveyed in this study were engaged in the agronomic activity of upland rice production, however, the ethnic backgrounds differed among the regions (Table 1). Officially, Lao is composed of 49 ethnic groups that are geographically categorized into three groups of Lowland Lao, Midland Lao and Highland Lao (State Planning Committee 1997). Generally, Lowland Lao is engaged in lowland rice production, whereas Midland and Highland Lao are engaged in upland rice production. In Lao, the staple food is glutinous rice; the exception is the Hmong group, a major ethnic group of Highland Lao who eat non-glutinous rice as a staple food (Roder 1996).

Germplasm collection

The germplasm survey was conducted during the dry season from December 2014 to March 2015. In each village, we collected all the upland rice varieties and interviewed farmers regarding the agronomic traits and cultivation frequency for all of the collected varieties. In this survey, we collected a total of 244 varieties from 26 villages. With regard to ethnic groups, Midland Lao comprises the Khum in the Luang Prabang and Vientiane Provinces, whereas Sekong Province comprises the Katou in the Galun district and the Kriang in the Dak Chun district. Lowland Lao and Highland Lao include the Lao Loum and Hmong, respectively.

Table 1. Village list for rice genotype collection.

District Name	Village Name	Number of households ¹⁾				% of upland farmers	Num of genotypes	
		Lowland Lao	Midland Lao	Highland Lao	Total		Glutinous	Non-glutinous
Lurang Prabang (Northern Lao)								
Xiengngeun	Kiew mai lo	2	59	4	65	100	12	3
	Phonexay	26	81	0	107	60	13	1
	Kiew Ya	0	100	26	126	100	18	4
Phonexay	Houay King	0	130	96	226	100	15	3
	Houa Meuang	0	87	0	87	100	17	2
	Tha pho	42	113	0	155	60	15	1
Pakou	Latthahae	116	0	0	116	50	8	2
	Houay lo	43	139	33	215	75	11	5
	Houay kang	0	70	0	70	70	9	2
Vientiane (Central Lao)								
Meun	Namphuoun	100	400	10	510	30	11	3
	Namphone	12	1	505	518	40	3	3
	Phonseng	126	0	308	434	40	2	3
Met	Houay pa mak	2	182	0	184	80	9	0
	Nadi	132	19	0	151	10	1	0
	Seesaath nua	50	11	0	61	50	8	1
	Na do kun	0	53	0	53	40	11	1
Vanvieng	Na mou nua	3	0	176	179	20	4	3
	Nan part	35	30	0	65	40	5	0
	Keo kun	43	64	0	107	30	3	0
Fuang	Nameuang	38	105	0	143	65	18	4
Sekong (Southern Lao)								
Galun	Chun	0	33	0	33	100	5	1
	Chok	0	45	0	45	95	5	1
	Sonkong	0	69	0	69	90	5	0
Dak Chun	Dak doum	0	43	0	43	95	4	2
	Cha lun	0	114	0	114	80	3	4
	Dao doum	0	14	0	14	100	4	2
(Average)								
Luang Prabang		25	87	18	129.7	79.4	13.1	2.6
Vientiane		49	79	91	218.6	40.5	6.8	1.6
Sekong		0	53	0	190	42	6	3

Interview survey and grain shape measurement

In the interview survey, agronomic traits were collected as categorical parameters with two to five classes: 1) endosperm type (non-glutinous, glutinous), 2) maturity (very early, early, moderate, late, very late), 3) plant height (short, moderate, long), 4) tiller number (small, moderate, many), 5) lodging (non-resistant, moderate, resistant), 6) soil adaptability (poor soil, moderate soil, good soil), 7) aroma (non-aromatic, moderate, strong), and 8) grain color (white, colored (black or red)). We also identified the varieties with the highest cultivation frequency in each village. In total, 27 varieties were identified from 26 villages because two varieties were selected in one of these villages. These 27 varieties were categorized as the primary genotype group.

For all the collected varieties, grain shape and grain weight were measured at the Rice Research Center, Vientiane, Lao. For grain weight determination, the total weight and moisture

content of 500 grains were measured twice and then averaged with a moisture correction of 14%. For the grain shape measurement, we estimated the parameters of grain width and grain length using the image analysis software “SmartGrain” developed by Tanabana et al. (2012); 70 to 100 grains were scanned at a resolution of 400 dpi. Based on the morphological data, we categorized each variety as one of three subspecies (tropical japonica, indica or temperate japonica) using the categorization method proposed by Matsuo (1952).

Statistical analysis

To identify the key agronomic traits of the farmers’ selection of varieties, we compared the frequency distribution between the primary genotype group ($n = 27$) and the total population ($n = 244$) for each trait. When the primary genotype group exhibited a different frequency distribution of, for example, trait “A” in comparison with that of the total population, the primary genotypes were interpreted as intentionally selected from the perspective of trait “A”, indicating that trait “A” is a key trait in the farmers’ selection of variety. Based on this concept, we used Pearson’s chi square test to compare the frequency distribution between the two groups using JMP 10.1 software (SAS, Inc., Tokyo).

Results and Discussion

As Table 1 shows, the availability of upland rice varieties was very different among the regions. The number of varieties was highest in Luang Prabang (15.7 varieties per village), where upland rice is still cultivated widely, followed by Vientiane (8.5 varieties per village) and Sekong (6.0 varieties per village). Generally, the number of glutinous varieties was much greater than the number of non-glutinous varieties of rice; although the non-glutinous varieties were popular in particular locations, in the highland Lao population, which consumes non-glutinous rice as a staple food (Namphone village, etc.), or villages located near the Vietnam border such as villages in the Dak Chun district, which sell non-glutinous rice to Vietnam, non-glutinous varieties were in the majority. Even in Vientiane Province, the villages with large populations in midland Lao, particularly the Khum tribe, also exhibited a larger number of varieties. These results indicate that genotype availability could be affected by both regional factors and ethnic factors.

Each variety was categorized as one of three subspecies using the categorization method of Matsuo (1952) (Fig. 1). The majority of varieties were categorized as tropical japonica, whereas indica ($n = 22$) and temperate japonica ($n = 1$) were in the minority. This result was consistent with the results of previous studies (Sato 1996, Roder et al. 1996), which reported that upland rice in Lao is mainly composed of the tropical japonica type, suggesting that the ecotype may be similar among the three regions.

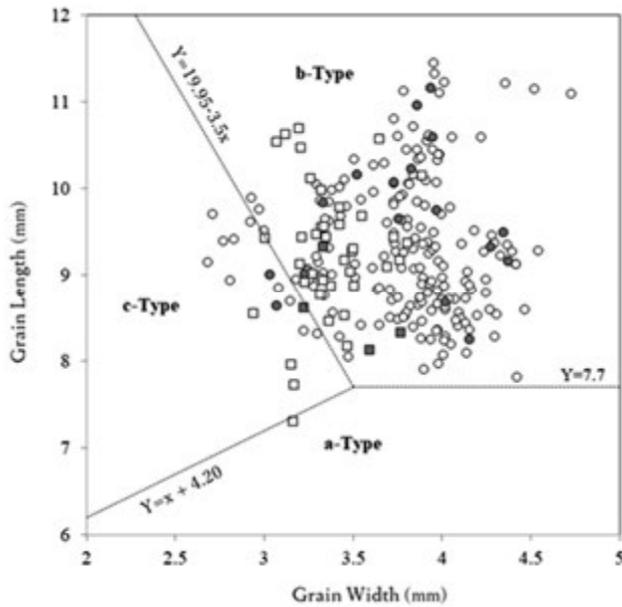


Fig. 1. Grain shape of glutinous and non-glutinous genotypes. Each accession was categorized as one of three types using the method developed by Matsuo (1952): a-type (temperate japonica), b-type (tropical japonica) and c-type (indica). The circle and square symbols represent the glutinous and non-glutinous genotypes, respectively. The closed symbols are the primary genotypes in each village (Asai et al. 2016).

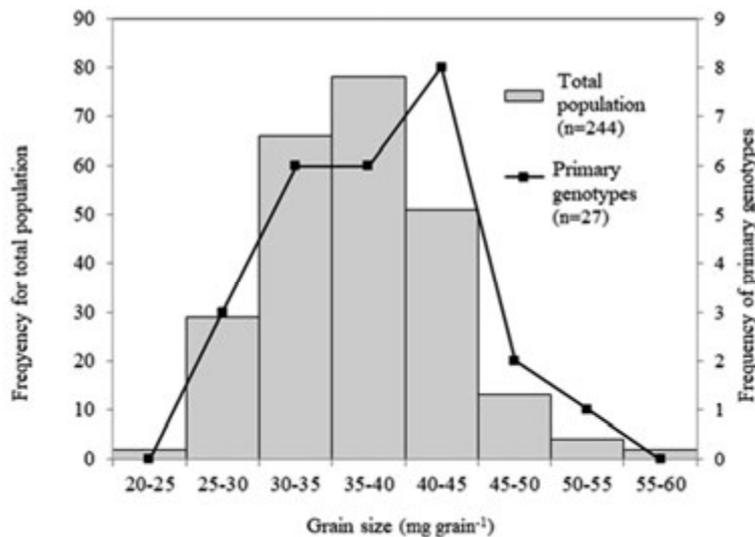


Fig. 2. Frequency distribution of grain size for the total population and primary genotypes. Pearson's chi-squared test indicated that the frequency distributions were not significantly different between the two groups (Asai et al. 2016).

The genotypic variation in grain weight was very large, ranging from 22 mg to 58 mg (Fig. 2). A statistical comparison of grain shape between glutinous and non-glutinous varieties showed that glutinous rice has a greater grain width (3.8 mm vs 3.4 mm) but no difference in grain length,

and thus glutinous rice has a higher grain weight. With regard to the ecotype, there were no significant differences in grain weight and grain shape (length and width) among three regions.

The frequency distributions for each parameter are presented in Table 2. With the exception of the parameters of endosperm and grain color, these categorical parameters should be carefully interpreted. Because the categorization was conducted within each village, the criteria may differ among the villages. This implies that the variation within a single category might be high. Rao et al. (2006) noted a similar issue in that the varieties categorized as early maturity exhibited variation in the number of days to harvesting (from 90 days to 120 days). To determine the reliability of the dataset, we focused on the relationship between plant height and lodging resistance. It is well known that plant height is a key parameter determining lodging resistance (Jones et al. 1997). Thus, we evaluated whether this relationship was observable within our dataset. The results clearly showed that the proportion of lodging-resistant varieties was higher in the short plant height group than in the high plant height group, as the hypothesis predicts (Fig. 3), indicating that our dataset might be not sufficiently robust to precisely identify the phenotypes of each individual variety, but it could be applied to identify the overall trends for each parameter.

Table 2. Frequency distribution of each characteristic for the total population and the primary genotypes based on Pearson's chi-squared test.

Interview topic	Answer	Total population (n=244)		Primary genotypes (n=27)		Probability ratio (Pearson's chi-squared test)
		Frequency (accessions)	Ratio (%)	Frequency (accessions)	Ratio (%)	
Endspem type	Non-glutinous	48	19.7	5	18.5	0.96 ns
	Glutinous	196	80.3	22	81.5	
Maturity	Very Early	5	2.0	0	0.0	<0.05
	Early	42	17.3	2	7.4	
	Moderate	72	29.6	3	11.1	
	Late	98	40.3	15	55.6	
	Very Late	26	10.7	7	25.9	
Plant height	Short	29	11.9	3	11.1	0.66 ns
	Moderate	92	37.7	8	29.6	
	Long	123	50.4	16	59.2	
Tiller number	Small	8	3.2	1	3.7	0.25 ns
	Moderate	148	60.7	12	44.4	
	Many	88	36.1	14	51.8	
Lodging	Non-resistant	51	20.9	7	25.9	0.77 ns
	Moderate	134	54.9	13	48.1	
	Resistant	59	24.2	7	25.9	
Environment adaptability	Poor environment	68	27.9	4	14.8	<0.01
	Moderate environment	107	43.9	6	22.2	
	Good environment	69	28.2	17	63.0	
Aroma	Non-aromatic	29	11.8	1	3.8	0.34 ns
	Moderate	177	72.2	23	85.2	
	Strong	39	15.9	3	11.5	
Grain color	White	192	78.3	24	88.9	0.38 ns
	Black or Red	53	21.6	3	11.1	

*ns, not significant.

The results from the Pearson's chi-square test indicated a significant difference in the frequency distribution between the primary genotype group and total population for environment adaptability ($p < 0.01$) and maturity ($P < 0.05$). For environment adaptability, the proportion of "poor environment", "moderate environment" and "good environment" was 28%, 44% and 28% for the total population, respectively, but 15%, 22% and 63% for the primary genotype group, respectively. For maturity, the proportion of "very early and early", "moderate" and "very late and late" was 19%, 30% and 51% for the total population, respectively, but 7%, 11% and 82% for the primary genotype group, respectively. These results indicated that the primary genotype group has the traits of late maturity and adaptability to good soil. This result is consistent with a case study in Vientiane (Asai et al. 2017) that reported that in Nameuang village, where shifting cultivation was widely practiced, 78% of the total seed sown was categorized as the late maturity type and 70% of the total seed sown was adapted to good soil. These findings suggest that late

maturity and adaptability to good soil play a key role in upland rice production in Lao.

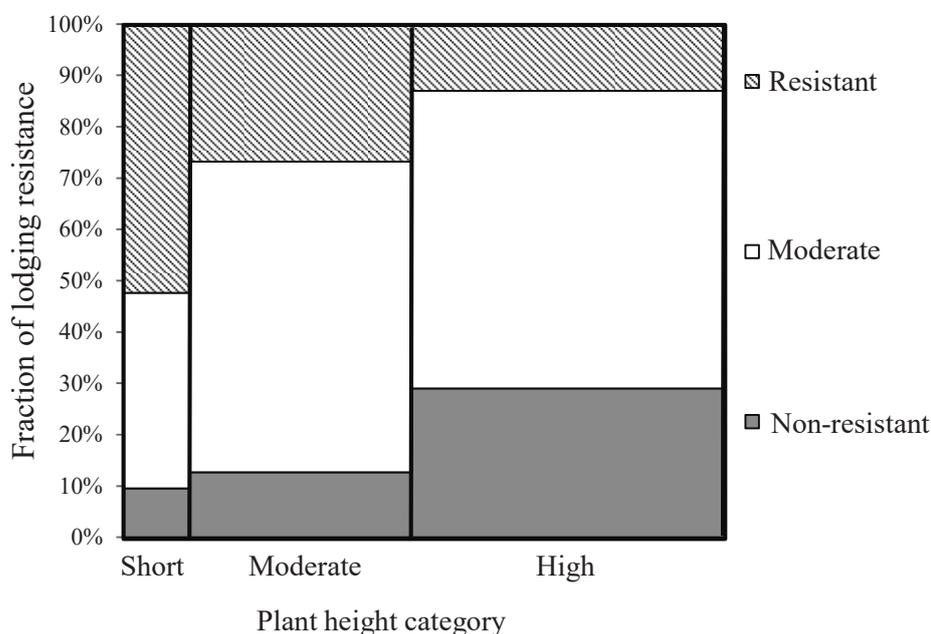


Fig. 3. Relationship between lodging resistance and plant height (Asai et al. 2016).

This trend in the farmers' selection of varieties should be interpreted in context of the socioeconomic situation, particularly the land allocation policy of the 1990s and the subsequent expansion of the market economy in the 2000s. When the land application policy was put into effect, farmers actively adopted varieties with adaptability to poor soil and early maturity to overcome the soil degradation and to shorten the period of rice insufficiency (Songyikhangthour et al. 2002; Saito et al. 2006b). However, our results demonstrate that the trend in farmer variety selection shifted from early maturity and adaptability to poor soil in the 1990s to late maturity and adaptability to good soil in the present. The background for this shift is that the demand for early maturity and adaptability to poor soil has decreased, in part because land use intensity has decreased due to the declining trend in shifting cultivation and in part because the approaches to rice security have been diversified, even in rural areas, due to the expansion of the cash crop market and labor employment opportunities (Nakatsuji 2013). Upland farmers in Lao generally recognize that some of the cash crops such as sesame and Job's tears are more adaptable to poor soil environments than upland rice (Nakatsuji 2004; Saito et al. 2006b); this was also verified in on-farm trials (Asai and Pheunphit 2017). Under the current conditions of the expanding market for cash crops, local farmers may have concluded that under poor soil conditions, cash crop production could be more economically beneficial than upland rice production. In other words, the current high demand for adaptability to good soil implies that upland rice production should be practiced not under poor soil conditions, but under good soil conditions.

It is widely accepted that the late maturity trait is disadvantageous for rice production under

rainfed conditions where the rainfall pattern is generally erratic, because upland rice is susceptible to damage from terminal drought (Kamoshita et al. 2008). However, our results indicate that upland farmers preferentially grow the late maturity types. This is in part because local farmers believe that the long vegetative period of late maturity types could be beneficial for upland rice production, particularly under good soil conditions. A previous case study also reported that the late maturity type was mainly grown on fertile soils, whereas the early maturity type was grown on infertile soils (Asai et al. 2017). This suggests that the wide acceptance of late maturity was closely related to the environmental shift in rice cultivation from poor soils to good soils. With regard to the risk of drought damage, the majority of upland farmers believe that drought after sowing is more critical to rice production, leading to poor germination and often resulting in resowing. In contrast, there are few farmers who regard the terminal drought as a yield constraint. Although the reason is unclear, Kiyono et al. (2008) pointed out that in the mountainous areas of Lao, morning fog rises every day in the dry season and could supply a considerable amount of water to soil even without a precipitation event. Thus, it is possible that the damage from terminal drought is minimized by the water supply from morning fog.

In Lao, the Upland Agriculture Research Center has the functional roles of seed production and distribution. Four upland rice varieties selected in the late 1990s have been distributed to smallholders as recommended varieties. However, all of these varieties were categorized as early or middle maturity and adaptable to poor soil. Therefore, the new variety selection for the late maturity type needs to be advanced, particularly in good soil environments. The maturity trait is relatively easy to evaluate, whereas the evaluation of the soil adaptability trait is time-consuming and labor-intensive, because a multilocation trial is inevitable. Our dataset demonstrated that the trend for environment adaptability has a relationship with tiller number and grain weight (Fig. 4). The group with adaptability to poor soil has the higher proportion of varieties producing many tillers and small grain weight, whereas the group with adaptability to good soil shows the opposite trends. These trends were consistent with previous studies, which reported that the indica varieties, which have a high tillering capacity and small grain size, had better yield performance under poor soil conditions (Linguist et al. 2006; Saito et al. 2007; Asai et al. 2009, 2017). These results indicate that information on tiller number and grain size can be simple parameters for soil adaptability classification. Our study concluded that under the current socioeconomic conditions, farmers prefer to grow varieties with late maturity and adaptability to good soil. However, these desirable agronomic traits were not always sufficient to account for farmers' variety selection. Saito et al. (2007) reported that despite superior yield performance, several improved varieties were not highly valued because these varieties are non-glutinous and have poor grain texture. Therefore, grain quality needs to be considered in new variety selection.

In the upland study in progress, we focused not only the productivity but also value addition, especially on grain texture and functional metabolite in rice. In this presentation, we explained the research activities to identify the productive rice, the healthy rice and delicious rice (Fig.5) For productive rice, the participatory variety trial is in progress to identify the recommended variety for seed distribution. For healthy rice, we are using the latest mass spectrometry

technology to identify the healthy rice with high anti-oxidant effects. For delicious rice, the grain quality were measured by RVA (rapid viscosity analyzer) to understand the texture variation in Lao rice genotypes. Genome sequence with next generation sequencer was performed for all of these genotypes used for texture analysis and metabolite analysis in order to conduct the coupling analysis between phenotype and genotype. These genotypes, named as “Lao Upland Rice Core Collection” will be distributed for academic use with genome sequence data in future.

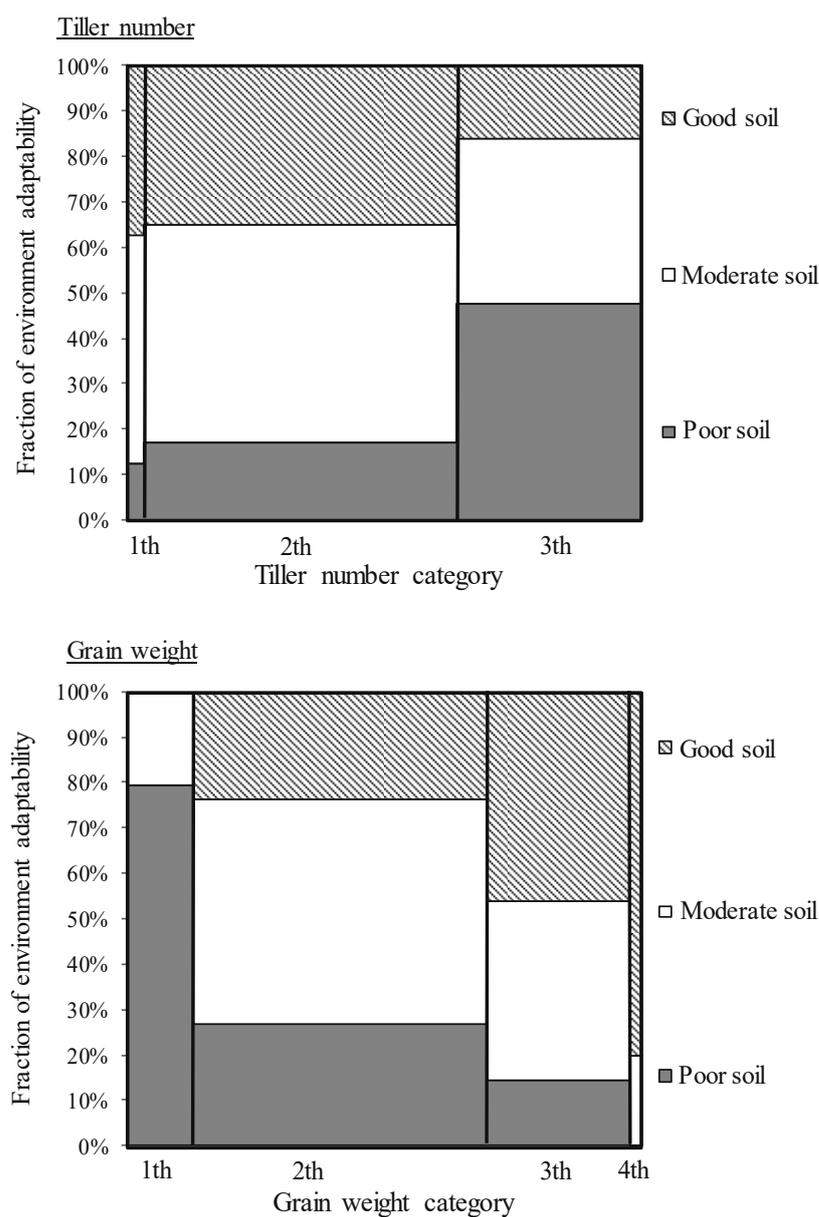


Fig. 4. Mosaic graphs of the relationship between soil adaptability and tiller number (above) and grain weight (below) (Asai et al. 2016).

For the tiller number categories, 1st indicates little tillering, 2nd indicates moderate tillering, and 3rd indicates high tillering. For the grain weight categories, 1st indicates 20 to 30 mg grain⁻¹, 2nd indicates 30 to 40 mg grain⁻¹, 3rd indicates 40 to 50 mg grain⁻¹, and 4th indicates 50 to 60 mg grain⁻¹.

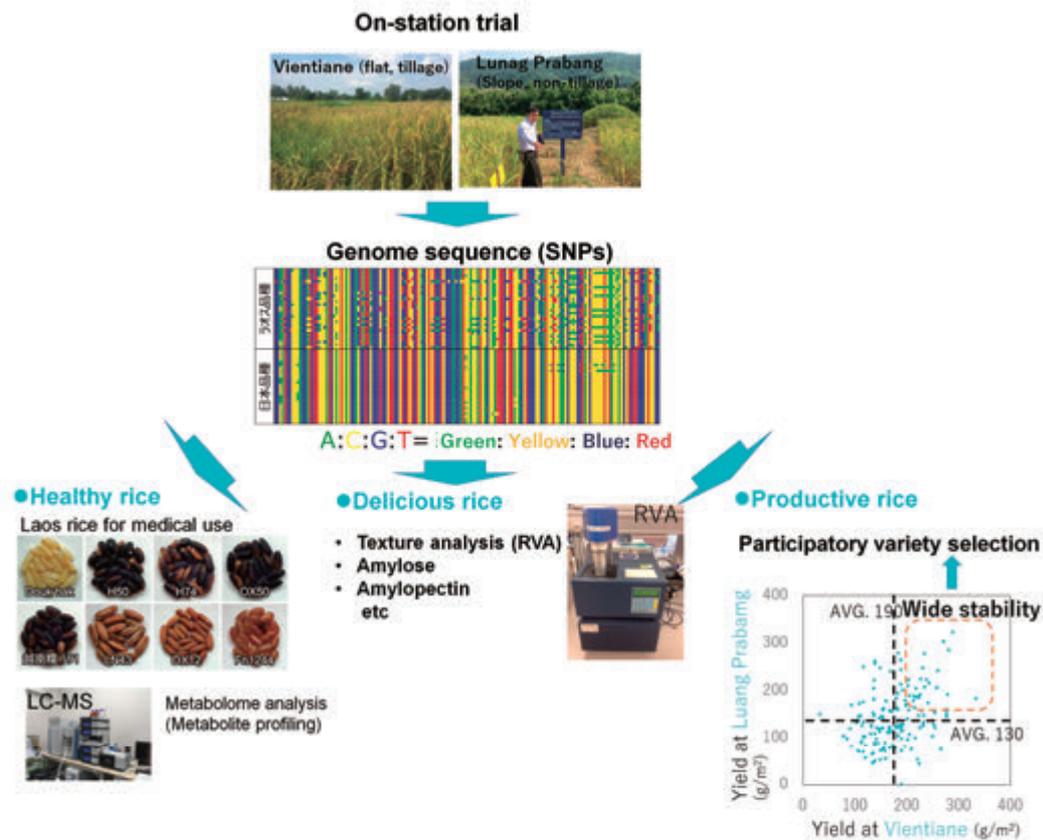


Fig. 5. Research activities for identification for productive, healthy and delicious rice.

Acknowledgements

I would like to thank Mr. Vilaxay Kabae and the research staff for assistance with the germination collection and the interview survey.

References

- Asai H, Saito K, Samson B, Songyikhangsuthor K, Homma K, Shiraiwa T, Kiyono Y, Inoue Y, Horie T (2009) Yield response of indica and tropical japonica genotypes to soil fertility conditions under rainfed uplands in northern Laos. *Field Crop Res* 112:141-148
- Asai H, Soisouvanh P (2017) Yield performance of upland rice, maize and Job's tears in a rainfed upland ecosystem in mountainous Laos. *Jap Aqril Res Quart* 51:309-318
- Asai H, Soisouvanh P, Sengxua P, Kimura K, Vongphuthine B, Ando M (2017) Labour-saving practices for the external expansion of swidden agriculture for upland rice production in a mountainous area of Laos. *Tropic Agri Dev* 61:166-178
- Asai H, Soisouvanh P, Vongphuthine B, Sengxua P (2016) Identification of key agronomic traits for farmer's variety selection in rainfed upland rice systems of Laos. *Res Tropic Agricul* 9:52-58
- Committee for Planning and Investment (2006) 1975–2005 Basic statistics, The Socio-economic development in the Lao P.D.R. Vientiane

- Ducourtieux O, Castella J.C. (2006) Land reforms and impact on land use in the uplands of Vietnam and Laos: Environmental protection or poverty alleviation? In: Proceedings of Colloque international “Les frontieres de la question fonciere – At the frontier of land issues”, Montpellier. pp. 2
- Global Rice Science Partnership (GRiSP) (2013) Rice almanac, 4th edition. International Rice Research Institute, Los Baños
- Hurni K, Hett C, Heinimann A, Messerli P, Wiesmann U (2013) Dynamics of shifting cultivation landscapes in Northern Lao PDR between 2000 and 2009 based on an analysis of MODIS time series and Landsat images. *Human Ecol* 41:21-36
- Jones MP, Mande S, Aluko K (1997) Diversity and potential of *Oryza glaberrima* steud in upland rice breeding. *Breed Sci* 47:395-398
- Kamoshita A, Babu RC, Boopathi NM, Fukai S (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crop Res* 109:1-23
- Kiyono Y, Chiba Y, Asai H, Shiraiwa T, Inoue Y (2008) Growth rates of slash-and-burn fallowed forests and humidity before the sunrise in the dry season in mountainous areas of northern Lao People’s Democratic Republic. *Kanto Shinrin Kenkyuu* 59:149-152 (in Japanese with English abstract)
- Kuroda Y, Rao SA, Bounphanousay C, Kongphanh K, Iwata A, Tanaka K, Sato YI (2006) Diversity of wild and weedy rice in Laos. In: Chanphengxay S et al. (eds) *Rice in Laos*. International Rice Research Institute, Los Baños. pp. 215–234
- Linquist B, Saito K, Keoboualapha B, Phengchan S, Phanthaboon K (2006) Improving upland rice-based cropping systems in Laos. In: Chanphengxay S et al. (eds) *Rice in Laos*. International Rice Research Institute, Los Baños. pp. 391–407
- Linquist B, Trosch K, Pandey S, Phouynyavong K, Guenat D (2007) Montane paddy rice: its development and effects on food security and livelihood activities of highland Lao farmers. *Mountain Res Dev* 27:40–47
- Matsuo T (1952) Bulletin of the National Institute of Agricultural Sciences D3.
- Ministry of Planning and Investment (2013) *Statistical Yearbook 2013*.
- Nakatsuji S (2004) Changes in slash-and-burn agriculture after the introduction of cash crops in the hilly areas of Laos: a case study of number 10 village, Xiengngeun District, Luang Prabang Province. *Human Geography* 56:449–469
- Nakatsuji S (2013) Differences in the importance of shifting cultivation among villages in northern Laos: a case study of 14 villages in Xieng Ngeun District, Luang Prabang Province. *Human Geography* 65:339–356
- Rao SA, Bounphanousay C, Schiller JM, Alcantara AP, Jackson MT (2002) Naming of traditional rice varieties by farmers in the Lao PDR. *Gen Res Crop Evol* 49:83–89
- Rao SA, Schiller JM, Bounphanousay C, Jackson MT (2006) Diversity within the traditional rice varieties of Laos. In: Chanphengxay S et al. (eds) *Rice in Laos*. International Rice Research Institute, Los Baños. pp. 123–140
- Roder W (2001) Slash-and-burn cropping systems in northern Lao; description, challenges and opportunities. International Rice Research Institute, Los Baños

- Roder W, Keobounlapha B, Vannalath K, Phouravanh B (1996) Glutinous rice and its importance for hill farmers in Laos. *Economic Botany* 50:401–408
- Saito K, Atlin GN, Linguist B, Phanthaboon K, Shiraiwa T, Horie T (2007) Performance of traditional and improved upland rice cultivars under nonfertilized and fertilized conditions in northern Laos. *Crop Sci* 47:2473–2481
- Saito K, Linguist B, Keobualapha B, Phanthaboon K, Shiraiwa T, Horie T (2006a) Cropping intensity and rainfall effects on upland rice yields in northern Laos. *Plant and Soil* 284:175–185.
- Saito K, Linguist B, Keobualapha B, Shiraiwa T, Horie T (2006b) Farmers' knowledge of soils in relation to cropping practices: A case study of farmers in upland rice based slash-and-burn systems of northern Laos. *Geoderma* 136:64–74
- Sato Y (1991) Variation in spikelet shape of the indica and japonica rice cultivars in Asian origins. *Jap J Breed* 41:121–134
- Songyikhangsuthor K, Atlin GN, Phengchanh S, Linguist B (2002) Participatory varietal selection: Lessons learned from the Lao upland programme. *Breeding rainfed rice for drought-prone environments: Integrating conventional and participatory plant breeding in South and Southeast Asia*. In: *Proceedings of a DFID Plant Sciences Research Programme/IRRI Conference, Los Baños*, pp. 49–52
- State planning Committee (1997) *Results from the Population Census 1995*
- Steering Committee for the Agricultural Census (2012) *Lao census of Agriculture 2010/11*. National Statistic Center, Vientiane
- Tanabata T, Shibaya T, Hori K, Ebana K, Yano M (2012) SmartGrain: high-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiol* 160:1871–1880

Toward efficient farm management with remote sensing technologies in lowland rice fields in Laos

Kensuke Kawamura¹, Hiroshi Ikeura¹, Naruo Matsumoto¹, Hidetoshi Asai¹, Sengthong Phongchanmixay², Phanthasin Khanthavong³, Soukasdachanh Souvannasing⁴, Thavone Inthavong⁴

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Rice Research Center, National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

³ Maize and Cash Crop Research Center, National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

⁴ Research Centre for Climate Change Resilience in Agriculture, National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

This paper reports our current activities in Laos for monitoring rice production with remote sensing technologies in lowland field toward efficient farm management at small farm scale (<1 km²) or local scale (village, <100 km²). At farm scale (Research 1), the grain yield of lowland rice was evaluated from field hyperspectral data of paddy fields during the reproductive stage to the ripening stage in conjunction with iterative stepwise elimination partial least squares (ISE-PLS) regression. At local scale (Research 2), we established a land infrastructure data set in a lowland rice field at Koudkher village combining with UAV images and geographic information system (GIS) in order to assess the potential yield and its environmental effects. In Research 1, the highest R^2 values and the lowest root mean squared error of cross-validation (RMSECV) values were obtained from the ISE-PLS model at the booting stage ($R^2 = 0.873$, RMSECV = 22.903); the residual predictive deviation was >2.4. Selected hyperspectral (HS) wavebands in the ISE-PLS model were identified in the red-edge (710–740 nm) and near-infrared (830 nm) regions. These results confirmed that the booting stage might be the best time for in-season rice grain assessment and that rice yield could be evaluated accurately from the HS sensing data via the ISE-PLS model. In Research 2, a GIS-based land infrastructure information dataset was established in rain-fed paddy field (Koudkher village) from UAV images and digital terrain model. Using the data set, Ikeura et al. (2019) assessed the relationship between rice yield and water/soil conditions in the rainfed rice fields in Koudkher village.

Introduction

Lao People's Democratic Republic (Laos) is one of the major rice (*Oryza sativa* L.)-consuming countries in South-East Asia (Partnership, 2013). While Laos achieved a self-sufficient rice production status in the late 1990s, and the national economy has continuously

grown, rice remains the main staple food for people in Laos, and its demand is continuously growing (World Bank 2012). For example, the government of Laos plans to increase rice production not only for domestic consumption but also for export, with the goal of producing 4.7 million tons in 2020 and 5.0 million tons in 2025 (MAF 2015). Thus, improvements of rice yield and efficient management are important for rice sector and farmers.

Nowadays, remotely sensed imageries from satellite or aircraft become a practical tool for monitoring agricultural production at regional or global scales (Atzberger 2013; Doraiswamy et al. 2003; Sakamoto et al. 2013). However, the satellite platforms have limited ability to assess crop production at a farm or local scale applications because of coarse spatial (pixel) resolutions, infrequent coverage, clouds, and slow delivery of information to users. Such difficulties related to spatial and temporal resolutions can recently be overcome using low-altitude platform remote sensing technologies, such as balloon, unmanned aerial vehicle (UAV).

Currently, a large number of studies are underway to realize precision farming with UAV based remote sensing technologies (Zhang and Kovacs 2012). Moreover, recent advances in sensor technologies provide large opportunities for assessing crop production and nutritive status (Inoue et al. 2012; Wang et al. 2014). In this paper, we report our current activities in Laos, including two topics as Research 1: rice grain assessment at ground scale with canopy hyperspectral measurements (Kawamura et al. 2018b); and Research 2: establishment of land infrastructure data set at local scale combining with UAV images and digital terrain model (DTM) based on a geographic information system (GIS) (Kawamura et al. 2018a).

In site specific fertilizer management, in-season assessment of rice grain yield could benefit for farmers to improve productivity, and for rice-processing industries by quantifying produce supply and market prices. Therefore, Research 1 tried to clarify the optimal timing for assessing rice yield from field hyperspectral (HS) measurement at an experimental lowland rice field (Kawamura et al. 2018b). In order to scaling up from farm to local scale, in Research 2, land infrastructure information was established in a rain-fed paddy field in a selected village (Koudkher village) (Kawamura et al. 2018a).

Materials and methods

Study area

Fig. 1 shows study areas of the Rice Research Center (RRC) and Koudkher village for Researches 1 and 2, respectively, with the UAV images. Research 1 was conducted in an experimental field at the RRC of National Agriculture and Forestry Research Institute (NAFRI), in the central part of Vientiane in Laos (Fig. 1b). This area has a hot, humid summer season and belongs to a tropical climate ('Aw' in Köppen's climate classification). The mean annual temperature is 25°C, and the annual precipitation is 1,622 mm. The soil type is characterized by clay loam (0–30 cm) and light clay (40–60 cm).

Research 2 was conducted in Koudkher village, Outhoumphone District, Savannakhet Province, Laos (Fig. 1c). The Koudkher village locates approximately 300 km to the southeast of

the Vientiane capital, and 34 km to the east of Savannakhet city (Ikeura et al. 2019). The mean annual temperature is 26.1°C (average from 2014, 2016 and 2017), and the annual precipitation is 1,533 mm (average from 2001 to 2013). The soil type is classified as sand and loamy sand at the surface layer (0–20 cm), and sand, loamy sand, sandy loam, sandy clay loam, and sandy clay at the subsurface layer (20–40 cm).

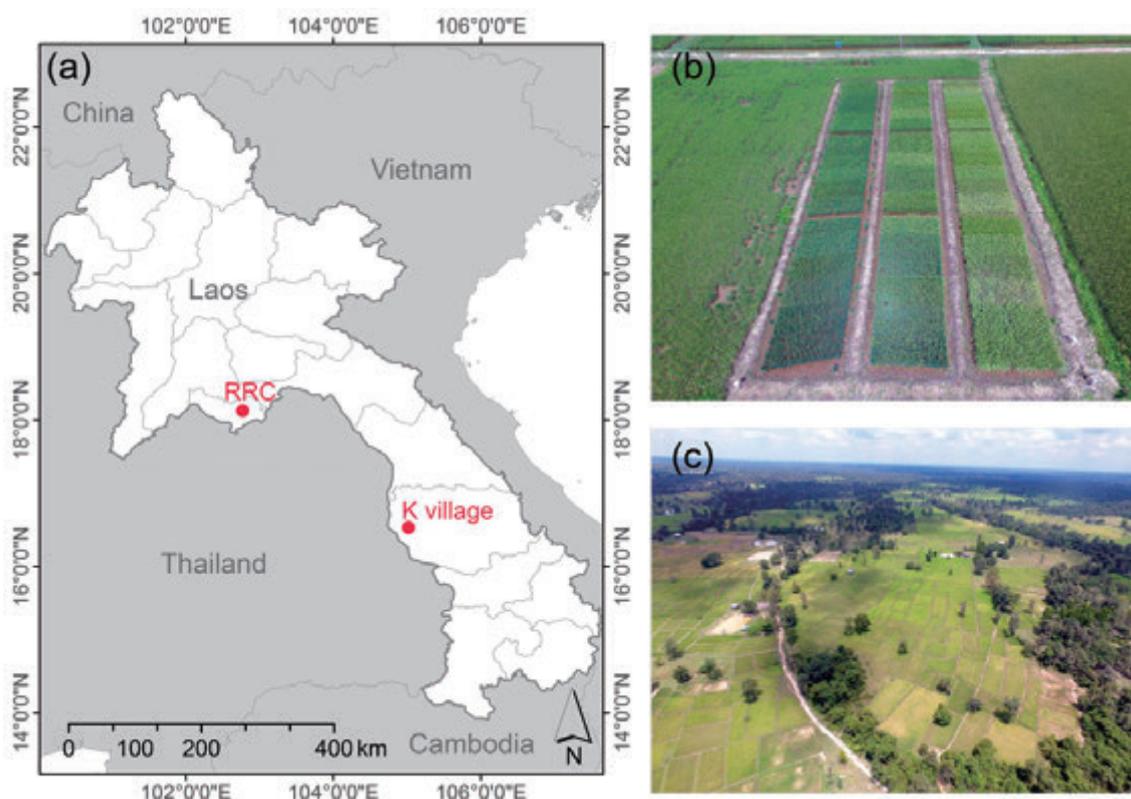


Fig. 1. Locations of the rice research center (RRC) and Koudkher village (a); and the UAV images for RRC (Research 1) (b) and Koudkher village (Research 2) (c).

Research 1: Plot design, canopy HS measurements and statistical analysis

The experiment was performed during the growing period in 2017 using a randomized complete block design with three replications (R1–R3). Each plot size was 5 m × 2 m. The treatments included three different transplanting dates (T1: July 12, T2: July 26, and T3: August 8) and six rice cultivars (cv.) (V1: cv. Tadokkham [TDK] 8, V2: cv. TDK11, V3: cv. Tasano 7 (TSN7), V4: cv. Homsavanh [HSV], V5: cv. Khaophorbane [KPB], and V6: cv. Khaokongkane [KKK]); V4, V5 and V6 were planted in Koudkher village in southern Laos, V1, V2 and V3 were improved varieties expected to be introduced to southern rain-fed paddy fields in the future. Plants were sampled on the harvest dates: T1 was harvested from October 18–27 (day of year [DOY] = 291–300), T2 was harvested from October 27–November 10 (300–314), and T3 was harvested from November 6–16 (314–320) in 2017. For rice yield, the rice grain weights were calculated with a moisture content of 14%.

Canopy HS measurements were carried out on October 2, 2017 using a portable MS-720

spectroradiometer (EKO Instruments, Tokyo, Japan). On that date, the rice growth stages at T1, T2, and T3 included the ripening (with the exception of V4), booting and panicle initiation stages, respectively. The spectroradiometer has a spectral sampling wavelength of 3.3 nm in the 350–1050 nm range, which was reproduced as a 1 nm-resolution wavelengths for outputting the data using MS720 software (EKO Instruments).

Standard full-spectrum PLS (FS-PLS) and ISE-PLS were performed using smoothed reflectance data to evaluate the biomass (BM) and grain yield for all combined data ($n = 54$) and for the T1, T2, and T3 data sets ($n = 18$), respectively. To assess the predictive abilities of the FS-PLS and ISE-PLS models, the coefficient of determination (R^2), RMSECV, and residual predictive deviation (RPD) in conjunction with leave-one-out (LOO) cross-validation were used in this study. High R^2 and low RMSECV values indicate the best model for predicting grain yield. The RPD was defined as the ratio of the standard deviation (SD) of the reference data for predicting the RMSECV (Williams 2001). For the performance ability of the calibration models, the RPD is suggested to be at least 3 for agricultural applications; RPD values between 2 and 3 indicate a model with a good predictive ability, $1.5 < \text{RPD} < 2$ indicates an intermediate model needing improvement, and an $\text{RPD} < 1.5$ indicates that the model has a poor predictive ability (D'Acqui et al. 2010). All the data handling and linear regression analyses were performed using Matlab software ver. 9.0 (MathWorks, Sherborn, MA, USA).

Research 2

A small consumer UAV, the DJI Phantom 4 (DJI, Shenzhen, China), was used to capture the red-green-blue (RGB) color images in May 30–June 1 in 2017 and June 1 in 2018 at a flight altitude of 100 m, to cover the entire Koudkher village area. The UAV flights followed autonomous flight plan using DJI Ground Station Pro application (DJI, Shenzhen, China) to ensure substantial overlap (85% forward and 60% side). Using 3D modeling software, Agisoft Photoscan Pro ver. 1.4.3 (Agisoft LLC, St. Petersburg, Russia), the 3D point clouds, ortho-mosaic images and DSMs were constructed from 4,344 images taken by the UAV with the geographic coordinates of 52 ground control points (GCPs) (UTM 48N). The spatial resolution (pixel size) was 5 cm.

In order to analyze the water resources environment, calculate watersheds and surface water flow was calculated using high-resolution digital terrain model (DTM) data from AW3D (<https://www.aw3d.jp/>) and GRASS software (<https://grass.osgeo.org>).

Using ArcGIS software ver. 10.3.1 (ESRI, USA), shape files for ground surface features (paddy field, irrigation pond, building and road) were created manually by visual discrimination of ortho-mosaic image. For paddy fields, the area and topographic environment (elevation, slope, direction) of each field were calculated.

Results and discussion

Research 1: Grain yield evaluations from field HS data with PLS models

Table 1 shows cross-validated calibration results between canopy HS reflectance spectra and grain yields via FS-PLS and ISE-PLS (Kawamura et al. 2018b). Based on ISE-PLS model, the selected number of wavebands (NW) and the selected NW as a percentage of the full spectrum ($NW\% = NW / \text{whole waveband [531 bands]} \times 100$) ranged from 2–131 (0.4–24.7%). In most cases (combined data set, T1, T2 and T3), the ISE-PLS models showed better predictive accuracy than the FS-PLS models. These findings support previous results indicating that the performance of FS-PLS models can be improved via waveband selection (Cho et al. 2007; Kawamura et al. 2017).

Fig. 2 shows the relationships between the observed and cross-validated prediction values of grain yield in each data set as predicted by the ISE-PLS models (Kawamura et al. 2018b). Overall, the best R^2 and lowest RMSECV values were obtained with ISE-PLS at the booting stage of the T2 data set ($R^2 = 0.843$, RMSECV = 22.903). Moreover, low predictive accuracy from the combined data could indicate that the in-season rice yield assessment depends on the appropriate growth stage for canopy HS measurements. The RPD value indicated that the T2 data set used in the ISE-PLS model accurately predicted the rice grain yield (RPD = 2.437).

Table 1. Optimum number of latent variables (NLV), coefficient of determination (R^2), root mean squared errors of cross-validation (RMSECV), and residual predictive values (RPD) from full-spectrum partial least squares (FS-PLS) and iterative stepwise elimination PLS (ISE-PLS) models with a selected number of wavebands (NW) and their percentages of the full spectrum (NW%) (Kawamura et al. 2018b).

Data set	Regression	NLV	R^2	RMSECV	RPD	NW	NW%
Combined	FS-PLS	3	0.113	68.927	1.050		
	ISE-PLS	2	0.157	66.734	1.085	2	0.4
T1	FS-PLS	1	0.009	86.114	0.875		
	ISE-PLS	2	0.023	80.216	0.939	84	15.8
T2	FS-PLS	3	0.078	58.480	0.944		
	ISE-PLS	10	0.843	22.903	2.437	11	2.1
T3	FS-PLS	8	0.301	66.418	1.068		
	ISE-PLS	7	0.479	54.088	1.316	131	24.7

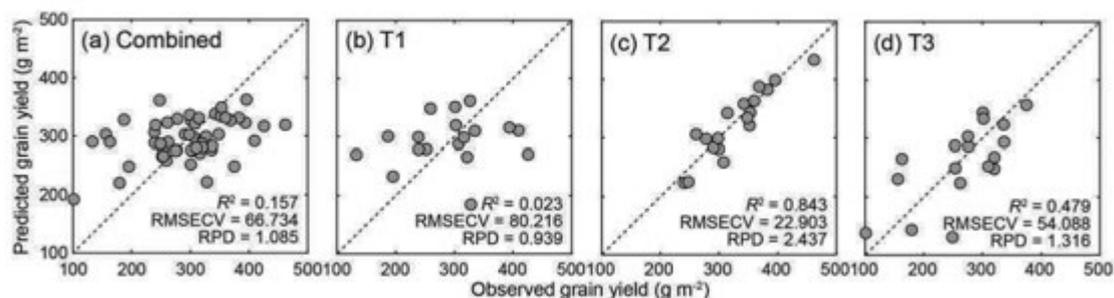


Fig. 2. Observed and cross-validated predicted values of rice grain yield (a, b, c, d) using ISE-PLS regression for the combined ($n = 54$) and T1, T2 and T3 data sets ($n = 18$), respectively (see Table 1) (Kawamura et al., 2018b).

Research 2: Establishment of GIS-based land infrastructure information

Using UAV images and DTM, land infrastructure information was established for efficient farming management in rain-fed paddy field (Koudkher village) (Fig. 3). Based on the GIS dataset, there were a total of 4,952 paddy fields (total area 1.26 km², 27.6% of Koudkher village). The average area of single field was 253.84 m², and the size was varied (2.27–8,924.44 m²). From the analysis of topography and catchment area using DTM data, the area was divided into five catchment areas. A large proportion of paddy field were found in the catchment area E including the village's residential area and lowland, and catchments C and D facing the road with low elevation. These basic knowledge for land infrastructure is principal for efficient farm management. Moreover, combining with field data (Ikeura et al. 2019), yield prediction and efficient fertilizer management could be expected by assessing environmental factors at local scale.

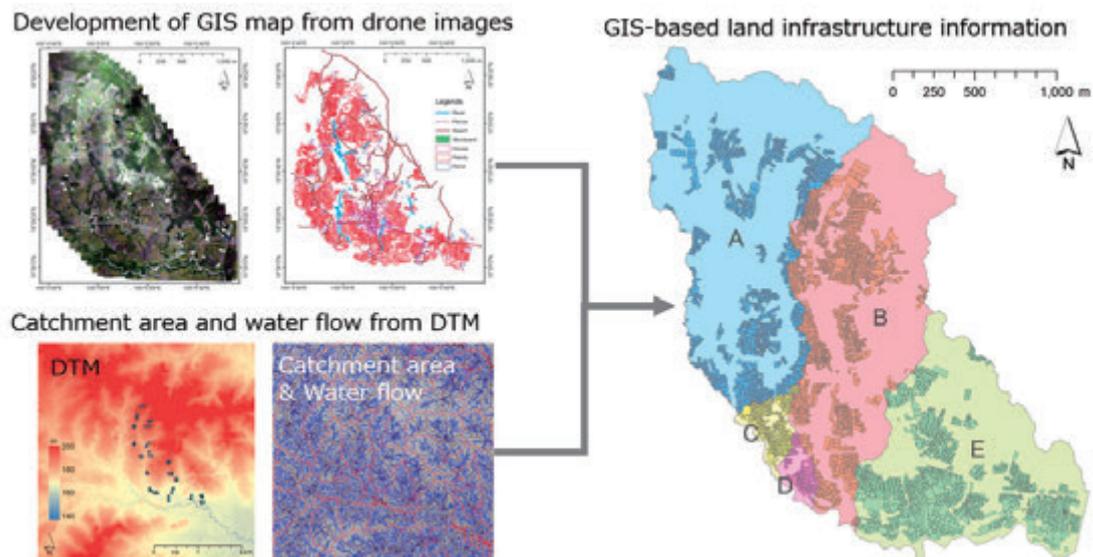


Fig. 3. Graphical flow for developing a GIS-based land infrastructure information data set in K village, Savannakhet.

Conclusion

Remote sensing technologies are being important field assessment tool in many agricultural applications. This paper summarized our on-going research activities in lowland rice field in Laos. In Research 1, we evaluated the feasibility of using canopy HS data for in-season grain yield evaluations at the reproductive phase of rice. Our results indicated that rice grain yield can be assessed by the ISE-PLS model, and the booting stage was identified as the best time for in-season evaluations via canopy HS assessments. These findings suggest that it is possible to evaluate rice yield from rice canopy reflectance approximately one month prior to harvest which could be useful information not only for famers but also for rice processing industries to quantify

rice supply and market prices. Moreover, the wavebands selected with ISE-PLS in the booting stage could be tested by HS sensors or multi-spectral cameras onboard an UAV. In Research 2, to realize efficient farming management on rain-fed paddy fields in Koudkher village, Savannakhet, a land infrastructure information was established using UAV image and DTM. Based on the GIS dataset with field data (rice yield and soil chemical status), it is expected that the rice yield prediction and efficient fertilizer management could be possible in the catchment area or each unit scale.

Acknowledgements

This study was conducted as the part of the collaborative research project between Japan International Research Center for Agricultural Sciences (JIRCAS), Japan and National Agriculture and Forestry Research Institute (NAFRI), Laos, sponsored by the JIRCAS on the “Formation of food value chain through value addition of food resources to support sustainable rural development”. We are grateful for the helpful assistance of Outhoumpone District Agriculture and Forestry Office, Savannakhet Province, Laos and all the residents of Koudkher village for working with us.

References

- Atzberger C (2013) Advances in remote sensing of agriculture: Context description, existing operational monitoring systems and major information needs. *Remote Sens* 5:949–981. <https://doi.org/10.3390/rs5020949>
- Cho MA, Skidmore A, Corsi F, van Wieren SE, Sobhan I (2007) Estimation of green grass/herb biomass from airborne hyperspectral imagery using spectral indices and partial least squares regression. *Int J Appl Earth Obs Geoinf* 9:414–424
- D’Acqui LP, Pucci A, Janik LJ (2010) Soil properties prediction of western Mediterranean islands with similar climatic environments by means of mid-infrared diffuse reflectance spectroscopy. *Eur J Soil Sci* 61:865–876
- Doraiswamy PC, Moulin S, Cook PW, Stern A (2003) Crop yield assessment from remote sensing. *Photogramm Eng Remote Sens* 69:665–674
- Ikeura H, Phongchanmixay S, Chomxaythong A, Matsumoto N, Kawamura K, Homsengchanh L, Inkamseng S (2019) Variation in lowland rice yield and its determinants in a rainfed area in Savannakhet Province, Laos. *Paddy Water Environ* (<https://doi.org/10.1007/s10333-019-00704-7>)
- Inoue Y, Sakaiya E, Zhu Y, Takahashi W (2012) Diagnostic mapping of canopy nitrogen content in rice based on hyperspectral measurements. *Remote Sens Environ* 126: 210–221
- Kawamura K, Ikeura H, Matsumoto N, Asai H, Phongchanmaixay S, Khanthavong P, Souvannasing S, Inthavong T, Chomxaythong A (2018a) Gathering basic land information on Laos’s rain-fed rice field using drone, in: *Proceedings of JASS 2018 Autumn Meeting*. Japanese Agricultural Systems Society, Fukuyama, pp. 29–30
- Kawamura, K, Ikeura H, Phongchanmaixay S, Khanthavong P (2018b) Canopy hyperspectral sensing of paddy fields at the booting stage and PLS regression can assess grain yield. *Remote Sens* 10:1249

- Kawamura K, Tsujimoto Y, Rabenarivo M, Asai H, Andriamananjara A, Rakotoson T (2017) Vis-NIR spectroscopy and PLS regression with waveband selection for estimating the total C and N of paddy soils in Madagascar. *Remote Sens* 9. <https://doi.org/10.3390/rs9101081>
- Ministry of Agriculture and Forestry (MAF) (2015) Laos, Agriculture Development Strategy to 2025 and Vision to the Year 2030. Ministry of Agriculture and Forestry, Vientiane
- Partnership GRS (2013) Rice Almanac, 4th edition. International Rice Research Institute, Los Banos
- Sakamoto T, Gitelson AA, Arkebauer TJ (2013) MODIS-based corn grain yield estimation model incorporating crop phenology information. *Remote Sens Environ* 131:215–231
- Wang L, Tian Y, Yao X, Zhu Y, Cao W (2014) Predicting grain yield and protein content in wheat by fusing multi-sensor and multi-temporal remote-sensing images. *Field Crop Res* 164:178–188
- Williams PC (2001) Implementation of near-infrared technology. In: Williams PC, Norris, KH (eds), *Near-Infrared Technology in the Agricultural and Food Industries*. American Association of Cereal Chemists Inc., St. Paul, Minnesota. pp. 145–169
- World Bank (2012) Lao People's Democratic Republic Rice Policy Study
- Zhang C, Kovacs JM (2012) The application of small unmanned aerial systems for precision agriculture: a review. *Prec Agric* 13:693–712

Soil–water conditions and efficient fertilization strategies to increase rice grain yield in rainfed lowland fields in Savannakhet Province

**Hiroshi Ikeura¹, Sengthong Phongchanmixay², Amphone Chomxaythong³,
Bounson Bounyavong², Phanthasin Khanthavong⁴, Thanouphone Khanthavong⁵,
Naruo Matsumoto¹, Kensuke Kawamura¹, Laeh Homsengchanh², Somphone Inkhamseng³**

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Rice Research Center (RRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

³ Faculty of Water Resources, National University of Laos, Vientiane, Lao PDR

⁴ Maize and Cash Crop Research Center (MCRC) , National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

⁵ Faculty of Natural Science, National University of Laos, Vientiane, Lao PDR

Abstract

Most lowland rice fields in Savannakhet Province, the main rice-producing region in Laos, are rainfed and cover both lowlands as well as gradual hilly terrain with sandy soils. The water and nutrient conditions of the lowland rice fields likely vary by location and geological conditions. Additionally, high risk of nutrient leaching from applied fertilizer is expected in such sandy rainfed fields. The objectives of this study are 1) to assess rice yield and water and soil conditions in the rainfed rice fields of Savannakhet Province, 2) to identify the determinants of variation in rice yield, and 3) to evaluate rice plant growth and nutrient leaching under different split chemical-fertilizer application patterns. The rice grain yield varied from 0.6 to 3.6 t ha⁻¹, and the yield in the lower position fields was significantly higher than in the upper position fields. Surface water in the observed fields was mostly maintained above ground level throughout the planting period. Total carbon, total nitrogen, available phosphorus, and exchangeable potassium were extremely low, while the exchangeable calcium and exchangeable magnesium were relatively high. Exchangeable calcium was selected as the highest influential factor to rice grain yield, followed by total carbon. The grain yield from six split fertilization was higher than from three split fertilization (standard) and basal fertilization only. The split fertilization is advantageous in that it supplies nitrogen for panicle initiation and maturing stages in sandy paddy fields.

Introduction

Rice is a staple food in the Lao People's Democratic Republic (hereinafter referred to as Laos). The net rice production, 0.54 million tons in 1961, has increased to 4.15 million tons in 2016 (Food and Agriculture Organization (FAO) 2018). The government of Laos aims to increase

rice production for domestic consumption as well as export and plans to increase production to 4.7 million tons in 2020 and 5.0 million tons in 2025 (Ministry of Agriculture and Forestry, Laos (MAF) 2015). Only 4% of the total Laos land area is cultivated, with rice constituting 70% of the net cropped area (World Food Program (WFP) 2007). The irrigated areas in Laos constitute only 12% of the total agricultural land (FAO 2018), and most lowland rice is cultivated under rainfed conditions.

Savannakhet Province is the main rice-producing region in Laos; the province produced 25% of the country's total lowland rice (MAF 2016). Generally, the rice fields in the province are laid out on flat or almost flat land, with slopes ranging from 0–8% (Inthavong et al. 2011). Soils in most rainfed lowland areas in Laos are highly weathered, moderately acidic sandy loams, loams, and loamy sands; the soils in Savannakhet have low levels of nitrogen (N), phosphorus (P), and sometimes potassium (K). Low organic matter and cation exchange capacity (CEC) are also common (Lathvilayvong et al. 1994). When combined with sandy soil textures that have high permeability and low fertility, these geological conditions may affect water and nutrient conditions of each field. In addition, a considerable part of applied fertilizer may be lost by the leaching due to high permeability of the soil.

The objectives of this study are 1) to assess the rice yield and water and soil conditions in the sloped rainfed rice fields of Savannakhet Province, 2) to identify the factors affecting difference in rice yield among field positions, considering environmental factors, and 3) to evaluate rice plant growth and leaching loss of chemical-fertilizer from sandy soil by pot experiment.

Materials and methods

Field survey

Field surveys were conducted in Koudkher Village, Outhomphone District, Savannakhet Province, Laos (Fig. 1). The topography gently slopes downward from north to south at a 2.8% mean slope gradient. The elevation varies from 153 to 183 m above sea level from the lowest to the highest field (Fig. 2). Soil sampled from twenty fields in the village was classified as sand and loamy sand at the surface layer (0–20 cm), and sand, loamy sand and sandy loam at the subsurface layer (20–40 cm) according to the International Society of Soil Science Method. Saturated hydraulic conductivity was 10^{-3} – 10^{-5} cm s⁻¹, and 10^{-4} – 10^{-8} cm s⁻¹ at the surface and subsurface layers, respectively. The annual precipitation in Seno meteorological station, 10 km away from the village, is 1,560 mm (average from 2001 to 2013); almost 85% of precipitation occurs between May and September (Ikeura et al. 2019). Weather data in the village was measured from 2014 to 2017.



Fig. 1. Location of survey site
This figure was modified to reference
Ikeura et al. (2019) and
<http://www.freemapviewer.com>.

Twenty fields were selected to cover the entire lowland field area in the target village (Fig. 2). Located on five sequential slopes, the fields were classified into upper, middle, and lower groups by their toposequential locations. There were 5, 7, and 8 fields in the upper, middle, and lower groups, respectively. Three sampling quadrats (1×1 m) were set in each field in the middle of July 2017 (20 fields \times 3 replicates = 60 quadrats). In one-quarter of fields only basal fertilizer was applied in low amounts (e.g., $4\text{--}5$ kg N ha^{-1} , $1\text{--}3$ kg P_2O_5 ha^{-1} , $0\text{--}2$ kg K_2O ha^{-1}). In the other fields fertilizer was not applied, and the only manure was supplied by browsing cows and buffalos during the dry season. Transplanting began in mid-June and was completed before making the quadrats. Six cultivars were included in the samples. Rice samples were harvested from each quadrat at maturity, and grain weight and moisture content were measured after drying, threshing, and winnowing (Ikeura et al. 2019).

The surface water depth in each plot was measured using gauge rods on July 17, August 24, September 10, October 7 and 21 in 2017. To evaluate soil fertility, a topsoil sample (0–10 cm depth) was collected from each quadrat before flowering at the end of August 2017. Soil samples were air-dried and passed through a 2-mm mesh screen. Soil pH (1:2.5), total nitrogen (total N), total carbon (total C), available phosphorus (available P) and exchangeable cations [potassium (exchangeable K); magnesium (exchangeable Mg); calcium (exchangeable Ca)] were determined (Ikeura et al. 2019).

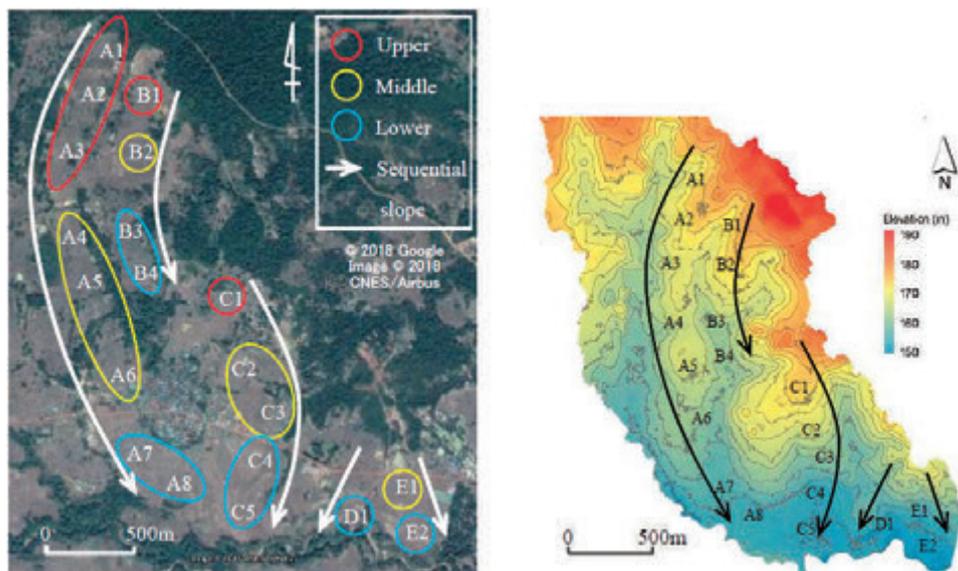


Fig. 2. Location of surveyed fields and topographic condition of survey site (Ikeura et al. 2019).

The difference in yield, water depth, and soil chemical properties among the upper, middle, and lower groups were analyzed by Tukey–Kramer HSD test using JMP 10 statistical software (SAS Institute Inc., Cary, NC, USA.). To investigate the factors affecting differences in rice grain yield, linear regression analyses were performed using R statistical software v. 3.4.1 (R Core Team 2016), as per the following steps (Ikeura et al. 2019):

Step 1: Multiple linear regression (MLR) analysis was conducted to determine the major factors as shown in Eq. 1; the response variable was rice grain yield (GY), and explanatory variables were environmental factors (V) such as average water depth in the rice growing period, soil chemical properties (pH, total N, total C, available P, exchangeable K, exchangeable Mg, and exchangeable Ca), and variety of rice.

$$GY = a + b_1V_1 + b_2V_2 + \dots + b_nV_n \quad \text{Eq. 1}$$

where GY is the rice grain yield, a is constant, V_1 to V_n are the explanatory variables selected by stepwise variable selection (here, V_n is the factor with the highest correlation to GY), n is the number of selected variables, and b_1 to b_n are coefficients of variables V_1 to V_n , respectively.

Step 2: To specify the determinants of difference in rice grain yield, a generalized linear mixed model (GLMM) incorporating location (upper, middle, and lower positions) as a fixed effect (L_i) was applied. The explanatory variables include the variables selected by Step 1 and the locational factor as continuous variables ($i = 1$ [lower] to 3 [upper]).

$$GY = a_{Li} + b_1V_1 + b_2V_2 + \dots + b_{nLi}V_n + L_i \quad \text{Eq. 2}$$

where a_i is constant based on the i th fixed effect of the locational factor (L_i).

Pot experiment

A pot experiment was conducted at the Rice Research Center, National Agriculture and Forestry Research Institute, Laos from January to April 2018. The soil used in the experiment was topsoil (0 to 20 cm depth) collected in the dry season (March 2017) from a field in Koudkher Village (near Field E2). Fifteen (15) kg of air-dried soil were placed in 20 Wagner pots (surface

area = 1/2,000 a). The soil was loamy sand composed of 4% clay, 4% silt, and 92% sand. It contained 141 mg N kg⁻¹ of total N, 6.04 mg P kg⁻¹ of available P and 6.93 mg K kg⁻¹ of exchangeable K. At the beginning of the experiment the soil had an estimated 2,115 mg N of total N, 90.6 mg P of available P and 104 mg K of exchangeable K in each pot (Phongchanmixay et al. 2019). A glutinous rice variety (cv. TSN-7) used in this study was developed for sandy paddy fields in Savannakhet Province. One hill (three seedlings) of 30-day-old seedlings was transplanted in each pot on January 9, 2018. Linqvist and Sengxua (2001) proposed supplying 60 kg N ha⁻¹, 20 kg P₂O₅ ha⁻¹, and 20 kg K₂O ha⁻¹ to rice planted in rainfed fields with sandy soil. They also suggested three-split fertilization, with 20 kg N–20 kg P₂O₅–20 kg K₂O ha⁻¹ basal fertilization at transplanting and 20 kg N ha⁻¹ applied prior to active tillering and panicle initiation stages as topdressings. This was defined as the standard fertilization (control: C). Three more treatments were set as follows: 6-split fertilization applied at 12-day intervals (SF); basal fertilization in which all fertilizer was applied at transplanting (BF), and no fertilization (NF). The contents of application for each treatment are shown in **Table 1**. The four treatments were tested with five replicates.

Table 1. Fertilizer treatments in the pot experiments

	0 DAT	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT
C	0.1 g N					
	0.1 g P ₂ O ₅	—	0.1 g N	—	0.1 g N	—
	0.1 g K ₂ O					
SF	0.05 g N	0.05 g N				
	0.05 g P ₂ O ₅	0.05 g P ₂ O ₅	0.05 g N	0.05 g N	0.05 g N	0.05 g N
	0.05 g K ₂ O	0.05 g K ₂ O				
BF	0.3 g N	—	—	—	—	—
	0.1 g P ₂ O ₅					
	0.1 g K ₂ O					
NF	—	—	—	—	—	—

DAT: Days after transplanting

Total applied fertilizer amount of each C, SF and BF were equal to 60 kg N–20 kg P₂O₅–20 kg K₂O ha⁻¹.

Compound fertilizer (N-P₂O₅-K₂O:15-15-15%) and urea (CO(NH₂)₂, N:46%) was applied for basal (0 and 12 DAT) and topdressings (24 - 60 DAT), respectively.

This table was modified to reference Phongchanmixay et al. (2019).

To simulate rainfed conditions, pots were placed under a roof and irrigation was conducted as controlled rainfall. The irrigation amounts and intervals were determined based on rainfall patterns from the 2016 rainy season in Koudkher Village; 27 mm of water was applied at 3-day intervals until 81 DAT, then 12 mm of water was applied at 10-day intervals until harvest. The drain plug of each pot was opened 24 hours before the next irrigation to measure the drainage amount and collect samples.

Plant height and number of tillers were measured every 6 days after transplanting until harvest. After harvesting, the air-dried weights of grain and rice straw were measured. Total N, total P and dissolved potassium (dissolved K) were measured in the drainage and irrigation water and drained N, P and K were calculated from the concentrations of total N, total P, dissolved K and drained water volume (Phongchanmixay et al. 2019).

Results and Discussion

1. Field survey of the factors affecting yield differences

Precipitation during the observed period

Table 2 shows monthly and annual precipitation. The annual precipitation in 2017 was 1,847 mm; 300 mm more than the 2001 to 2013 average and 500 mm more than the 2014 and 2016 average observed in the target village; particularly, the precipitation doubled in July 2017.

Table 2. Monthly precipitation (mm) in the field survey site

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Growing period* ¹	Annual
2001-2013* ²	3	17	43	69	201	242	324	330	239	81	9	3	1,416	1,560
2014* ³	0	0	0	62	86	342	439	355	231	6	0	0	1,460	1,521
2016* ³	6	0	0	14	181	191	205	194	286	60	25	1	1,115	1,161
2017* ³	0	1	136	27	195	246	644	185	313	83	14	3	1,667	1,847

This table was modified to reference Ikeura et al. (2019).

*1: Rice growing period from May to October (including a seedling period in nursery) (drawn as green color).

*2: Average data observed at Seno meteorological station (obtained from Department of Meteorology and Hydrology).

*3: Observed at Koudkher Village (data in 2015 was missing).

Rice grain yield

The rice grain yield in 2017 varied from 0.6 to 3.6 t ha⁻¹, averaging 1.9 t ha⁻¹. Compared to the national average yield in 2016 (4.26 t ha⁻¹; FAO 2018) and the yield in a plot-to-plot irrigation system in the semi-mountainous village in Vientiane Province (3.54 t ha⁻¹; Ikeura et al. 2016), the yield in Koudkher Village was extremely low. Fig. 3 shows the average grain yield for each toposequential position. The yield in the upper fields was significantly lower than in the lower fields (Ikeura et al. 2019).

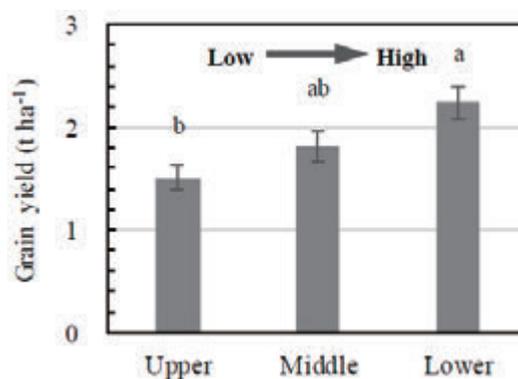


Fig. 3. Average rice grain yields in upper, middle and lower position fields

This figure was modified to reference Ikeura et al (2019).

Error bar showed standard error.

Surface water depth in the fields

Fig. 4 shows the average surface water depth for each toposequential position. Throughout the observation period, the surface water depth in the lower fields was larger than those in the upper and middle position fields. However, even in high and middle position fields, surface water was mostly maintained above ground level except before harvesting. In the upper and middle fields, harvesting was completed by mid-October, suggesting that drought stress in 2017 may have been slighter than usual (Ikeura et al. 2019).

Soil chemical properties

Fig. 5 shows the soil chemical properties in the upper, middle, and lower fields. The values of total C and total N were the highest in the lower fields but the lowest in the middle fields. The available P was the lowest in the lower fields; this was the opposite trend as the rice grain yield. Exchangeable K showed the highest content in the upper fields and the lowest in the middle fields.

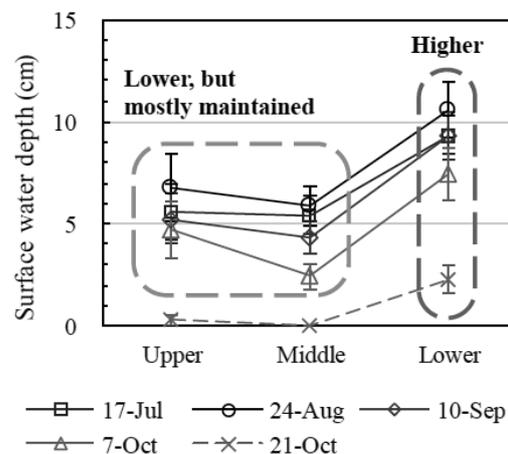


Fig. 4. Average surface water depths in upper, middle and lower position fields. This figure was modified to reference Ikeura et al (2019). Error bar showed standard error.

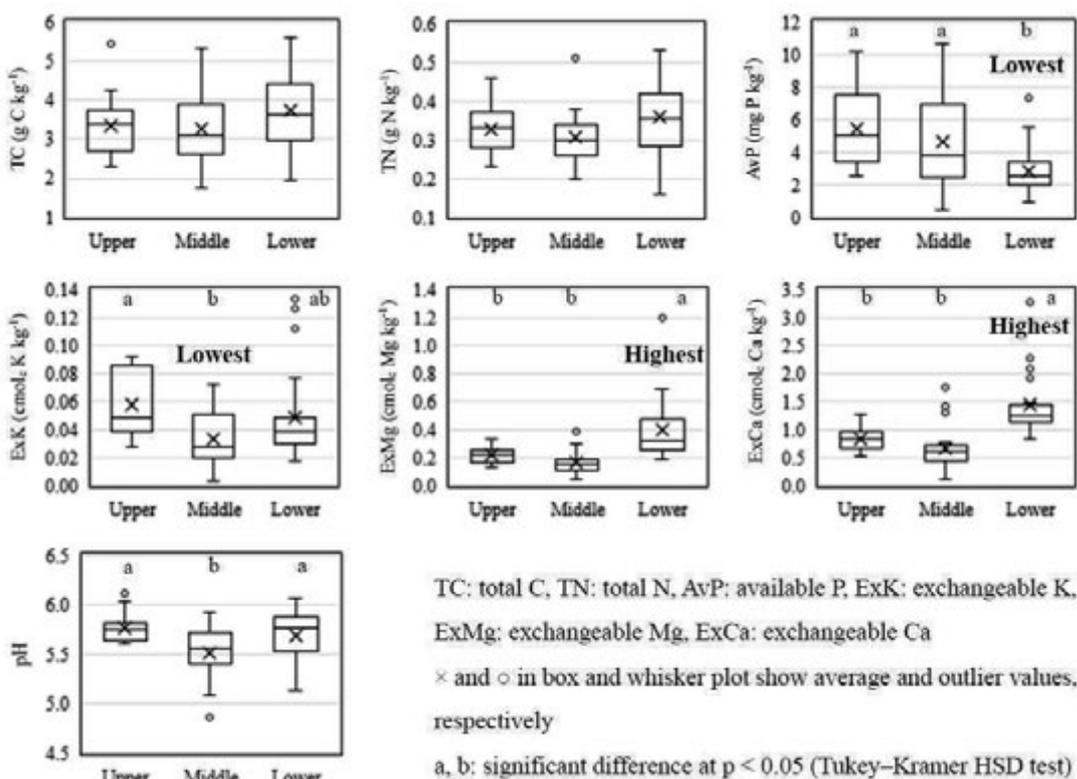


Fig. 5. Soil chemical properties in upper, middle and lower position fields. This figure was modified to reference Ikeura et al. (2019).

Exchangeable Mg and exchangeable Ca were the highest in the lower fields, but were the lowest in the middle fields; these two soil factors were higher than exchangeable K. Soil pH was 5.4–5.9, and the middle fields had lower pH than upper and lower field positions.

Determinant analysis

Table 3 shows the MLR results using all available variables (Model 1) and selected variables (Model 2). Through the stepwise variable selection procedure, the average water depth, total C, total N, available P, exchangeable K, exchangeable Mg, and exchangeable Ca were the selected variables for Model 2; total C, available P, exchangeable K, and exchangeable Ca were selected next. Exchangeable Ca showed the strongest significance ($p < 0.001$); suggesting that exchangeable Ca was the main factor for predicting *GY* (Ikeura et al. 2019).

Using the four variables selected in Model 2, a GLMM with a locational factor was applied. The slope of the exchangeable Ca was dependent on the locational factor. The results of Models 3 and 4 are shown in Table 4. Model 3 showed $R^2 = 0.541$, which more significant than Model 2. In Model 4, available P was not selected for the final model. Although Model 4 had lower R^2 (0.529) than Model 3 (0.541), the lowest Akaike Information Criteria (AIC) value shown in Model 4 suggested that it was the most appropriate model to predict *GY*. These results indicated that the main factor affecting difference in rice grain yield was exchangeable Ca, followed by total C, exchangeable K (Ikeura et al. 2019).

Table 3. Constant and coefficient of each model (MLR; Eq. 1)

	Constant		Regression coefficient (<i>b</i>)						R^2	AIC	RMSE
	(a)		<i>V1</i>	<i>V2</i>	<i>V3</i>	<i>V4</i>	<i>V5</i>	<i>V6</i>			
Model 1		WDavg	TC	TN	AvP	ExK	ExMg	ExCa			
	70.0*	0.545	26.3	-98.0	4.65	-871.0*	14.7	74.5*	0.345	668.1	54.5
Model 2		TC	AvP	ExK	ExCa						
	67.0	18.3*	4.47	-878.5*	81.0***				0.378	662.4	54.7

WDavg: average water depth, TC: total C, TN: total N, AvP: available P, ExK: exchangeable K, ExMg: exchangeable Mg, ExCa: exchangeable Ca

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. This table was modified to reference Ikeura et al. (2019).

Table 4. Constant and coefficient of each model (GLMM; Eq. 1)

	Constant (a_i)			Regression coefficient (b)						R^2	AIC	RMSE
				<i>V1</i>	<i>V2</i>	<i>V3</i>	Vn_{Li}					
	L_1	L_2	L_3				L_1	L_2	L_3			
Model 3				TC	AvP	ExK	ExCa					
	72.6	37.2	177.1	22.0**	3.66	-689.9*	64.9	118.4	-93.6	0.541	664.6	48.2
Model 4				TC	ExK		ExCa					
	86.9	44.8	196.9	23.0**	-609.6*		58.4	120.8	-104.6	0.529	664.3	48.9

TC: total C, AvP: available P, ExK: exchangeable K, ExCa: exchangeable Ca

*: $p < 0.05$, **: $p < 0.01$. This table was modified to reference Ikeura et al. (2019).

The surface water depth was not selected as a major factor affecting differences in rice yield in this study. Although the average water depths of the upper and the middle fields were lower than those of the lower fields, water was mostly maintained above ground level. In the year of our study, the low yields in the upper fields were not caused by drought stress (Ikeura et al. 2019).

Exchangeable Ca was selected as the first factor of yield difference. One (1) g of rice grain and rice straw contained 0.5 mg Ca and 3.5 mg Ca, respectively (Dobermann and Fairhurst 2002). The grain and straw weights were 300 g m⁻² and 400 g m⁻², respectively (Figs. 9 and 10), and Ca requirement was 1.55 g Ca m⁻². The amounts of exchangeable Ca in topsoil (0–10 cm depth, bulk density: 1.5 g cm⁻³) was 25.2, 19.2, and 44.4 g Ca m⁻² in the upper, middle, and lower fields, respectively. This suggests that exchangeable Ca contained in the field soil in the target village was not negligible for rice growth, and the difference in exchangeable Ca contents may have affected rice yield (Ikeura et al. 2019).

Total C, selected as the second factor, was before total N as the determinant of this analysis because it had higher variation than total N. Total C showed strong collinearity with total N ($R^2 = 0.94$). Therefore, total N was a similar factor to total C for affecting differences in yield (Ikeura et al. 2019).

The average total N, available P, and exchangeable K contents in topsoil (0–10 cm depth) were calculated using the soil analysis results as 495 kg ha⁻¹, 6.24 kg ha⁻¹, and 26.9 kg ha⁻¹, respectively. When available N was defined as 3% of the total N (Murata et al. 1997a, 1997b), available N was 14.9 kg ha⁻¹. N and P were deficient in the rainfed fields of the target village and K barely met the required minimum (60 kg ha⁻¹ of N, 13 kg ha⁻¹ of P, 25 kg ha⁻¹ of K; Linquist and Sengxua 2001). Considering the poor soil conditions, N, P, and K fertilization is essential for increasing rice yield in sandy rainfed paddy fields of the target village (Ikeura et al. 2019).

2. Pot experiment for efficient fertilization of sandy fields

Fig. 6 shows the rice plant height for each treatment. There were similar growth trends for standard fertilization (C), 6-split fertilization (SF), and basal fertilization (BF). The maximum plant heights in C, SF and BF were 87.3 cm, 87.4 cm and 87.4 cm at 78 DAT, respectively. No fertilization (NF) grew slower than C, SF, and BF until 18 DAT, then grew at a similar speed until heading (Phongchanmixay et al. 2019).

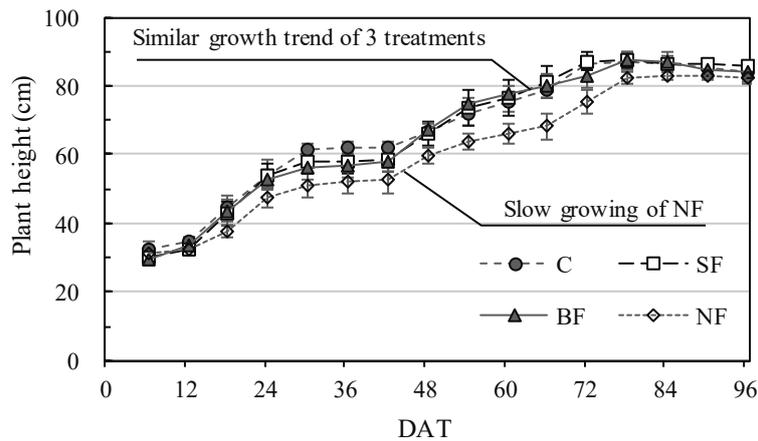


Fig. 6 Rice plant height

This figure was modified to reference Phongchamixay et al. (2019).

Error bar shows standard deviation.

Fig. 7 shows the number of tillers. In all treatments, tiller increase stopped at 42 DAT. At 84 DAT, the number of tillers in all treatments decreased rapidly; this timing coincided with changes to the irrigation intervals (3-day interval to 10-day interval). Therefore, drought stress may have affected this decreased tillering. At this point, C and BF had lost half of their tillers, while SF had retained 70% of tillers. The final topdressing fertilizer for SF contributed to maintaining the number of tillers (Phongchanmixay et al. 2019).

Fig. 8 shows the volume of drainage water. The drainage water volume for NF was higher than the other groups. Drainage from C, SF, and BF mostly stopped after 54 DAT, while NF drained continuously over the 3-day irrigation interval period. Because NF showed the lowest plant height and number of tillers among the four treatments, it can be concluded that evapotranspiration was lower than for the other groups, causing higher drainage volumes (Phongchanmixay et al. 2019).

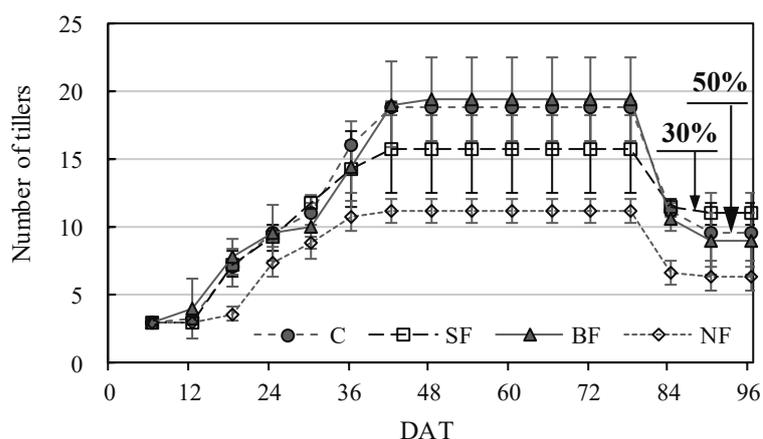


Fig. 7. Number of tillers

This figure was modified to reference Phongchanmixay et al. (2019).
Error bar shows standard deviation.

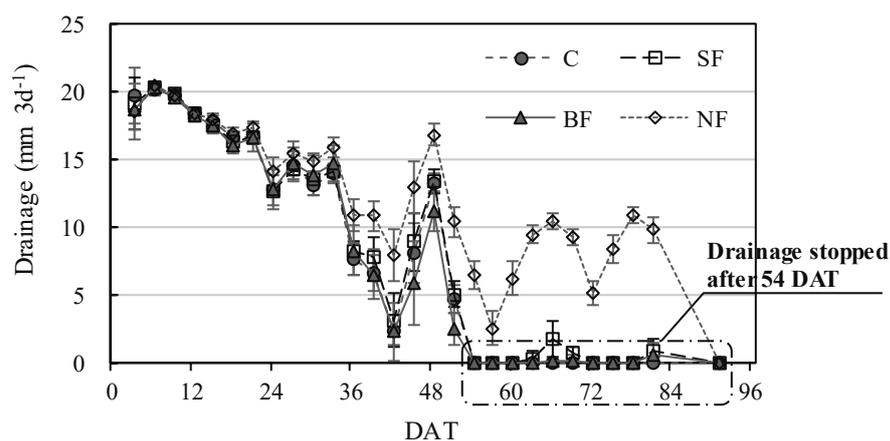


Fig. 8. Drainage water volume

This figure was modified to reference Phongchanmixay et al. (2019).
Error bar shows standard deviation.

Fig. 9 shows the rice grain yield for each fertilizer treatment. SF had the highest yield of all treatments, although there were no significant differences between yields with the C and BF treatments. Our results also indicated that the SF treatment had the potential to increase yield by 70%, 50%, and 30% above NF, BF, and C, respectively. Fig. 10 shows the weight of rice straw. The weight of straw in NF was significantly lower than the other three treatments. There were no significant differences in the weight of straw among C, SF, and BF treatments. These results suggest that SF used nutrients most efficiently to increase grain yields (Phongchanmixay et al. 2019).

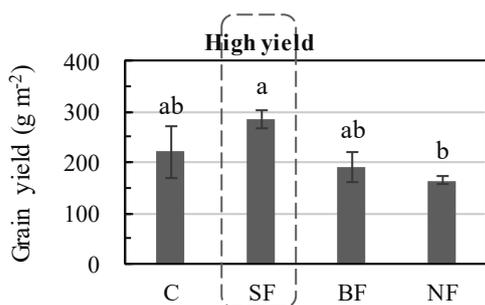


Fig. 9. Rice grain yield

This figure was modified to reference Phongchanmixay et al. (2019).

Error bar shows standard error.

a, b: significant difference ($p < 0.05$).

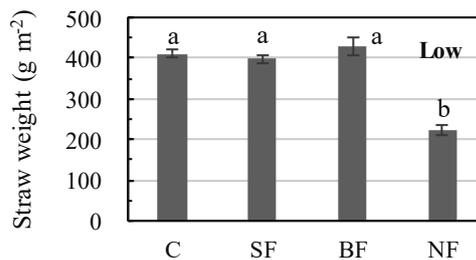


Fig. 10. Rice straw weight

This figure was modified to reference Phongchanmixay et al. (2019).

Error bar shows standard error.

a, b: significant difference ($p < 0.05$).

Table 5 shows the N, P and K amounts in the applied fertilizer, irrigation water and drainage. The N in drainage water of BF was significantly higher than in C, SF, and NF. Total N inputs by fertilizer and irrigation were 300 mg N and 14.4 mg N, respectively. The percentage of leached N from fertilizer and irrigation inputs in C, SF, and BF were 13.2%, 12.1%, and 22.7%, respectively. The drained N in NF plot came from irrigation water and the soil. Drained P was less than 2% of the fertilizer input. Drained P in NF was significantly higher than those in C, SF, and NF. The total K inputs were 83.0 mg K from fertilizer and 20.8 mg K from irrigation during the rice-growing period. The K leaching loss was 24-31% of the total K input. The drained K in SF plot was lower than that in BF plot; split K application seems to increase K uptake by rice and to decrease K leaching (Phongchanmixay et al. 2019).

Table 5. N, P and K amount of fertilizer application, irrigation and drainage

		Fertilizer (mg pot ⁻¹)	Irrigation (mg pot ⁻¹)	Drainage (mg pot ⁻¹)	Leaching ratio*
N	C	300	14.4	41.5 ± 2.9 ^b	0.132
	SF	300	14.4	37.9 ± 3.2 ^b	0.121
	BF	300	14.4	71.3 ± 12.9 ^a	0.227
	NF	0	14.4	40.9 ± 5.1 ^b	2.838
P	C	43.7	0	0.418 ± 0.028 ^b	0.0096
	SF	43.7	0	0.469 ± 0.097 ^b	0.0107
	BF	43.7	0	0.542 ± 0.105 ^b	0.0124
	NF	0	0	0.765 ± 0.097 ^a	-
K	C	83.0	20.8	30.0 ± 1.3 ^{ab}	0.289
	SF	83.0	20.8	25.0 ± 2.3 ^c	0.241
	BF	83.0	20.8	32.4 ± 2.3 ^a	0.312
	NF	0	20.8	27.0 ± 2.2 ^{bc}	1.297

Fertilizer and irrigation are NPK input. Drainage is NPK output.

* Leaching ratio: drainage / (fertilizer + irrigation).

a, b, c: different characters show a significant difference ($p < 0.05$) among treatments.

Phongchanmixay et al. (2019).

Conclusion

A field survey was conducted in the village in Savannakhet Province to assess rice yield and water and soil conditions in sloped rainfed rice fields with sandy soil, as well as to identify determinants of variation in rice yield. Additionally, to reduce nutrient loss from leaching and improve rice yields in sandy paddy fields, efficient six split fertilization (SF) was tested to compare rice plant growth and leaching loss. We obtained the following results:

- 1) The rice grain yield was higher in the lower fields than in the upper and middle fields (Ikeura et al. 2019).
- 2) Although the average depth of surface water was lower in the upper and middle fields than in the lower fields, standing water was sustained during the rice-growing period; surface water depth was not selected as the determinant variable for the observed year (Ikeura et al. 2019).
- 3) Contents of total C, total N, available P, and exchangeable K in rainfed lowland field soil in the target village were extremely low; fertilization is essential for improving rice yield. As the results of multiple linear regression analysis and generalized linear mixed model, Exchangeable Ca was selected as the highest influential factor to rice grain yield, followed by total C. (Ikeura et al. 2019).
- 4) The results of the pot experiment indicate that, although the difference was not significant, rice grain yield of SF (six split fertilization) was higher than C (standard fertilization: control), BF (basal fertilization), and NF (no-fertilization). The leaching ratios of N and K in SF pots were lower than in C and BF pots. SF was also advantageous for supplying N for panicle

initiation and maturity. This result shows that split fertilization is a useful method for increasing rice grain yield (Phongchanmixay et al. 2019).

Acknowledgments

This study was conducted as part of the collaborative research project of Japan International Research Center for Agricultural Sciences (JIRCAS), National Agriculture and Forestry Research Institute and National University of Laos funded by JIRCAS. We are grateful for the helpful assistance of Outhoumpone District Agriculture and Forestry Office, Savannakhet Province, Department of Meteorology and Hydrology, Laos, and all the residents of Koudkher Village for working with us.

References

- Dobermann A and Fairhurst TH (2002) Rice Straw Management. *Better Crops International* 16, Special Supplement:7-11
- FAO (2018) FAOSTAT. <http://www.fao.org/faostat/en/#data/QC>. Accessed 24 July 2018
- Ikeura H, Pongchanmixay S, Chomxaythong A, Matsumoto N, Kawamura K., Homsengchanh L and Inkhamseng S (2019) Variation in Lowland Rice Yield and its Determinants in a Rainfed Area in Savannakhet Province, Laos. *Paddy and Water Environment* 17:121–130
- Ikeura H, Phongchanmixay S, Phonsangone S, Xaypanya P, Inkhamseng S, Soubat S (2016) Factors affecting differences in the rainy season rice yield in a lowland area of a mountainous village in Lao PDR. *Paddy and Water Environment* 14:343–353
- Inthavong T, Fukai S, Tsubo M (2011) Spatial variations in water availability, soil fertility and grain yield in rainfed lowland rice: a case study from Savannakhet Province, Lao PDR. *Plant Production Sci* 14:184–195
- Lathvilayvong P, Schiller JM, Phommasack T, Chanphengsay M (1994) Soil fertility management in the rainfed lowland environment of the Lao PDR, soil organic matter for sustainable agriculture in Asia. In: *ACIAR Proceedings No. 56*. Ubon Ratchathani. Pp. 91–97
- Linquist B, Sengxua P (2001) Nutrient management in rainfed lowland rice in the Lao PDR. International Rice Research Institute, Los Baños
- Ministry of Agriculture and Forestry (2015) Laos, Agriculture Development Strategy to 2025 and Vision to the Year 2030
- Ministry of Agriculture and Forestry (2016) Laos, Agricultural statistics year book 2015
- Murata T, Tanaka H, Sakaguch K, Asaka D, Hamada R (1997) The influence on microbial biomass, available soil N and neutral sugar composition induced by wheat straw and manure application. *Jap J Soil Sci Plant Nutr* 68:249–256 (in Japanese)
- Phongchanmixay S, Bounyavong B, Khanthavong P, Khanthavong T, Ikeura H, Matsumoto N, Kawamura K (2019) Rice plant growth and nutrient leaching under different patterns of split chemical fertilization on sandy soil using a pot. *Paddy and Water Environment* 17:91–99
- R Core Team (2016) R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>

WFP (2007) Lao PDR: Comprehensive food security & vulnerability analysis. World Food Program,
Vientiane

Current status of paddy field agriculture and efficient water use in existing reservoirs for fish aquaculture in a semi-mountainous village in Vientiane Province, Laos

Hiroshi Ikeura¹, Toshihiko Anzai¹, Amphone Chomxaythong², Khaykeo Keokhampui², Salermphon Phonsangone², Phetyasone Xaypanya², Sengthong Phongchanmixay³, Somphone Inkhamseng², Somnuck Soubat⁴, Haruyuki Fujimaki⁵

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Faculty of Water Resources, National University of Laos, Vientiane, Lao PDR

³ Rice Research Center (RRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

⁴ Maize and Cash Crop Research Center (MCRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

⁵ Arid Land Research Center, Tottori University, Tottori, Japan

Abstract

Rice is a staple food in Laos; its production has increased eightfold over the last fifty years. In hilly and mountainous areas, rainy season lowland rice is cultivated in a valley between mountains with undeveloped irrigation facilities. The area used for agricultural production is limited during the dry season due to water shortages, and most lowland fields are used as grazing land. We conducted a survey to examine the status of lowland rice production in a semi-mountainous village in Vientiane Province, then evaluated the feasibility of increasing rice yield and introducing dry season crop production using water stored in existing reservoirs for fish aquaculture. During the rainy season, low rice grain yield was observed in the fields where water shortages early in the season had delayed transplanting. In the dry season, supplemental irrigation is essential for soybean production. We determined the water requirements for preparatory irrigation for early transplanting in rainy season and for supplemental irrigation through the field experiments. An irrigation plan was formulated utilizing 8,700 m³ of water stored in six reservoirs in the surveyed area. The potential area of the preparatory irrigation for lowland rice in the rainy season and the supplemental irrigation for soybean cultivation in the dry season using reservoir water were 10.4-11.0 ha and 3.2-3.5 ha, respectively. By irrigating with water stored in existing reservoirs, the income of the entire surveyed area is expected to increase by 80 million kip.

Introduction

Rice is a staple food in the Lao People's Democratic Republic (hereinafter referred to as Laos). The net rice production, which was 0.54 million tons in 1961, had increased to 4.15 million

tons by 2016 (Food and Agriculture Organization (FAO) 2018). However, the level of rice-based self-sufficiency varies among provinces. (World Food Program (WFP) 2007). In addition to variations in rice productivity at the national level, there are discrepancies at local levels (e.g., district and village levels) as well, and 18.5% of the total Laos population was undernourished in 2014 - 2016 (FAO 2015).

Rainy season lowland rice comprises 78.3% (755,243 ha) of the total rice cultivation area (Ministry of Agriculture and Forestry, Laos (MAF) 2016). In hilly and mountainous areas, rainy season lowland rice is cultivated in low-lying zones between mountains (Anzai et al. 2019a). The differences in rice productivity are caused by farming techniques, water accessibility, planting environment, and other factors (Ikeura et al. 2016). To increase rice production in each village's low-productivity fields, the factors influencing yield reduction must be examined and necessary measures, such as irrigation and fertilization, should be considered.

Dry season lowland rice comprises 10.3% (99,018 ha) of the rainy season lowland rice area (MAF 2016). Although some paddy fields are used for dry season cropping after the rainy season rice harvest, the area is limited by water shortage; remaining fields are used mainly as grazing land for livestock during the dry season. The area equipped with irrigation facilities is only 13% of the total agricultural land (FAO 2015). Although irrigation facilities have not been enough developed, farmers have small reservoirs for fish aquaculture (Anzai et al. 2017). Supplemental irrigation using water resources stored in existing aquaculture reservoirs may be considered to extend dry season cropping.

The objectives of our study are 1) to elucidate the factors driving reduced lowland rice yield that is occurring in a semi-mountainous village, 2) to evaluate the potential for dry season cropping and water requirements for supplemental irrigation, 3) to evaluate the the volume of water available for irrigation in existing aquaculture reservoirs, and 4) to design an irrigation plan using reservoir water. This paper summarizes our results.

Materials and Methods

Field description

Field surveys were conducted in the Nameuang Village, Feuung District, Vientiane Province, Laos, which is 88 km northwest of the Vientiane Capital (Fig. 1). Paddy fields are distributed in the lowland (lowland fields: 81 ha) and mountainous areas (12 ha). This study investigated the lowland fields (Ikeura et al. 2016).



Fig. 1. Location of survey site

This figure was modified to reference Ikeura et al. (2016) and <http://www.freemapviewer.com>.

Field survey on water conditions and farming activities for rainy season rice planting

Fig. 2 shows the study site in Nameuang Village. Field surveys were carried out in Areas A, B, and C in River Basins A and B from June 9 to August 11, 2013. Total number of fields is 55 in the basins. Surface water depth and farming activities such as nursery establishment, plowing, puddling, transplanting or direct seeding were recorded weekly for each field. To evaluate water conditions during the rice-growing period, automatic water level gauges were installed in 6 fields. Sampling quadrats (1 m × 1 m) were installed in 47 field blocks in Areas A, B, and C in September 2013. Three large field blocks were divided into two parts, with three quadrats installed in each. At maturity, rice samples were harvested from each quadrat; grain weight and moisture content were measured after drying, threshing, and winnowing. To examine the effect of soil fertility on grain yield, soil samples were collected from the upper 10 cm of soil in each quadrat at the end of September 2013, just before flowering. Total nitrogen and available phosphorus were measured for the collected soil samples (Ikeura et al. 2016).

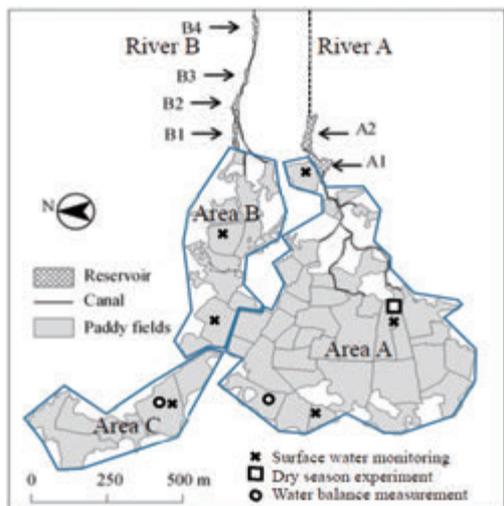


Fig. 2. Surveyed fields and reservoirs

This figure was drawn to reference Ikeura et al. (2016), Ikeura et al. (2017) and Anzai et al. (2019a).

Field experiments for dry season cropping

Rainfed (Exp. 1, December 2013 to March 2014) and irrigated (Exp. 2, December 2014 to March 2015) cropping experiments were conducted in the field shown in Fig. 2 (Ikeura et al. 2017). Soybean, maize, mung bean and upland rice were planted in Exp. 1, and soybean and maize were planted in Exp. 2. Of the 4 test crops, only soybean was grown until harvesting, and its yield and root system were measured. Groundwater depth, soil moisture content, soil moisture potential, and meteorological data were also measured. Finally, the soybean water requirements and irrigation timings were determined based on the data obtained from water balance analysis (Ikeura et al. 2017) and the evapotranspiration rate calculated by Penman-Monteith Equation (Allen et al. 1998).

Reservoir capacity surveys

To evaluate the capacity of the existing reservoirs, 2 reservoirs on River A and 4 reservoirs on River B (shown in Fig. 2) were surveyed. These 6 reservoirs, which were owned by 5 farmers, were constructed for fish aquaculture, and water overflows from the reservoirs flow into the paddy fields located downstream. Although the stored water is not used for paddy field irrigation, it is pumped out (drainage operation) at the end of each April to harvest fish. Cross section measurements were used to calculate the reservoir capacity (Anzai et al. 2017).

Irrigation planning for rainy season rice and dry season cropping

The objectives of the irrigation plan using existing reservoirs are 1) to irrigate paddy fields with low rice yield during the rainy season, and 2) to provide irrigation for dry season cropping using the remaining water. We analyzed possible water withdrawal from 6 reservoirs located on Rivers A and B (shown in Fig. 2) for Cases 1 to 4. The water balance of each reservoir, aquaculture cultivation period, minimum water level for fish survival, and timing of the drainage operation

were considered (Anzai et al. 2019a).

(Case 1) Year-round fish cultivation without drainage operation.

(Case 2) Fish cultivation with drainage operation conducted on 1 April.

(Case 3) Fish cultivation with drainage operation conducted at the same time as final irrigation for dry season cropping.

(Case 4) No fish cultivation, all water can be used for irrigation.

In cases with irrigation operations, a minimum water level of 50 cm was maintained except in Case 4. Meteorological and hydrological data measured from July 2014 to June 2015 were used for water balance analysis. To estimate the water requirements prior to transplanting, soil moisture sensors and automatic water level gauges were installed into 2 of the lower fields (shown in Fig. 2). Soybean was selected as the target crop, and water requirement obtained from dry season cropping tests was applied to the irrigation plan. The water application efficiency of furrow irrigation (0.7; Ali 2011) as well as conveyance losses measured during both the rainy and dry seasons, were considered. The potential irrigation area for the rainy and dry seasons was calculated based on possible water withdrawal. Finally, we calculated the potential benefits of the proposed irrigation plan (Anzai et al. 2019a).

Results and Discussion

Water conditions and farming activities for rainy season rice planting

Water from Rivers A and B flowed into the upper fields located in Areas A and B, respectively, then flowed downward in each area. Water was supplied to Area C from the lowest fields of Areas A and B; in other words, Area C was received water from both Rivers A and B. Fig. 3 shows the surface water depth and farming practices for each field. Rainy season rice is transplanted from early July to mid-August. Although transplanting was taking place in the upper and middle parts of the lowland area, there was still no water by mid-July, and even plowing had not begun in the lower fields. In such fields, transplanting started from the beginning of August (Ikeura et al. 2016). The results of surface water monitoring for 6 fields located in the upper, middle and lower positions indicated that no serious water shortage was expected in the entire lowland field areas after the 2013 transplant. As shown in Fig. 4, the fields with greater than 4.0 t ha⁻¹ (400 g m⁻²) yields were mainly located in the upper and middle areas. In contrast, the fields with less than 2.0 t ha⁻¹ yields were located in the lower areas (Ikeura et al. 2016). No correlations were found between rice grain yield and total nitrogen ($R^2=0.004$) or available phosphorus ($R^2=0.08$) (Ikeura et al. 2016).

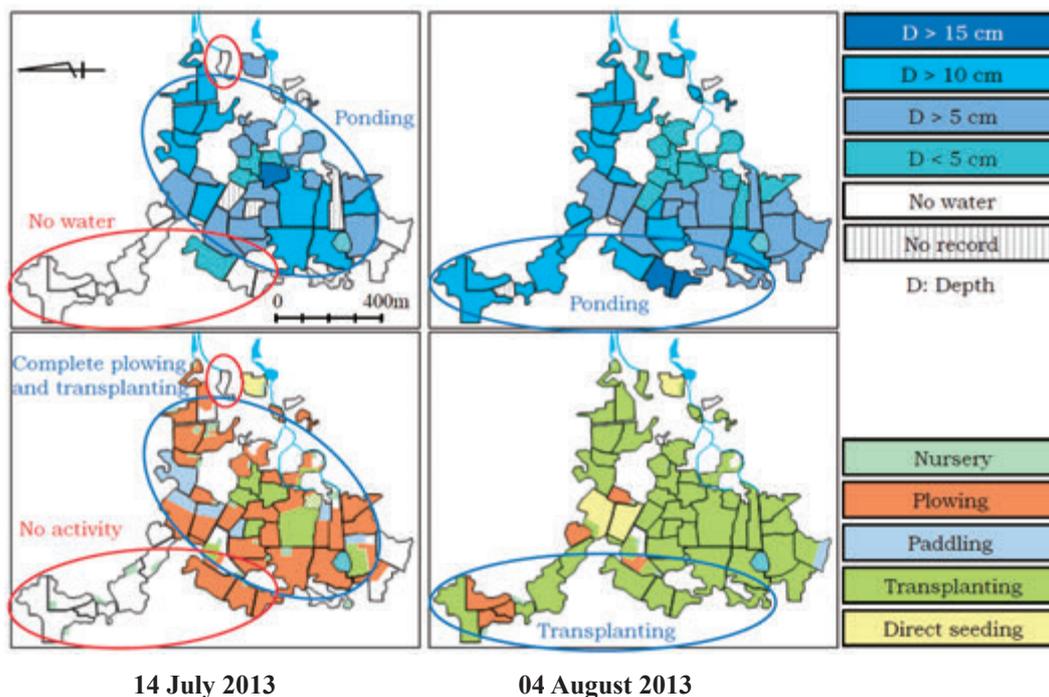


Fig. 3. Surface water depth (upper) and practiced farming activities (lower) in each field
 This figure was modified to reference Ikeura et al. (2016).

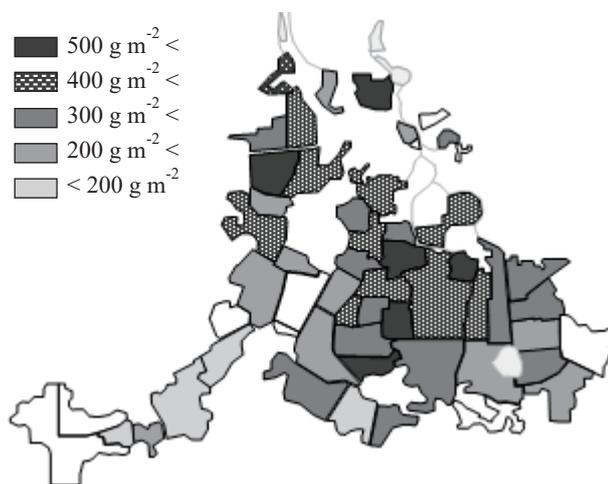


Fig. 4. Grain yield in each field
 Grain yield with 14% of moisture content
 Ikeura et al. (2016).

Table 1 shows the relationship between grain yield and the start times for ponding and transplanting in 137 plots. The grain yield was significantly higher in fields with early ponding (before July 20) and early transplanting (before July 28) than in fields with late ponding (after July 21) and late transplanting (after July 29) (Ikeura et al. 2016). The results suggest that late transplanting due to water shortage resulted in reduced yields in the lower fields of the plot-to-plot irrigated area. To increase grain yield in the lower fields, transplanting should be completed

by mid-July, and preparatory irrigation is needed to accelerate water supply to the lower fields (Ikeura et al. 2016).

Table 1. Relationship between grain yield and starting times of ponding, transplanting

Classification		N*	Avg. grain yield (t ha ⁻¹)	Note
Start time of ponding	Before Jul 20	108	3.87 ^a	Significant difference between a and b at $p < 0.05$ according to t test
	After Jul 21	29	2.22 ^b	
Start time of transplanting	Before Jul 14	28	4.20 ^a	Significant difference between a and b at $p < 0.05$ according Tukey-HSD test
	Jul 15 –Jul 28	64	3.68 ^a	
	Jul 29 – Aug 11	45	2.88 ^b	

This table was modified to reference Ikeura et al. (2016)

* The 13 samples (4 plots harvested by farmers before sampling and 9 plots in direct seeding field) were excluded from the analysis ($n = 137$).

Dry season cropping potential and water requirements

Soil moisture content at the surface layer (0 – 20 cm depth) was 0.45 cm³cm⁻³ at the beginning of rainfed cropping experiment (Exp. 1) and decreased to 0.34 cm³cm⁻³ within 3 weeks, almost depletion of moisture content for optimum growth. The soil moisture became saturation after rain and decreased again to 0.33 cm³cm⁻³ within three weeks. These results suggest that drought stress occurred after three weeks without rain or irrigation. Based on the results of changing soil moisture and soil moisture retention characteristics, we determined the water requirement for irrigated cropping experiment (Exp. 2), shown in Table 2. Before sowing began in Exp. 2, 17.6 mm of available moisture still remained in the surface layer; therefore 16 mm of water was supplied for the first irrigation. The third irrigation was skipped, because the field had been saturated by rainfall prior to the determined irrigation time (Ikeura et al. 2017).

Table 2. Water requirement and supplied water for soybean in Exp. 2

Irrigation	Date	Water requirement (mm)	Supplied water (mm)	Evapotranspiration (mm) ^{*1}
Remaining available moisture		—	17.6	—
1st	01 Dec 2014	33.6	12.8	38.3
	06 Dec 2014		3.2	
2nd	27 Dec 2014	50.4	51.2	52.5
3rd	17 Jan 2015	50.4	— ^{*2}	76.0
4th	11 Feb 2015	82.2	78.4	52.4
Total		216.6	163.2	219.2

This table was modified to reference Ikeura et al. (2017).

*1: Evapotranspiration was calculated by Penman-Monteith Equation using climate data observed in the period of Exp. 2.

*2: Third irrigation was not practiced because the field was saturated by rainfall before irrigation.

Table 3 shows the rooting ratio, dry matter weight and yield for soybean. Rooting ratio means the ratio of survived plant before harvesting. The soybean rooting ratio was 64% under rainfed conditions and 85% under irrigated conditions; rainfed conditions had a lower rooting ratio because of water shortage during the initial growth stage (Ikeura et al. 2017). Dry matter weight and yield under irrigated conditions (Exp.2) increased up to 4.5 and 7 times, respectively, compared with rainfed conditions (Exp. 1). Although the yield increased with irrigation, the yield was still 1/5 of the national average (1.4 t ha^{-1} : FAO 2017).

Table 3. Rooting ratio, dry matter weight and yield of soybean

	Rooting ratio	Dry matter weight (t ha^{-1})	Yield (t ha^{-1})
Exp. 1 (rainfed)	0.637	0.201	0.0404
Exp. 2 (irrigated)	0.854	0.894	0.285

This table was modified to reference Ikeura et al. (2017).

Fig. 5 shows precipitation, irrigation, and changes in soil suction during the planting period. Soil moisture in the surface layer decreased mainly at the beginning stage, while at the 30 cm depth it decreased 3 weeks after sowing. Although the soil moisture increased following irrigation, field capacity ($-63 \text{ cm H}_2\text{O}$) did not effectively recover. Increased groundwater levels were observed after irrigation, suggesting that part of the irrigation water flowed into cracks caused by soil shrinkage and got through the root zone. This caused water shortage and lower soybean yields than the national average (Ikeura et al. 2017).

From the experimental results, we concluded that: 1) irrigation is essential for dry season cropping, 2) the total water requirement estimated by the soil moisture balance mostly agrees with evapotranspiration calculated from meteorological data, and 3) soil cracks caused loss of infiltration and decreased soybean yield; infiltration loss must be considered for irrigation planning.

Capacity of existing aquaculture reservoir

The reservoirs had earth-type dykes 1.2 to 2.5 m high. Water outlets at the upper part of the dikes were opened, and water-controlling gates were not installed all reservoirs. Overflow

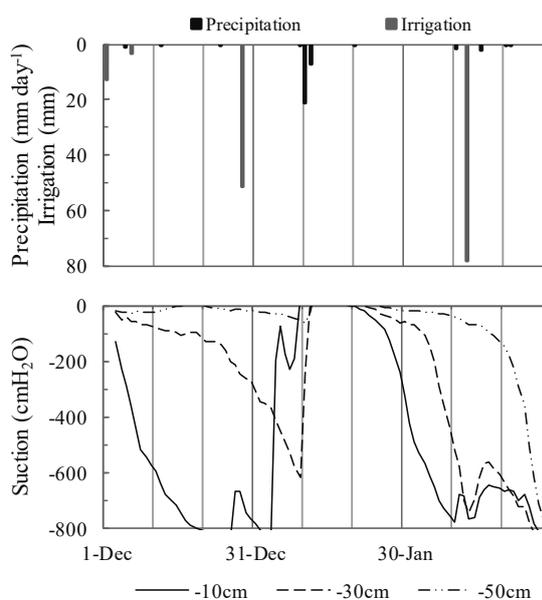


Fig. 5. Precipitation, irrigation and change in suction of field soil in soybean planting period.

This figure was modified to reference Ikeura et al. (2017).

occurred naturally when the water level was higher than the bottom level of the water outlets; an engine pump or syphon were required to take stored water below the outlet level (Anzai et al. 2017). Table 4 shows the reservoir storage capacities, which were obtained using cross section measurements. The maximum and minimum volumes were 2,441 m³ in A2 and 368 m³ in B3. The total volumes of Rivers A and B were 4,361 m³ and 4,352 m³, respectively; a total of 8,700 m³ of stored water remained due to structural issues with the reservoir outlet (Anzai et al. 2017).

Table 4. Storage capacity of reservoirs

River	Reservoir	Water surface area (m ²)	Storage capacity (m ³)	
			Each reservoir	Each river
River A	A1	2,633	1,920	4,361
	A2	3,085	2,441	
River B	B1	1,363	1,271	4,352
	B2	1,294	765	
	B3	698	368	
	B4	1,902	1,945	

This table was modified to reference Anzai et al. (2017).

Potential water withdrawal and irrigation area

Preparatory irrigation for rainy season rice (PIR) and supplemental irrigation for soybean cultivation in the dry season (SID) were planned based on the rainy season rice planting survey results and the dry season cropping experiments. Fig. 6 shows the PIR target fields; the fields were selected based on two conditions; (1) less than 3.0 t ha⁻¹ grain yield and (2) transplanting was not practiced at the end of July, when it was completed for a large proportion of the upper and middle parts of lowland rice fields. The target field areas were 7.8 ha and 6.3 ha in River basins A and B, respectively (Anzai et al. 2019a). PIR

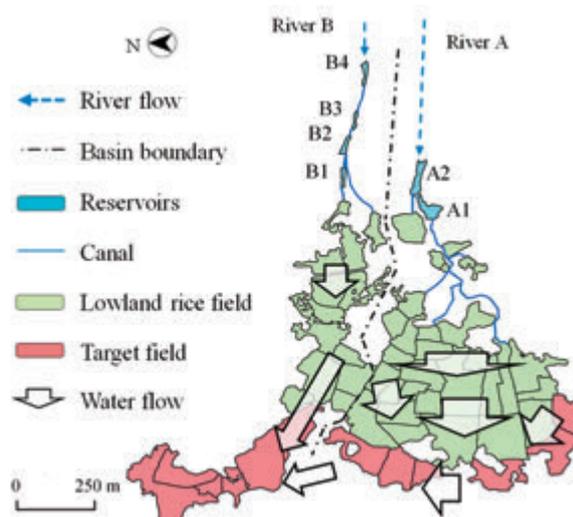


Fig. 6. Target fields of preparatory irrigation
This figure was modified to reference Anzai et al. (2019a) and Anzai et al. (2019b).

was planned from July 1-15, and water requirements were determined as 57.5 mm and 32.9 mm in the target fields in the River Basins A and B, respectively, based on water balance observed in two fields (Anzai et al. 2019a). Based on dry season experiments, the soybean cultivation period was planned for the three months from December to February. Four SID times were planned, as

follows. First: 38.3 mm on December 1 at the time of sowing; second: 52.5 mm on December 28, four weeks after sowing; third: 76.0 mm on January 18, three weeks after the second; and fourth: 52.4 mm on 11 February, three weeks after the third. For the first irrigation event, the remaining soil moisture at sowing time was considered 17.6 mm (Ikeura et al. 2017; Anzai et al. 2019a). A reservoir water management was formulated as shown in Fig. 7 (Anzai et al. 2019a; Anzai et al. 2019b).

Table 5 shows possible water withdrawal for PIR (PW_{PIR}) and for SID (W_{SID}). In Rivers A and B, the PW_{PIR} was the same for Cases 1–3 because the stored water volume at the beginning of PIR was equal to net inflow in the PIR period. In Case 4, PW_{PIR} increased because all of the stored water could be used for PIR. For River A, W_{SID} was also same for Cases 1–3. W_{SID} was higher in Case 4 than in Cases 1–3 because the maintenance volume was zero at the third irrigation time, when the water requirement was highest. In River B, W_{SID} was same for Cases 1, 2 and 4. In Case 3, W_{SID} in Case 3 was the largest of all cases, because all of the stored water was used at the final irrigation time (Anzai et al. 2019a).

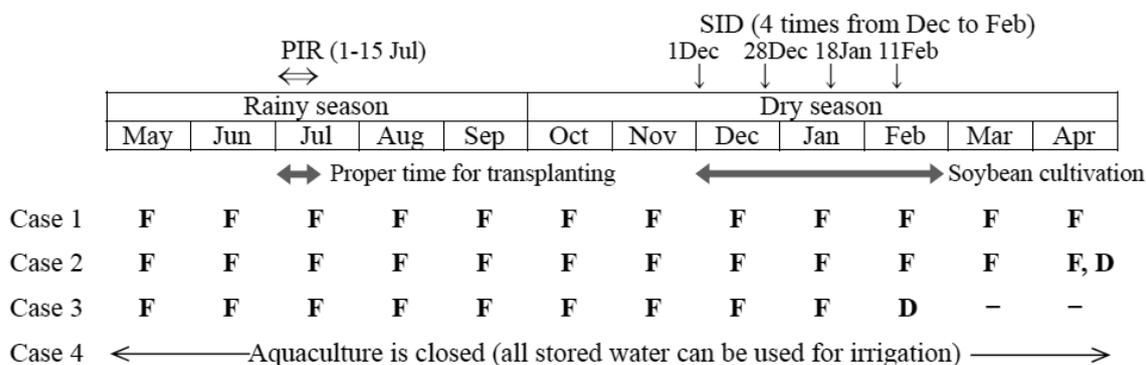


Fig. 7. Water management utilizing the reservoirs constructed for aquaculture
 F: fish growing month, D: drainage operation for harvesting fish, ⇔: period PIR, ↓: time of SID.
 The figure was drawn to reference Anzai et al. (2019a) and Anzai et al. (2019b).

Table 5. Water withdrawal for PIR and SID.

Case	Possible water withdrawal for PIR (m ³)		Water withdrawal for SID (m ³)*	
	River basin A	River basin B	River basin A	River basin B
1	13,235	9,331	29,379	3,614
2	13,235	9,331	29,379	3,614
3	13,235	9,331	29,379	4,306
4	15,004	11,424	34,072	3,614

* Water withdrawal for SID is the total amount of first to fourth irrigations.

This table was modified to reference Anzai et al. (2019a).

Table 6. Potential area of PIR and SID

Case	Potential area for PIR (ha)				Potential area for SID (ha)	
	River basin A		River basin B		River basin A	River Basin B
	Potential area	Deficit*	Potential area	Deficit*	A	B
1	4.10	3.70	8.61	-2.31	2.10	1.07
2	4.10	3.70	8.61	-2.31	2.10	1.07
3	4.10	3.70	8.61	-2.31	2.10	1.27
4	4.65	3.15	10.54	-4.24	2.44	1.07

This table was modified to reference Anzai et al. (2019a).

* Deficit is the difference of potential area to the area of target fields. When it shows minus, the potential area covered target area, and surplus water remained.

Table 7. Preliminary calculation of benefit obtained by PIR and SID

Case	Potential irrigation area* ¹		Rice production		Soybean	Benefits from irrigation (1,000 Kip)		
	PIR ^a	SID ^b	Grain	Increment in	product	Total income	Compensation	Net
			yield* ²	product (t)		e = c×2,500KIP kg ⁻¹	for feeding and	income
			(t ha ⁻¹)	c = a×(3.9-2.2)	d = b×1.4* ³	+ d×8,000KIP kg ⁻¹ * ⁴	loss of income ^f	e - f
Current	No irrigation		2.2	0	0	—	—	0
Case 1	10.40	3.17	3.9	17.68	4.44	79,704	0	79,704
Case 2	10.40	3.17	3.9	17.68	4.44	79,704	0	79,704
Case 3	10.40	3.37	3.9	17.68	4.72	81,944	720* ⁵	81,224
Case 4	10.95	3.52	3.9	18.62	4.92	85,850	6,108* ⁶	79,742

This table was modified to reference Anzai et al. (2019a) and Anzai et al. (2019b).

*1: Potential irrigation area is summation of potential areas of River Basins A and B. In the case of PIR of River Basin B, potential area overs target area; therefore target area is used for calculation of total potential irrigation area in two river basins.

*2: As referred to Ikeura et al. (2016), the grain yield of rainy season rice increases from 2.2 t ha⁻¹ to 3.9 t ha⁻¹ due to on-time transplanting through PIR.

*3: The average yield of soybean due to SID is assumed to be 1.4 t ha⁻¹ (FAO 2017).

*4: The grain rice price was the selling price from farmer to trader obtained by interview survey in Nameuang Village in 2015 (Anzai et al. 2019b), and soybean price was the selling price from farmer to the local market in Khammouane Province in 2015 (Ikeura et al. 2017).

*5: In Case 3, reservoir owners are compensated with feeding fees.

*6: In Case 4, gross income from aquaculture is paid to reservoir owners as “loss of income compensation”.

Table 6 shows potential areas for PIR (A_{PIR}) and SID (A_{SID}). A_{PIR} was calculated as 4.1 ha (Cases 1–3) and 4.7 ha (Case 4) in River basin A, and 8.6 ha (Cases 1–3) and 10.5 ha (Case 4) in River basin B. A_{PIR} did not cover the entire target field area in River basin A, however, it covered all the target field area (6.3 ha), with a surplus area of 2.3–4.2 ha in River basin B (Anzai et al. 2019a). PIR enabled on-time transplanting for about 75% of target fields (14.1 ha) (Table 7) (Anzai et al. 2019b). A_{SID} was calculated as 2.1 ha (Cases 1–3) and 2.4 ha (Case 4) in River basin A, and 1.1 ha (Cases 1, 2, and 4) and 1.3 ha (Case 3) in River basin B (Table 6) (Anzai et al. 2019a). The total A_{SID} of the two river basins were 3.17, 3.17, 3.37, and 3.52 ha for Cases 1, 2, 3 and 4, respectively (Table 7).

To calculate the benefits of irrigation, income from the increased rice and soybean yields from irrigation and aquaculture were considered, as were aquaculture expenditures such as feed costs, compensation paid for losses caused by a shortened fish cultivation period in Case 3, and compensation costs for closing aquaculture in Case 4. The results showed expected revenue growth in all Cases (Table 7). It is desirable to maintain aquaculture (Cases 1, 2 and 3) to secure animal protein resources (Anzai et al. 2019b).

Conclusion

We conducted a survey about the current state of rice production in lowland fields located in a semi-mountainous village in Vientiane Province, Laos. Then, we studied the potential for increasing rice yield and introducing dry season crops by using water stored in existing reservoirs used for fish aquaculture. The findings were as follows;

- 1) Low rice grain yield was observed in fields where transplanting was delayed due to water shortages at the beginning of the rainy season. The results suggest that the yield was reduced because of delayed transplanting. Preparatory irrigation is needed to accelerate transplanting and improve the rice yield.
- 2) Supplemental irrigation is essential for soybean cropping in the dry season. A total of 220 mm of water is needed during the soybean planting period (December to February). The water should be divided for 4 applications at 3-week intervals.
- 3) Within the survey area, 6 existing reservoirs used for fish aquaculture were storing a total of 8,700 m³ water with the potential for use in irrigation.
- 4) The potential area of preparatory irrigation for rainy season lowland rice and supplemental irrigation for dry season cropping were 10.4-11 and 3.2-3.5 ha, respectively. A total of 80,000,000 kip of additional income was expected through the practice of irrigating with the water stored in existing reservoirs in the surveyed river basins.

The results of this research can be applied to Nameuang Village as well as to other villages where rainy season lowland rice cultivation and aquaculture have been conducted. However, potential irrigation areas should be calculated based on each village's water resources and land use. Additionally, if engine pumps or syphons are needed to withdraw water from reservoirs, equipment costs and fuel should be considered in the calculations.

Acknowledgments

This study was conducted as part of the collaborative research project of Japan International Research Center for Agricultural Sciences (JIRCAS), National Agriculture and Forestry Research Institute and National University of Laos funded by JIRCAS. We are grateful for the helpful assistance of Agriculture and Forestry Office of Feuang District, Vientiane Province, Department of Meteorology and Hydrology, Laos, and all the residents of Nameuang Village for working with us.

References

- Ali MH (2011) Practices of irrigation and on-farm water management, volume 2. Springer:113
- Allen RG, Pereira LS, Raes D, Smith M (1998) Crop Evapotranspiration. FAO Irrigation and Drainage Paper 56
- Anzai T, Chomxaythong A, Ikeura H (2017) Storage capacities on fish aquaculture reservoirs and considerations of challenges on irrigation practice by using the reservoirs at a village in a semi-mountainous area, Lao PDR. *J Rainwater Catchment Syst* 23:51–58 (in Japanese)
- Anzai T, Ikeura H, Chomxaythong A, Keokhampui K, Inkhamseng S (2019a) Estimation of potential area of preparatory irrigation for rainy season lowland rice and supplemental irrigation for dry season cropping using existing reservoir constructed for aquaculture: a case study in semi-mountainous village, Lao PDR. *Paddy and Water Environment*. <https://doi.org/10.1007/s10333-018-00685-z>
- Anzai T, Ikeura H, Chomxaythong A, Keokhampui K, Inkhamseng S, Fujimaki H (2019b) Increasing rainy season lowland rice and dry season crop yields in the semi-mountainous villages of Laos using stored water intended for aquaculture. *Research Highlights of 2018, Japan International Research Center for Agricultural Sciences*. https://www.jircas.go.jp/en/publication/research_results/2018_c04, Accessed 24 July 2018
- Food and Agriculture Organization (2015) *The State of Food Insecurity in the World 2015*
- Food and Agriculture Organization (2017) FAOSTAT. <http://faostat3.fao.org/faostat-gateway/go/to/download/E/EL/E>. Accessed 15 September 2017
- Food and Agriculture Organization (2018) FAOSTAT. <http://www.fao.org/faostat/en/#data/QC>. Accessed 24 July 2018
- Ikeura H, Keokhamphui K, Fujimaki H, Anzai T, Inkhamseng S (2017) Effects of remaining soil moisture and irrigation on dry season cropping in lowland rice fields in Lao PDR. *Irrig Drain Rural Engin J* 304: II_25–II_33 (in Japanese)
- Ikeura H, Phongchanmixay S, Phonsangone S, Xaypanya P, Inkhamseng S, Soubat S (2016) Factors affecting differences in the rainy season rice yield in a lowland area of a mountainous village in Lao PDR. *Paddy and Water Environment* 14:343–353
- Ministry of Agriculture and Forestry, Laos (2016) *Agricultural statistics year book 2015*
- WFP (2007) *Lao PDR: Comprehensive food security & vulnerability analysis*. World Food Program, Vientiane

Section II

Agricultural management in mountainous slope area



Teak forest in Laos



Laotian mango varieties

Soil suitability map for planted teak (*Tectona grandis* L.f.) stand growth in the mountainous area of northern Lao People's Democratic Republic

Akihiro Imaiya¹, Simone Vongkhamho², Phutthavong Sikhot²

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Forestry Research Center (FRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

A soil suitability map was developed for teak (*Tectona grandis* L.f.) plantations in northern Lao People's Democratic Republic based on 110 sample sites located along a 40 km transect line running northwest–southeast in southwestern Luang Prabang Province, 63 of which included teak stands. The soils were observed to a depth of 1 m using a soil auger at 59 sites and by obtaining a soil profile at 51 sites, and the soil physicochemical properties were summarized for the 0–20 cm depth layer. In addition, the height and diameter at breast height of the teak trees and stand age were measured in a 20 × 20 m plot at each teak stand. The stand age ranged from 3 to 31 years, and the average dominant tree height ranged from 5.1 to 27.7 m. The site index (SI), which was defined as the dominant tree height at base age (20 years), was computed based on a height–growth model using the Richards function and ranged from 12.6 to 27.0. A predictive model SI based on soil physicochemical properties, site conditions, and terrain characteristics was developed using the decision tree method, which showed that CN ratio, electrical conductivity, convergence index, coarse sand fraction, slope gradient, and 22 additional factors contributed to the SI. This model was then used to construct a soil suitability map, which showed that suitable land covered approximately 50% of the study area, 8% of which was optimal land, while unsuitable land covered approximately 30% of the area.

Introduction

Nearly two-thirds of the territory of Lao People's Democratic Republic (PDR) is covered by forest. However, the majority of these land areas have been deforested and/or rapidly degraded. In particular, serious deforestation is occurring in the northern mountainous region, largely due to the expansion of shifting cultivation practices by local people living in poverty. Indigenous tree plantations are expected to mitigate the impacts of deforestation by helping to restore forest cover and providing an alternative income for local people who would otherwise depend solely on unsustainable shifting cultivation practices (MAF 2005). However, the development of sustainable and profitable tree plantations in the hilly terrain, which is characterized by high precipitation, requires that special attention be given to soil conservation measures. Therefore, the aim of this project was to propose suitable silvicultural technologies that could be used by local

communities in northern Lao PDR to manage indigenous tree plantations while taking soil conservation into consideration.

Teak (*Tectona grandis* L.f.) is one of the most important tropical hardwood species in the international market for high-quality timber and is also one of the most valuable indigenous tree species for smallholder woodlots in the mountainous region of Lao PDR. Teak is native to South Asian regions that have a monsoon climate and develops on fertile soils of alluvial, limestone, and basalt origin (Kaosa-ard 1989). However, teak has also been planted across a wide variety of site conditions both within and outside its native area across the tropics, including at sites with extreme climates and soils (Kollert and Kleine 2017). Since the site/soil characteristics that are optimal for teak growth may vary according to the climate, geology, topography, etc. of a particular region, site selection is the most critical issue for the successful establishment and management of this important species (Kollert and Kleine 2017). JIRCAS and the Royal Forest Department developed a series of soil suitability maps for teak plantations in northeast Thailand. These maps were established based on soil group maps and field observations of landform and soil properties in order to describe actual soil features and limitations (Sukchan and Noda 2012). This method could be used as a basis for establishing soil suitability maps in neighboring Lao PDR. The soil suitability maps in NE Thailand were targeted toward flat and gently sloping lands because farmers were growing teak on flat land in Thailand. In contrast, teak plantations were established on sloping land in Lao PDR. For this reason, it is necessary to develop a technology adapted to sloping land. Furthermore, both countries use different soil classification systems, so we cannot directly compare the soils and soil properties.

The objective of this study was to develop a land evaluation method for teak growth in northern Lao PDR. To do this, the site index (SI), which was defined as the dominant tree height at base age, was estimated using the decision tree method, which allows taxonomic units to be subdivided into areas of similar productivities or site qualities. The resulting model was then used to establish a soil suitability map for teak plantations in northern Lao PDR based on the SI.

Materials and methods

Study sites

A transect line was established in a northwest–southeast direction from the village of Timsom in the city of Luangprabang, which is located on the Mekong River (19°48'11" N, 101°59'54" E), to the village of Nammok, Xiengngeun District, which is located on the mountain ridge of Phou Kham (19°31'32" N, 102°16'20" E) in Luangprabang Province. This transect was approximately 40 km long and approximately 300–1500 m above sea level (a.s.l.). It crossed three mountains that are divided by the Mekong and Khan rivers and their tributaries and consist of Paleozoic sedimentary rocks, limestone, and volcano-sedimentary rocks (JICA-DGEO-DOM 2008a, 2008b). The soils along the transect were primarily Acrisols and Alisols with associated Cambisols and Leptosols (NAFRI 2000).

Our study examined 110 sites along this transect, 63 of which included stands of planted teak at elevations of 287 to 1057 m a.s.l. The land uses of the remaining 47 sites were comprised

of crop land and fallow forest resulting from slash-and-burn cultivation, secondary forest, and natural forest.

Tree measurement and SI assessment

A tree survey was undertaken in a 20 × 20 m plot at each teak stand site. During this survey, the diameter at breast height (DBH) was measured using a diameter tape, and the total height (H) was measured using an ultrasound distance measure (Vertex IV; Haglöf, Sweden). In addition, three sample trees were felled in each plot and the number of tree rings at ground level was counted to determine the stand age.

SI was defined as the dominant tree height at base age in each plot, which was selected as 20 years. SI was computed based on a height–growth model using the Richards function (Ishibashi et al. 2002) according to the following equations:

$$H_o = A_o \cdot (1 - \exp(-k_o \times t))^{m_o} \quad (1)$$

$$A_i = \frac{H_i}{(1 - \exp(-k_o \times t_i))^{m_o}} \quad (2)$$

$$SI_i = A_i \cdot (1 - \exp(-k_o \times A_{SI}))^{m_o} \quad (3)$$

$$SI_i = H_i \cdot \left(\frac{1 - \exp(-k_o \times A_{SI})}{1 - \exp(-k_o \times t_i)} \right)^{m_o} \quad (4)$$

where H_o is the dominant tree height (m); t is the stand age (years); A_o , k_o , and m_o are the parameters of the guide curve; A_i , H_i , t_i , and SI_i are the parameters of the height curve, dominant tree height, stand age, and SI of the i th plot, respectively; and A_{SI} is the base age. The dominant tree height at the time of measurement (DTH) was taken as the average tree height of the 15 tallest trees in each plot. Each parameter in the Richards function for the SI curve [Equation (1)] was estimated based on the height and age of each felled tree at the time of measurement using nonlinear regression (JMP 12.0.1, SAS Inst). Although Ishibashi et al. (2010) improved the height–growth model based on the Mitscherlich function by adding more elder stand data, our study used the Richards function because the range of sample ages was similar to that of Ishibashi et al. (2002).

Soil sampling and chemical analysis

Soil samples were obtained at 20 cm depth intervals to a depth of 1 m using a soil auger at 59 sites along the transect and from each individual soil horizon in a 1 m depth soil profile at 51 sites on the slopes around the transect. At each site, the coordinate and elevation were recorded using a handheld global positioning system receiver (GPSMAP64; Garmin), and the slope aspect and gradient were measured using a clinometer.

The soil samples were air-dried and passed through a 2 mm mesh sieve, after which any visible organic fragments were manually removed before analysis. The soil pH was measured in water and in a 1 mol L⁻¹ KCl suspension using a 1:2.5 w/v soil/solution ratio with the glass electrode method. Delta pH was calculated as the difference between pH(KCl) and pH(H₂O). The

electrical conductivity (EC) of the soil was measured in a 1:5 soil/water ratio using the Pt electrode method. The total soil carbon (TC) and nitrogen (TN) content were determined using the dry combustion method with an NC analyzer (Sumigraph NC-220; Sumika Chemical Analysis Service, Ltd.). The particle size distribution was assessed according to the International Society of Soil Science classification (coarse sand [CoS], ≥ 0.2 mm; fine sand [FS], ≥ 0.02 mm; silt, ≥ 0.002 mm; and clay, < 0.002 mm) using the pipette method after dispersion and oxidation of the organic matter (van Reeuwijk 2002).

The cation exchange capacity (CEC) and exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were extracted using a 1.0 M ammonium acetate solution adjusted to pH 7.0, while exchangeable aluminum (ex.Al) was extracted with 1 M KCl. The concentrations of these cations were then determined using an inductively coupled plasma emission spectrometer (ICPE-9000; Shimadzu Inc.). The total base cations (TBC) was calculated as the sum of exchangeable Ca, Mg, K, and Na, and the effective CEC (ECEC) was calculated as the sum of TBC and ex.Al. Base saturation (BS) and effective base saturation (EBS) were then calculated as TBC divided by CEC and ECEC, respectively. In addition, CEC_{clay} was calculated based on the CEC and clay content. Available phosphorus (P) was determined according to the Bray 2 method, while the P concentration was determined using the ascorbic acid-molybdenum blue method. Finally, the bulk density (BD) and fine earth density (< 2 mm) of the soil profile were measured using the core method (Soil Survey Laboratory 1996).

The concentration/value of each soil physicochemical property at a depth of 0–20 cm in the soil profile was calculated from the integrated mass of each material in each soil horizon.

Spatial prediction of soil suitability based on SI

The spatial prediction covered the area of the city of Luangprabang and Xiengneun District (coordinate range: 19.35–20.03° N, 101.80–102.60° E).

Terrain analyses of the topographic wetness index (TWI), topographic position index (TPI), terrain ruggedness, curvatures, channel networks, slope position and heights, and landforms were performed using the NASA Shuttle Radar Topography Mission Digital Elevation Model (SRTM DEM) with a spatial resolution of 30 m in SAGA 5.0.0 (Conrad et al. 2015). The spatial diversity of each soil physicochemical property at a depth of 0–20 cm was predicted by the decision tree method in Python 3.7 using Scikit-learn v.0.21.3 (Pedoregosa et al. 2011) based on soil cross-section surveys, physicochemical analyses, topographical data, and the survey area, which was divided into the three mountains in the study area. The model for prediction of SI was established based on the analysis of the actual tree census data responsibility to variables of field-observed soil and terrain features using decision tree classification. A soil suitability map for teak plantations was then produced based on the predicted SI model, which incorporated the predicted soil and terrain spatial diversities.

Results and discussion

Tree census and SI of teak plantations

The age of the teak stands in the 63 plots ranged from 3 to 31 years, with nearly all of these stands having been established between 1995 and 1999 (41%) or 2006 and 2010 (43%). The

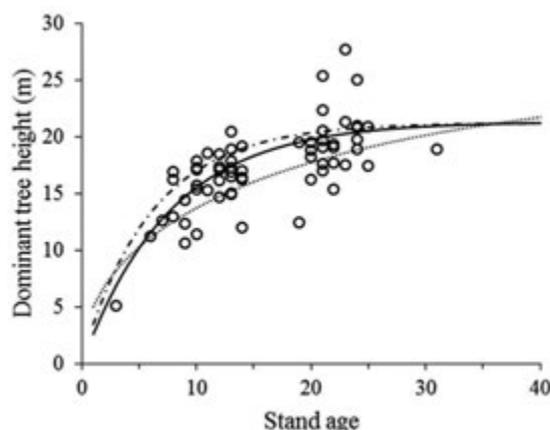


Fig. 1. Dominant tree height curve for teak (*Tectona grandis*) stands in northern Lao People's Democratic Republic estimated using the Richards function (solid line) and raw data (open circles). The chain line and dotted line represent dominant tree height curves that were constructed using the parameters estimated by Dieters et al. (2014) and Ishibashi et al. (2002), respectively.

average number of trees in the plots was 1113 ± 357 trees per hectare (mean \pm SD) (range = 425 to 2128). The average DBH was 16.0 ± 3.4 cm (range = 6.0 to 25.1 cm), the average H was 15.0 ± 3.1 m (range = 6.3 to 23.5 m), and the average DTH was 17.3 ± 3.6 m (range = 5.1 to 27.7 m).

The estimated guide curve is shown in Figure 1. The parameters A_o , k_o , and m_o were estimated as 21.33, 0.131, and 1.009 (RMSE = 2.911), respectively, while the mean estimated SI for the plots was 19.3 ± 2.8 (range = 12.6 to 27.0). Using the census of young teak trees (5–16 years) in smallholder woodlots in Luangprabang Province (Dieters et al. 2014), the parameters A_o , k_o , and m_o were estimated as 21.28, 0.155, and 0.934 (RMSE = 2.238), respectively, with a mean SI of 20.4 ± 2.7 (range = 14.6 to 25.9). By contrast, in a study in northeast Thailand, Ishibashi et al. (2002) estimated the parameters A_o , k_o , and m_o as 25.59, 0.030, and 0.465 (RMSE = 2.925), respectively. Since the parameter A_o represents the upper limit of tree height growth, k_o represents the response to time (i.e., the initial growth rate), and m_o represents the shape form of the SI curve (Teraoka 1995), the study sites had a lower A_o value as compared to northeast Thailand, despite their initial height growth being faster (Figure 1). By contrast, the initial height growth of trees at the study sites was slightly slower than that of young teak trees in Luangprabang, but their upper limits of height growth were similar. These differences in the shape of the SI curves can be explained by the fact that the stand studied by Ishibashi et al. (2002) was 5–48 years of age, making it wider and older than the stands in our study sites. This suggests that ranges of sample age are needed for alignment when comparing the growth model to the other study. In Lao PDR, observation of older teak stands is needed for improvement of the height–growth model.

Table 1. Soil physicochemical properties in the 0–20 cm depth layer at the study sites ($n = 110$).

	TC g kg ⁻¹	TN	CN ratio	Mechanical composition				pH		EC mS m ⁻¹	Available P P ₂ O ₅ mg kg ⁻¹	
				CoS %	FS	Silt	Clay	H ₂ O	KCl			
Mean	26.6	2.6	9.8	11.9	14.0	27.5	41.6	6.0	4.7	4.6	9.5	
Standard deviation		31.8	2.9	1.8	8.1	6.7	7.2	11.9	0.7	0.8	5.6	21.5
Median		20.8	2.2	9.7	10.1	12.5	27.4	40.8	6.0	4.6	3.2	3.1
Upper 95% confidence interval		32.6	3.2	10.2	13.4	15.3	28.9	43.9	6.1	4.8	5.6	13.5
Lower 95% confidence interval		20.6	2.1	9.5	10.4	12.8	26.1	39.4	5.8	4.5	3.5	5.4

	Exchangeable					TBC	ECEC	CEC	BS %	EBS	CEC _{clay} cmolc kg ⁻¹ clay	
	Ca cmolc kg ⁻¹ soil	Mg	K	Na	Al							
Mean	14.6	3.1	0.34	0.04	0.67	18.1	18.8	19.8	75.0	86.6	27.8	
Standard deviation		34.1	3.2	0.26	0.06	1.18	35.2	35.0	13.4	57.3	24.0	30.0
Median		6.1	2.4	0.28	0.02	0.24	10.3	10.6	16.4	67.9	98.9	22.3
Upper 95% confidence interval		21.1	3.8	0.39	0.06	0.89	24.8	25.4	22.3	85.8	91.1	33.5
Lower 95% confidence interval		8.2	2.5	0.29	0.03	0.45	11.5	12.2	17.2	64.2	82.1	22.1

TC: total carbon; TN: total nitrogen; CN ratio: carbon-nitrogen ratio; CoS: coarse sand; FS: fine sand; KCl potassium chloride; EC: electrical conductivity; Available P: available phosphorus; Ca: calcium; Mg: magnesium; K: potassium; Na: sodium; Al: aluminum; TBC: total base cations; ECEC: effective cation exchange capacity; CEC: cation exchange capacity; BS: base saturation; EBS: effective base saturation; CEC_{clay}: cation exchange capacity per clay

Soil physicochemical properties in the 0–20 cm depth layer

Table 1 shows the average soil physicochemical properties in the 0–20 cm depth layer across the 110 study sites. Half of the study sites had poor soil humus (TC < 20 g kg⁻¹), and nearly all of the sites contained soils that had clayey textures, were slightly acidic, and had a moderate CEC and a high BS and EBS.

A comparison of soil characteristics associated with high teak productivity in Brazil (Kollert and Kleine 2017) showed that the percentages of study sites that had optimum and unsuitable soil characteristics were 64% and 11% for Ca, 4% and 15% for Al, 36% and 0% for organic matter content, 45% and 5% for clay content, and 37% and 25% for Mg, respectively. Thus, soils with unsuitable physicochemical properties for teak growth appear to have a limited distribution in the study area, with the exception of Mg limitation.

Soil suitability map for teak plantation growth

A total of 66 factors associated with site and terrain characteristics (plan curvature, profile curvature, convergence index, TWI, LS-factor, channel network base level, channel network distance, valley depth and relative slope position in the basic analysis, curvature classification, geomorphons, clusters, landforms based on Iwahashi and Pike, convexity, terrain surface texture, TPI-based landforms (100 to 1000 m, 200 to 3000 m, and 500 to 5000 m), convergence index, convergence index (search radius), gradient (downslope distance), gradient difference, mass balance index, multi-scale TPI (maximum scale: 8 or 27), surface area, slope height, valley depth, normalized height, standardized height, mid-slope position, general curvature, profile curvature, plan curvature, tangential curvature, longitudinal curvature, cross-sectional curvature, minimal curvature, maximal curvature, total curvature, flow line curvature, terrain ruggedness index (1 cell, 10 cell), TPI (0 to 100 m, 0 to 500 m, 0 to 2000 m), local curvature, upslope curvature, local upslope curvature, downslope curvature, local downslope curvature, vector terrain ruggedness (VRM; 1 cell, 5 cell, 10 cell), positive openness, negative openness, flow accumulation, specific

catchment area, flow path length, slope length, flow direction, altitude, slope gradient, slope aspect, geological area) and 33 factors associated with soil physicochemical properties [pH(H₂O), pH(KCl), delta pH, EC, TC, TN, CN ratio, CEC, ex.Ca, ex.Mg, ex.K, ex.Na, TBC, BS, ex.Al, ECEC, EBS, CoS, FS, silt, clay, CECclay, P₂O₅, Ca saturation, Mg saturation, Ca/Mg ratio, Ca+Mg, Ca+Mg saturation, Al saturation, Ca/Al ratio, fine earth density, BD, and thickness of A horizon] were used to predict SI values for the 63 sites containing teak plantations using a decision tree analysis with 10-fold cross validation by hyperparameter tuning with the GridSearchCV module. The model with the best estimated parameters divided the observed teak sites into 35 units according to the following 27 factors: CN ratio, EC, convergence index, CoS, slope gradient, profile curvature, channel network distance, terrain surface texture, pH(KCl), pH(H₂O), CECclay, geomorphons, normalized height, local upslope curvature, gradient difference, fine earth density, landforms based on Iwahashi and Pike, ex.Na, slope length, VRM using 10cell, slope height, specific catchment area, TPI-based landforms, plan curvature, clay content, BS, and delta pH (tree maximum depth = 8, R² = 0.508, generalization performance: 53.6%). Among these, the CN ratio, EC, convergence index, CoS, and slope gradient contributed approximately 9.1, 9.0, 6.7, 6.4 and 6.0%, respectively, to the model. The sites with the highest SI values (over 22.2) had 1) a high conversion index (>-0.178), medium CN ratio (9.7 to 11.4), short channel network distance (≤68.3), low CoS (≤31.1%), high pH(KCl) (>4.3), high fine earth density (>0.84 kgm⁻³), and a low BS (≤50%); 2) a low convergence index (≤-0.178), high EC (>2.4), large slope gradient (>9.2%), low pH(H₂O) (<6.4), low CoS (≤15.7%), low ex.Na (≤0.014 cmol_ckg⁻¹), and a low VRM (≤0.099); and 3) a low convergence index (≤-0.178), high EC (>2.4), small slope gradient (≤9.2%), and a low CECclay (≤43.7 cmol_ckg⁻¹). The percentages of samples and precision of these three leaves were 1.1, 4.9, and 9.3%, and 75, 72, and 82%, respectively. By contrast, the sites with the lowest SI values (under 15.2) had 1) a high conversion index (>-0.178), high CN ratio (>11.4), and a rough terrain surface texture (>18.8); 2) a high conversion index (>-0.178), low CN ratio (<11.4), long channel network distance (>68.3), and a long slope length (>43.5); 3) a high conversion index (>-0.178), low CN ratio (<11.4), short channel network distance (<68.3), low CoS (<31.1%), high pH(KCl) (>4.3), and a low fine earth density (<0.84 kgm⁻³); and 4) a low convergence index (≤-0.178), high EC (>2.4), large slope gradient (>9.2%), high pH(H₂O) (>6.4), and a large gradient difference (<-0.035). The percentage of samples and precisions of these four leaves were 4.9, 1.9, 1.6, and 1.9% and 89, 43, 50, and 57%, respectively.

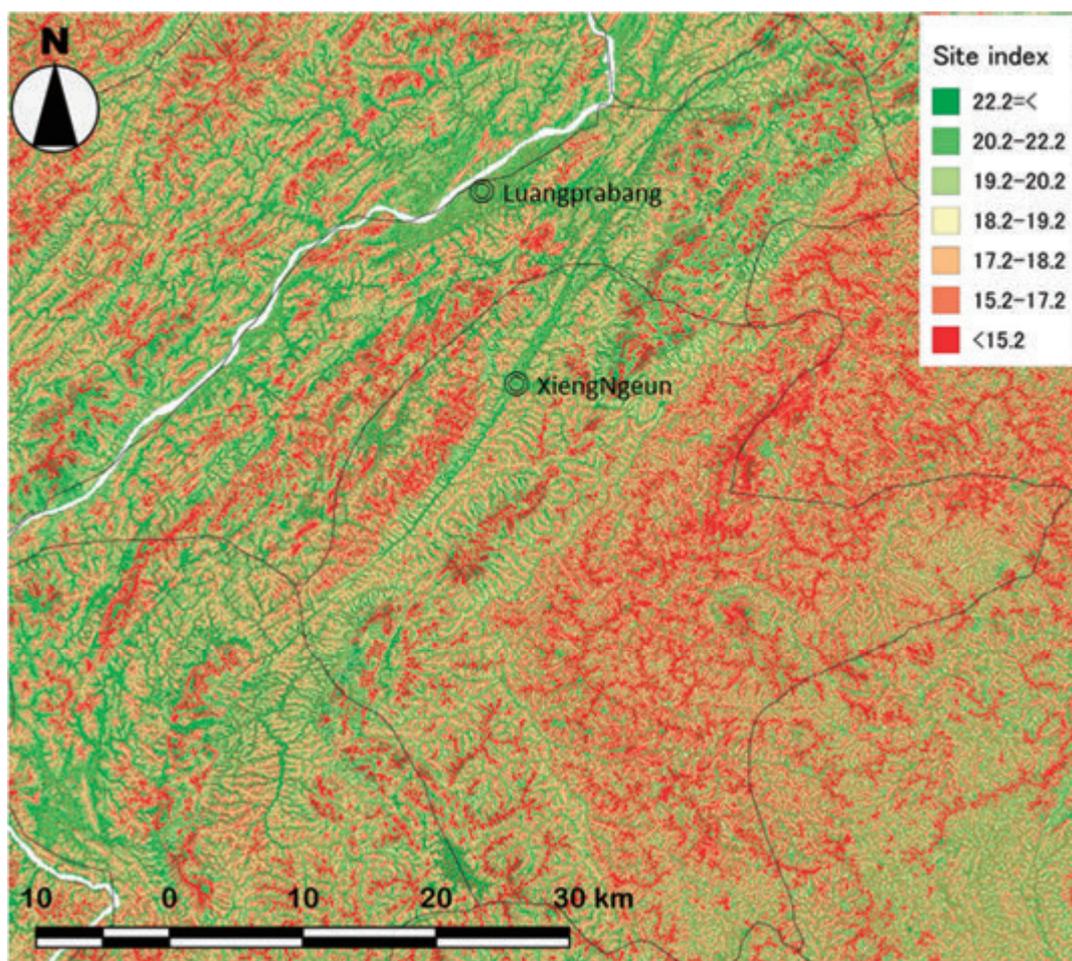


Fig. 2. Soil suitability map for teak plantations in the city of Luangprabang and Xiengngeun District, Luangprabang Province.

Table 2. Coverage of each site index range in the predicted area

Site index	<15.2	15.2–17.2	17.2–18.2	18.2–19.2	19.2–20.2	20.2–22.2	>22.2
km ²	1266.12	389.23	1028	185.27	831.88	1644.09	435.32
%	21.9	6.7	17.8	3.2	14.4	28.4	7.5

A soil suitability map was developed for teak plantations in the study area by applying the results of the above decision tree analysis to the spatially predicted soil physicochemical properties (CN ratio, EC, CoS, pH(H₂O), pH(KCl), CECclay, ex.Na, clay content, BS, and ΔpH), the results of the terrain analysis (convergence index, profile curvature, channel network distance, terrain surface texture, geomorphons, normalized height, local upslope curvature, gradient difference, landforms, slope length, VRM, slope height, specific catchment area, and plan curvature), and the geomorphological characteristics (slope gradient) using GIS (Figure 2). This showed that approximately one-third of the study area had a low SI (<17.2), and only 8% of the study area had a high SI (≥22.2) (Table 2). A large area of flat land around the river basin had a higher SI value, with various parts having low SI values. By contrast, the SI value changed rapidly

over short distances on the sloping lands of the mountain area, following the complicated topography. These findings suggest that teak growers must be careful to select appropriate land even within a small area when establishing teak plantations.

It should be noted that this soil suitability map did not consider the effects of surface geology as a parent material for the soil, as the study area was divided into only three areas along the mountain range. In the study area, limestone forms outstanding karst topography. During the field survey, limestone was seen to form the upper part of the mountain range that is located near the Mekong River and next to the southeast mountain range, but some of the limestone that occurs on the slopes of these mountains is mixed with other kinds of rocks, such as sandstone. In general, soils that are derived from limestone have unique characteristics, such as large amounts of Ca, small amounts of Al, high BS levels, high pH, and clayey textures (Imaya et al. 2005). Consequently, the soil physicochemical properties on the mountain slopes in the study area might be controlled by the contamination ratio of soil materials derived from limestone and other parent materials. Therefore, the prediction accuracy of soil that is suitable for teak growth could be improved in the future by clarifying the relationship between soil physicochemical properties and the ratio of contaminated limestone-origin materials. Moreover, another study should be conducted to validate the predicted soil suitability map.

Conclusion

A soil suitability map for establishing teak plantations in a part of southwestern Luangprabang Province was constructed based on soil cross-section surveys, soil physicochemical analyses, tree census plots, and topographical analyses. This map indicates that the average height of dominant trees was 20 years old, which is the age at which planted teak is harvested in Luangprabang. Thus, use of this map will allow suitable sites to be selected for establishing teak plantations, improving productivity, and reducing the risk of plantation failure or poor growth.

Acknowledgements

We thank the Luangprabang provincial forestry office, the office of agriculture and forestry Luangprabang District, and Xiengngeun agriculture and forestry office for the cooperate with field survey. The authors deeply appreciate the assistance of the following individuals in conducting the field surveys: Mr. Sengchanh Phengsavanh, Mr. Moukdam Southammavong at LPB PAFO, Mr. Sisouphanh Teukasri at LPB DAFO, Mr. Toulee Thou, and Mr. Sengphet Chanhthasoutthi at XN DAFO. The authors give special thanks to Dr. Satoshi Nakamura, Ms. Kumiko Yoshihara and the staff for the soil analysis. The authors thank Mr. Vongvilay Vongkhamsao at FRC, Dr. Gen Takao and Dr. Hiroyasu Oka at JIRCAS for the management with the project. The transport of soil samples from Lao PDR to Japan was approved by the Plant Quarantine Authority of Lao PDR (000128/16/0001, 000146/16/0001, 000001/17/0001, 000003/17/0001, 000004/17/0001, 000005/17/0001, 000001/18/0001, 000003/18/0001) and the Plant Protection Station of Ministry of Agriculture, Forestry and

Fisheries of Japan (28Y926, 29Y353, 29Y1468, 30Y351). The authors would like to thank Enago (www.enago.jp) for the English language review.

References

- Conrad O, Bechtel B, Bock M, Dietrich H, Fischer E, Gerlitz L, Wehberg J, Wichmann V, Boehner J (2015) System for Automated Geoscientific Analyses (SAGA) v. 2.1.4. *Geosci Model Dev* 8, 1991-2007. doi:10.5194/gmd-8-1991-2015
- Dieters M, Newby J, Cramb R, Sexton G, McNamara S, Johnson M, Vongphacsouvanh S (2014) Final report: Enhancing on-farm incomes through improved silvicultural management of teak in Luang Prabang Province of Lao PDR. Australian Centre for International Agricultural Research, Canberra
- Imaya A, Ohta S, Tanaka N, Inagaki Y (2005) General chemical properties of brown forest soils developed from different parent materials in the submontane zone of the Kanto and Chubu Districts, Japan. *Soil Sci Plant Nutr* 51:873–884
- Ishibashi S, Ishida H, Okabayashi M, Sirilak S, Wanussakul R, Klaikaew N, Intuman C, Cha-umpol C (2002) Yield prediction table on *Tectona grandis* (Teak) in Northeast Thailand. (Study Report) 11th July 2002. The Reforestation and Extension in the Northeast of Thailand Phase II (REX II)
- Ishibashi S, Sakai M, Noda I, Vacharangkura T, Krongkitsiri V, Kamolpanit D, Himmapan W (2010) Yield Prediction Table on *Tectona grandis* (Teak) in Northeast Thailand (Revised edition). RFD-JIRCAS Joint Research Project, Bangkok
- Japan International Cooperation Agency, Department of Geology, Ministry of Energy and Mines, Department of Mines, Ministry of Energy and Mines (2008a) Explanatory notes for the geological and mineral resources map of Lao People's Democratic Republic, 1:1,000,000: the geological mapping and mineral information service project for promotion of mining industry in the Lao PDR (2006-2008). JICA, Tokyo
- Japan International Cooperation Agency, Department of Geology, Ministry of Energy and Mines, Department of Mines, Ministry of Energy and Mines (2008b) Geological and mineral resources map of Lao People's Democratic Republic, 1:1,000,000. JICA, Tokyo
- Kaosa-ard A (1989) Teak (*Tectona grandis* Linn. f), its natural distribution and related factors. *Nat His Bull Siam Soc* 29:55–74
- Kollert W, Kleine M (2017) The global teak study. Analysis, evaluation and future potential of teak resources. IUFRO World Series Volume 36. Vienne
- Ministry of Agriculture and Forestry (2005) Forestry strategy to the year 2020 of the Lao PDR. 89pp.
- NAFRI (2000) Soil Map of Lao PDR. Ministry of Agriculture and Forestry (MAF), National Agriculture and Forestry Research Institute (NAFRI), Soil Survey and Land Classification Center (SSLCC), Vientiane
- Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay E (2011) Scikit-learn: Machine Learning in Python. *J Machine Learning Res* 12:2825–2830
- Soil Survey Laboratory (1996) Bulk density cores field moist (4A3a). In: Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42 ver. 3.0. Natural Resources Conservation Service, US Department of Agriculture, Washington DC. pp.137–138

- Sukchan S and Noda I (2012) Improvement of soil suitability mapping for teak plantations in Northeast Thailand. JIRCAS Working Report No.74:27–32
- Teraoka Y (1995) The development of polymorphic site index curves: using a varying shape form parameter model. Bull Kyushu Univ Forest 72:135–142 (in Japanese)
- Van Reeuwijk LP (2002) Procedures for Soil Analysis sixth edition. Technical paper 9, International Soil Reference and Information Centre, Wageningen

Collections and genetic diversity analyses of SSR markers for mango native genetic resources in Lao PDR

Shinsuke Yamanaka¹, Tatsushi Ogata¹, Phaythoune Mounsena², Nouphone Khamsohou², Hiroshi Matsuda¹, Bounneang Douangboupha²

¹ Tropical Agriculture Research Front, Japan International Research Center for Agricultural Sciences (JIRCAS), Ishigaki, Japan

² Horticulture Research Center (HRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

Exploration and collection of mango native genetic resources have been conducted in southern and central parts of Laos. A total of 24 accessions of mango local varieties were collected, and successfully propagated and conserved by grafting at Horticultural Research Center as the first collection of mango genetic resources in Laos. Collected genetic resources were employed for DNA analysis of SSR markers to estimate genetic relationship and diversity among accessions. From the results, it was interesting that some accessions were identified as same genotype even from different regions and different local variety names. Compared with world standard varieties, Laos mango genetic resources have potentially unique genotypes to be conserved and utilized.

Introduction

Tropical fruits are important potential cash crops for small-scale farmers in Laos and demand for these products has been increasing as a result of the economic development. Tropical fruits sell at a higher price than that of other crops, allowing farmers to reap greater profits from their limited land. Fruit trees can also be grown on the slopes that dominate much of the Laotian landscape.

Despite the market demand in Laos for tropical fruits, most are imported from neighboring countries, with domestic commercial production remaining low. Laos exported 151 tons of tropical fresh fruits in 2017, while importing 4,762 tons (the sum of the following items: [Fruit, tropical fresh nes] and [Mangoes, mangosteens, guavas] in FAOSTAT Database, <http://www.fao.org/faostat/en/#data>). This is partly due to the lack of established cultivation techniques and cultivars suitable for growth in Laos. Many tropical fruit trees in Laos are propagated by seed, resulting in varying inferior fruit quality compared with improved varieties (Dubbeldam 2005). Meanwhile, a number of fruit tree seedlings are being imported from neighboring countries such as Thailand (Boulom 2019), thus providing farmers with improved cultivars; however, the adaptability of these imported cultivars to local conditions remains unknown. Therefore, to effectively promote fruit tree cultivation in Laos, it is necessary to

evaluate the adaptability of potential commercial cultivars. Because of the limited availability of imported cultivars, an analysis of native genetic resources in Laos is of great importance.

Mango (*Mangifera indica* L.) is considered one of the most important fruit trees in Laos. In this study, we focused on genetic resources of underutilized mango trees native to Laos were collected and analyzed to provide a foundation for the promotion of domestic fruit production in Laos.

Materials and methods

Collection of native mango genetic resources in Laos

Explorations of native mango were conducted in Champasak province in southern Laos (Fig. 1A), the main fruit production area, in November 2016, and in Savannakhet and Bolikhamxai provinces (central part of Laos) in December 2017. During these visits, surveys and collections of mango genetic resources were carried out and interviews with farmers and residents were conducted in order to obtain additional information (Fig. 1B). Collected samples were propagated and conserved via grafting at the Horticulture Research Center (HRC) in Vientiane to provide a collection of mango genetic resources in Laos.

DNA analysis of diversity and phylogeny

Genomic DNA was isolated from dried mango leaves using a DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Seventeen simple sequence repeat (SSR) markers from a previous study (Yamanaka et al. 2019) were then used for PCR amplification. A phenogram was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) based on the similarities between genotypes estimated by Dice's coefficient,

$$Dc = 2n_{xy} / (n_x + n_y),$$

where n_x and n_y represent the number of putative SSR alleles for materials X and Y , and n_{xy} represents the number of putative SSR alleles shared between X and Y . A phenogram was then drawn using NTSYS-pc software v. 2.1 (Rohlf 1998). To determine genetic diversity, the genetic distance between accessions was calculated from the allele size of each SSR locus using GenAIE software v. 6.5 (Peakall and Smouse 2012), followed by principal coordinates analysis (PCoA).

Results and discussion

Collection and DNA analysis of native mango genetic resources in Laos

From our survey and collection of mango genetic resources in southern and central Laos in 2016 and 2017, 24 accessions were conserved and propagated via grafting in the HRC experimental nursery (Figure 1C). These accessions were used for DNA analysis along with 7 other varieties conserved at the HRC comprising mainly imported varieties.



Fig. 1. Collection of mango native genetic resources in Laos.

A: a typical location of native mango genetic resources observed in Champasak province. Trees of local mango varieties remaining over decades were frequently found at temple gardens, public squares of villages, private backyards, so on, B: interview with farmers and residents to collect information about local mango varieties, C: conservation of collected genetic resources by grafting at the HRC.

The phenogram created based on the SSR genotypes revealed 22 identical genotypes in the 31 accessions. Moreover, accessions showing the same genotype were found in different collection areas (Figure 2). These duplicated accessions were considered the results of independent selection of common desirable characteristics in different areas (MG012 and MG042, MG015 and MG043, MG016 and MG041(MG048), and MG009 and MG040).

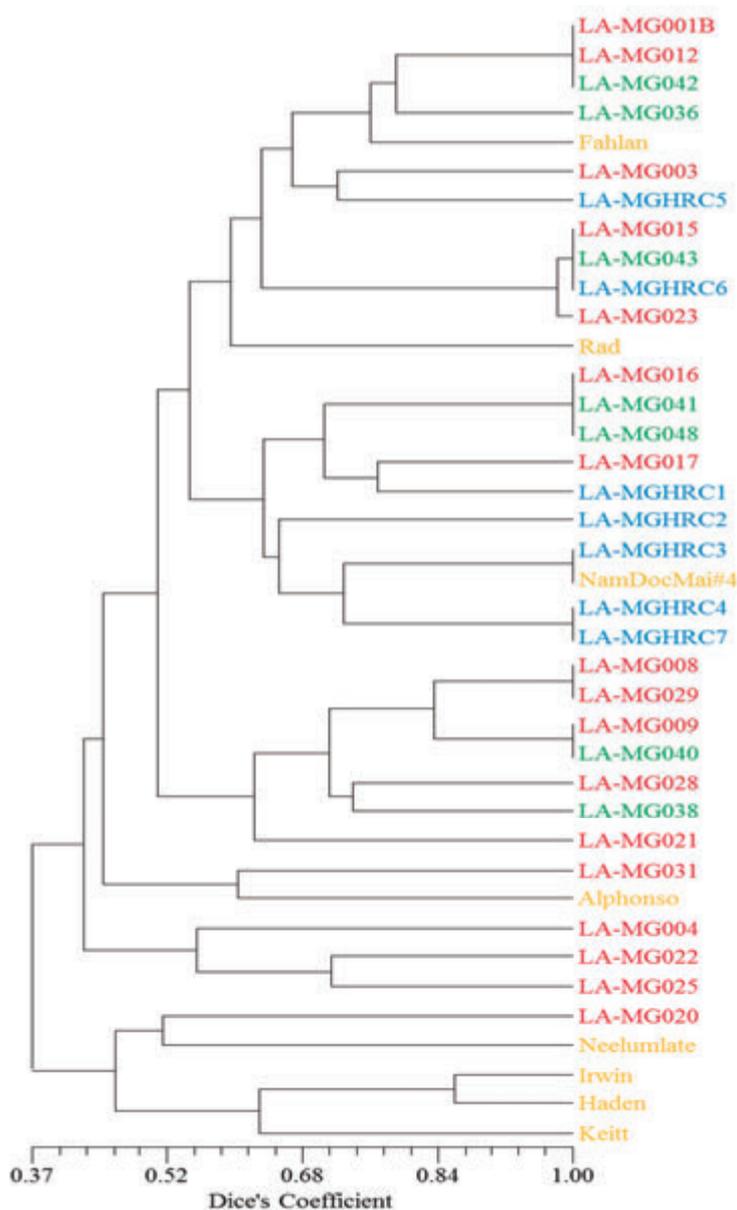


Fig. 2. Phenogram of mango native genetic resources in Laos obtained via SSR analysis. Red, green, and blue indicate the collection areas (southern and central Laos, and the HRC collections, respectively). Those highlighted in yellow represent major global varieties for comparison.

To examine genetic diversity, we also conducted PCoA using the SSR genotype data. First and second principal coordinates explained 16.88% and 15.59% of the variation, respectively, with the 31 Laos accessions distributed sparsely on the scatter plot, except for the duplicate genotypes mentioned above. These findings suggest that genetic resources in Laos possess a certain level of genetic diversity in terms of SSR variation compared with the 8 most popular global varieties (Figure 3). Further detailed analyses such as elucidation of possible pedigrees and variety identification of DNA markers are currently being conducted based on the SSR marker genotypes (Yamanaka *et al.* in preparation).

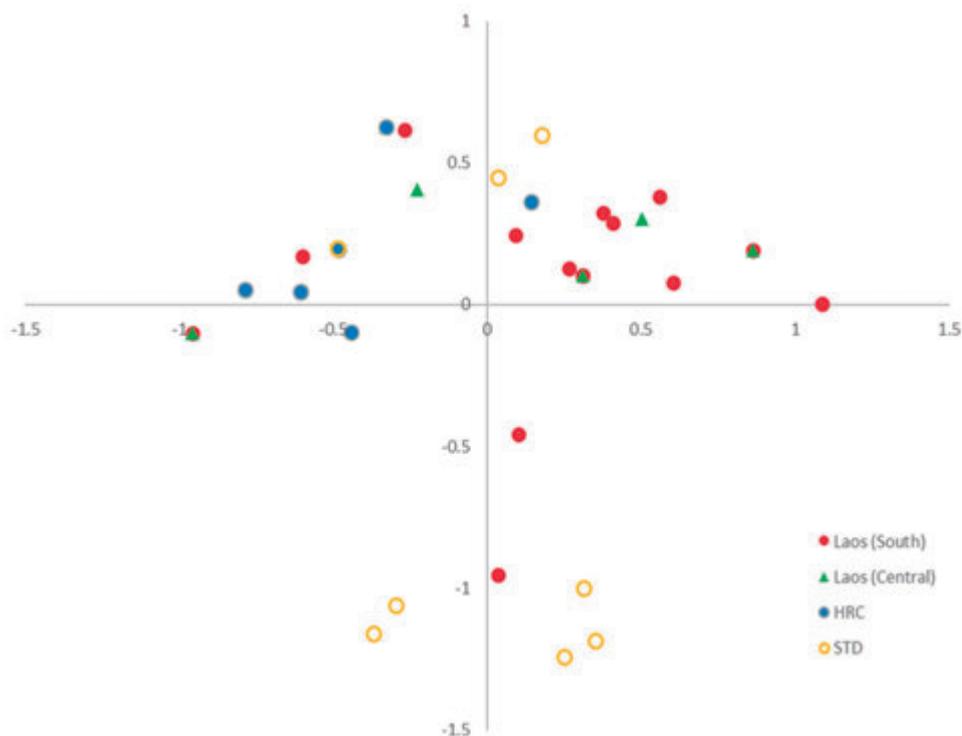


Fig. 3. Principal coordinate analysis (PCoA) of the 31 Laos accessions and 8 major global varieties (STD).

Conclusion

Mango is one of the most popular and potentially economically important fruit trees in Laos; however, at present, most fresh fruits and seedlings consumed in Laos are imported from other countries. Promotion of domestic production is therefore vital to the Laotian economy. In this study, we provided a basis for the utilization of native mango resources by collecting and conserving underutilized local varieties. In doing so, assessment of the genetic relationships between samples was carried out along with field conservation of the collected accessions at the HRC. Evaluation of important traits such as the fruit quality of these domestic mango varieties will be the subject of future research.

Acknowledgements

We would like to thank Dr. S. Goto, Ms. F. Hosaka, and Dr. Y. Sawamura (Institute of Fruit Tree and Tea Science, NARO, Japan) for collaboration in the SSR analysis.

References

- Boulom S (2019) Laos still reliant on imported plant supplies. *Vientiane Times* 11 July 2019
- Dubbeldam R (2005) Fruit growing as an alternative to slash-and-burn agriculture: findings and discussion. In: *Proceedings of NAFRI Workshop on "Poverty Reduction and Shifting Cultivation Stabilization*

in the Uplands of Lao PDR: Technologies, approaches and methods for improving upland Livelihoods”. National Agriculture and Forestry Research Institute, 27-30 January 2004, Luang Prabang. pp. 449–454

Food and Agriculture Organization of the United Nations (2018) FAOSTAT Database. Rome. <http://www.fao.org/faostat/en/#data>

Peakall R and Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28:2537–2539

Rohlf FJ (1998) NTSYS, numerical taxonomy and multivariate analysis system, Ver. 2.01. Exeter Publishing, Ltd., Setauket, New York

Yamanaka S, Hosaka F, Matsumura M, Onoue-Makishi Y, Nashima K, Urasaki N, Ogata T, Shoda M, Yamamoto T (2019) Genetic diversity and relatedness of mango cultivars assessed by SSR markers. *Breed Sci* 69:332–344

Section III

Nutritional improvement in rural areas and value addition of fishes



Daily meal in rural village of Lao



Laotian fermented fish *pa-deak*

***Padaek* distribution and business management in Vientiane capital, Lao PDR**

**Katsumi Hasada¹, Phonesanith Phonhnachith², Phonevilay Sinavong³,
Manivanh Phimpachanvongsod², Shintaro Kobayashi¹, Junichiro Marui¹**

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Research Center to Climate Change Resilience in Agriculture (RCRA), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

³ Planning and Cooperation Division (PCD), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

This study presents distribution characteristics of a fermented freshwater fish paste product called *padaek* and the business management of *padaek* stakeholders in Vientiane capital in Lao PDR. *Padaek* stakeholders such as farmers, middlemen, and retailers were interviewed using a semi-structured questionnaire. The stakeholders' responses demonstrate that there are seven types of distribution spread over three patterns. A typical distribution channel takes place with a middleman-intervention pattern in which middlemen mediate between farmers and retailers. Regarding business conditions, farmers and retailers claim a higher gross margin than do middlemen. Furthermore, 70% of farmers face difficulties in obtaining fish, whereas 39% and 31% of retailers, 31% and 23% of middlemen, respectively, confront difficulties of insufficient funds and market competition in *padaek* business management.

Introduction

In Lao PDR, fermented freshwater fish paste product, called *padaek* in Laotian, is used daily as a seasoning for soups, salads, stews, and other dishes. *Padaek* is not only used as a seasoning but also as a half-decomposed fish meat from which people derive animal protein. Moreover, *padaek* contains lysine, an essential amino acid, which is seemingly otherwise deficient in the Laotian diet (Marui et al. 2018). Consequently, *padaek* is a necessary food product in the Laotian diet.

Padaek was domestically produced and consumed in most households at one time. Recently, the demand for *padaek* in the markets has nearly doubled compared with the demand 10 years ago (Ministry of Planning and Investment 2015). *Padaek* is expected to be a processed food using local resources as a product of the “One District One Product (ODOP)”¹⁾ (Marui et al. 2019). In addition, *padaek* has potential as an export food product because it is consumed in the neighboring countries of Thailand, Vietnam, Cambodia, and Myanmar (Adams et al. 1991). Nevertheless, few studies have examined *padaek* distribution and its business management. Important information is required for the further establishment and enforcement of a *padaek* supply chain.

The objectives of this study, therefore, are to examine the patterns, types, and channels of *padaek* distribution in the market and to examine gross margins and trade issues that stakeholders confront in the *padaek* business management. This information can elucidate the characteristics of *padaek* distribution and *padaek* business management affecting the market.

Materials and Methods

Vientiane capital and the two adjacent districts of Vientiane Province were selected as research sites (Fig. 1). A survey was administered from June 2016 through February 2017, and data were collected using snowball sampling. First, interviews were conducted with retailers who sold *padaek* at main markets in Vientiane capital to obtain information about their *padaek* suppliers such as middlemen and farmers. Then, the middlemen introduced by the retailers were interviewed to obtain information about their *padaek* suppliers, the farmers. A semi-structured questionnaire was used for the interviews allowing multiple answers in some questions. Finally, 49 farmers, 12 middlemen, and 46 retailers were sampled (Table 1). They were interviewed about their *padaek* suppliers and customers, their activities in *padaek* distribution, their trading price, and their *padaek* business management issues.



Fig. 1. Location of the research sites

Source: Hasada et al. (2019) with modification

Table 1. Targeted stakeholders

Stakeholder	Number of interviewees	Research sites
Farmer	49	2 districts in the Vientiane capital (Naxaithong district and Pakngum district)
Middleman	12	3 districts in the Vientiane capital (Naxaithong district, Pakngum district, and Sikhottabong district) 2 districts in the Vientiane Province (Phonhong district and Thoulakhom district)
Retailer	46	16 markets in the central area of the Vientiane capital

Results and Discussion

Padaek distribution patterns and types in Vientiane capital

On the basis of interview results from the respondents, stakeholders and their activities in *padaek* distribution were elucidated. There are four main stakeholders in the *padaek* distribution of Vientiane capital, namely, farmers, middlemen, retailers, and consumers. *Padaek* distribution in Vientiane capital has six main activities: collecting fish and producing, fermenting, transporting, retailing, and consuming *padaek*. *Padaek* distribution is categorized into three patterns by focusing on stakeholders that mediate between producers and consumers: middleman-intervention, no-middleman-intervention, and farmer direct sales. Furthermore, four stakeholders were combined with six activities to describe seven distribution types (Fig. 2). For instance, in type 1, farmers collect fish at the waterside, middlemen produce *padaek* and transport it to retailers in a few weeks, retailers ferment the *padaek* received from the middlemen for a period between a few months and 6 months before sale, and consumers buy the *padaek* at the markets. This type is categorized as the middleman-intervention pattern (pattern 1) because middlemen mediate between farmers and retailers.

Among respondents, 28 farmers belonged to type 2 and 31 farmers belonged to type 3 in the middleman-intervention pattern (pattern 1). In the no-middleman-intervention pattern (pattern 2), 1 farmer belonged to type 5 and 2 farmers belonged to type 6. Lastly, 14 farmers belonged to type 7 in the farmer direct sales pattern (pattern 3). No farmer was found who belonged to type 1 or type 4. This finding implies that types 2 and 3 in the middleman-intervention pattern (pattern 1) are more popular in Vientiane capital.

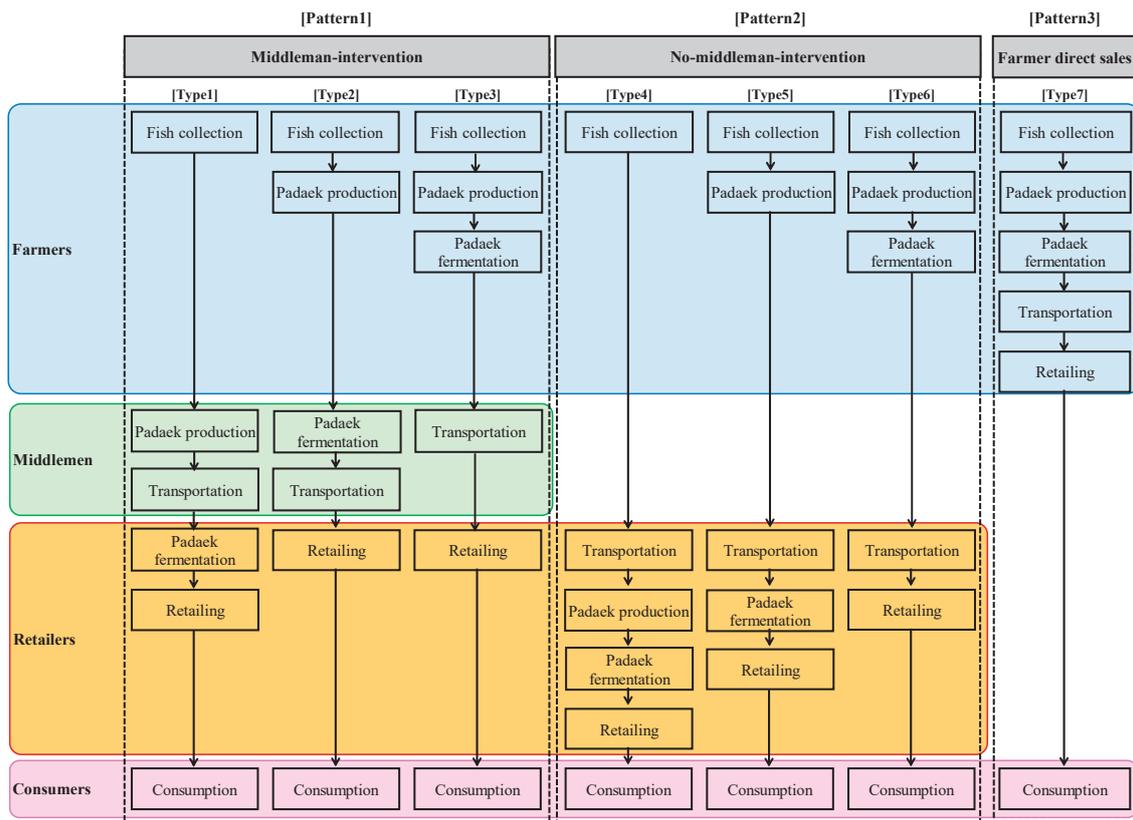


Fig. 2. Padek distribution patterns and types

Padaek distribution channels

Vientiane capital has three main distribution channels characterized by the above-mentioned types except types 1 and 4. Types 1 and 4 are excluded because farmers in these types do not produce *padaek* by themselves but merely sell fish to middlemen, or retailers. This kind of distribution is not targeted in this analysis. The distribution channels including supply and demand areas are described as distribution channels A, B, and C.

Distribution channel A (Fig. 3) is a typical channel characterized by types 2 and 3 in the middleman-intervention pattern. In this distribution channel, middlemen mainly purchase *padaek* from farmers living in the suburbs of Vientiane capital and sell it to retailers in big markets in Vientiane capital. Retailers in big markets sell *padaek* to retailers in small markets, or consumers in the big markets.

Distribution channel B (Fig. 4) is a typical channel characterized by types 5 and 6 without middleman-intervention. Retailers doing business in big markets purchase *padaek* directly from farmers living in the suburbs of Vientiane capital and sell it to small market retailers and general consumers.

Lastly, in distribution channel C (Fig. 5), characterized by type 7, farmers make and sell *padaek* by themselves in their villages, or at a market near the village.

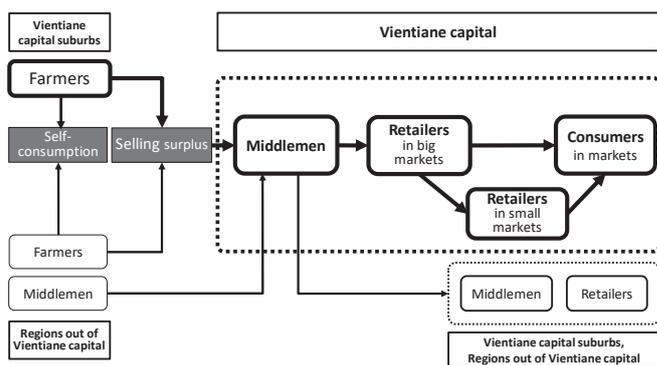


Fig. 3. Distribution channel A
Source: Hasada et al. (2019)

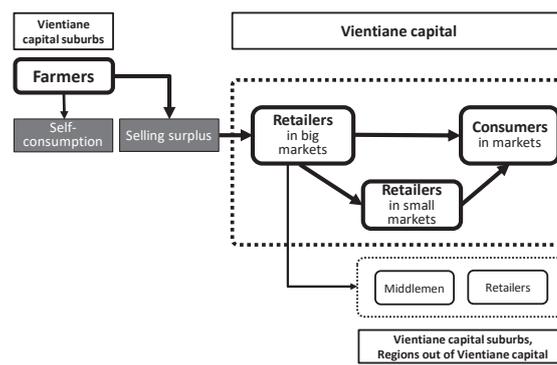


Fig. 4. Distribution channel B
Source: Hasada et al. (2019)

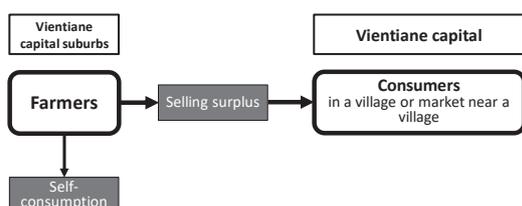


Fig. 5. Distribution channel C
Source: Hasada et al. (2019)

Trading prices and gross margins

Trading prices and gross margins²⁾ of the respective stakeholders are the indicators used to evaluate the *padaek* value chain. Table 2 presents trading prices and gross margins of typical *padaek* fermented with mixed fish, salt, and rice bran in the three distribution channels. It shows the average buying price, average selling price, gross margin, and ratio of gross margin to consumer price, or retailers' selling price at the market.

In distribution channel A, the average selling price of farmers to middlemen, or the gross margin, was 5,657 kip/kg, whereas the gross margin of middlemen was only 708 kip/kg, substantially lower than that of farmers. The greater part of the gross margin of middlemen was apparently the distribution cost because they merely collected and distributed *padaek* without processing the *padaek* for adding value. The gross margin of retailers was 5,316 kip/kg, which is 7.5 times as much as that of middlemen. Regarding the ratio of gross margin to consumer price, the ratios for farmers and retailers were 46% and 43%, respectively, whereas that of middlemen was only 6%. This finding implies that farmers and retailers take a higher gross margin than middlemen do.

In distribution channel B, the average farmer selling price to retailers, or the gross margin, was 8,333 kip/kg, higher than the gross margin of farmers in distribution channel A. The ratio of gross margin to consumer price reached 71%. In contrast, the gross margin of retailers was 5,565 kip/kg, and the ratio of gross margin to consumer price was 48%, 5 points higher than that in distribution channel A. This means that there is no large difference in the gross margin of retailers, or its ratio to consumer price between these two distribution channels.

In distribution channel C, the average selling price of farmers to customers, or the gross

margin, was 9,833 kip/kg, the highest among all the distribution channels. Sales of *padaek* directly to customers are more profitable for farmers. However, considering the opportunity cost of seeking customers, or the cost of transportation to the markets, the sale of *padaek* to middlemen, or retailers might have been economically rational.

Table 2. Trading prices of *padaek* and gross margins

	Farmer			Middleman				Retailer			
	Selling price (kip/kg)	Gross margin (kip/kg)	Gross margin/Consumer price (%)	Buying price (kip/kg)	Selling price (kip/kg)	Gross margin (kip/kg)	Gross margin/Consumer price (%)	Buying price (kip/kg)	Selling price (kip/kg)	Gross margin (kip/kg)	Gross margin/Consumer price (%)
Distribution channel A	5,657	5,657	46	4,887	5,595	708	6	7,000	12,315	5,316	43
Distribution channel B	8,333	8,333	71	—	—	—	—	6,055	11,620	5,565	48
Distribution channel C	9,833	9,833	100	—	—	—	—	—	—	—	—

Source: Hasada et al. (2019) with modification

Note:

1. The sample size of *padaek* for the respective channel is as follows:

Distribution channel A: farmer 28, middleman 7, retailer 17.

Distribution channel B: farmer 3, retailer 9.

Distribution channel C: farmer 14.

Some stakeholders treated various *padaek* made from different types of fish.

2. The kip is the currency of Lao PDR. The exchange rate was 8,130 kip/US\$ as of 2016 (Ministry of Planning and Investment 2017).

Issues of padaek business management

Concerning stakeholders' business management, the survey data showed that 39 out of 49 farmers, 9 out of 12 middlemen, and 24 out of 46 retailers reported some issues related to *padaek* business management. Figure 6 presents the issues that these stakeholders faced.

Seventy percent of farmers faced lack of fish, the *padaek* raw material (resource issue). The population in Vientiane capital has increased, whereas rice-planted areas, which generally include some waterside areas such as canals, ponds, and streams, have remained almost unchanged. The population increase led to an increase of fish self-consumption in households, and fish for producing *padaek* decreased. Therefore, *padaek* sold to middlemen might have decreased (Hasada et al. 2019).

Lack of *padaek* (resource issue), lack of customers (market competition issue), and insufficient capital (capital/cost issue) were important issues for the middlemen. For middlemen, 31%, 23%, and 23%, respectively, confronted those issues. It is difficult for them to buy *padaek* from farmers if *padaek* production by farmers decreases because of the small fish catch. Middlemen cannot make consistent sales to retailers because they lack sales contracts with them. In addition, retailers occasionally refuse to buy their *padaek* because of its low quality. Middlemen struggle with capital because of significantly small gross margins from *padaek* trading and fewer opportunities to borrow capital from financial institutions (Hasada et al. 2019).

Main issues for the retailers were insufficient capital and the cost of sales (capital/cost issue). Thirty-nine percent of retailers confronted these issues. Another difficulty for retailers was the competition among retailers at markets (market competition issue). Most retailers sell *padaek* at markets. Consumer prices will rise if markets have few *padaek* supplies. However, retailers

cannot raise the price of *padaek* because of intense sales competition of *padaek* at the markets. The competition leads to low profits that cannot be invested in *padaek* business expansion. Thirty-one percent of retailers faced this issue.

These results indicate that the resource issue is most important in the upstream (production) phase of *padaek* distribution, whereas capital/cost issue and market competition issue are much more severe in the downstream (sales) phase of the distribution.

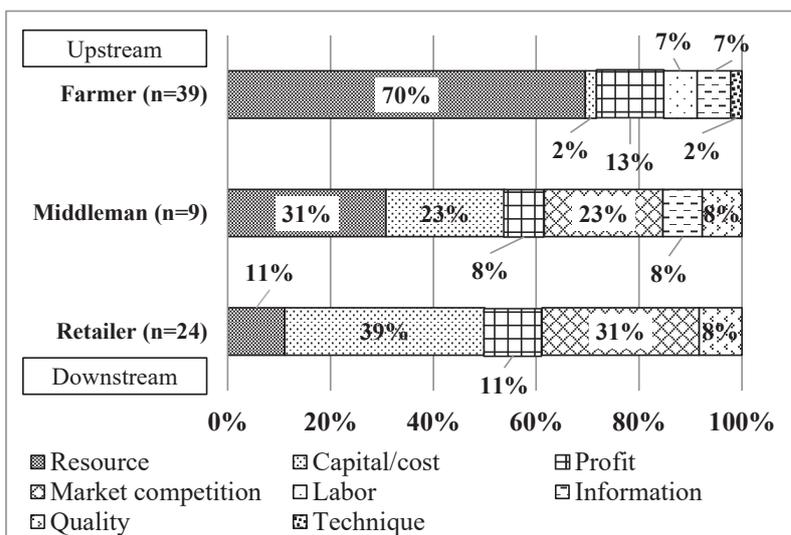


Fig. 6. Stakeholder issues in *padaek* business management

Conclusion

The details concerning *padaek* distribution and its business management that are described in this study can be summarized as follows:

1. *Padaek* distribution in Vientiane capital was categorized into three patterns with seven types. A representative distribution pattern is a middleman-intervention pattern.
2. The gross margin for farmers and retailers was higher than the gross margin for middlemen in distribution channel A, whereas the gross margin for farmers was higher than gross margin for retailers in distribution channel B. The gross margin for farmers in distribution channel C was the highest among all the distribution channels.
3. In *padaek* business management, farmers faced resource issues, middlemen mainly confronted resource, market competition, and capital/cost issues. Retailers mainly confronted capital/cost and market competition issues. In summary, the resource issue is the most important issue in upstream distribution, whereas the market competition and capital/cost issues are much more severe in downstream distribution.

End Notes

- 1) ODOP is a priority project by Laos government. It was started in December 2008 by the Laos government with the support of the Japan International Cooperation Agency in order to

promote local small businesses and to thereby improve the life of residents.

2) Gross margin is found using the following equation.

$$\text{Gross margin} = \text{Selling price} - \text{Buying price} = \text{Distribution cost} + \text{Profit}$$

Acknowledgements

The authors are grateful for the support from the district and village offices of Vientiane capital and Vientiane Province, the market managers, *padaek* stakeholders, and all the staff of the Research Center to Climate Change Resilience in Agriculture, the Planning and Cooperation Division, and the National Agriculture and Forestry Research Institute. The study was funded by the Japan International Research Center for Sciences.

References

- Adams MR, Cooke RD, Rattagool P (1991) Fermented fish products of South East Asia. Technological working paper No. 4/1991, Fish Technological Development Institute, Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok
- Hasada K, Kobayashi S, Marui J (2019) Current situation and issues of fermented freshwater fish paste product “*Padaek*” distribution in Vientiane, Lao PDR. *J Agricul Devel Stud* 30:65–74 (in Japanese)
- Marui J, Hasada K, Boulom S (2018) Salt-fermented fish products in Lao PDR, an inland country in Southeast Asia. *Bull Soc Sea Water Sci* 72:279–287 (in Japanese)
- Marui J (2019) Science of *pa daek*, a traditional fermented seasoning of Laos. *Kagaku* 89:824–829 (in Japanese)
- Ministry of Planning and Investment (2015) Statistical 40 years 1975–2015 Lao PDR. Lao Statistics Bureau, Vientiane
- Ministry of Planning and Investment (2017) Statistical Yearbook 2016. Lao Statistics Bureau, Vientiane

Assessment of food access in Laos from macro and micro perspectives

**Katsumi Hasada¹, Souny Phomdouangsy², Phonevilay Sinavong³,
Phonesanith Phonhnachith⁴, Manivanh Phimpachanvongsod⁴**

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Policy and Legal Department, Ministry of Agriculture and Forestry (MAF), Vientiane, Lao PDR

³ Planning and Cooperation Division (PCD), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

⁴ Research Center for Climate Change Resilience in Agriculture (RCRA), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

Abstract

This study assesses the current status of food access in Laos from both macro and micro perspectives. Food security in Laos is evaluated from a macro perspective utilizing the Food and Agriculture Organization (FAO) data. In terms of accessibility of food security, Laos faces acute issues among the Southeast Asian countries. From a micro perspective, we analyze a case study of food access of households in a semi-mountainous village in central Laos, with a focus on plant and animal foodstuffs. Plant foodstuffs were mainly acquired through production (cultivation) and collection during the wet season. Plant foodstuffs during the dry season were primarily obtained through collection and making purchases at a market. By contrast, animal foodstuffs were mainly acquired by collection and purchase in both dry and wet seasons. When comparing other methods for acquiring foodstuffs, during both seasons, villagers were highly dependent on the collection methods irrespective of seasons and kinds of foodstuffs. People appeared to rely on collecting foodstuffs from nature to secure access to food. Collecting from nature was supplemented by production and purchasing foodstuffs at a market. To generalize these results, further research targeting larger areas is required to analyze factors effecting food access by household.

Introduction

At the World Food Summit of 1996, food security was defined as a situation that exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. This definition points to the four dimensions of food security: availability, accessibility, utilization, and stability (FAO 2006). Since the 1980s, food security has been recognized not only as an issue of supply but also of demand. This new recognition has led researchers to specifically examine the food access of households and individuals (Peng and Berry 2019). This shift in focus has influenced the second goal of the Sustainable Development Goals (SDGs), Zero Hunger, which targets all people, including poor people and infants, in vulnerable situations (UNDP 2015).

Among all Southeast Asian countries, Laos struggles the most with food security issues. The country achieved self-sufficiency in rice production in 1999, and food security has improved from the energy supply perspective (FAO 2011). Nevertheless, about 1.5 million people still remain undernourished (FAO 2014) and the rate of stunted growth for child under five is 44.2% (Moh and LSB 2012). Many undernourished households live in semi-mountainous villages located in some of the poorest areas of Laos. Therefore, undernourishment is assumed to be intimately related to physical issues such as lack of infrastructure and economic issues such as poverty (WFP 2013).

The purpose of this study is to assess food access of Laos from both macro and micro perspectives. Therefore, this report was designed (1) to clarify the situation of food access in Laos in comparison with other Southeast Asian countries utilizing the Food and Agriculture Organization (FAO) data, and (2) to elucidate food access in a semi-mountainous village in central Laos by analyzing survey data.

Food access in Laos as regards accessibility of food security

Four dimensions of food security

Food security can be analyzed according to four dimensions of food security suggested by the World Food Summit of 1996: availability, accessibility, utilization, and stability. Each of these four dimensions is further evaluated at the national, household, and individual levels as follows (FAO 2006; Peng and Berry 2019).

Availability: The availability of sufficient quantities and appropriate quality of food, supplied through domestic production or imports, including food aid (national level).

Accessibility (food access): Access by individuals to adequate resources (entitlements) for acquiring appropriate foods for a nutritious diet. Entitlements are defined as the set of all commodity bundles over which a person can establish command given the legal, political, economic, and social arrangements of the community in which they live, including traditional rights such as access to common resources (household level).

Utilization: Utilization of food through adequate diet, clean water, sanitation, and health care to reach a state of nutritional well-being where all physiological needs are met. This brings out the importance of non-food inputs in food security (individual level).

Stability: To be food secure, a population, household or individual must have access to adequate food at all times. They should not risk losing access to food as a consequence of sudden shocks (e.g., an economic or climatic crisis) or cyclical events (e.g., seasonal food insecurity). The concept of stability can therefore refer to both the availability and access dimensions of food security (It may be considered as a time dimension that affects all levels.).

Food security in different Asia regions

The FAO analyzed food security in developing regions using the indicators classified along the four dimensions of food security mentioned above (FAO 2014). Fig. 1 shows the state of food security in different Asia regions when analyzed according to these dimensions.

East Asia has experienced rapid progress in all four dimensions over the past two decades. South Asia has displayed slower progress in raising the availability and utilization levels, whereas accessibility has progressed rapidly. Southeast Asia has shown moderate progress but has been unable to equal the progress of East Asia; however, Southeast Asia has shown more growth than South Asia. Nevertheless, the accessibility in Southeast Asia is lower than in South Asia, implying that Southeast Asia has not made sufficient progress in improving food access at the household level.

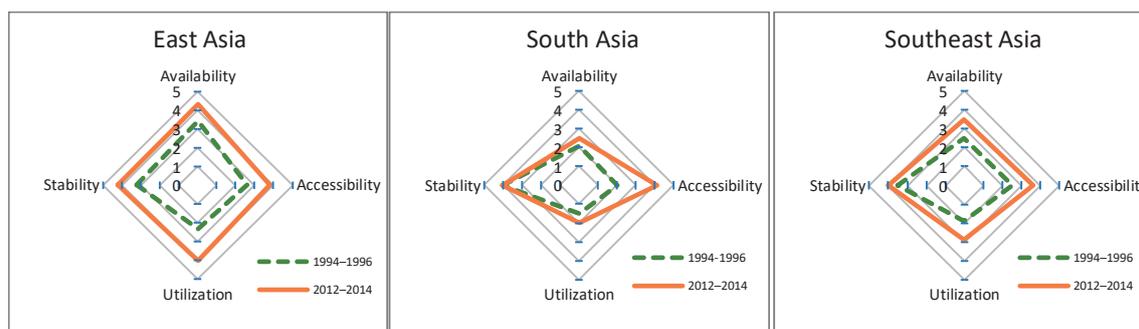


Fig. 1. Evolution of food dimensions in Asia

Source: FAO (2014) with modification

Note: East Asia includes China, Democratic People's Republic of Korea, Mongolia, and Republic of Korea. Southeast Asia includes Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Philippines, Thailand, Timor-Leste, and Vietnam. South Asia includes Afghanistan, Bangladesh, India, Iran, Maldives, Nepal, Pakistan, and Sri Lanka.

Food accessibility of Southeast Asian countries

Table 1 provides information on food accessibility of Southeast Asian countries with several indicators related to the accessibility of food security. These indicators conform to the set of food security indicators suggested by the FAO. In Laos, the percentage of paved roads over total roads and road density as physical access indicators are 13.7% and 16.7%, respectively. These figures are the lowest and second lowest percentages, respectively, among all the countries considered. Food access is restricted in Laos because of underdeveloped infrastructure. Its domestic food price index as an economic access indicator is the worst among all countries (8.62), despite their gross domestic product (GDP) per capita being moderate (5,079 US\$ based on the purchasing power parity [PPP]), comparatively. This implies that economic food access in Laos is considerably limited or constrained. Although the prevalence of undernourishment in Laos has decreased since 2000 (FAO 2014), it still remains relatively high when compared to other countries. The average dietary energy supply adequacy from 2011 to 2013 in Laos was 104% (FAO 2019); Laos supplied sufficient food on caloric basis at national level. By contrast, as indirect indicators, the depth of food deficit and prevalence of food inadequacy, which emerged as the result of food access, are the second highest after Timor-Leste. This implies that a gap still exists between dietary energy supply and dietary energy demand in Laos. Consequently, Laos faces severe food access issues that cause nutritional insecurity.

Table 1. Food accessibility assessment of Southeast Asian countries

Indicator	Cambodia	Indonesia	Laos	Myanmar	Philippines	Timor-Leste	Vietnam	Brunei Darussalam	Thailand	Malaysia
<i>Physical access</i>										
Percentage of paved roads over total roads (%) (2009)	n/a	56.9	<u>13.7</u>	49.7	n/a	n/a	47.6 ^d	79.9	n/a	80.9
Road density (per 100 km ² of land area) (2009)	21.9	24.9	<u>16.7</u>	4.8	n/a	n/a	48.3 ^d	53.1	35.1 ^c	40.9
Rail-lines density (per 100 km ² of land area) (2013)	n/a	0.2	n/a	n/a	n/a	n/a	0.7	n/a	1	0.7
<i>Economic access</i>										
Gross domestic product per capita (PPP, US\$) (2013)	2,964	9,652	<u>5,079</u>	4,423	6,282	8,887	5,066	79,323	14,771	23,412
Domestic food price index ^a (2013)	7.63	6.38	<u>8.62</u>	8.43	6.75	n/a	n/a	3.06	4.33	2.86
<i>Outcome indicators</i>										
Prevalence of undernourishment (%) (2012–2014)	18.4	9	<u>17.8</u>	12.9	14.2	26.5	11.4	2.7	8.2	3.2
Share of food expenditure of the poor (%) (2008)	84 ^c	22	<u>84^d</u>	n/a	61 ^c	n/a	65	n/a	n/a	n/a
Depth of food deficit ^b (kcal/capita/day) (2012–2014)	122	51	<u>126</u>	102	101	185	89	17	62	20
Prevalence of food inadequacy (%) (2012–2014)	24.9	14.7	<u>30</u>	25.2	21.3	36.7	19.9	6.1	16.9	7.4

Source: FAOSTAT (2019), Land Portal (2012)

Notes:

a: Domestic food price index is calculated by dividing the food purchasing power parity (FPPP) by the general PPP.

b: Depth of food deficit indicates how many calories would be required to improve the state of the undernourished, everything else being constant.

c: Data from 2006.

d: Data from 2007.

e: Data from 2009.

Food access in a semi-mountainous village in central Laos

The preceding section demonstrated that Laos still faces food security issues from dimension of accessibility. In this section, food access in a semi-mountainous village in central Laos is assessed using survey data.

1. Research area

Nameuang village in Feuang district, one of the poorer districts in the Vientiane province (Lao PDR 2004), was selected as the research site (Fig. 2). This village is located about 100

kilometers (km) from Vientiane capital. It consists of a residential area, paddy fields, upland fields, fallow land, and forests. At the time of the survey in 2012, the village had 140 households (53 Lao ethnic group households and 87 Khmu ethnic group households) inhabited by a total of 650 people (Kimura et al. 2014). In this village, the wet season lasts from May to October, whereas the dry season from November to April. Most households subsist by planting paddy rice in the flat lands and cultivating upland rice and Job's tears in upland fields.

Methods of foodstuff acquisition (food access) in the village consist of collection, making purchases, production (rearing livestock), exchange, and receiving as gifts. Villagers collect plant and animal foodstuffs in and around residential area, paddy fields, ponds, rivers, upland fields, and forests. Certain kinds of vegetables are planted in gardens while livestock is mainly allowed to feed in and around the paddy fields and residential area. Pot herbs such as green onion and garlic as well as eggs, dry beef meat, and tinned fish are sold at village grocery stores. Additionally, various types of vegetables and fruits, cultured fish (such as tilapia and catfish), some varieties of meats, and other foods are available at a permanent market located 6 km from the village. Villagers rarely exchange items or foodstuffs for other foodstuffs. Foodstuffs are often gifted by relatives.

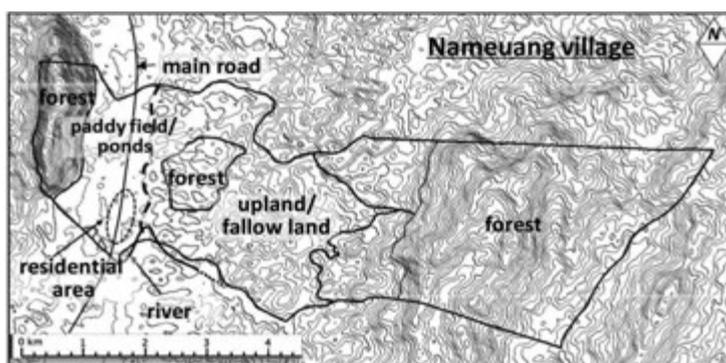


Fig. 2. Location of the research site
Source: Kimura et al. (2014) with modification

2. Methodology

Four households that owned a paddy field and four households that did not own a paddy field were selected among households that planted rice and had an average family size. Existing plant and animal foodstuffs as well as the methods for acquiring foodstuffs for meals throughout the day were recorded, excluding instances of dining out. The survey was administered in September 2014, during a period of rice shortage, and then in early February to early March of 2015, during a period of rice abundance. Table 2 presents attributes of selected households.

Table 2. Attributes of selected households

Attribute	Household A	Household B	Household C	Household D	Household E	Household F	Household G	Household H
Ethnicity	Khmu	Lao	Lao	Lao	Khmu	Khmu	Khmu	Khmu
Number of household members	5	4	5	6	6	4	4	3
Income (1,000 KIP/capita/year)	2,600	1,612	2,857	5,287	4,021	1,363	600	3,637
Paddy field area (ha)	0.64	0.64	0.80	1.20	-	-	-	-
Upland rice field area (ha)	2.80	0.96	0.48	-	2.00	3.50	3.00	4.44
Rice yield (kg) (paddy rice + upland rice)	9,620	3,010	4,860	5,364	4,660	8,155	6,990	10,345
Number of livestock (cattle and buffalo/pig/poultry)	0/0/41	0/0/25	0/2/15	0/0/30	2/6/5	0/1/0	0/0/4	0/0/15
Income aside from selling farm products	Tree trimming, agricultural labor	Agricultural labor, weaving, carpentry	Agricultural labor, selling medical tree	Tree trimming, crop transportation	Tree trimming, crop transportation	Agricultural labor	Agricultural labor	Agricultural labor
Main cultivated crops	Paddy rice, upland rice, Job's tears	Paddy rice, upland rice, Job's tears	Paddy rice, upland rice	Paddy rice, Job's tears	Upland rice, Job's tears	Upland rice, Job's tears	Upland rice	Upland rice, Job's tears

Source: Hasada and Yamada (2017) with modification

Notes:

- 1: Income represents the total of on-farm income and off-farm income from January 2014 to early March 2015. The exchange rate was 8,049 kip/US\$ as of 2014. GDP per capita of Lao PDR in 2014 was 1,725 US\$ (MPI 2015).
- 2: Values are based on data from 2014.

3. Survey Results

Plant foodstuff acquisition

Table 3 shows the use frequency and ratios of plant foodstuffs in different acquisition methods during the wet season. Among the eight households, six (households A, B, C, E, F, and G) achieved their highest ratio of acquisition by production during the wet season. This accounted for 45.4% of all acquisitions. The second highest ratio of acquisition was collection, which accounted for 39.3%. All households acquired more than 75% of plant foodstuffs through wild vegetables and homegrown vegetables. This might account for the high degree of food diversity displayed.

Table 3. Use frequency and ratios of plant foodstuffs in different acquisition methods during the wet season

Household	Collection	Purchase	Exchange	Received	Production	Collection + Production
A	16 (30.8)	2 (3.8)	0 (0.0)	10 (19.2)	24 (46.2)	40 (76.9)
B	37 (35.9)	0 (0.0)	0 (0.0)	16 (15.5)	50 (48.5)	87 (84.5)
C	25 (35.2)	4 (5.6)	0 (0.0)	13 (18.3)	29 (40.8)	54 (76.1)
D	41 (53.2)	1 (1.3)	0 (0.0)	8 (10.4)	27 (35.1)	68 (88.3)
E	13 (33.3)	4 (10.3)	0 (0.0)	5 (12.8)	17 (43.6)	30 (76.9)
F	32 (43.2)	1 (1.4)	0 (0.0)	0 (0.0)	41 (55.4)	73 (98.6)
G	19 (26.8)	2 (2.8)	0 (0.0)	5 (7.0)	45 (63.4)	64 (90.1)
H	33 (53.2)	4 (6.5)	0 (0.0)	9 (14.5)	16 (25.8)	49 (79.0)
Total	216 (39.3)	18 (3.3)	0 (0.0)	66 (12.0)	249 (45.4)	465 (84.7)

Source: Hasada and Yamada (2017) with modification

Notes:

- 1: Values represent use frequency; those in brackets denote the use ratio (%) for all methods.
- 2: Dark gray denotes the first highest ratio. Light gray denotes the second highest ratio among all methods of plant foodstuff acquisition.

The ratio of plant foodstuffs acquired by collection was the highest, followed by the ratio of foodstuffs purchased during the dry season. The total ratios of collection and purchase to all acquisitions were 33.4% and 29.6%, respectively (Table 4). Households A and D relied heavily on cultivated vegetables because they had gardens near a river or a well as water sources for cultivation. Households A and D acquired about 70 to 80 percent of their plant foodstuffs through collection and production, whereas the other households acquired about 60 to 80 percent through collection and making purchases (Table 4). Collection and production for households A and D and collection and purchase for households C, F, G, and H, respectively, showed mutually complementary relationships.

Table 4. Use frequency and ratios of plant foodstuffs in different acquisition methods during the dry season

Household	Collection	Purchase	Exchange	Received	Production	Collection + Production	Collection + Purchase
A	14 (21.5)	12 (18.5)	0 (0.0)	2 (3.1)	37 (56.9)	51 (78.5)	26 (40.0)
B	57 (61.3)	5 (5.4)	0 (0.0)	19 (20.4)	12 (12.9)	69 (74.2)	62 (66.7)
C	20 (27.8)	37 (51.4)	0 (0.0)	10 (13.9)	5 (6.9)	25 (34.7)	57 (79.2)
D	21 (28.4)	9 (12.2)	0 (0.0)	14 (18.9)	30 (40.5)	51 (68.9)	30 (40.5)
E	3 (8.8)	18 (52.9)	0 (0.0)	9 (26.5)	4 (11.8)	7 (20.6)	21 (61.8)
F	21 (36.2)	22 (37.9)	0 (0.0)	4 (6.9)	11 (19.0)	32 (55.2)	43 (74.1)
G	15 (37.5)	8 (20.0)	5 (12.5)	5 (12.5)	7 (17.5)	22 (55.0)	23 (57.5)
H	16 (25.0)	37 (57.8)	0 (0.0)	5 (7.8)	6 (9.4)	22 (34.4)	53 (82.8)
Total	167 (33.4)	148 (29.6)	5 (1.0)	68 (13.6)	112 (22.4)	279 (55.8)	315 (63.0)

Source: Hasada and Yamada (2017) with modification

Notes:

1: Values represent use frequency; those in brackets denote the use ratio (%) for all methods.

2: Dark gray denotes the first highest ratio. Light gray denotes the second highest ratio among all methods of plant foodstuffs acquisition.

Animal foodstuff acquisition

Table 5 shows the use frequency and ratios of animal foodstuffs in different acquisition methods during the wet season. The ratio of acquiring foodstuffs by collection was highest followed by the ratio by purchase during the wet season, which respectively accounted for 53.7% and 28.9% of all acquisitions. Indeed, the ratio of collection is almost twice that of purchase, implying that the collection for animal foodstuffs is more important than that of plant foodstuffs. Among eight households, five (households B, C, D, F, and G) achieved the highest ratio of acquisition through collection, whereas the other households had the highest ratio of acquisition by purchase. Particularly, Lao ethnic group households (households B, C, and D) collected more fish at waterside areas. Khmu ethnic group households (households F, G, and H) collected more small mammals, such as mice and squirrels, in upland areas and forests.

Although some households raised livestock such as poultry, they had little use for their livestock as animal foodstuffs. This implies that livestock is not expected to provide a daily source of food, but is instead used as assets during celebrations such as wedding ceremonies, housewarmings, birthday celebrations, and religious ceremonies.

Table 5. Use frequency and ratios of animal foodstuffs in different acquisition methods during the wet season

Household	Collection	Purchase	Exchange	Received	Production
A	1 (3.0)	21 (63.6)	0 (0.0)	7 (21.2)	4 (12.1)
B	75 (83.3)	8 (8.9)	0 (0.0)	3 (3.3)	4 (4.4)
C	45 (70.3)	12 (18.8)	0 (0.0)	3 (4.7)	4 (6.3)
D	26 (48.1)	16 (29.6)	0 (0.0)	10 (18.5)	2 (3.7)
E	2 (4.7)	25 (58.1)	0 (0.0)	11 (25.6)	5 (11.6)
F	22 (62.9)	11 (31.4)	0 (0.0)	2 (5.7)	0 (0.0)
G	15 (71.4)	4 (19.0)	0 (0.0)	2 (9.5)	0 (0.0)
H	5 (31.3)	6 (37.5)	0 (0.0)	3 (18.8)	2 (12.5)
Total	191 (53.7)	103 (28.9)	0 (0.0)	41 (11.5)	21 (5.9)

Source: Hasada and Yamada (2017) with modification

Notes:

- 1: Values represent use frequency; those in brackets denote the use ratio (%) for all methods.
- 2: Dark gray denotes the first highest ratio. Light gray denotes the second highest ratio among all methods of animal foodstuff acquisition.

The ratio of animal foodstuff acquisition by collection and purchase was also higher during the dry season. Similar to the wet season data, the ratio of collection was almost twice that of purchase, i.e., 53.6% and 24.1%, respectively. The ratios of acquiring animal foodstuffs by collection were highest in households A, B, C, E, F, and G, whereas the ratio by purchase was highest in households D and H (Table 6). Household members can easily catch small mammals, such as mice, weasels, and squirrels, because many of them appear in rice fields after harvesting in search for fallen rice. As regards livestock, the use frequency and its ratio for all methods displayed little variation between the wet and dry seasons.

Table 6. Use frequency and ratios of animal foodstuffs in different acquisition methods during the dry season

Household	Collection	Purchase	Exchange	Received	Production
A	49 (69.0)	6 (8.5)	0 (0.0)	15 (21.1)	1 (1.4)
B	41 (60.3)	2 (2.9)	0 (0.0)	14 (20.6)	11 (16.2)
C	39 (73.6)	11 (20.8)	0 (0.0)	1 (1.9)	2 (3.8)
D	28 (42.4)	31 (47.0)	0 (0.0)	3 (4.5)	4 (6.1)
E	16 (34.8)	13 (28.3)	0 (0.0)	7 (15.2)	10 (21.7)
F	15 (53.6)	11 (39.3)	0 (0.0)	2 (7.1)	0 (0.0)
G	15 (44.1)	5 (14.7)	0 (0.0)	14 (41.2)	0 (0.0)
H	15 (36.6)	19 (46.3)	0 (0.0)	5 (12.2)	2 (4.9)
Total	218 (53.6)	98 (24.1)	0 (0.0)	61 (15.0)	30 (7.4)

Source: Hasada and Yamada (2017) with modification

Notes:

- 1: Values represent use frequency; those in brackets denote the use ratio (%) for all methods.
- 2: Dark gray denotes the first highest ratio. Light gray denotes the second highest ratio among all methods of animal foodstuff acquisition.

4. Summary

Among the households we surveyed, the ratios of foodstuff acquisition by collection were highest, excluding plant foodstuff in the wet season, which indicates that collection is an extremely important foodstuff acquisition method affecting food access. Regarding animal foodstuffs, more than 50% of acquisition depended on collection, probably because this village

consists of plain land, upland, fallow land, and forest that supply villagers with various types of natural foods (Hasada and Yamada 2017). The second most important method was purchasing foodstuffs, especially when considering access to animal foodstuffs, irrespective of income limitation and access restriction to the market. This method had a mutually complementary relation with collection. Acquisition methods of collection and purchase are readily influenced by external factors. Unstable climate and land use changes can decrease opportunities for collecting fish and mammals. Purchasing foodstuffs is contingent on market price fluctuations and household income (MAF 2013). Accordingly, households seem to have acquired animal foodstuffs by employing a mixed method of collection and purchase to avoid the vulnerability of depending on a single method of acquisition (Hasada and Yamada 2017).

Conclusion

In this paper, we attempted to clarify uncertainties concerning the situation of food access in Laos. First, we determined that Laos faces severe food security issues among all the Southeast Asian countries from the viewpoint of accessibility. Second, the findings from analysis of food access (accessibility) data by households in a semi-mountainous village in central Laos indicated that households strongly depended on foodstuff collection for both plant and animal foodstuffs. This is likely related to specific characteristics of the village such as diverse land types. In addition, people seem to be primarily dependent on nature for food security while supplementing collection with foodstuffs that were either purchased or produced.

To generalize the results obtained from this study, we call for research on food access targeting villages with different environments. Moreover, research must be conducted from a perspective not only of food quantity but also of food quality.

Acknowledgements

The authors are grateful for the support of the Feuang District Office, the Nameuang Village Office, Nameuang villagers, and all the staff at the Agriculture and Forestry Policy Research Center, the Research Center for Climate Change Resilience in Agriculture, the Planning and Cooperation Division, and the National Agriculture and Forestry Research Institute. This study was funded by the Japan International Research Center for Sciences.

References

- Food and Agriculture Organization (FAO) (2006) Policy Brief: Food Security. FAO, Rome
- Food and Agriculture Organization (FAO) (2011) Country Profile: Food Security Indicators, Country: Lao PDR. FAO, Rome
- Food and Agriculture Organization (FAO) (2014) The State of Food Insecurity in the World 2014: Strengthening the Enabling Environment for Food Security and Nutrition. FAO, Rome
- Food and Agriculture Organization (FAO) (2019) FAOSTAT, Suite of Food Security Indicators. <http://www.fao.org/faostat/en/#data/FS>
- Hasada K, Yamada R (2017) Current status and issue on food access in a semi-mountainous village of

- central Laos. *Water Land Environ Eng* 85:469-474 (in Japanese)
- Kimura K, Kobayashi S, Yoneda R (2014) The economic evaluation on the non-timber forest products collected in the rural areas in central Laos – case study on N village in Feuang District, Vientiane Prefecture. *Paper Environ Inform Sci* 28:55-58 (in Japanese)
- Land Portal (2012) Domestic food price level index. <https://landportal.org/book/indicators/indfaofsec1>
- Lao People's Democratic Republic (Lao PDR) (2004) National growth and poverty eradication strategy (NGPES). Vientiane
- Ministry of Agriculture and Forestry (MAF) (2013) Lao PDR: risk and vulnerability survey 2012/13 analysis report. MAF, Vientiane
- Ministry of Planning and Investment (MPI) (2015) Statistical Yearbook 2014. Lao Statistics Bureau, Vientiane
- Ministry of Health (MoH) and Lao Statistics Bureau (LSB) (2012) Lao social indicator survey LSIS 2011-2012. MoH/LSB, Vientiane
- Peng W, Berry EM (2019) The concept of food security. In: Ferranti P, Berry EM, Anderson JR (eds), *Encyclopedia of Food Security and Sustainability 2*. Elsevier, Amsterdam. pp. 1–7
- United Nations Development programme (UNDP) (2015) *Transforming Our World: The 2030 Agenda for Sustainable Development*. New York
- World Food Programme (WFP) (2013) *Food and Nutrition Security Atlas of Lao PDR*. Rome

Assessment of usage and fermentation-related components of *pa daek*, a salt-fermented freshwater fish paste, for household use in a semi-mountainous village in Laos

Junichiro Marui¹, Katsumi Hasada¹, Souphachay Phouphasouk², Sayvisene Boulom²

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Faculty of Agriculture, National University of Laos, Vientiane, Laos

Abstract

A salt-fermented freshwater fish paste, locally called *pa daek* in Laos, has been popularly used countrywide as an all-purpose seasoning as well as a protein-rich preservative food. Although commercial products are currently available in markets, the in-house production and consumption of *pa daek* utilizing various indigenous freshwater fish species from surrounding water environments such as rivers and paddy ponds continues today in rural households in the country. An interview survey of 137 households in a semi-mountainous rural village revealed frequent use of the *pa daek* in the daily diets. The concentrations of lactic and glutamic acids, which are closely related to the preservability and palatability of *pa daek*, varied widely among the homemade products examined, indicating the need for the widespread dissemination of knowledge and techniques of the *pa daek* production between rural households to manage the long-term fermentation and use of the product.

Introduction

A salt-fermented freshwater fish paste, locally referred to as *pa daek* in Laos (Fig. 1A), has been popularly used countrywide as an all-purpose seasoning as well as a protein-rich preservative food. Similar types of fermented fish products have traditionally been produced and consumed in Southeast and East Asian countries. Although commercial products are currently available in markets, the in-house production and consumption of *pa daek* utilizing various indigenous freshwater fish species from surrounding water environments such as rivers and paddy ponds continues today in rural households in Laos. In the production of *pa daek*, well-washed fish is mixed with salt and rice bran. The mixed material is stuffed in a covered container followed by fermentation at an ambient temperature. According to producers, although *pa daek* is deemed to be edible after 2–3 months of fermentation, 6–12 months are required to enhance palatability. Fermented products are usually stored without refrigeration while being used and can last for a year or longer. Halophilic lactic acid bacteria species such as *Tetragenococcus halophilus* and *muriaticus* have been detected in *pa daek* products with salt concentrations of 15–20% (Marui et al. 2015). In our experimental *pa daek* production, lactic acid started to increase after 20 days of fermentation, reaching a plateau after 2 months to create acidic environment that should be advantageous for preventing the potential growth spoilage bacteria in the

highly salted products (Marui et al. 2019a, b). A fermentation period-dependent increase of a variety of free amino acids derived from fish protein hydrolysis to enhance the taste and nutritional benefits of the product is another distinct feature of *pa daek* fermentation (Marui et al. 2019a,b). To strengthen and utilize fully such benefits of *pa daek* for sustainable nutrition supply in rural areas, the present study aimed to clarify the current status of *pa daek* use in households, as well as its fermentation-related quality components such as lactic and glutamic acid.

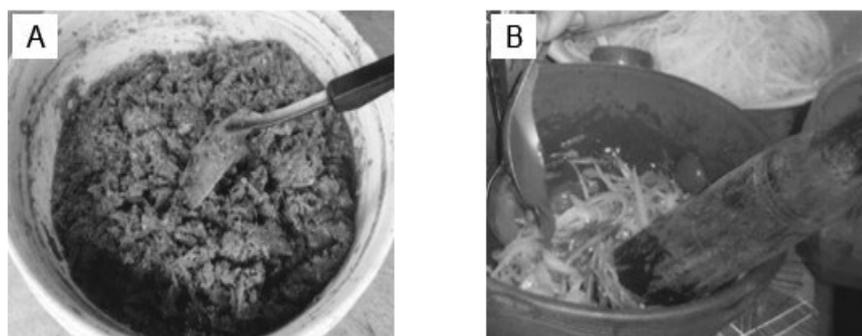


Fig. 1. *Pa daek* made in a rural household in Laos (A), and Lao-style papaya salad seasoned with *pa daek* (B).

Materials and methods

Usage survey of pa daek in a village and sample collection

An interview survey of 137 households on the use of *pa daek* was conducted from August to October 2016 in a semi-mountainous rural village in Feuang District, Vientiane Province, Laos. The homemade *pa daek* products analyzed in the present study were collected from the same village in May 2017. Information about the age (months since the start of the fermentation) of each sample was provided by the producer.

Measurement of the fermentation-related quality components of homemade pa daek

The collected *pa daek* samples were minced by a blender and stored in a freezer at $-20\text{ }^{\circ}\text{C}$ until analysis. For the salt, pH, and lactic acid measurements, each minced sample was mixed vigorously in 10 mL of sterilized water. The mixture was then centrifuged at $15,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$, followed by the collection of supernatant for the analyses. The salt concentration and pH values were measured using a LAQUA twin compact salt and pH meter (Horiba Ltd., Kyoto, Japan), respectively. Lactic acid content was measured using a D-/L-lactic acid enzymatic test kit (R-Biopharm AG, Darmstadt, Germany).

For the glutamic acid contents, 2 g of each minced sample was diluted to 50 mg/mL with 3% sulfosalicylic acid. The mixture was cooled on ice for 1 h, followed by centrifugation. The glutamic acid content in the supernatant was measured using a fully automated amino acid analyzer (JLC-500/V2; JEOL Ltd., Tokyo, Japan).

Results and discussion

Frequent use of pa daek in rural households

The interview survey conducted in the present study revealed that *pa daek* was used by more than 70% of the households (Fig. 2A). Among the *pa daek* users, approximately 70% used it at least once a week (Fig. 2B). It is noteworthy that 30% of the households used it every day (Fig. 2B). *Pa daek* is used for seasoning a variety of Lao dishes, such as papaya salad (Fig. 1A), soups, and dipping sauces in daily diets (data not shown). The fish body in *pa daek* is also grilled or deep fried to eat (data not shown). These results suggest the importance of *pa daek* as a palatable seasoning to enhance appetite as well as a source of nutrients such as protein and amino acids. Despite such a high frequency of use, more than half of the users said they obtained the *pa daek* by purchasing or from relatives instead of in-house production. Although *pa daek* production methods have been passed down for generations among families and local communities, it may become increasingly difficult to maintain such important traditional food utilization techniques because of changes in living environments and lifestyles. Meanwhile, the respondents who did not report using *pa daek* mentioned not only a disliked flavor, but also experiences of physiological disorders, presumably caused by eating spoiled products, as unfavorable factors (data not shown). Analytical assessments of the fermentation-related components that determine both the palatability and preservability of *pa daek* are needed to clarify the current status of homemade products for developing a scientific approach to promote the production and use of *pa daek* further for the improvement of rural livelihoods.

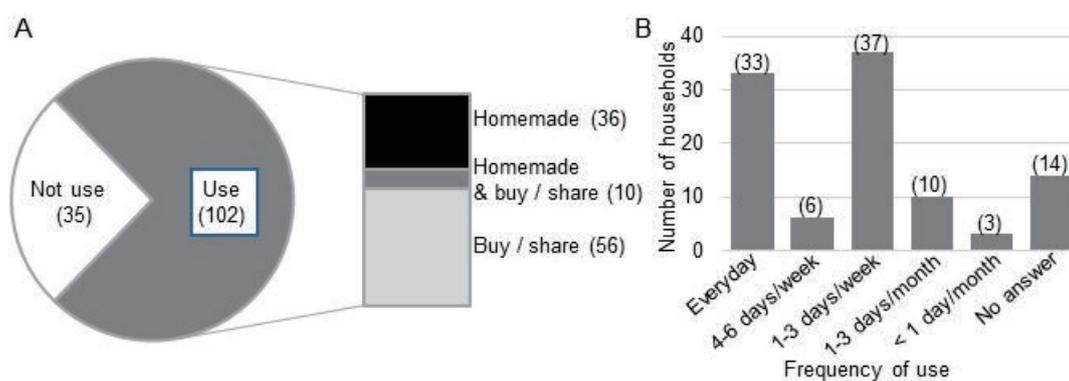


Fig. 2. Schematic representation of the usage (A) and frequency (B) of *pa daek* in rural households. The quantity of responses to each option is presented in parentheses.

Table 1. Age, pH, salt, lactic and glutamic acid concentrations of homemade *pa daek* products.

Sample number	Age* ¹ (months)	Salt (%)	pH* ²	Lactic acid* ³ (%)	Glutamic acid (mg/100g)
1	8	11	6.2	1.56	115
2	8	11	6.2	1.06	Not detected
3	8	11	6.7	1.33	Not detected
4	13	20	5.9	0.48	616
5	2	11	5.0	1.81	609
6	2	18	5.3	0.72	183
7	8	6.6	4.9	1.81	921
8	2	11	6.0	1.60	815
9	2	15	5.0	2.08	623
10	5	25	5.6	0.08	275
11	13	15	5.6	1.33	689
12	12	12	7.5	Not detected	Not detected
13	13	22	5.4	0.40	316
14	6	10	6.8	0.70	1286
15	7	8.4	6.8	0.10	688
16	3	16	6.5	0.43	694
17	7	17	6.8	0.85	465
18	15	12	7.2	0.06	Not detected
19	13	14	6.6	0.30	626
20	14	15	6.0	0.57	515
21	4	15	5.8	0.96	404
22	2	15	6.1	0.11	442
23	13	13	5.5	0.73	673
24	5	4.4	6.6	0.22	Not detected

*¹ Months since start of fermentation is presented

*² pH values of sample extracts prepared as describe in the section of Materials and Methods are presented

*³ Total amount of D- and L-lactic acids is presented

Variability of fermentation-related quality components among homemade pa daek products

In the present study, 24 *pa daek* products made and used in village households were collected to analyze the fermentation-related components (Table 1). The time since the start of fermentation ranged from 2 to 15 months, indicating the usefulness of the traditional *pa daek* fermentation technique for furnishing a seasonally available indigenous freshwater fish with long shelf life as well as palatability. Salt is thought to be an important factor to control microbial growth in *pa daek* products by lowering the water activity, even though the salt concentration in *pa daek* products varied widely from 4.4% to 25% (Table 1). The minimum inhibitory water activity values for the growth of microorganisms differ between species (Grant 2004). In our previous study, halophilic lactic acid bacteria species such as *Tetragenococci* spp. were commonly

detected in *pa daek* products with a salt concentration of not less than 11%, while lactobacilli were common in those with a salt concentration of less than 10% (Marui et al. 2015). Further studies are now in progress to investigate the preservability and microbial diversity of *pa daek* products with relatively low salt concentrations.

Lactic acid is a characteristic component of *pa daek*. It is made by halophilic lactic acid bacteria in the initial phase of fermentation, accompanied by a pH decrease in the fermenting materials (Marui et al. 2019a, b). The creation of acidic conditions in such products should be advantageous to secure preservability. Among the *pa daek* samples examined in the present study, a wide range of variation in lactic acid concentration was observed (average: 0.80%, standard deviation: 0.61%), as well as a negative correlation with the pH of the product (average: 6.1, standard deviation: 0.70) ($r=-0.58$, $p<0.01$, $n=24$) (Fig. 3), indicating that lactic acid is an important factor to determine the product pH. It is necessary to determine the causes of the low lactic acid concentration and neutral pH observed in some products. Microbial profiling of the halophilic lactic acid bacteria and other species involved in the variation of lactic acid concentration and pH are required to secure the long shelf life of *pa daek*.

During *pa daek* fermentation, varieties of proteogenic amino acids are released in the product (Marui et al. 2019a, b). The free amino acids generated mainly by protein hydrolysis in fish meat during long-term fermentation emphasize the uniquely delicious taste as well as the nutritional benefits of *pa daek* products. Among the free amino acids, the present study focused on glutamic acid, which was found to exist at relatively high levels in *pa daek* products (Marui et al. 2019a, b). Glutamic acid is commonly abundant in fermented Asian fish or soybean seasonings such as fish sauce/paste, soy sauce, and miso, and is well known as a natural taste element that makes a variety of foods palatable (Yamaguchi et al. 2000). Dishes cooked with such kinds of glutamic acid-rich seasonings enhance appetite and go well with rice, which is a staple food in Asian countries. The glutamic acid concentration of the 24 *pa daek* samples examined in the present study varied from undetectably low levels to 1% (Table 1), with an average of 0.4% and standard deviation of approximately 0.3%. Although a fermentation period-dependent increase of glutamic acid was observed in our *pa daek* fermentation analysis (Marui et al. 2019a, b), no correlation was observed between the glutamic acid concentration and age (period since the start of fermentation) in the product examined in the present study (data not shown). Endogenous proteolytic enzymes such as lysosomal cathepsins and proteases in fish muscle are considered to be involved in protein hydrolysis, preferably in the acidic environment of the *pa daek* fermentation process, because such enzymes are released from fish muscle lysosomes and function preferably in an acidic environment (Mukundan et al. 1986). On the other hand, the non-enzymatic conversion of glutamic acid to insipid pyroglutamic acid was previously found in soy sauce fermentation and its associated storage period (Hori et al. 1956); this could also happen in long-term *pa daek* fermentation and daily household use. It is also interesting to consider the possible involvement of microbial metabolism in the increase and decrease of glutamic acid concentrations in *pa daek* products.

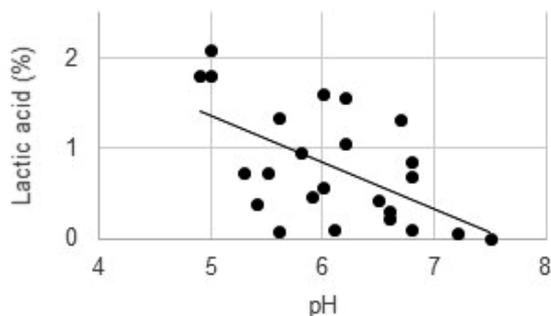


Fig. 3. Scatter diagram of the lactic acid concentration and pH of homemade *pa daek* products. The linear approximation is indicated.

Conclusion

The present study revealed the frequent use of *pa daek* in the daily diets of rural households in Laos. The results emphasize the continued importance of traditional fermented freshwater fish products for fully utilizing indigenous fishery resources for sustainable nutrition supply in rural areas. The concentrations of lactic and glutamic acids, which are closely related to the palatability and preservability of *pa daek*, varied widely among the homemade products examined, indicating the need for the widespread dissemination of knowledge and techniques between rural households to manage the long-term fermentation and use of the product. Microbiological and biochemical research and education should facilitate further efforts to gain a better understanding of traditional fermentation techniques to achieve the stable production of *pa daek* with good quality.

References

- Grant WD (2004) Life at low water activity. *Phil Trans R Soc Lond B* 359(1448):1249–1267
- Hori S, Ogawa T, Aoki R, Kondo Y, Ota Y (1956) Studies on the behavior of L-glutamic acid in shoyu. Part. I. Studies on the various types of L-glutamic acid and their formation mechanism in shoyu. *Nippon Nogeikagaku Kaishi* 30:519–523 (in Japanese)
- Marui J, Boulom S, Panthavee W, Momma M, Kusumoto K, Nakahara K, Saito M (2015) Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos. *Biosci Microbiota Food Health* 34:45–52
- Marui J (2019a) Science of *pa daek*, a traditional fermented seasoning of Laos. *Kagaku* 89:824–829 (in Japanese)
- Marui J, Giavang Y, Phouphasouk S, Yiale Y, Boulom S (2019b) Fermentation period-dependent changes of lactic and amino acid concentrations in *pa daek*, a salt fermented freshwater fish paste in Laos. *JIRCAS Working Report 90*, Japan International Research Center for Agricultural Sciences, Tsukuba. pp. 95–100
- Mukundan MK, Antony PD, Nair MR (1986) A review on autolysis in Fish. *Fish Res* 4:259–269
- Yamaguchi S, Ninomiya K (2000) The use and utility of glutamates as flavoring agents in food. *J Nutr* 130:921S–926S

Fermentation period-dependent changes of lactic and amino acid concentrations in *pa daek*, a salt-fermented freshwater fish paste in Laos

Junichiro Marui¹, Yethao Giavang², Souphachay Phouphasouk²,
Yanglao Yiale², Sayvisene Boulom²

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Faculty of Agriculture, National University of Laos, Vientiane, Laos

Abstract

Pa daek is a salt-fermented freshwater fish paste that can be made and consumed at home and is popularly used as an all-purpose seasoning in Laotian cooking. Nowadays, the products made by small and medium-sized manufacturers are also becoming popular in local markets. The traditional *pa daek* fermentation technique furnishes a seasonally available indigenous freshwater fish with palatability as well as long shelf life. In our *pa daek* fermentation conducted using small clupeid freshwater fish (locally called *pa keo* in Laos), the increase in lactic acid was observed after 20 days of fermentation, reaching approximately 1% after 2 months, and maintaining the same level at after 6 months. The pH decreased from 6.3 to 5.4 as the lactic acid fermentation proceeded, showing the importance of the early phase of the *pa daek* fermentation to secure the preservability. The total amount of 20 free proteogenic amino acids increased in a time-dependent manner throughout the observation period. Significant increases in glutamic acid and lysine in the fermentation could well explain the advantages of the long-term fermentation for making *pa daek* products tasty and nutritious.

Introduction

Pa daek is a salt-fermented freshwater fish paste that can be made and consumed at home and is popularly used as an all-purpose seasoning in Laotian cooking. Nowadays, the products made by small and medium-sized manufacturers are also becoming popular in local markets. The *pa daek* products are usually stored without refrigeration while being used and can last for a year or longer, illustrating the benefits of a traditional fermentation technique that furnishes a seasonally available indigenous freshwater fish with palatability as well as long shelf life. We previously reported the importance of salt concentration to determine the representative lactic acid bacteria in such products (Marui *et al.* 2015). Halophilic lactic acid bacteria species such as *Tetragenococcus halophilus* and *Tetragenococcus muriaticus* have been detected in *pa daek* products with salt concentrations of 15–20% (Marui *et al.* 2015). The long period of *pa daek* fermentation is also of scientific interest for making products that meet consumer preferences. According to the producers, although *pa daek* is deemed to be edible after 2–3 months of

fermentation, 6–12 months are required to enhance palatability. Here, we describe time-dependent changes in lactic and amino acid concentrations in our experimental *pa daek* fermentation procedure, established with small clupeid freshwater fish, locally referred to in Laos as *pa keo*.

Materials and Methods

Pa daek fermentation

In the present study, small clupeid freshwater fish (*pa keo*) were collected from Nam Ngum Reservoir in Laos for use in the *pa daek* fermentation. The length of the fish used in the present study was around 40 mm that was reported to be the standard lengths of the matured *pa keo* in the Nam Ngum Reservoir (Morioka *et al.* 2019). First, the fish were washed well with water, followed by draining in a strainer. The washed fish were mixed with salt and rice bran at a ratio by weight of 3:1:1. The salt concentration in the liquid portion of the mixture was measured using a LAQUA twin compact salt meter (Horiba Ltd., Kyoto, Japan) and adjusted to approximately 15% by adding water. The mixed material was stuffed tightly into plastic containers to remove the air and sealed with screw caps. The fermentation took place at an ambient temperature in the laboratory.

Serial measurements of pH, lactic acid, and amino acids

For the serial measurements of pH and lactic acid, the *pa daek* fermentation was conducted as described above from February to August 2017. The samples were collected after 0, 1, 3, 7, 10, and 20 days, and after 1, 2, 4, and 6 months of fermentation. The collected samples were minced by blender and stored in the freezer at $-20\text{ }^{\circ}\text{C}$ until analysis. For the measurements, each minced sample was mixed vigorously in 10 mL of sterilized water. The mixture was centrifuged at $15,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$, followed by the collection of supernatant for the analyses. The pH was measured using a LAQUA twin compact pH meter (Horiba Ltd.). The lactic acid content was measured using a D-/L-lactic acid enzymatic test kit (R-Biopharm AG, Darmstadt, Germany).

For the free amino acid measurements, fermentation was conducted from December 2015 to June 2016. After 0, 1, 2, 4, and 6 months of fermentation, the samples were collected and stored as described above. Next, 2 g of each minced sample was homogenized in 10% trichloroacetic acid, followed by centrifugation. The glutamic acid content in the supernatant was measured using a fully automated amino acid analyzer (JLC-500/V2; JEOL Ltd., Tokyo Japan).

Results and Discussion

Lactic acid production in the early phase of pa daek fermentation

As shown in Fig. 1, an increase in lactic acid was observed after 20 days of fermentation, reaching approximately 1% after 2 months, and maintaining the same level at after 6 months. The pH decreased from 6.3 to 5.4 as the fermentation proceeded. In accordance with the accumulation pattern of lactic acid, the ratio of the pH decrease between 10 days to 2 months of fermentation was more significant compared with an earlier phase of fermentation, suggesting that lactic acid

was a major factor in creating the acidic conditions in *pa daek*. In addition to the high salt concentration, such acidic conditions in *pa daek* products should be effective for preventing the potential growth of spoilage microorganisms that preferably grow in neutral or alkaline pH conditions.

Since the salt concentration in the samples was approximately 15% throughout the fermentation period, the lactic acid in the *pa daek* samples was thought to have been produced by halophilic lactic acid bacteria species such as *T. halophilus* and *T. muriaticus*. Lactic acid

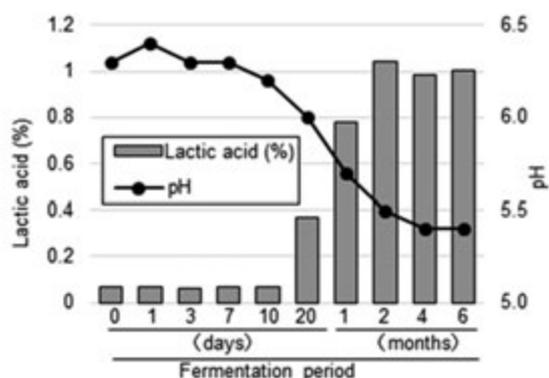


Fig. 1. Lactic acid concentration and pH levels in *pa daek* fermentation (Adapted from Marui, 2019).

concentration reached a plateau after 2 months of fermentation, presumably because of the pH sensitivity of the species. These species were reported to grow and produce lactic acid preferably in the growth media with initial pH values of 6.5 and 7.5, while the media with an initial pH value of 5.8 negatively affected the growth (Kobayashi *et al.* 2004). It is also important to note that the *Tetragenococcus* spp. are facultative anaerobic bacteria. The *T. halophilus* strain achieved a higher growth rate than aerobic cultures in oxygen-free anaerobic cultures (Gürtler *et al.* 1998). Taken together, in practical *pa daek* fermentation, it is deemed desirable to minimize the inclusion of air in the fermenting materials particularly for the first few months of the fermentation process to create and maintain anaerobic conditions favorable for the growth of halophilic lactic acid bacteria species. *Pa daek* producers can estimate the status of lactic acid fermentation in their products by monitoring pH with a measuring device or test paper.

Free amino acids in *pa daek* fermentation

The concentration of 20 free proteogenic amino acids in *pa daek* was analyzed until 6 months of fermentation (Table 1). The total amount of free amino acids increased in a time-dependent manner throughout the observation period. The amount in the sample fermented for 6 months (3,194 mg/100 g) reached 16.7 times that of the amount observed at the beginning of *pa daek* fermentation (191 mg/100 g). The free amino acids generated by long-term fermentation should be important factors to furnish *pa daek* products with the characteristic delicious taste and nutritional benefits. For instance, the *pa daek* prepared in the present study contained glutamic acid at the highest level in the amino acids examined (Table 1). Glutamic acid is a well-known natural taste element that makes a variety of foods palatable. It is commonly abundant in fermented Asian fish or soybean seasonings such as fish sauce/paste, soy sauce, and miso

(Yamaguchi *et al.*, 2000). Dishes cooked with such kinds of glutamic acid-rich seasonings enhance appetite and go well with rice, which is a staple food in Asian countries. Lysine was detected at a comparable level to glutamic acid, also suggesting the usefulness of *pa daek* in the Laotian rice-based diet. Although lysine is an essential amino acid for humans, it is the first limiting amino acid in rice (STFCJ, 2015). The frequent use of *pa daek* in daily diets might partly complement the possible shortage of dietary lysine.

Table 1. Concentrations of free proteogenic amino acids in *pa daek* fermentation [adapted from Marui *et al.* (2018), Marui (2019)].

Amino acids (mg/100 g)	Fermentation period (months)				
	0	1	2	4	6
Glutamic acid	16	160	230	320	420
Lysine	24	180	250	330	410
Leucine	14	150	190	270	340
Alanine	18	100	140	200	260
Valine	9	85	120	180	230
Aspartic acid	5	53	80	130	190
Threonine	8	55	80	130	170
Isoleucine	6	55	80	130	170
Serine	9	58	81	120	160
Phenylalanine	7	53	72	120	150
Tyrosine	3	42	55	110	140
Methionine	4	49	65	95	120
Asparagine	4	44	59	81	100
Proline	7	31	43	69	95
Glycine	7	27	39	63	85
Histidine	26	45	56	70	82
Glutamine	7	58	65	47	34
Tryptophan	1	7	10	20	26
Arginine	16	130	21	7	12
Cysteine	ND	ND	ND	ND	ND
Total	191	1,382	1,736	2,492	3,194

ND: Not detected

In our preliminary analysis, glutamic acid and lysine were detected at relatively high levels among the free amino acids examined in homemade *pa daek* samples made from various fish species (data not shown). The free amino acids in *pa daek* are derived mostly from protein hydrolysis occurring in the fermenting fish; thus, the free amino acid profile of *pa daek* products

might vary depending on the fish species used for production. Amino acid profiling of the various freshwater fish species in Laos will be beneficial not only for understanding the nutritional benefits, but also for developing *pa daek* products that are rich in valuable free amino acids.

Further research should explore the detailed mechanisms of the fermentation time-dependent increase in free amino acids that enhances both the taste and nutritional benefits of *pa daek*. In general, endogenous proteolytic enzymes such as lysosomal cathepsins and proteases in fish muscle are considered to be involved in protein hydrolysis in fermented fish production. Such enzymes are released from fish muscle lysosomes and function preferably in an acidic environment (Mukundan *et al.* 1986); thus, the pH decrease attributed to halophilic lactic acid bacteria in *pa daek* fermentation might be one of the necessary factors to increase the free amino acid concentration by promoting fish protein hydrolysis. It would also be interesting to investigate the possible direct involvement of the metabolic systems of halophilic lactic acid bacteria and other species in the generation of beneficial amino acids in *pa daek* products.

Conclusion

The importance of long-term fermentation of *pa daek* for product quality has been empirically recognized by producers for a long time. The present study confirmed such ancient wisdom by revealing a fermentation period-dependent increase in lactic and free amino acid concentrations in *pa daek*. Such a scientific basis is useful for encouraging both homemade and commercial *pa daek* producers to manage the fermentation period carefully to enhance palatability and ensure lactic acid fermentation. Furthermore, information regarding free amino acids should have a positive impact on the promotion of *pa daek* consumption. It is also noteworthy that *pa daek* fermentation conducted using small clupeid freshwater fish (*pa keo*) as the material is useful for a laboratory model of *pa daek* production. *Pa keo* is now popularly used for commercial *pa daek* production in Laos. It is available all year round and easily processed into *pa daek* because of its small size. Further promotion of microbial and biochemical research on *pa daek* with this fermentation model is encouraged for *pa daek* product development, as well as for progress in fermented food science research and education in Laos.

References

- Gürtler M, Gänzle MG, Wolf G, Hammes WP (1998) Physiological diversity among strains of *Tetragenococcus halophilus*. *System Appl Microbiol* 21:107–112
- Kobayashi T, Kajiwara M, Wahyuni M, Hamada-Sato N, Imada C, Watanabe E (2004) Effect of culture conditions on lactic acid production of *Tetragenococcus* species. *J Appl Microbiol* 96:1215–1221
- Marui J (2019) Science of *pa daek*, a traditional fermented seasoning of Laos. *Kagaku* 89:824–829 (in Japanese)
- Marui J, Boulom S, Panthavee W, Momma M, Kusumoto K, Nakahara K, Saito M (2015) Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos. *Biosci Microbiota Food Health* 34:45–52

- Marui J, Hasada K, Boulom S (2018) Salt-fermented fish products in Lao PDR, an inland country in Southeast Asia (*written in Japanese*). *Bull Soc Sea Water Sci Jpn* 72:279–287
- Morioka S, Vongvichith B, Marui J, Okutsu T, Phomikong P, Avakul P, Jutagate T (2019) Characteristics of two populations of Thai river sprat *Clupeichthys aesarnensis* from man-made reservoirs in Thailand and Laos with aspects of gonad development. *Fish Sci* 85:667–675
- Mukundan MK, Antony PD, Nair MR (1986) A review on autolysis in Fish. *Fish Res* 4:259–269
- Standard tables of food composition in Japan (STFCJ, 7th revised version) (2015) Ministry of Education, Culture, Sports, Science and Technology, Japan. http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/1374030.htm
- Yamaguchi S, Ninomiya K (2000) The use and utility of glutamates as flavoring agents in food. *J Nutr* 130: 921S–926S

Nutritional analysis of freshwater fish and shellfish for improving human nutrition in rural area in Laos

Kaori Fujita¹, Philavanh Boutsavath², Xaypunya Mahathilath²

¹Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

²Planning and Cooperation Division (PCD), National Agriculture and Forestry Research Institute (NAFRI), Vientiane capital, Lao PDR

Abstract

The nutritional composition of nine species of fish and three types of shellfish found in Nameuang Village and the capital city of Laos, Vientiane, were analysed to investigate their value as sources of food with the aim of improving nutritional status in rural Laos. These fish, except the Flying fish, had a high protein content (19–20 g/100 g sample) and quality (e.g., lysine content of 1,750–1,870 mg/100 g sample and 93.8– 97.9 mg/g protein). Their protein qualities were endorsed by amino acid score (amino acid score 100). In addition, the “small” group tended to have higher protein content than the “large” group across the fish species. In contrast, the fat content was higher in the “large” group than in the “small” group. Seasonal fluctuations in fat contents in three species, except the Walking catfish, were relevant to the reproductive cycle of the species. These results suggest that aquatic animals have high nutrient quality and are efficient/indispensable food sources for improving the nutritional status of the rural population in Laos.

Introduction

Freshwater aquatic animals such as fish and shellfish are reliable sources of essential nutrients for humans in inland countries. In the southeast Asian inlands, the major source of animal-food is obtained from freshwater aquatic animals in the Mekong River basin, which provide essential nutrients for people that subsist on cereal crops (Garaway et al. 2013; Halwart and Bartley 2014; Hortle 2007; Jams 2006). They are an excellent source of protein, fat, and micronutrients such as vitamins and minerals in an otherwise rice-based diet.

The Lao People's Democratic Republic (PDR) is one of the countries that are most dependent on these sources for daily nutrition (Pedersen et al. 2014; Phonvisay 2013). However, health challenges, such as malnutrition, are pervasive in Laos; one in four children (27%) under the age of 5 years is moderately underweight and 7% are severely underweight; of these, nearly half (44%) are moderately stunted and 19% are severely stunted (United Nations Children's Fund [UNICEF] 2012). People living in rural Laos account for more than 75% of the total population and depend on freshwater fish and other aquatic animals as a reliable source of animal proteins (Phonvisay 2013). Since there is little information about the nutritional value of the typical diet

in rural Laos, nutritional information of the available fish and shellfish is required. This study had two major objectives: to estimate the nutritional value of these foods based on previously published reports and food composition databases and to obtain preliminary information on the nutritional status of the rural population of Laos, including amino acid score.

Materials and Methods

Sample collection

This study about basic information on macro-nutritional values in major-freshwater fish and shellfish of Laos was conducted in a village, Nameuang, and two local markets in Vientiane, the capital of Laos. We selected eight groups of fish and shellfish based on the following criteria: common species found all over Laos, available throughout the year /or seasonally, and major species in the village (Table 1). The size of the samples depended on the feeding habit and supply to the village. The weight of samples varied with the species but was approximately 1 kg (on a wet weight basis) for each pooled sample from the markets. In the village, it was difficult to collect large amounts of a single pooled sample, and approximately 400 g was used for the analysis (Fujita et al. 2019).

There were differences in sizes of fish during growth. Species of fish studied included: Walking catfish (*Clarias batrachus*), Broadhead catfish (*Clarias macrocephalus*), Climbing perch (*Anabas testudineus*), and Striped snakehead (*Channa striata*). These were divided into three groups for analysis over four seasons (March, June, September, and January) throughout the year from March 2017 to January 2018. The seasons covered were the rainy season (beginning of May to end of September) and the dry season (beginning of October to end of April). Sampling was conducted at three local markets in Vientiane, which were selected due to abundance of natural fish traded directly between villagers and traders (Fujita et al. 2020). The target sample size was divided into three, and the number of fish was dependent on the sample size for each species but a minimum of 600 g (wet weight) was collected from samples pooled from each market.

Sample pre-treatment

Fresh or live samples were collected and transported on ice to the Living Aquatic Resources Research Center and stored frozen at -30 °C. Thereafter, the frozen samples were transported to the Japan International Research Center for Agricultural Sciences (JIRCAS) for nutrient composition analysis. The edible parts of fish may or may not include the head, viscera, scales, bones, and other parts according to traditional practices and depending on the species. Fillets with skin were analysed in all species except the small fish Such as, Flying barb (Pa siew) and Swamp barb C (Pa khao) which were analysed whole. Clam (Hoy kii) was analysed without the shell. Snails were prepared as follows: River snail (Hoy choup) was analysed after removal of the shell and eggs if present whereas, the Apple snail (Hoy pakkouang) was analysed after removal of the shell and internal organs (viscera).

Table 1. Name of the nutrition analysis sample¹.

No	Group name (in Lao)	Lao	English	Scientific name
<i>Fish group</i>				
1	Pa duk	Pa duk en	Walking catfish	<i>Clarias batrachus</i>
2		Pa duk oui	Broadhead catfish	<i>Clarias macrocephalus</i>
3	Pa keng	Pa keng	Climbing perch	<i>Anabas testudineus</i>
4	Pa khao	Pa khao	Swamp barb A	<i>Henicorhynchus siamensis</i>
5		Pa khao	Swamp barb B	<i>Puntius brevis</i>
6		Pa khao	Swamp barb C	<i>Rasbora aurotaenia</i>
7	Pa kor	Pa kor	Striped snakehead	<i>Channa striata</i>
8		Pa kor kan	Dwarf snakehead	<i>Channa gachua</i>
9	Pa siew	Pa siew	Flying barb	<i>Esomus metallicus</i>
<i>Shell fish (Snails and Clams) group</i>				
10	Hoy pakkouang	Hoy pakkouang	Apple snail	<i>Pomacea spp.</i>
11	Hoy choup	Hoy choup	River snail	<i>Viviparidae spp.</i>
12	Hoy kii	Hoy kii	Margaritiferidae (Freshwater bivalve)	<i>Unionidae spp.</i>

1) Sources: Fujita et al. (2019)

Proximate composition analysis

Essential food properties, such as moisture, protein, fat, carbohydrates, and ash contents, were evaluated according to the Standard Tables of Food Composition in Japan (STFCJ) (2015) based on the Association of Official Agricultural Chemists methods (Fujita et al. 2019). Each measurement was performed in duplicate. The protein content was analysed using the Kjeldahl method, and the quantity was calculated as 6.25 times the N content. Energy was calculated using Atwater's coefficient as a conversion factor: 4 for proteins and carbohydrates and 9 for fat. The total carbohydrate was calculated by subtracting measured moisture, protein, fat and ash from total weight while content was calculated from the total weight of each of the other components with the following formula: Carbohydrate (g/100 g)=100-(total moisture [g/100 g]+protein [g/100 g]+fat [g/100 g]+ash [g/100 g]).

Amino acid analysis

The amino acid composition of each sample was determined according to STFCJ (2015) using an automated amino acid analyser except for tryptophan, which was analysed by HPLC with a RF-20Axs spectrofluorometric detector (Fujita et al. 2019). The contribution of each species to the recommended nutrient intake of amino acids at each age was determined by comparisons to reference data. The amino acid score determines the effectiveness with which absorbed dietary N meets the essential amino acid requirement at a safe level of protein intake. It is based on the amount of limiting amino acid in 1 g of protein in a sample relative to the required amount (FAO/WHO/UNU 2007) using the following formula: Amino acid score = (mg of amino acid in 1 g test protein)/ (mg of amino acid requirement pattern) × 100.

Results and Discussion

Overview of proximate composition of fish and shellfish in Laos

Table 2 shows the proximate food composition, including crude protein, fat, carbohydrate, ash, moisture, and energy content of the edible parts in the 12 species studied. Total protein contents in the species of fish ranged between 19–20 g/100 g, except in Swamp barb A and Flying barb. The latter two had the lowest protein content among all fish due to inclusion of all parts of the fish, such as the head, bones, and viscera in the analysis. In contrast, the protein contents in the three types of shellfish ranged between 7.8–12.3 g/100 g, with no significant differences among species.

Focussing on specific species of fish, Climbing perch, Swamp barbs, and Flying barb, had lower water and protein contents and higher fat content (2.5–10.1 g/100 g) than the other species. With reference to previous data (James 2006; ASEAN FCD 2014), the fat content in these fish were two to seven times lower than was observed in the present study. Although the reason is not clear, previous research may have used cultured fish known to have more fat than the wild fish used in this study. Total energy varied widely among species from 79 kcal/100 g to 168 kcal/100 g according to their fat contents. The following species: Swamp barb C and Flying barb that were studied had high ash content; this may be due to the fact that their viscera, bones, and head were included in the analysis. Similarly, the high ash content of shellfish (River snail and *Margaritifera* members) was likely due to the inclusion of their viscera in the analysis.

Table 2. Proximate nutritional compositions in fish, snail and clam¹.

Kind	Sample name	Energy (kcal) ^{***}		Moisture (g) ^{**}		Protein (g) ^{**}		Fat (g) ^{**}		Carbohydrate (g) ^{**}		Ash (g) ^{**}	
		Village	VTE	Village	VTE	Village	VTE	Village	VTE	Village	VTE	Village	VTE
Fish	Walking catfish	101.5 ± 2.1	79.0	76.7 ± 0.6	80.0	20.6 ± 0.6	19.1	2.4 ± 0.1	0.3	0.0	0.0	1.1 ± 0.1	1.2
Fish	Broadhead catfish	—	94.0 ± 14.1	—	77.4 ± 1.7	—	19.9 ± 0.1	—	1.6 ± 1.5	—	0.2 ± 0.2	—	1.2 ± 0.1
Fish	Climbing perch	118.5 ± 0.7	125.0	74.5 ± 0.6	74.0	19.4 ± 0.2	19.2	4.3 ± 0.1	5.3	0.6 ± 0.8	0.0	1.6 ± 0.1	1.5
Fish	Swamp barb A*	—	97.0	—	77.7	—	17.8	—	2.5	—	0.7	—	1.3
Fish	Swamp barb B*	—	108.0	—	74.8	—	19.5	—	3.1	—	0.5	—	2.1
Fish	Swamp barb C*	—	168.0	—	65.5	—	19.1	—	10.1	—	0.2	—	5.1
Fish	Striped snakehead	88.0 ± 0.0	83.5 ± 3.5	77.3 ± 0.1	79.1 ± 0.8	20.7 ± 0.3	19.9 ± 0.6	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	1.4 ± 0.1	1.2 ± 0.0
Fish	Dwarf snakehead	87.0	—	77.6	—	20.9	—	0.4	—	0.0	—	1.4	—
Fish	Flying barb	93.5 ± 17.7	114.0 ± 11.3	76.8 ± 0.9	74.8 ± 2.0	14.7 ± 1.3	14.9 ± 1.7	3.7 ± 2.6	5.8 ± 0.4	0.4 ± 0.0	0.6 ± 0.3	4.5 ± 0.4	4.0 ± 0.4
Snail	River snail	59.0 ± 2.8	66.0	82.7 ± 0.6	80.4	8.7 ± 0.1	9.6	0.5 ± 0.0	0.6	4.9 ± 0.9	5.6	3.2 ± 0.1	3.8
Snail	Apple snail	55.5 ± 10.6	70.0 ± 7.1	85.0 ± 3.0	80.8 ± 1.2	10.3 ± 1.6	12.3 ± 1.1	0.5 ± 0.1	0.5 ± 0.1	2.6 ± 0.9	4.4 ± 0.6	1.8 ± 0.4	2.3 ± 0.5
Clam	Margaritiferae	52.0	83.0	85.1	77.1	7.8	8.8	0.7	0.9	3.7	10.0	2.7	3.2

Values are the mean ± standard deviation, except some samples, which are shown as a value n = 1. The all value show g or kcal/100g (wet weight basis). * Swamp barb A: *Henicorhynchus siamensis*; B: *Puntius brevis*; C: *Rasbora aurotaenia*. ** The value shows mg/100g sample (wet weight basis). *** The value shows kcal/100g sample (wet weight basis).

1) Sources: Fujita et al. (2019)

Seasonal and size- differences of nutrition composition in four fish species

There were differences in proximate composition relative to size and season. Significant increases in protein and ash content were recorded as fish size changed from large to small. In contrast, fat content increased with increasing fish size (Fig. 1). These findings suggest a significant relationship between these proximate compositions and fish size.

The seasonal differences in the proximate compositions of the four fish species are shown in Fig. 1. The tendencies towards seasonal differences were weak compared to those of size-dependent differences. Filet protein content was lowest in March, while fat content of the filet was highest in March, except for Striped snakehead.

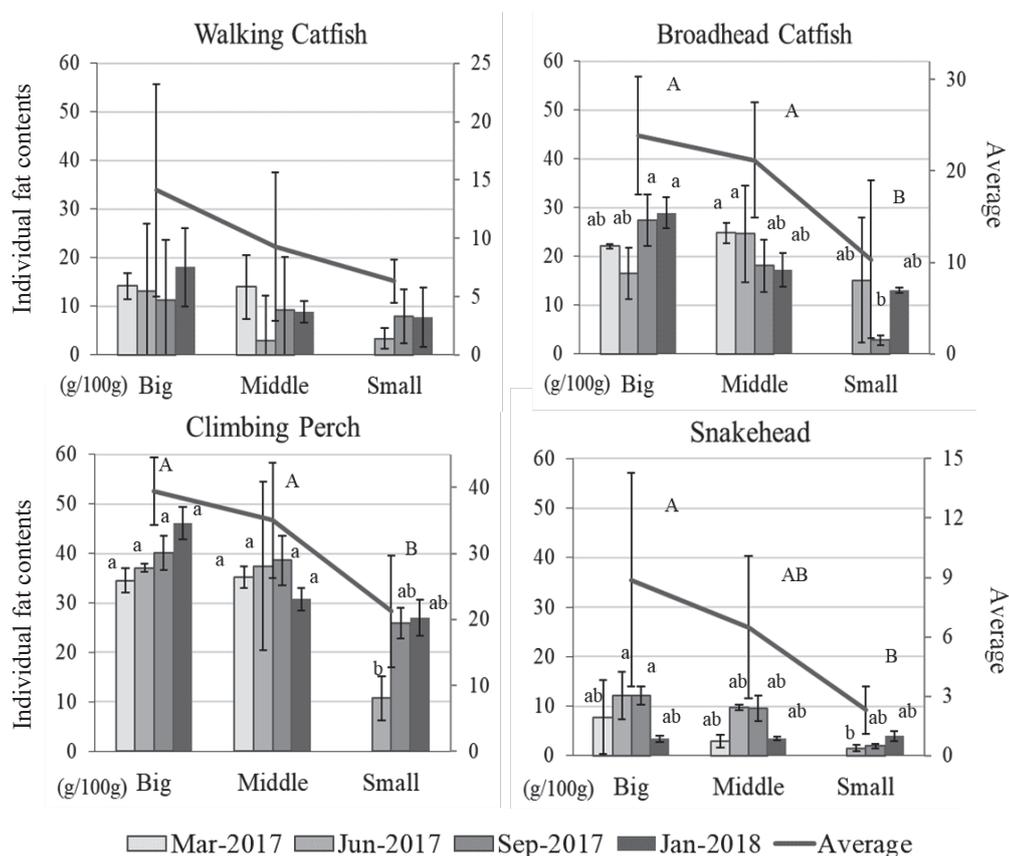


Fig. 1. Comparison of fat content (g/100 g) in the filets of four fish species (dry weight basis) (mean \pm SD) by seasons and sizes¹.

The different characters show significant differences ($P < 0.01$); uppercase letters show significant differences in the averages, lowercase letters show significant differences in individual seasons. 1) Sources: Fujita et al. (2020)

Amino acids properties of fish and shellfish

The amino acid composition of each sample is shown in Table 3 (Fujita et al. 2019). Lysine is an essential amino acid for human, especially in rural areas of Asia where there is predominance of rice farming and consumption, which may cause dietary deficiency in this amino acid (Vasal, 2002). Notably, the lysine content of Walking catfish and Striped snakehead were higher than the previously reported values by Nurhasan et al. (2010). In this study, the lysine content of fish (1,330–1,870 mg/100 g sample, 82.6–97.9 mg/g protein) was higher than that of shellfish (580–680 mg/100 g sample, 59.6–76.1 mg/g protein). It is also clarified that the lysine content of fish is higher than in livestock animal proteins such as rib roast of lean and fat meat in beef (83–87 mg/g), loin of lean and fat meat in pork (88 mg/g) and chicken portions (78–99 mg/g) (STFCJ 2015). As regarding to the other amino acids, these sample had also rich essential amino acids,

such as leucine, valine and threonine, and they were also richly non-essential amino acids such as testy components, in particular high amount of glutamic acid and aspartic acid.

Protein quality can be predicted based on the indispensable amino acid score from a comparison of the pattern of its amino acid composition to the pattern of human amino acid requirement (FAO 2013). The scores were calculated for each age category based on the new amino acid scoring pattern (WHO/FAO/UNU 2007). The analysed fish and shellfish samples in the present study were rich in essential amino acids and showed a good balance of protein in all categories for each age with appropriate amino acid scores (>100, data not shown), particularly for children from the age of 1 to 18 years. As previously mentioned, lysine is a critical component of the typical diet in rural Asia where milled rice—which is low in lysine, an essential amino acid in protein synthesis—is the staple food (STFCJ 2015). Thus, aquatic animals consumed by the people of Nameuang village can potentially prevent malnutrition.

Table 3. Amino acid compositions in fish, snail and clam¹.

Sample name	Walking catfish		Climbing perch		Striped snakehead		Flying barb		River snail		Apple snail		Margaritiferidae	
	mg/g*	mg/100g**	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g
<i>Essential amino acids</i>														
Histidine	22.5	430	23.4	450	24.6	480	28.6	460	22.9	220	18.4	210	20.5	180
Isoleucine	46.1	880	42.2	810	44.1	860	41.0	660	38.5	370	38.6	440	43.2	380
Leucine	83.2	1590	78.6	1510	80.5	1570	74.5	1200	76.0	730	80.7	920	76.1	670
Lysine	97.9	1870	91.1	1750	93.8	1830	82.6	1330	60.4	580	59.6	680	76.1	670
Methionine	29.8	570	28.6	550	30.3	590	26.7	430	19.8	190	17.5	200	25.0	220
Cysteine	9.9	190	9.9	190	10.3	200	9.3	150	12.5	120	10.5	120	12.5	110
Phenylalanine	44.0	840	43.2	830	43.1	840	41.0	660	40.6	390	37.7	430	37.5	330
Tyrosine	34.0	650	31.8	610	33.8	660	31.7	510	34.4	330	32.5	370	34.1	300
Threonine	49.2	940	45.3	870	45.1	880	41.6	670	46.9	450	44.7	510	45.5	400
Tryptophan	9.9	190	9.9	190	10.3	200	9.3	150	12.5	120	10.5	120	11.4	100
Valine	50.3	960	47.4	910	47.2	920	45.3	730	46.9	450	45.6	520	46.6	410
<i>Non-essential amino acids</i>														
Arginine	67.5	1290	65.6	1260	64.1	1250	61.5	990	76.0	730	85.1	970	72.7	640
Alanine	66.0	1260	69.3	1330	64.6	1260	65.8	1060	55.2	530	61.4	700	51.1	450
Glycine	73.3	1400	74.0	1420	60.5	1180	70.8	1140	60.4	580	70.2	800	46.6	410
Proline	44.0	840	44.8	860	38.5	750	44.7	720	45.8	440	49.1	560	35.2	310
Glutamic acid	161.8	3090	152.1	2920	155.9	3040	144.1	2320	144.8	1390	160.5	1830	145.5	1280
Aspartic acid	109.4	2090	102.6	1970	104.1	2030	96.3	1550	105.2	1010	102.6	1170	95.5	840
Serine	43.5	830	41.7	800	42.1	820	39.8	640	45.8	440	47.4	540	47.7	420

* mg/g protein, ** mg/100g sample

1) Sources: Fujita et al. (2019)

Conclusions

The purpose of this study was to evaluate the nutrient composition of fish and shellfish species consumed in Laos with a specific focus on rural areas. The results demonstrate that the aquatic animals analysed here, in particular fish such as the Broad catfish, Climbing perch, and Striped snakehead, had high protein contents and quality with appropriate amino acid scores. These types of fish also had high lysine contents. Although their supply in rural areas is currently considered insufficient, increase in supply through promotion of fishery/aquaculture may contribute to reduction in malnutrition caused by protein deficiency.

Moreover, because these freshwater fish and shellfish species are widely consumed inland globally, these data may also be useful from a nutritional public health standpoint for improving the nutritional status in rural areas in other developing countries. In addition, the Climbing perch had the highest fat content compared to the other three species. This information is useful from a public health perspective for residents in the Indochinese regions where these species are frequently consumed. Besides the four species above, there are other small-sized trash fishes, e.g., *Esomus metallicus*, *Rasbora spp.* and *Puntius brevis* of *Cyprinidae* (K. Fujita, unpubl. data). Others include *Parambassis siamensis* of *Ambassidae* and *Trichopsis spp.* of *Osphronemidae* (S. Morioka, unpubl. data). They are all important daily food sources particularly in mountainous areas of rural Laos, but their nutritional value is yet to be analysed. Since insufficient animal protein intake for residents of rural Laos is a major concern, nutritional evaluations of these trash fishes are also important for improving nutritional status in these areas.

Acknowledgements

The authors express our sincere thanks to Dr. Shinsuke Morioka and Mr. Katsumi Hasada, JIRCAS, and Mr. Bounsong Vongvichith, LARReC, for their valuable supports of the research management and sample collections. We are also grateful to the staff of NAFRI and LARReC for their assistance at sample collection.

References

- ASEAN Food Composition Database (2014) ASEAN Food Composition Database. ASEANFOODS Regional Center and INFOODS Regional Database Center, Institute of Nutrition, Mahidol University, Nakhon Pathom
- Food and Agriculture Organization (FAO) (2013) Dietary protein quality evaluation in human nutrition. Food and Nutrition Paper 92, FAO, Rome
- Fujita K, Saito M, Vongvichith B, Hasada K, Boutsavath P, Mahathilath X, Morioka S (2019) Analysis of the nutritional composition of aquatic species toward nutritional improvement in a Lao PDR rural area. *Jap Agricul Res Quart* 53:191–199
- Fujita K, Saito M, Vongvichith B, Hasada K, Boutsavath P, Mahathilath X, Morioka S (2020) Variations of basic nutritional compositions by seasons and sizes in four freshwater fishes as common food sources in Lao P.D.R. *Jap Agricul Res Quart* 54:53-61
- Garaway C J, Photitay C, Roger K, Khamsivilay L, Halwart M (2013) Biodiversity and nutrition in rice-based ecosystems; the case of Lao PDR. *Human Ecology* 41:547–562
- Halwart M, Bartley D (2014) Aquatic biodiversity in rice-based ecosystems. Studies and reports from Indonesia, Lao PDR and the Philippines. FAO, Rome
- James DG (2006) The impact of aquatic biodiversity on the nutrition of rice farming households in the Mekong Basin: consumption and composition of aquatic resources. *J Food Comp Anal* 19:756–757
- Nurhasan M, Maehre HK, Malde MK, Stormo SK, Halwart M, James D, Elvevoll EO (2010) Nutritional composition of aquatic species in Laotian rice field ecosystems. *J Food Comp Anal* 23:205–213
- Phonvisay S (2013) An introduction to the fisheries of Lao PDR. Mekong Development Series No.6.

Mekong River Commission, Phnom Penh

Standard Tables of Food Composition in Japan (STFCJ, 7th revised version) (2015) Ministry of Education, Culture, Sports, Science and Technology, Japan. http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/1374030.htm

United Nations Children's Fund (UNICEF) (2012) Lao PDR - social indicator survey 2011-12. UNICEF, New York

Vasal SK (2002) The role of high lysine cereals in animal and human nutrition in Asia. In: Protein Sources for the Animal Feed Industry, Expert Consultation and Workshop. FAO, Rome. pp. 167–183

World Health Organization and Food and Agriculture Organization and United Nations University (WHO/FAO/UNU) (2007) Protein and amino acid requirements in human nutrition 2007. WHO Technical Report Series 935, WHO, Geneva

Section IV

Sustainable aquaculture and fisheries



Indigenous freshwater shrimp *Macrobrachium dolatum* in Laos



Indigenous cyprinid *Barbonymus gonionotus* in Laos

A study on larval rearing of the local freshwater shrimp, *Macrobrachium dolatum*, in Laos: a potential target species for aquaculture

Tomoyuki Okutsu¹, Aloun Khounthongbang², Phonenaphet Chanthasone², Phoutsamone Phommachan², Sayaka Ito³

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Living Aquatic Resources Research Center (LRReC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR

³ Hokkaido National Fisheries Research Institute, Japan Fisheries Research and Education Agency (FRA), Kushiro, Japan

Abstract

Freshwater shrimp are a valuable aquatic product, in terms of their high economic value, for the people living along the rivers of Laos. However, no freshwater shrimp farming system has thus far been established in Laos. With the aim of developing such technique in Laos, the present study found four local species, *Macrobrachium dolatum*, *M. eriocheirum*, *M. amplinus*, and *M. dienbienphuense*, inhabiting Luang Prabang province. *M. dolatum* was selected for further study because of its relatively greater body size and fecundity, which are desirable features for aquacultural purposes. Hatched larvae were obtained from wild broodstock reared in an experimental tank. Next, a larval rearing trial was conducted on *M. dolatum*, using freshwater and brown pond water, and survival rates were observed for 4 weeks. The survival rate at 4 weeks of rearing was 1.8% and 20.5% when the larvae were reared in freshwater and brown pond water (collected from a fish-rearing pond), respectively. Further studies, such as using salt water as rearing water, are in progress to elucidate preferable rearing conditions for the larvae of *M. dolatum*.

Introduction

Freshwater shrimp are particularly important and in high demand in Southeast Asian countries as a source of protein and income. A larvae production system has been established for the giant freshwater prawn, *Macrobrachium rosenbergii*, and this species has now become the most commercially farmed freshwater prawn in the world (New 1990; New 2002). Although the Laos government has a policy of supporting the culture of indigenous aquatic animals, sustainable aquaculture of local shrimp has yet to be established in Laos. Since Laos is the only inland country in Southeast Asia, landlocked fluvial shrimp species are only available in this country (Hanamura et al. 2011). And since the ecological and biological features of amphidromous (e.g., *Caridina leucosticta*) and landlocked (e.g., *Caridina denticulata ishigakiensis*) shrimp species differ greatly

(Shokita 1976; Leberer and Cai 2003; Yatsuya et al. 2013), the most advanced knowledge and techniques for freshwater shrimp culture, such as those developed for *M. rosenbergii*, which has an amphidromous life cycle, cannot be applied directly to the landlocked fluvial shrimp inhabiting Laos. Especially in the larval stage, different biological characteristics, such as salinity tolerance, are exhibited even among the same species, such as *M. nipponense* (Ogasawara et al. 1979). The present study, then, was conducted with the aim of developing a larval rearing technique specifically suitable for landlocked local shrimp in Laos.

Materials and methods

Animal collection and species identification

Local freshwater shrimp were collected from the Mekong River, or purchased in the local market in Luang Prabang province, Laos, in October 2016. For species identification, the collected shrimp were morphologically examined according to the criteria in Cai et al. (2004) and Hanamura et al. (2011). Among the various species examined, *M. dolatum* (Fig. 1) was selected for further study because of its relatively greater body size and fecundity, which are desirable characteristics for aquacultural purposes.

Collection and rearing of M. dolatum

M. dolatum was regularly (roughly each month) collected from the Mekong River in Xanghai village (20°00'12" N, 102°13'50" E), Pak Ou district, Luang Prabang province (Fig. 2). The specimens were kept in outdoor concrete tanks (dimensions: 1 m (W) × 1 m (D) × 0.5 m (H)), with 250 L of freshwater and an aeration, circulation, and filtration system, in the Living Aquatic Resources Research Center (LARReC) in Chansavang village, Sikodtabong district, Vientiane Capital (Fig. 2), until used for the experiments. They were maintained by feeding to satiation with commercial pellets once a day.

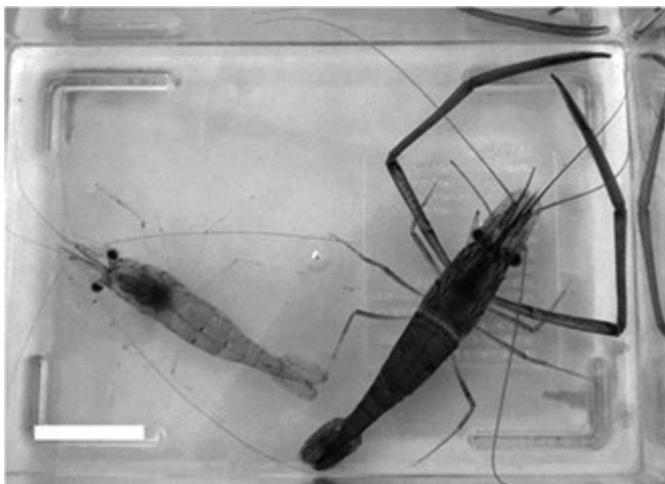


Fig. 1. Female (left) and male (right) *M. dolatum* captured from the wild (Bar: 2 cm).



Fig. 2. Laos; solid circle: Xanghai village, collection site for *M. dolatum*; open circle: Vientiane Capital, location of the Living Aquatic Resources Research Center (LARReC, 18°01'05.3" N, 102°31'07.2" E).

Obtaining and rearing the M. dolatum larvae

Some of the *M. dolatum* specimens gathered in the abovementioned collection site were found to be carrying eggs. In time, *M. dolatum* larvae were successfully hatched in the experimental tank in LARReC. After hatching, the larvae from one mother were separated into two groups of 10 to 20 larvae, with one group reared in freshwater, and one in brown pond water, each in a 1-L plastic box. The freshwater was prepared by aerating tap water for at least 24 hours to eliminate the chlorine, and the brown pond water was collected from a LARReC fish-rearing pond and used immediately. The water temperature ranged from roughly 23 to 27 °C in this study. Both groups were fed to satiation with commercial powder feed (Gold prawn, Higashimaru; manually grounded before use) once a day, and the larval survival rates in each group were examined for four weeks after hatching. Six replicates, each using larvae from a different mother, were run.

Results

Species identification and basic biological criteria

The morphological examination in the present study revealed that four species, *M. dolatum* (Fig. 1), *M. eriocheirum*, *M. amplinus*, and *M. dienbienphuense*, inhabited Luang Prabang province. The maximum carapace length (CL) of *M. dolatum* was 18.0 mm, and its clutch size ranged from 135 to 502 eggs, both larger than those of the other species: *M. eriocheirum* (15.7 mm and 31-125 eggs), *M. amplinus* (17.8 mm and 22-68 eggs), and *M. dienbienphuense* (17.7 mm and 96 eggs) (Table 1). As aforementioned, *M. dolatum*'s relatively greater body size and fecundity, which are particularly important biological features for aquacultural purposes, were the reason why this species was selected for the present study and used in the following experiments.

Table 1. Maximum carapace length (CL) and clutch size of the four shrimp species identified in the present study.

Species	Maximum CL (mm)	Clutch size (eggs)	
<i>M. dolatum</i>	18.0	135-502	(n = 8)
<i>M. eriocheirum</i>	15.7	31-125	(n = 5)
<i>M. amplinus</i>	17.8	22-68	(n = 5)
<i>M. dienphuensis</i>	17.7	96	(n = 1)

Larval rearing of *M. dolatum*

The water temperature ranged from roughly 23 to 27 °C in the present study. Based on the results of the six replicates, the average survival rate of the group reared in freshwater was 29.5, 1.8, 1.8, and 1.8% at 1, 2, 3, and 4 weeks after hatching, respectively (Fig. 3); and the average survival rate of the group reared in brown pond water was 85.7, 75.9, 58.0, and 20.5% at 1, 2, 3, and 4 weeks after hatching, respectively (Fig. 3). The total larval length at hatching was around 3 mm, and reached a maximum of 11.2 mm at 4 weeks of rearing.

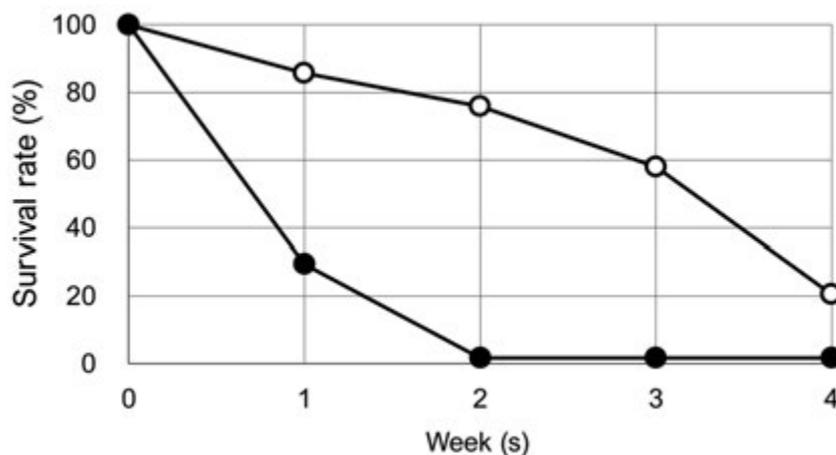


Fig. 3. The survival rate of *M. dolatum* larvae reared in freshwater (solid circles) and brown pond water (open circles). Each point indicates the weighted average survival rate of the larvae from six different mothers (“Week 0” indicates the onset of the experiment).

Discussion

Overall, the survival rate of *M. dolatum* larvae obtained in this study, in the case of both freshwater and brown pond water, was not good enough for larvae production in aquacultural practice. That said, the survival rate (at 4 weeks of rearing) in brown pond water (20.5%) was greater than in freshwater (1.8%), perhaps because the brown pond water may include edible components such as *Moina* and rotifers, which may contribute, as feed, to improved survival for the larvae. In larvae culture of the giant freshwater prawn *M. rosenbergii*, the most popular freshwater shrimp for aquacultural production, live planktonic feed such as *Artemianauplii* is widely used in hatcheries (New 1990), and *Moina* or rotifers can be used as supplemental or substitutional feed (Lovett and Felder 1988; Alam et al. 1993). Microalgae contained in the brown pond water might also play a role in improving survival, similar to that of the edible components above, by providing less stressful culture conditions for the larvae, as reported in detail by Lober and Zeng (2009) in the case of larval rearing of *M. rosenbergii*. The maximum total larval length at 4 weeks of rearing was 3.7 times that at hatching; however, it is unclear whether this growth rate is good or not, since there is, at present, no reported data on *M. dolatum* larval growth. In other *Macrobrachium* species, the growth and/or development of larvae can be improved by modifying the feed, salinity, and/or water temperature (Anger and Hayd, 2010; Lal et al., 2012; Chand et al. 2015). Our previous study showed that the salinity of rearing water was a critical factor for the survival of the larvae of *M. yui* (Okutsu et al. 2018), a species reported as landlocked fluvial shrimp found locally in Laos (Kounthongbang et al. 2015). In that study, the cumulative mortality rates of *M. yui* larvae at the end of the experimental period (from hatching to settling to the bottom, 3-4 weeks) were 58.9, 14.8, 8.3, and 35.4% when reared in freshwater, 1.7 ppt, 3.5 ppt, and 10.5 ppt artificial seawater, respectively. The study clearly showed that salt water was a better environment for the survival of *M. yui* larvae. However, in general, it is known that the

larvae of landlocked fluvial shrimp species (e.g., *Caridina mccullochi*, *Macrobrachium lanchesteri*, *Caridina formosae*) do not require salt water, but can be reared and develop normally in freshwater (Benzie 1982; Wong 1994; Shy et al. 2001; Lai and Shy 2009). In contrast, the larvae of amphidromous shrimp need to be reared in salt water (Hunte 1979a, b; Hayashi and Hamano 1984; Bauer and Delahoussaye 2008; Boudour-Boucheker et al. 2016). Though the reasons for the anomalous results in the *M. yui* study are unclear, a possible reason is that the streams in the habitat of *M. yui* flow through karst formations that contain electrolytes derived from an ancient sea, and the larvae of *M. yui* may utilize these (detailed discussion in Okutsu et al. 2018). The *M. dolatum* larvae investigated in the present study may also require saline water for their survival and development, since *M. dolatum* originates from the same region as *M. yui*. Further studies are underway to explore favorable feed and salinity conditions for survival and growth in *M. dolatum* larval production.

Acknowledgements

We are grateful to the staff in LARReC and Luang Prabang province for supporting this study. This study was supported by Management Expenses Grants from the Ministry of Agriculture, Forestry and Fisheries to the Japan International Research Center for Agricultural Sciences (JIRCAS)

References

- Alam MJ, Ang KJ, Cheah SH (1993) Weaning of *Macrobrachium rosenbergii* (de Man) larvae from *Artemia* to *Moina micrura* (Kurz). *Aquaculture* 112:187–194
- Anger K, Hayd L (2010) Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. *Aquat Biol* 9:251–261
- Bauer RT, Delahoussaye J (2008) Life history migrations of the amphidromous river shrimp *Macrobrachium ohione* from a continental large river system. *J Crustac Biol* 28:622–632
- Benzie JAH (1982) The complete larval development of *Caridina mccullochi* Roux, 1926 (Decapoda, Atyidae) reared in the laboratory. *J Crustac Biol* 2:493–513
- Boudour-Boucheker N, Boulo V, Charmantier-Daures M, Anger K, Charmantier G, Lorin-Nebel C (2016) Osmoregulation in larvae and juveniles of two recently separated *Macrobrachium* species: Expression patterns of ion transporter genes. *Comp Biochem Physiol A Mol Integr Physiol* 195:39–45
- Cai Y, Naiyanetr P, Ng PKL (2004) The freshwater prawns of the genus *Macrobrachium* Bate, 1868, of Thailand (Crustacea: Decapoda: Palaemonidae). *J Nat Hist* 38:581–649
- Chand BK, Trivedi RK, Dubey SK, Rout SK, Beg MM, Das UK (2015) Effect of salinity on survival and growth of giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquaculture Rep* 2:26–33
- Hanamura Y, Imai H, Lasasimma O, Souliyamath P, Ito S (2011) Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea, Decapoda, Palaemonidae) from Laos. *Zootaxa* 3025:1–37
- Hayashi K, Hamano T (1984) The complete larval development of *Caridina japonica* De Man (Decapoda, Caridea, Atyidae) reared in the laboratory. *Zool Sci* 1:571–589

- Hunte W (1979a) The complete larval development of the freshwater shrimp *Micratya poeyi* (Guérin-Méneville) reared in the laboratory (Decapoda, Atyidae). *Crustaceana Suppl* 5:153–166
- Hunte W (1979b) The complete larval development of the freshwater shrimp *Atya innocous* (Herbst) reared in the laboratory (Decapoda, Atyidae). *Crustaceana Suppl* 5:231–242
- Kounthongbang A, Lasasimma O, Souliyamath P, Iguchi K, Ito S (2015) Life history characteristics of the fluvial shrimp *Macrobrachium yui* (Holthuis, 1950) (Decapoda, Palaemonidae) spawning in the cave streams in northern Laos. *Crustaceana* 88:164–183
- Lai HT, Shy JY (2009) The larval development of *Caridina pseudodenticulata* (Crustacea: Decapoda: Atyidae) reared in the laboratory, with a discussion of larval metamorphosis types. *Raffles B Zool Suppl* 20:97–107
- Lal MM, Seeto J, Pickering TD, Hodge S (2012) Salinity and temperature requirements for larviculture of the Monkey River prawn *Macrobrachium lar* (Fabricius, 1798) (Decapoda: Caridea: Palaemonidae). *Aquaculture* 366–367:1–8
- Leberer T, Cai Y (2003) Shrimp of the family Atyidae from Guam, Mariana Islands. *Micronesica* 35–36:353–358
- Lober M, Zeng C (2009) Effect of microalgae concentration on larval survival, development and growth of an Australian strain of giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture* 289:95–100
- Lovett LD, Felder DL (1988) Evaluation of the rotifer *Brachionus plicatilis* as a substitute for *Artemia* in feeding larvae of *Macrobrachium rosenbergii*. *Aquaculture* 71:331–338
- New MB (1990) Freshwater prawn culture: a review. *Aquaculture* 88:99–143
- New MB (2002) A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). FAO Fisheries Technical Paper 428
- Okutsu T, Chanthasone P, Phommachan P, Kounthongbang A, Lasasimma O, Hamada K, Morioka S, Ito S (2018) Use of artificial seawater in the rearing of the fluvial prawn *Macrobrachium yui* larvae. *Aquacult Int* 26:325–335
- Ogasawara Y, Koshino S, Taki Y (1979) Responses to salinity in larvae from three local populations of the freshwater shrimp, *Macrobrachium nipponense*. *Bull Japan Soc Scient Fish* 45:937–943
- Shokita S (1976) Early life-history of the land-locked atyid shrimp, *Caridina denticulata ishigakiensis* Fujino et Shokita, from the Ryukyu Islands. *Res Crustac* 7:1–10
- Shy JY, Lai HT, Yu HP (2001) On the larval development of *Caridina formosae* (Decapoda, Atyidae) reared in the laboratory. *Crustaceana* 74:1159–1168
- Wong J (1994) Larval development of the Palaemonid prawn, *Macrobrachium lanchesteri* (De Man) reared in the laboratory (Decapoda, Caridea). *Crustaceana* 67:296–315
- Yatsuya M, Ueno M, Yamashita Y (2013) Life history of the amphidromous shrimp *Caridina leucosticta* (Decapoda: Caridea: Atyidae) in the Isazu river, Japan *J Crustac Biol* 33:488–502

Importance of resources of small-sized fishes as fundamental components of food resources and fish diversity in Lao PDR

Shinsuke Morioka¹, Bounsong Vongvichith²

¹ Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

² Living Aquatic Resources Research Center (LRReC), National Agriculture and Forestry Research Institute of (NAFRI), Vientiane, Lao PDR

Abstract

Growth, reproduction, and lifespan were reviewed in three indigenous small-sized fishes in Lao PDR belonging to different taxa, *Parambassis siamensis* (Ambassidae), *Rasbora rubrodorsalis* (Cyprinidae), and *Clupeichthys aesarnensis* (Clupeidae). All three species were estimated to have short lifespans (< one year) and breed throughout the year with plural generation alternations within a year. Environment in high temperature accelerated initial growth in all species. In *C. aesarnensis*, while higher temperature was considered to lead to earlier maturation and downsizing of maturation size, evolutionary downsizing owing to overfishing was of another concern. As the recent economic development and population growth within the country has led to an increase in fish demand and deterioration of the environment, all the species are considered to be in danger of stock decline. Therefore, in the present study, some ideas for stock managements for these species based on biological aspects are discussed.

Introduction

Lao PDR is a country with rich resources of indigenous fish species, particularly in the basins of the Mekong River and its tributaries. Estimates of the numbers of indigenous fish species in this region range from 700 to more than 1,200 (Kottelat 2001; Sverdrup-Jensen 2002). Among these, various indigenous small-sized fishes are distributed across the country regardless of taxa. These small-sized fishes are important as one of the major food sources for farmers in rural areas owing to easy access, as they are abundantly present in agricultural water masses (e.g., rice paddies, irrigation canals, and reservoirs) in which assured catch is expected using simple fishing gears. Some, such as a non-common miniature fish *Brachygobius mekongensis* (Gobiidae) with a maximum size of ca. 14 mm in standard length (SL) are not valuable as fishery resources (Morioka and Sano 2009). However, species that are larger than this and smaller than 70 mm SL with short lifespans, sometimes called “trash fishes”, are important members supporting the rich diversity of regional fauna as prey of carnivorous fishes, birds, mammals, and reptiles. However, the increasing population of Lao PDR and over-fishing of natural fish resources since the 20th century (FAO 2006) have led to the introduction of substantial numbers of alien fishes for aquaculture, particularly the Nile tilapia *Oreochromis niloticus*, common carp *Cyprinus carpio*, and Chinese carps (e.g., the grass carp *Ctenopharyngodon idella* and silver carp

Hypophthalmichthys molitrix) (Phillips 2002; Welcomme and Vidthayanon 2003). Consequently, more than 20 invasive alien species are now considered to have established natural breeding populations in the Mekong region, and their numbers are increasing even now through continuous un-official introduction of alien fishes (e.g., *Paramisgurnus dabryanus* of Cobitidae) (S. Morioka, unpubl. data). The presence of these alien fishes is of concern because of a potential for decline in the region's native and endemic fish diversity and their stock levels.

With the above context, ecological investigation on several small-sized fishes in Lao PDR have been made and some information helpful for stock management have so far been obtained. In this report, the summarized results of these investigations are presented for important food fishes of different taxa, i.e., *Parambassis siamensis* of Ambassidae, *Rasbora rubrodorsalis* of Cyprinidae, and *Clupeichthys aesarnensis* of Clupeidae.

Materials and methods

Three Laotian indigenous small-sized indigenous fishes (Fig. 1) are discussed in the present report. *Parambassis siamensis* (locally called *Pa kapkohn*) belonging to the family Ambassidae, is widely distributed in lakes, swamps, and small-scale rivers and is a source of dried fish. *Rasbora rubrodorsalis* (called *Pa siew*) belonging to the family Cyprinidae is distributed in small-scale reservoirs and rivers and is eaten in the form of traditional fermented fish (called *Pa som noy*). *Clupeichthys aesarnensis* (locally called *Pa keo*) belonging to the family Clupeidae is distributed in mid/large-scale reservoirs and rivers and is eaten in dried and traditional fermented forms (*Pa deak*).



Fig. 1. Three Laotian small-sized fishes investigated and reviewed in this article., Morioka et al. (2014) and Morioka et al. (2019). a: *Parambassis siamensis* [Okutsu et al. (2011)], b: *Rasbora rubrodorsalis* [Morioka et al. (2014)], c: *Clupeichthys aesarnensis* [Morioka et al. (2019)].

Investigations on *P. siamensis* and *R. rubrodorsalis* were conducted in 2010 in Laos, and that on *C. aesarnensis* in 2016–2017 in Laos and in Thailand in 2012–2013. For all species, the daily ages were estimated using otolith daily increments, and the growth patterns and hatching periods of each species were then estimated. The sagittae were used for *P. siamensis* and *C. aesarnensis*, and the lapillus was used for *R. rubrodorsalis* for daily age determination (Fig. 2).

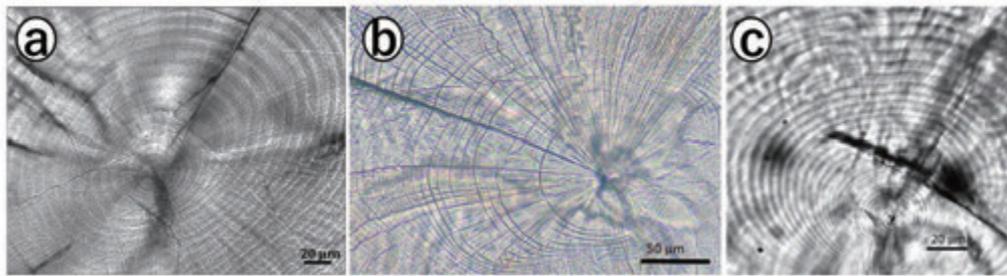


Fig. 2. Otoliths of *Parambassis siamensis* (a), *Rasbora rubrodorsalis* (b) and *Clupeichthys aesarnensis* (c).

In addition to the growth analyses, gonad development was also investigated with the application of gonadosomatic indices (GSI; weight of ovary or testis / body weight \times 100) for *R. rubrodorsalis* and *C. aesarnensis*, and histological observations of ovaries and testes for *P. siamensis*.

Results

Growth of each species was analyzed by seasons (warmer period from March to October (WP), cooler period from November to February (CP)) for *P. siamensis* and *R. rubrodorsalis*. In *P. siamensis*, growth (relationship between ages and standard length) was regressed using the logistic growth formulae for the specimens collected during the WP and CP as follows (Okutsu et al. 2011) (L_t and t in the formulae were the size at the age t and age in days, respectively):

$$\text{Warmer period: } L_t = 36.97 / (1 + \exp(-0.036 \cdot (t-47.09))) \quad (R^2 = 0.86, n = 85)$$

$$\text{Cooler period: } L_t = 40.98 / (1 + \exp(-0.026 \cdot (t-76.37))) \quad (R^2 = 0.91, n = 92)$$

The formulae were significantly different (F -test, $p < 0.01$), and growth during the WP was faster than that during the CP (Fig. 3). In this species, matured females appeared both during the WP and CP, and most of the matured females were > 30 mm SL (although a few females with < 30 mm SL seemed matured) (Fig. 4), indicating maturation age was estimated to be ca. 90 days in the WP and ca. 115 days in the CP (Fig. 3). These results suggest that *P. siamensis* breeds throughout the year and plural generation alternations within a year take place.

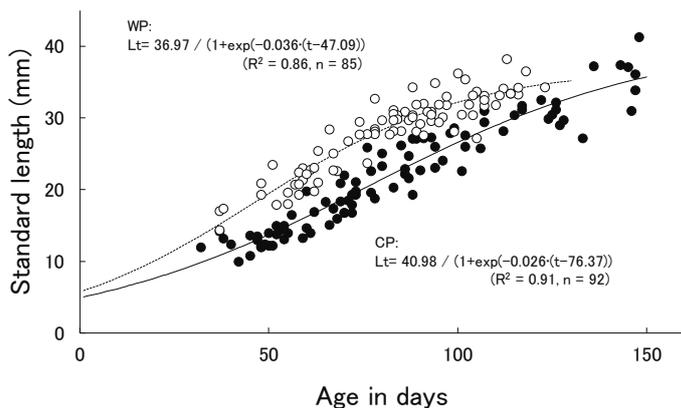


Fig. 3. Comparison in growths of *P. siamensis* collected during warmer period (open circles) and during cooler period (solid circles). [Figure cited from Okutsu et al. (2011) with modification].

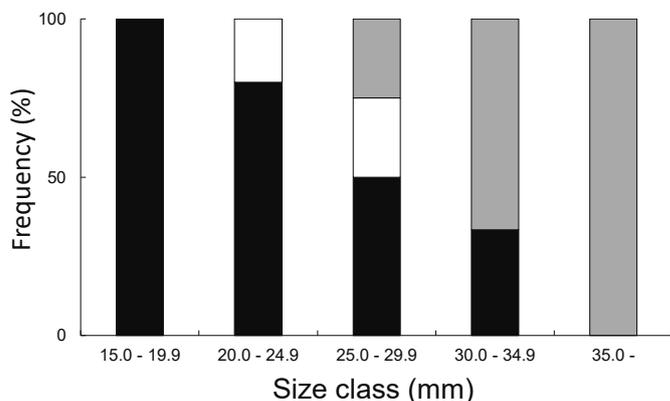


Fig. 4. Frequency distribution (%) of females with primary growth (black bar) previtellogenic (open bar) and vitellogenic oocytes (grey bar) observed in their ovaries in each size class. [Figure cited from Okutsu et al. (2011) with modification].

In *R. rubrodorsalis*, growth was analyzed separately by seasons and by sexes, because we identified that the females of this species grow larger than the males and the sex ratio was remarkably biased towards females (female : male = 1 : 0.43) (Fig. 5). Logistic growth formulae were used as follows (Morioka et al. 2014):

Warmer period

Females: $Lt = 29.38 / (1 + \exp(-0.050(t-38.40)))$ ($R^2 = 0.82, n = 185$)
 Males: $Lt = 24.91 / (1 + \exp(-0.054(t-31.61)))$ ($R^2 = 0.92, n = 80$)

Cooler period

Females: $Lt = 29.22 / (1 + \exp(-0.043(t-48.37)))$ ($R^2 = 0.87, n = 85$)
 Males: $Lt = 31.28 / (1 + \exp(-0.036(t-54.85)))$ ($R^2 = 0.95, n = 42$)

As observed in *P. siamensis*, growth in *R. rubrodorsalis* during the WP was also faster than that during the CP (Fig. 6). In addition, based on the GSI, this species also breeds during the CP in the females > ca. 20 mm SL (Fig. 7). Considering their short lifespan (< 150 days, Fig. 6), this species is considered to breed over a year and maturation age of ca. 50 days in the WP and ca. 60 days in the CP (Fig. 6).

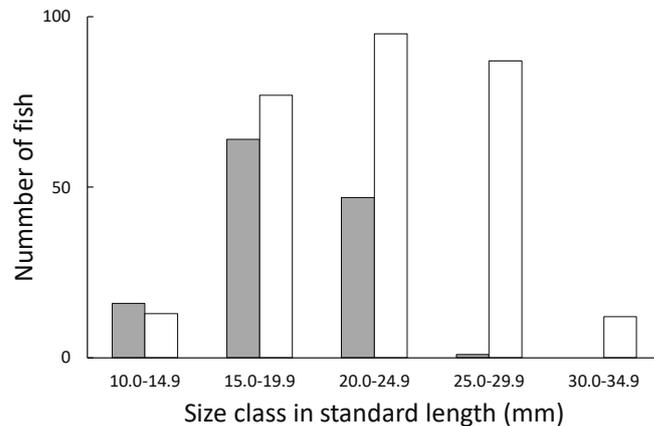


Fig. 5. Frequency distributions in standard length (mm) of *R. rubrodorsalis*. Open and solid bars indicate females and males. [Figure cited from Morioka et al. (2014) with modification].

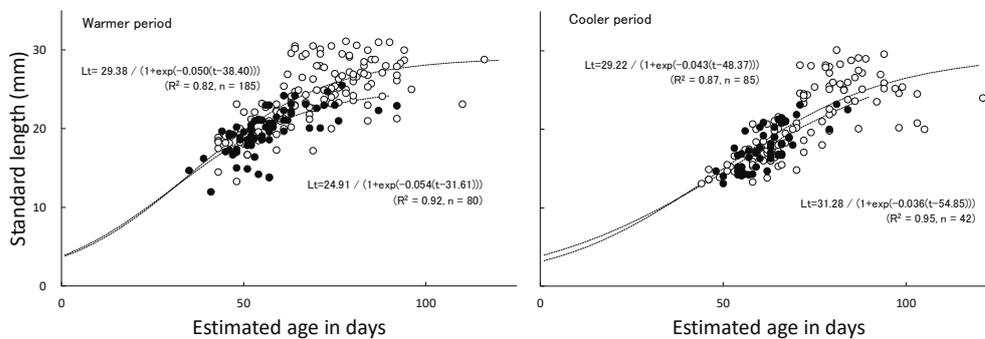


Fig. 6. Growths of *R. rubrodorsalis* collected during warmer period (left) and during cooler period (right). Open and solid circles are females and males. [Figure cited from Morioka et al. (2014) with modification].

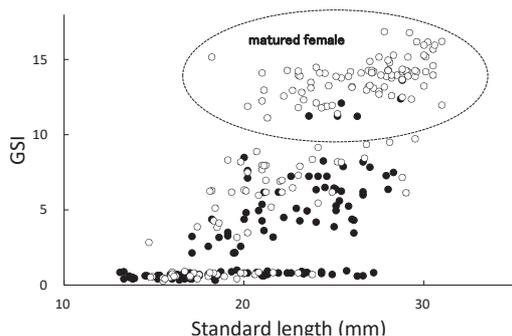


Fig. 7. Relationship between standard length (mm) and gonadosomatic index of female *R. rubrodorsalis*. Open and solid bars indicate females collected during the WP and CP, respectively. [Figure cited from Morioka et al. (2014) with modification].

In *C. aesarnensis*, growth of the populations in the Sirindhorn and Nam Ngum reservoirs was analyzed separately using the von Bertalanffy growth formula as follows (Morioka et al. 2019):

$$\begin{aligned} \text{Sirindhorn Reservoir: } Lt &= 36.72(-\exp(-0.02t)) & (R^2 = 0.85, n = 378) \\ \text{Nam Ngum Reservoir: } Lt &= 44.76(-\exp(-0.01t)) & (R^2 = 0.89, n = 486) \end{aligned}$$

The Sirindhorn population, chronically under higher temperature than that of the Nam Ngum Reservoir, tended to grow faster (Fig. 8). The GSI indicated that the gonads started to mature in the specimens ca. > 20 mm SL in the Sirindhorn population and > ca. 25 mm SL in the Nam Ngum population (Fig. 9). Furthermore, the growth models indicated the theoretical maximum size of the Sirindhorn population was smaller than that of the Nam Ngum population (Fig. 8).

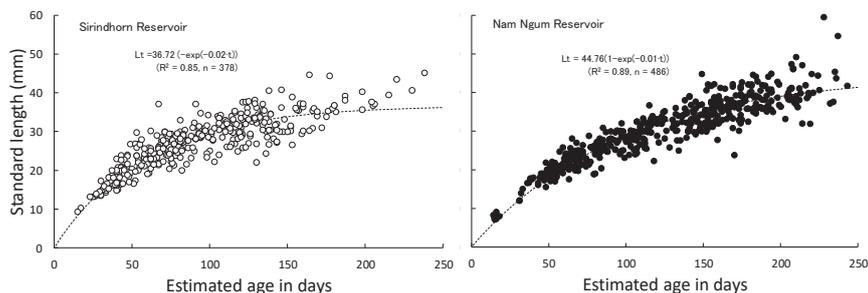


Fig. 8. Growths of *C. aesarnensis* collected from Sirindhorn Reservoir, Thailand (left) and from Nam Ngum Reservoir, Laos (right). [Figure cited from Morioka et al. (2019) with modification].

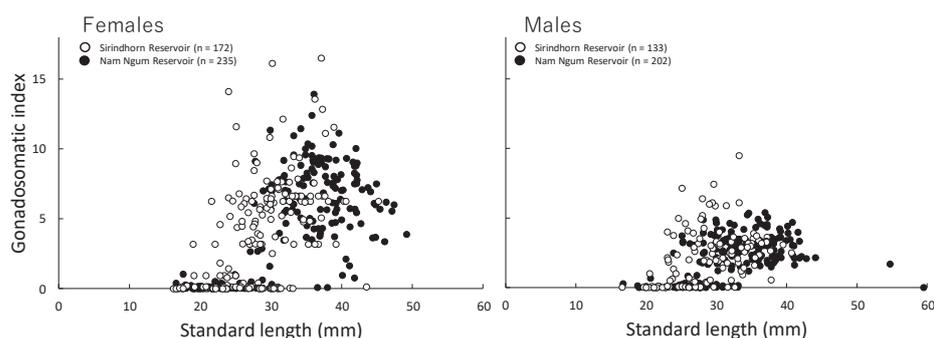


Fig. 9. Relationship between GSI and standard length (mm) of *C. aesarnensis* collected from Sirindhorn Reservoir, Thailand (open circles) and from Nam Ngum Reservoir, Laos (solid circles). [Figure cited from Morioka et al. (2019) with modification].

Discussion

The stocks of the three species, i.e., *Parambassis siamensis*, *Rasbora rubrodorsalis*, and *Clupeichthys aesarnensis*, have recently given cause for concern owing to a decline because of environmental changes (e.g., urbanization and land exploitation for cropping) and over-fishing. Accordingly, biological information on these species is required, with a view toward future stock assessment. Of additional concern, are the nearly 20 invasive exotic fish species (e.g., *Oreochromis niloticus*, *Clarias gariepinus*, and *Hypophthalmichthys molitrix*) that have recently been reported as having established breeding populations in the Mekong River basin (Phillips 2002; Welcomme and Vidthayanon 2003), emphasizing the need for conservation of the region's native/endemic fish species diversity. For fish diversity conservation as well as stock assessment, information on the life history of the targeted species, such as growth, sexual development, and generation time is a basic requirement. In this context, all the results presented here are useful information for future stock management leading to species conservation.

In the growth analyses for *P. siamensis*, *R. rubrodorsalis*, and *C. aesarnensis*, higher temperatures are considered to be one of the most important factors to accelerate growth of individual fish (Figs. 3, 6, 8) (Okutsu et al. 2011; Morioka et al. 2014; Morioka et al. 2019). Our results were consistent with previous reports illustrating that higher temperatures lead to faster growth in several fish species in semi-tropical areas (Morioka 2002; Morioka and Kaunda 2005). In addition, higher temperature can also cause earlier maturation (Dotsu 1982; Kon and Yoshino 2002) in the species led by faster growth. The earlier maturation under chronic higher temperature was typically observed in *C. aesarnensis* (Fig. 9), and the results from this species further indicate the downsizing by faster growth and earlier maturation (Figs. 8, 9) (Morioka et al. 2019).

In both the Sirindhorn and Nam Ngum reservoirs, the maximum sizes observed (45.2 mm SL during 2012-2013 and 59.5 mm SL during 2016-2017, respectively) tended to be smaller than those observed at the end of the 1990s (larger than 60 mm TL in the Sirindhorn reservoir and 70 mm SL in the Nam Ngum reservoir) (Baird et al. 1999; Jutagate et al. 2003). The decrease in maximum sizes may have been caused by overexploitation of each population leading to "evolutionary downsizing by overexploitation" as previously reported in several marine fish

species (Sharpe and Hendry 2009) in addition to the water temperature affecting maximum size in *C. aesarnensis*.

Considering the potential concerns of overexploitation and declines in stock levels of these species, appropriate methods of stock management should be proposed. Examples of classic methods for stock management in fishes are broadly summarized in Table 2.

Table 2. Examples of stock management manners for fishes.

Manner	Main target
1) Restriction / prohibition of fishing in spawning area	Adults (spawners)
2) Restriction / prohibition of fishing during breeding season	Adults (spawners)
3) Restriction / prohibition of fishing of small specimens	Larvae/juveniles, subadults
4) Restriction of annual catch amount	Whole population

For all the species presented in this report, spawning grounds are not known with breeding over a year, and hence the methods 1) and 2) described in Table 2 are not considered applicable. However, method 3) is applicable using mesh size control for restricting the catch of small specimens for future conservation of breeding specimens. Furthermore, to restrict the amount of annual catch as suggested by method 4), restriction in gear number is also considered practical. To realize these stock management strategies, related laws and/or ordinances by the central/local governments are to be ensured.

Acknowledgements

We thank the technical staff of the Living Aquatic Resources Research Center, Laos, for their assistance with collecting the samples.

References

- Baird IG, Inthaphaisy V, Kisouvannalath P, Phylavanh B, Mounsouphom B (1999) The fishes of southern Laos. Lao Community Fisheries and Dolphin Protection Project, Ministry of Agriculture and Forestry, Vientiane
- Dotsu Y (1982) The early life history of the combtooth blenny *Dasson trossulus* and spawnings of the laboratory-reared fish about three months after hatching. Bull Facul Fish, Nagasaki Univ 52:19–27 (in Japanese)
- Food and Agriculture Organization (FAO) (2006) Fishery country profile Lao P.D.R., Rome
- Jutagate T, De Silva SS, Mattson NS (2003) Yield, growth and mortality rate of the Thai river sprat, *Clupeichthys aesarnensis*, in Sirinthorn Reservoir, Thailand. Fish Mgmt Ecol 10:221–231
- Kon T, Yoshino T (2002) Extremely early maturity found in Okinawan gobioid fishes. Ichthyol Res 49:224–228
- Kottelat M (2001) Fishes of Laos. Wildlife Heritage Trust, Colombo
- Morioka S (2002) Otolith features and growth of juvenile *Opsaridium microcephalum* (Pisces: Cyprinidae) from the southwestern shoreline of Lake Malawi. Afr Zool 37:165–170

- Morioka S, Kaunda E (2005) Preliminary examination of hatching season and growth of *Engraulicypris sardella* (Pisces: Cyprinidae) larvae and juveniles in Lake Malawi. *Afr Zool* 40:9–14
- Morioka S, Koizumi N, Vongvichith B (2014) Seasonal growth and reproduction of *Rasbora rubrodorsalis*, a small-sized cyprinid fish from central Laos (Teleostei: Cyprinidae). *Ichthyol Explor Freshwaters* 25:277–287
- Morioka S, Sano K (2009) Growth and maturation of the bumble-bee goby *Brachygobius mekongensis* (Perciformes: Gobiidae) occurring in the Mekong basin, in Vientiane Province, Central Laos. *Ichthyol Explor Freshwater* 20:267–275
- Morioka S, Vongvichith B, Marui J, Okutsu T, Phomikong P, Avakul P, Jutagate T (2019) Characteristics of two populations of Thai river sprat *Clupeichthys aesarnensis* from man-made reservoirs in Thailand and Laos, with aspects of gonad development. *Fish Sci* 85:667–675
- Okutsu T, Morioka S, Shinji J, Chanthasone P (2011) Growth and reproduction of the glassperch *Parambassis siamensis* (Teleostei: Ambassidae) in Central Laos. *Ichthyol Explor Freshwaters* 22:97–106
- Phillips MJ (2002) Freshwater aquaculture in the Lower Mekong Basin. MRC Technical Paper No. 7, Mekong River Commission, Phnom Penh
- Sharpe DMT, Hendry AP (2009) Life history change in commercially exploited fish stocks: an analysis of trends across studies. *Evolution Appl* 2:260–275
- Sverdrup-Jensen S (2002) Fisheries in the lower Mekong Basin: status and perspectives. MRC Technical Paper 6:1–95
- Welcomme RL, Vidthayanon C (2003) The impact of introductions and stocking of exotic species in the Mekong basin and policies for their control. MRC Technical Paper No. 9, Mekong River Commission, Phnom Penh

Technical achievements of indigenous fish aquaculture in rice-paddy and pond in rural areas of Lao PDR: summarized results obtained by the JIRCAS-LARReC project

Bounsong Vongvichith¹, Shinsuke Morioka²

¹ Living Aquatic Resources Research Center (LRReC), National Agriculture and Forestry Research Institute of (NAFRI), Vientiane, Lao PDR

² Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

Abstract

Research achievements in aquaculture-related research techniques during the JIRCAS-NAFRI project with LARReC since 2007 are presented in this report. In particular, seed production of Laotian indigenous fishes and morphological development of several species are summarized. In addition, case studies of rice-paddy fish culture and pond fish culture that were conducted with farmers in rural areas are also presented. Based on the results we can propose practical applications for aquaculture using Laotian indigenous fishes by farmers in rural areas that can contribute to increase in fish production. In addition, information on morphological development of larval and juvenile stages of the concerned species is essential for consideration of their evolutionary ecologies and contribution to the conservation of indigenous species.

Introduction

Lao PDR is a country in which ca. 70 % of the population (total population 6.9 million) (Worldmeter 2019) is directly/indirectly engaged in the agricultural sector (Seo 2018). It is an inland country surrounded by China, Vietnam, Cambodia, Thailand, and Myanmar. The country is still categorized as a least developed country (LDC) as per economic status, although economic development has been accelerated in recent years (World Bank 2018). However, the national population of the country is growing at a high rate (ca. 1.45 % per year), and the further enhancement of food production for national food security is an important national subject. Moreover, the rate of malnutrition in the population, reported to be ca. 21 %, is much higher than that of Thailand (ca. 7 %), and animal protein intake of 14 g/day is much lower than that of Thailand (ca. 24 g/day) (FAOSTAT 2018). These nutritional deficiencies may cause high stunting rate (ca. 41.4 %) (WHO 2018) and shorter lifespan (ca. 67 years) (World Bank 2017) in the country, and, furthermore, such malnutrition was reported to be more serious in mountainous rural areas than in urban areas (WFP 2013). Hence, enhancement of animal protein intake for nutritional improvement is one of the important national aims.

Considering the above situation, the government of Lao PDR has attempted to promote aquaculture development, since capture fishery production has been stagnant at ca. 30,000 t/year

during the past few decades (Phonvisay 2013) suggesting it to have reached the upper limit of exploitation. Consequently, further increase in fish production is largely dependent on aquaculture development and has in fact, developed rapidly since the early 2000s and has reached ca. 100,000 t/year of production currently (Phonvisay 2013). However, at present, most aquaculture production depends on alien fishes, e.g., the Nile tilapia, Chinese carps, and African catfish (Welcomme and Vidthayanon 2003), and the production is mainly operated in/or near urban areas that have easy accessibility for commercial sales at markets. Thus, such an aquaculture system contributes less to the enhancement of protein intake in rural areas, and poor infrastructure of cold chains is another factor affecting market distribution of aquaculture products in rural areas.

To improve the above situation, technical development of aquaculture using Laotian indigenous fishes that are applicable in rural areas has been considered, and the Japan International Research Center for Agricultural Sciences (JIRCAS) and the National Agriculture & Forestry Research Institute (NAFRI) have conducted a joint project in cooperation with the Living Aquatic Resources Research Center (LARReC) since 2007. In this report, several technical developments useful for the aquaculture of Laotian indigenous fishes being established by the JIRCAS- NAFRI project with technical cooperation from the LARReC, are reviewed.

Technical development of seed production of indigenous fishes as potential targets for aquaculture

Seed production techniques have been established for nine Laotian indigenous fishes (Table 1) during the JIRCAS-NAFRI project with LARReC since 2007. Among these species, we evaluated the growth performance (slow, moderate, and rapid growth), for each species (Table 1). For all species, morphological development during larval and juvenile stages have been described as also behavioral features (e.g., Morioka et al. 2012b etc.) (Fig. 1). This information is helpful not only for stable seed production of the target species but also for consideration of evolutionary ecology contribution to species and biodiversity conservation of the target species.

Table 1. Laotian indigenous fishes for which seed production techniques were established by the JIRCAS-NAFRI project with LARReC.

Fish species	Max. size	Growth	Feeding	References
Cypriniformes				
Cyprinidae				
<i>Hypsibarbus malcolmi</i> / <i>Barbonymus gonionotus</i>	ca. 25 cm SL	moderate	omnivorous	Ogata et al. (2010)
<i>Cirrhinus microlepis</i>	> 70 cm SL	rapid	omnivorous	Morioka et al. (2012a)
Siluriformes				
Clariidae				
<i>Clarias macrocephalus</i>	ca. 30 cm SL	slow	carnivorous	Morioka et al. (2013a)
Bagridae				
<i>Hemibagrus filamentus</i>	ca. 30 cm SL	slow	carnivorous	Morioka & Vongvichith (2011)
Pangasiidae				
<i>Pangasianodon</i> <i>hypophthalmus</i>	> 100 cm SL	rapid	omnivorous	Morioka et al. (2010b)
Perciformes				
Anabantoidei				
Anabantidae				
<i>Anabas testudineus</i>	ca. 25 cm SL	moderate	carnivorous	Morioka et al. (2009)
Osphronemidae				
<i>Trichogaster pectoralis</i>	ca. 25 cm SL	slow	omnivorous	Morioka et al. (2010a)
<i>T. trichopterus</i>	ca. 15 cm SL	slow	omnivorous	Morioka et al. (2012b)
<i>Osphronemus gouramy</i> / <i>exodon</i>	> 70 cm SL	rapid	omnivorous	Morioka et al. (2013b)

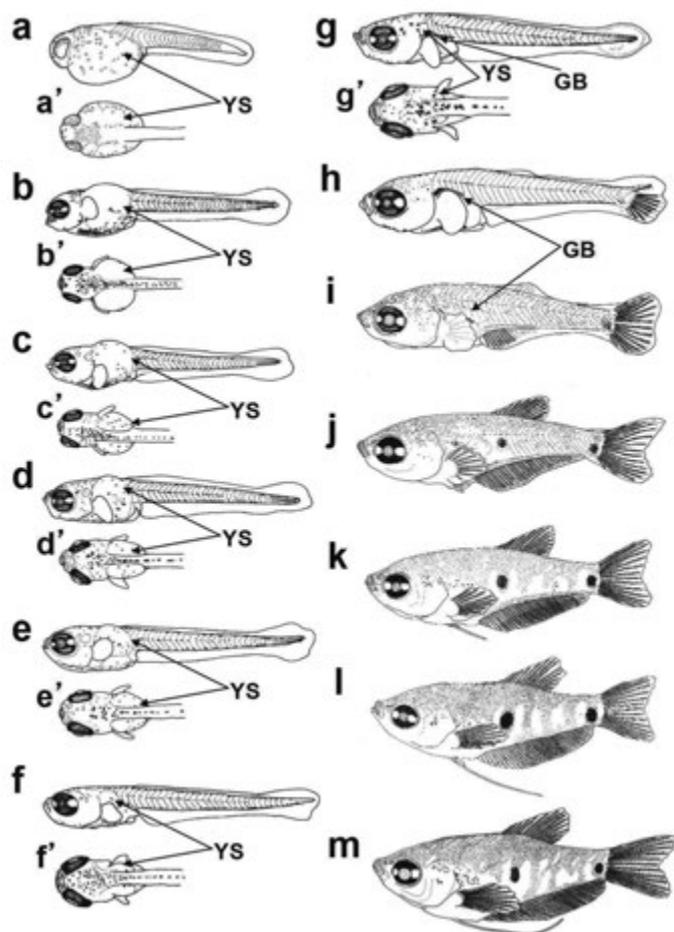


Fig. 1. Morphological development of *Trichogaster trichopterus*. **a** newly hatched larva (2.5 mm BL); **b** preflexion larva, day 1 (3.1 mm BL); **c** preflexion larva, day 2 (3.4 mm BL); **d** preflexion larva, day 3 (3.7 mm BL); **e** preflexion larva, day 5 (4.0 mm BL); **f** preflexion larva, day 7 (4.2 mm BL); **g** preflexion larva, day 9 (4.4 mm BL); **h** flexion larva, day 11 (5.5 mm BL); **i** postflexion larva, day 13 (6.7 mm BL); **j** postflexion larva, day 19 (8.6 mm BL); **k** postflexion larva, day 25 (10.6 mm BL); **l** juvenile, day 30 (12.5 mm BL); **m** juvenile, day 35 (14.0 mm BL). **a'** **b'**, **c'**, **d'** anterior dorsal view. **YS** yolksac, **GB** gas bladder [Figure modified from Morioka et al. (2012b)].

Applicable facilities of fish aquaculture and target species in rural areas

In rural areas, aquaculture operators (the farmers) are relatively economically disadvantaged in general and are not capable of building new facilities for aquaculture (e.g., cages and ponds). However, using existing agricultural water masses (i.e., rice-paddies and irrigation reservoirs), fish aquaculture is practically possible. In our trials, the basic design of a rice paddy for fish culture with the establishment of refuge canals in paddies is shown in Fig. 2.

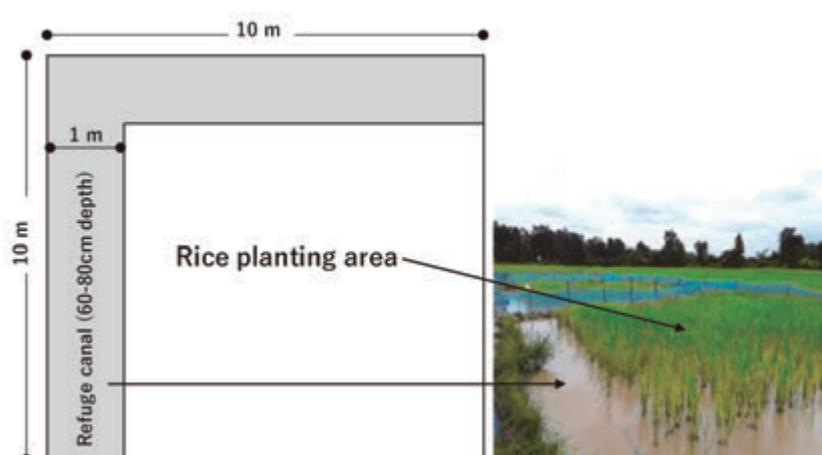


Fig. 2. Basic design of rice paddy for fish culture (left) and rice-paddy fish culture trial in Savannakhet Province, Lao PDR (right).

As suitable fishes for rice-paddy fish culture and pond fish culture, we selected four species (*Anabas testudineus*, *Barbonymus gonionotus*, *Pangasianodon hypophthalmus*, and *Osphronemus exodon*) from the species listed in Table 1 as shown in Table 2 and Fig. 3. As selection criteria, we considered the following aspects indicating suitability to rice-paddy and pond fish culture: 1) adaptability to rice-paddy and pond environment, 2) moderate and/or rapid growth, and 3) acceptable marketable values. All the species in Table 2 more or less satisfy the aspects 1) and 2), and three species, except *B. gonionotus*, have relatively higher market value among the fishes sold in the local market and satisfy aspect 3). The marketable value of *B. gonionotus* is indeed not as high as the other three species, but we identified this species as being ecologically well suited to the rice-paddy/pond culture system owing to ease of seed production and large fecundity that enables constant seed production and supply.

Table 2. Four indigenous fishes selected as applicable species for rice-paddy and pond fish culture.

Fish species	Seed production technique	Water mass
<i>Anabas testudineus</i>	semi-artificial propagation	rice-paddy / pond
<i>Barbonymus gonionotus</i>	semi-artificial propagation	rice-paddy / pond
<i>Pangasianodon hypophthalmus</i>	artificial propagation	rice-paddy
<i>Osphronemus gouramy / exodon</i>	natural propagation	rice-paddy

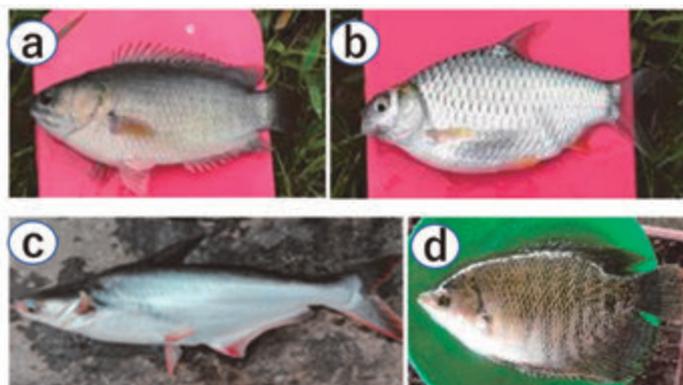


Fig. 3. Fishes applied for aquaculture in rural areas. a: *Anabas testudineus* (Pa kheng), b: *Barbonymus gonionotus* (Pa pak), c: *Pangasianodon hypophthalmus* (Pa sooai), d: *Osphronemus exodon* (Pa meng).

Productivity of rice-paddy fish and pond fish culture

We aimed to establish fish culture system(s) with low input (less input of artificial feeds) both in the rice-paddy and in pond. Rice-paddy fish culture trials were conducted in two sites of the Vientiane Province during 2013–2016 (Vongvichith et al. 2018). The results obtained indicate that low stocking density (< 0.5 fish/m²) could lead to a high biomass gain index (BGI = weight of stocked fish/weight of harvested fish) of 15–20 even without providing artificial feed. This BGI is considered acceptable for self-sufficiency of fish to the farmer's household.

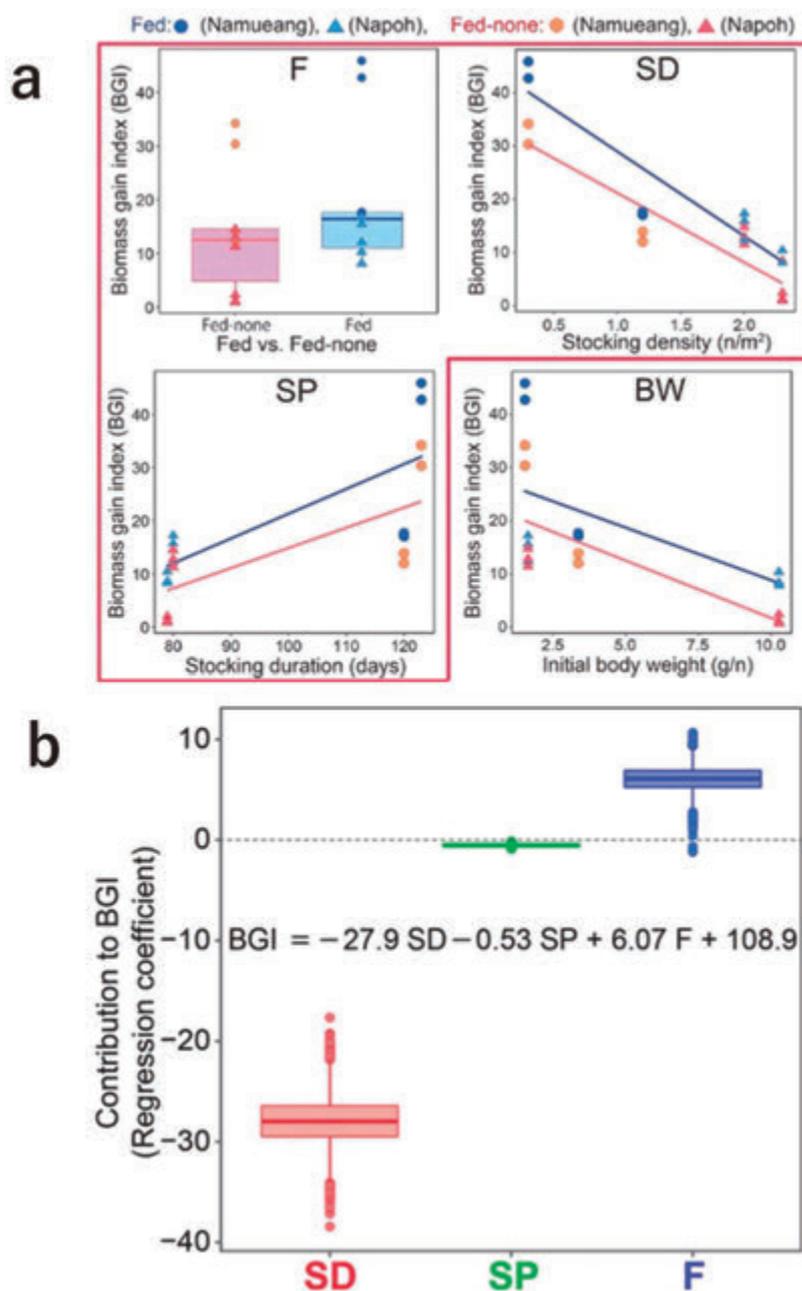


Fig. 4. Potential factors relevant to fish productivity (BGI) in rice paddy. [F: feeding or non-feeding, SD: stocking density of fingerlings, SP: stocking period, BW: weight of fingerling at stocking. F, SD & SP were selected as influential parameters by linear regression analyses, while BW was not selected] (a) and evaluation of contribution of selected parameters of stocking density (SD), stocking period (SP) and feeding condition (F) to fish productivity (BGI). [Figure modified from Vongvichith et al. (2018)] (b).

For pond fish culture, two trials were conducted during 2017–2018 in a village in Savannakhet Province to examine fish productivity, using an existing earthen pond of ca. 1,500 m² (ca. 1.5 m maximum depth). Applied fishes for pond culture were the four indigenous fish species as shown in Table 2 and Fig. 3.



Fig. 5. Pond fish culture trial in Savannakhet Province.

In the trial conducted in 2017, *A. testudineus* (2,000 juveniles) and *B. gonionotus* (4,000 juveniles) were stocked on June 18, 2017, and *P. hypophthalmus* (100 juveniles) and *O. exodon* (100 juveniles) were stocked on August 18, 2017 (overall stocking density was ca. 4 fish/m²). All fishes were harvested on February 22, 2018 (249 days of stocking for the former two species, and 188 days for the latter two). In the trial conducted in 2018, *A. testudineus* (1,200 juveniles), *B. gonionotus* (2,300 juveniles), and *P. hypophthalmus* (200 juveniles) were stocked on June 28, 2018 (ca. 2.5 fish/m²), and they were harvested on February 18, 2019. For the trial conducted in 2017, fish were provided commercial pellet feed intensively (ca. 4–5 % of biomass per day), whereas fish were fed agricultural byproducts (mainly rice bran with occasionally termite larvae) on an irregular basis by farmers for the trial in 2018.

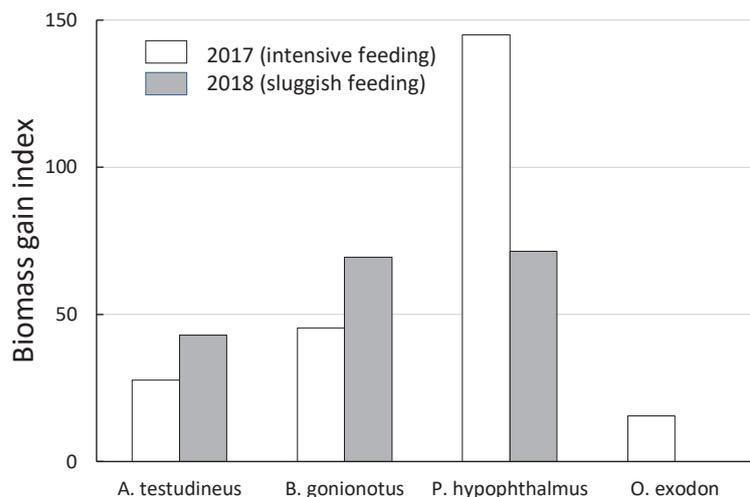


Fig. 6. Comparisons of biomass gain indices (BGI) of four indigenous fishes cultured in earthen pond in Savannakhet Province.

The results showed a remarkably high BGI in *P. hypophthalmus* observed in 2017; this high BGI probably being caused by its greater swimming ability than the others, leading to its

monopolistic feeding on commercial pellet feed. In contrast, distinctively low BGI of *O. exodon* was observed in 2017 and this is probably because of its inferior swimming ability with difficulty in feeding on commercial pellet feed. Considering this low BGI for *O. exodon*, polyculture of this species with the others having better swimming ability is probably not recommendable.

For *A. testudineus* and *B. gonionotus*, the BGIs were higher in 2018 than in 2017 indicating better productivities in 2018 regardless of the more limited feed condition in 2018, while the BGI for *P. hypophthalmus* was remarkably lower in 2018 than in 2017 (Fig. 6). Lower BGI in 2018 for *P. hypophthalmus* can be explained by the absence of artificial feed in 2018, but the higher BGIs for *A. testudineus* and *B. gonionotus* in 2018 were probably because of lower stocking densities in that year.

Future prospects for indigenous fish aquaculture in Lao PDR

In the present report, fish aquaculture using indigenous species, i.e., Pa kheng *Anabas testudineus*, Pa pak *Barbonymus gonionotus*, and Pa soai *Pangasianodon hypophthalmus* (rice-paddy fish culture for the first two species and pond fish culture for all three species), was mostly confirmed as a feasible system. Considering the BGIs in each system, the rice-paddy fish culture is more suitable for consumption at the household level because of its relatively lower BGI. However, the pond fish culture is considered to have more potential for commercial production of fishes because of higher BGIs even under limited artificial feeding conditions (Fig. 6). Currently, the major target for aquaculture in Lao PDR is the Nile tilapia *Oreochromis niloticus*, but the prices of many of the indigenous fishes are in general higher than the price of the Nile tilapia (B. Vongvichith pers. comm.). This situation also indicates that the culture of indigenous fish in ponds has a commercial potential.

However, we have found several serious problems that have affected the promotion of aquaculture across the country, including in rural areas of Lao PDR. These problems need to be urgently addressed for broad extension of fish culture techniques, as follows:

- 1) High dependency of imported fish feeds that are too expensive in economically disadvantaged areas of Lao PDR,
- 2) Insufficient seed production capacity and limited seed supply,
- 3) Quantitative and qualitative insufficiency of technical staff for seed production.

Concerning problem 1), we have been conducting trials in the use of the black soldier fly (BSF) larvae (Fig. 7) as fish feed. Nakamura et al. (2016) demonstrated a production system of the BSF larvae that is feasibly for small-scale farmers and the associated reduction in feed cost has mostly become realizable.



Fig. 7. Larvae of the black soldier fly produced in the LARReC.

For problems 2) and 3), infrastructural enhancement for seed production facilities as well as further capacity building of human resources are further required.

Acknowledgements

We thank the technical staff of the Living Aquatic Resources Research Center, Laos, for their assistance with collecting the samples.

References

- FAOSTAT (2018) Lao People's Democratic Republic. <http://www.fao.org/faostat/en/#country/120>
- Ogata Y, Morioka S, Sano K, Vongvichith B, Eda H, Kurokura H, Khonglaliane T (2010) Growth and morphological development of laboratory-reared larvae and juveniles of the Laotian indigenous cyprinid *Hypsibarbus malcolmi*. *Ichthyol Res* 57:389–397
- Morioka S, Ito S, Kitamura S, Vongvichith B (2009) Growth and morphological development of laboratory-reared larval and juvenile climbing perch *Anabas testudineus*. *Ichthyol Res* 56:162–171
- Morioka S, Ito S, Kitamura S (2010a) Growth and morphological development of laboratory-reared larval and juvenile snakeskin gourami *Trichogaster pectoralis*. *Ichthyol Res* 57:24–31
- Morioka S, Sano K, Phommachan P, Vongvichith B (2010b) Growth and morphological development of laboratory-reared larval and juvenile *Pangasianodon hypophthalmus*. *Ichthyol Res* 57:139–147
- Morioka S, Vongvichith B (2011) Growth and morphological development of laboratory-reared larval and juvenile *Hemibagrus filamentus* (Siluriformes: Bagridae). *Ichthyol Res* 58:245–254
- Morioka S, Cacot P, Moteki M, Thipvantong V, Philavong S, Pounvisouk L, Chantasone P, Thaphysy V (2012a) Ontogenetic development during changeover from an endogenous to exogenous nutritional source in Laotian cyprinid *Cirrhinus microlepis* larvae. *Fish Sci* 78:221–227
- Morioka S, Chantasone P, Phommachan P, Vongvichith B (2012b) Growth and morphological development of laboratory-reared larval and juvenile three-spot gourami *Trichogaster trichopterus*. *Ichthyol Res* 59:53–62
- Morioka S, Vongvichith B, Phommachan P, Chantasone P (2013a) Growth and morphological development of laboratory-reared larval and juvenile bighead catfish *Clarias macrocephalus* (Siluriformes: Clariidae). *Ichthyol Res* 60:16–25

- Morioka S, Vongvichith B, Phommachan P, Chantasone P (2013b) Growth and morphological development of laboratory-reared larval and juvenile giant gourami *Osphronemus goramy* (Perciformes: Osphronemidae). *Ichthyol Res* 60:209–217
- Nakamura S, Ichiki RT, Shimoda M, Morioka S (2016) Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. *Appl Entomol Zool* 51:161–166
- Phonvisay S (2013) An introduction to the Fisheries of Lao PDR. Mekong Development Series No. 6, Mekong River Commission, Phnom Penh
- Seo M (2018) Current situation of agricultural sectors in La PDR and economic integration of ASEAN. In: ASEAN Economic Community and Lao PDR (ed Suzuki M) pp 81–105. JICA Office in Lao PDR, Vientiane (in Japanese)
- Vongvichith B, Morioka S, Kawamura K, Mori A (2018) Factors influencing fish productivity in rice paddy aquaculture: a case study in Vientiane Province, central Laos. *JARQ* 52:359–366
- Welcomme RL, Vidthayanon C (2003) The impact of introductions and stocking of exotic species in the Mekong basin and policies for their control. Technical Paper No. 9, Mekong River Commission, Phnom Penh
- WFP (2013) Food and nutrition security atlas of Lao PDR. World Food Program, Rome. pp82
- WHO (2018) Nutrition dashboard for Lao People's Democratic Republic. https://ec.europa.eu/europeaid/sites/devco/files/laopdr_dashboard_2019.pdf
- World Bank (2017) Lao PDR. <https://data.worldbank.org/indicator/SP.DYN.LE00.IN?locations=LA>
- World Bank (2018) Lao PDR. <https://data.worldbank.org/country/lao-pdr>
- Worldmeter (2019) <https://www.worldometers.info/>

**Potential and Efficiency of Underused
Agricultural and Fishery
Resources in Laos**

President : Masaru IWANAGA

Editorial Secretary:

Masayoshi SAITO Director, Research Planning and Partnership Division
Seishi YAMASAKI Head, Information and Public Relations Office

Published by

Japan International Research Center for Agricultural Sciences

Tsukuba, Ibaraki, 305-8686 JAPAN

Tel. +81-29-838-6313

<https://www.jircas.go.jp/>

JIRCAS Working Report No.90

令和2年3月19日発行

発行者：国立研究開発法人 国際農林水産業研究センター
理事長 岩永 勝
〒305-8686 茨城県つくば市大わし 1-1
TEL 029(838)6313 (代表)

印刷：株式会社 イセブ

© by Japan International Research Center for Agricultural Sciences, 2020

© Reproduction and reprint of content herein are authorized only with prior written permission from JIRCAS

