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Factors Influencing Fish Productivity in Rice Paddy Aquaculture: A Case Study in Vientiane Province, Central Laos

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Abstract
To determine the factors influencing the productivity of fish in rice paddies (rice-cum-fish) in Laos, this study conducted aquaculture trials using an indigenous fish (*Anabas testudineus*) in Feuang and Nasaythong Districts, Vientiane Province, Laos. We evaluated stocking densities (*StockD*), initial body weights of fingerlings (*BWi*), stocking durations (*duration*), and feeding conditions (*fed*; feeding or non-feeding), which were considered the factors influencing fish productivity. The final model obtained by variable selection in multiple linear regression included *stockD* and *duration* with *fed* as a fixed effect. Based on a Bayesian framework, the 95% posterior probability intervals (PPIs) showed negative values for *stockD* (–32.75 to –23.36) and *duration* (–0.70 to –0.36), and positive values for *fed* (3.49 to 8.54). These results indicated that (1) stocking density was a primary factor, and higher stocking density reduced fish productivity, (2) longer stocking duration was less contributory to improving fish productivity compared with stocking density, and (3) feeding conditions improved fish productivity, which was suspected to support nutritional deficiency in the natural food items (e.g., aquatic insects, crustaceans) in paddies. Our findings suggested that fish productivity could be improved by lower stocking density, and that feeding had the potential to maintain high productivity with low stocking density and longer stocking duration.

Discipline: Fisheries
Additional key words: rice-cum-fish, low input, indigenous fish, *Anabas testudineus*

Introduction

Agriculture is the primary industry in Laos (Yamada 2014), and national food security relies largely on agricultural production. Although food self-sufficiency in Laos was achieved in 1999 (FAO 2011), Chaparro et al. (2014) reported that 26.7% of the population is still considered nutrient deficient. This is mainly due to the insufficient consumption of animal protein attributed to a limited supply of protein sources, resulting in an overdependence on rice in deprived areas (MAF 2013) such as semi-mountainous rural areas (Hasada & Yamada 2017).

In Laos, fish represent a major source of animal protein, with capture fisheries providing various kinds of fish for domestic consumption. The main fishing areas are the Mekong River, its tributaries, hydroelectric dams, and agriculture-related water masses (e.g., irrigation reservoirs and canals). However, natural fish resources in Laos are considered over-fished (FAO 2006), and the production of capture fisheries is insufficient to meet the ever-increasing demand fueled by population growth in recent decades (Worldometers 2017). This insufficiency in capture fishery production led to the need for aquaculture development (Phonvisay 2013). Fish aquaculture in Laos, particularly intensive cage culture, underwent remarkable development in the late 2000s and achieved annual production exceeding 50,000t (Phonvisay 2013), because of the successful commercial aquaculture of tilapia (*Oreochromis niloticus*). However,

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such commercial aquaculture activity is mainly operated in the suburbs of urban areas (e.g., Vientiane City). Moreover, most of the fish production is consumed in urban areas and thus does not contribute to the supply of protein in rural areas, where the supply of animal protein is limited.

Given the context described above, aquaculture development in rural areas is an important national issue in Laos (Cacaud & Latdavong 2008). However, farmers – the operators of aquaculture in rural areas – generally have economic difficulties and are unable to invest in constructing necessary facilities for intensive aquaculture systems. Hence, a more efficient use of existing agriculture-related water masses (e.g., rice paddies for aquaculture operation) has been considered. Although the potential for fish aquaculture in rice paddies (rice-cum-fish) has been examined in various areas/countries with various species in the Indochinese region, a majority of cases was examined using exotic/omnivorous species such as tilapia (*O. niloticus*), common carp (*Cyprinus carpio*), and silver carp (*Hypophthalmichthys molitrix*) (Chapman & Fernando 1994, Rothuis 1998, Berg 2002). In considering the conservation of biodiversity in rural areas that still have relatively well-conserved original biodiversity, and since Laos is a member country of the Convention on Biological Diversity (CBD), the utilization of indigenous species is recommended. Furthermore, carnivorous fishes have a generally preferred taste and are thus more valuable in the market, as well as contributory to increasing the supply of protein in rural areas.

In view of the background described above, we conducted four trials of rice-cum-fish in Vientiane Province, Central Laos during 2013-2016, using an indigenous/carnivorous fish species – the climbing perch (*Anabas testudineus*). This study focused on fish productivity and aimed to quantify the factors that are considered influential for fish productivity in rice-cum-fish systems (i.e., stocking densities, initial body weights of fingerlings, stocking durations).

Materials and methods

1. Study site and rice-cum-fish trials

Four rice-cum-fish trials were conducted during 2013-2016. The first two trials were conducted in Nameuang Village (18°32’44”N, 102°1’20”E), Feuang District, in 2013 and 2014. The last two trials were conducted in Napoh Village (18°16’8”N, 102°31’9”E), Nasaythong District in Vientiane province (Fig. 1). All four trials used the climbing perch (*Anabas testudineus*), which is a Laotian indigenous fish known locally as *‘Pa keng.*’ Paddy fields in Nameuang Village were irrigated, with water being supplied via a canal. Paddy fields in Napoh Village were non-irrigated, with the water supply being dependent on rainfall. The fingerlings used in the present study were artificially produced in the Living Aquatic Resources Research Center (LARReC), Vientiane, Laos. The first two trials were conducted in four separated paddies (Fig. 2 A); paddies Nos. 1, 2 and 4 were 100 m^2, and No. 3 was 160 m^2. Nos. 1 and 2 were operated without feeding, and Nos. 3 and 4 were operated with feeding (Fig. 2 A). The last two trials were conducted in six separated paddies, and the area of each paddy was 100 m^2. Nos. 1-3 were operated with feeding and Nos. 4-6 were operated without feeding (Fig. 2 B). For fish in paddies with feeding, commercial pellet feed (Centaco Co., Ltd., Thailand) containing 32% crude protein, 4% crude fat, and 6% crude fiber was provided at approximately 4% of total fish biomass per day. In each paddy, canals (1 m in width and 60-80 cm in depth) were made along two sides as fish refuges (Fig. 3). More detailed information [i.e., stocking density, size (length and weight) of fingerlings at stocking, and the average water temperature in each trial] are shown in Tables 1 and 2.

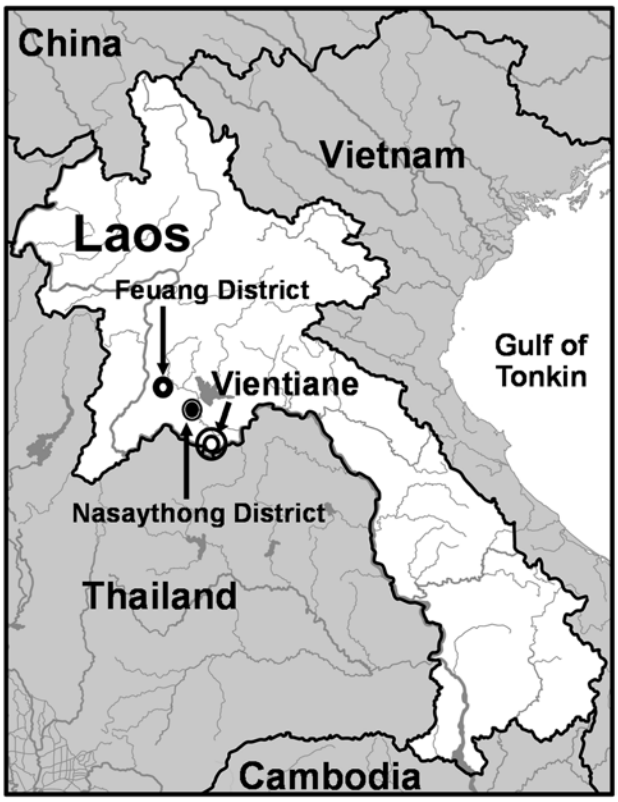

Fig. 1. Map of Laos showing two sites for rice-cum-fish trials in the present study

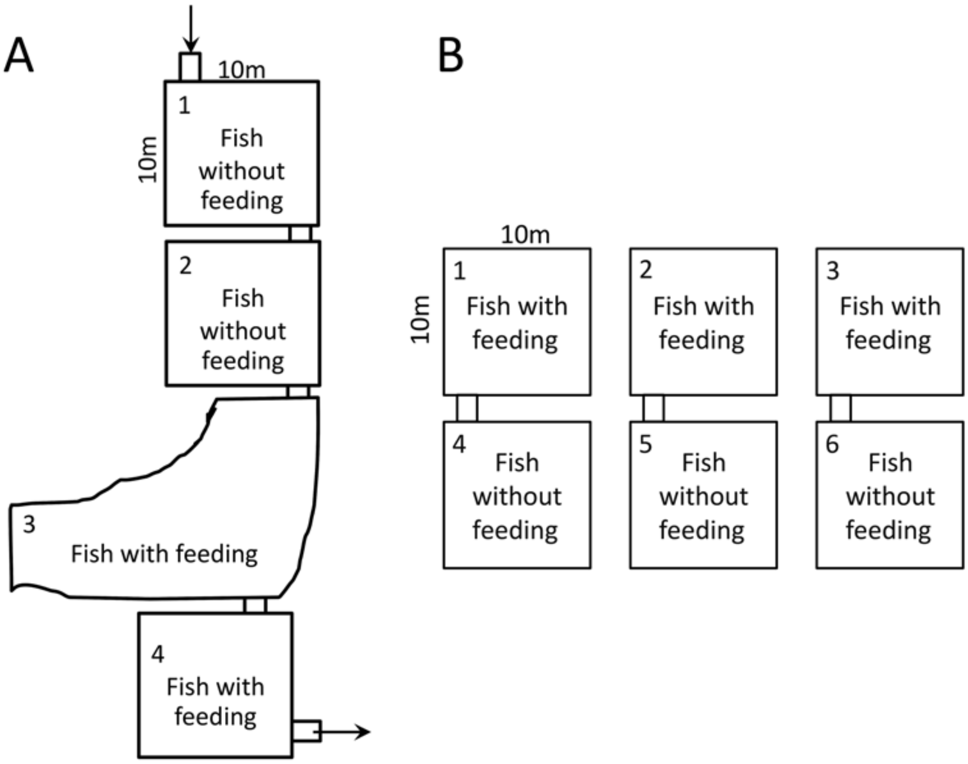

Fig. 2. Designs of rice paddies used for rice-cum-fish trials in the present study
(A: Nameuang Village, Feuang District, B: Napoh Village, Nasaythong District. Arrows in A indicate water inlet and outlet.)

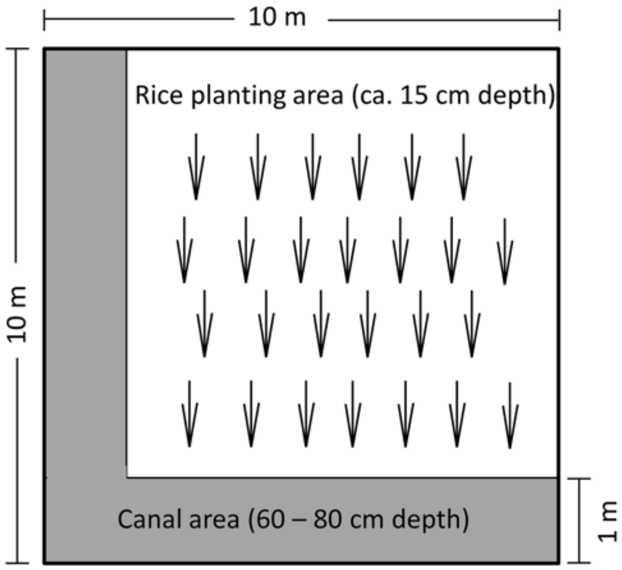

Fig. 3. Schematic description of rice paddies for rice-cum-fish trials in the present study

2. Statistical analyses

To evaluate the factors influencing fish productivity, we used a biomass gain index (BGI = total harvested biomass/total biomass at stocking) as an indicator. Each biomass was calculated based on the mean weight of fish at stocking/harvest and the number of fish at stocking/harvest. We also conducted assessment on fish productivity by developing regression models to estimate BGI from stocking densities (*stockD*), initial body weights (*BWi*) of fingerlings, stocking durations (*duration*), and feeding treatments (*fed*). First, to select and compare the explanatory variables *x* (factors), the models were established using all available combinations using multiple linear regression (MLR) analyses based on a generalized linear model (GLM) (see the regression equation in Table 3). Here, we selected an equation with the best predictive accuracy as the final model. Next, to assess the importance of selected variables (factors) in the final model, we estimated the optimal values of factors using the linear equation with a Bayesian framework.

Although MLR analysis is one of the most basic and commonly used prediction techniques, it requires prior assumptions, such as a linearity, multivariate normality, no-multicollinearity, and homoscedasticity. In GLM, the MLR model is estimated using maximum likelihood estimation, where we can determine the optimal values of parameters by maximizing the likelihood function. However, it is still dependent on the data set and site-specific results. The application of a Bayesian approach provides a more flexible strategy that expresses parameters as a probability distribution (Kubo 2009). In the Bayesian framework, the posterior probability distribution of parameters can be predicted using a Markov Chain Monte Carlo (MCMC) simulation to determine the importance of factors influencing fish productivity.

1) GLM and variable selection

MLR models were developed to estimate BGI using all available combinations of *stockD*, BW_i and *duration*. Here, *fed* was binary data (1: *fed* or 0: *fed-none*), so the regression equation included *fed* as a fixed effect:

$$y = a_i + b_j x_j + fed_i$$

where y is the response variables of *BGI*; a_i is the intercept based on ith fixed effect of *fed* (i = 0 [*fed-none*] and 1 [*fed*]); and b_j is the regression coefficients of the explanatory variables (x) for j (*stockD*, BW_i and *duration*). Akaike's information criterion (AIC) was used for explanatory variable selection to identify the optimal model (i.e., model with the lowest AIC value). The AIC value was calculated as a penalized log-likelihood with AIC = –2 × log-likelihood + 2 × p, where p is the number of parameters in the model. The predictive ability was also evaluated in the models based on the coefficient of determination (R^2) and the root mean squared error (RMSE) between observed and predicted values. The RMSE was calculated as:

Table 1. Basic information (years, village names, and durations of stocking) of rice-cum-fish trials in the present study

Year	Village	Date of stocking	Date of harvest	Duration of stocking (days)
2013	Nameuang	17th July	17th November	123
2014	Nameuang	19th July	16th November	120
2015	Napoh	7th August	26th October	80
2016	Napoh	5th August	23rd October	79

Table 2. Stocking density, size (length and weight) of fingerlings at stocking, and average water temperature in each trial

Year	Stocking density (fish number / m^2)	Average size at stocking (mean ± SD)		Average water temp. (mean ± SD, C°)
		Length* (mm)	Weight** (g)	
2013	0.3	36.8 ± 4.85	1.5 ± 0.58	28.2 ± 1.2
2014	1.2	62.3 ± 10.28	6.1 ± 1.66	27.2 ± 1.3
2015	2.0	34.1 ± 3.40	1.6 ± 0.44	29.0 ± 2.1
2016	2.0	71.1 ±12.1	5.7 ± 2.73	28.7 ± 1.9

*: standard length; **: wet body weight

Table 3. Linear equations and regression coefficients of regression analysis for estimating biomass gain index (BGI)

Model	Estimated equation (y)	Intercept (a)		Regression coefficients (b)			R^2	AIC	RMSE
		fed (0)	*fed* (1)	*stockD*	*BWi*	*duration*			
1	$a_i + b_1 stockD + fed_i$	36.51***	42.62*	–14.48***			0.85	125.48	4.56
2	$a_i + b_1 BWi + fed_i$	22.87***	28.98		–2.07**		0.45	151.78	8.81
3	$a_i + b_1 duration + fed_i$	–27.22*	–21.11*		*	0.42***	0.55	147.76	7.97
4	$a_i + b_1 stockD + b_2 BWi + fed_i$	36.34***	42.45*	–12.88***	–0.52		0.87	124.91	4.28
5	$a_i + b_1 stockD + b_2 duration + fed_i$	108.72***	114.83***	–27.95***		–0.53***	0.96	**99.94**	2.29
6	$a_i + b_1 BWi + b_2 duration + fed_i$	–10.82	–4.71		–1.26*	0.31**	0.68	143.14	6.75
7	$a_i + b_1 stockD + b_2 BWi + b3duration + fed_i$	106.43***	112.54***	–27.13***	–0.13	–0.51***	0.96	101.40	2.26

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i-y_i)^2}{n}}$$

where $\hat{y}_i$ and y_i denote the predicted and observed BGI values, respectively, and n is the number of samples (n = 20).

2) MLR model development using Bayesian approach

Based on a Bayesian framework, the final MLR model can be written in terms of a probabilistic model as follows:

$$\mu = a + b_j x_j$$

$$y \sim \mathrm{Normal}\ (\mu, \delta)$$

where response variable y (BGI) follows a normal distribution parametrized by mean μ_i, that is a linear function of x parametrized by α, β and by standard deviation σ. MCMC simulation was performed to estimate the posterior distribution. The length of the MCMC chain for this model was 3000 cycles after 1000 warm-up cycles, with samples saved every cycle. There were four chains. All data handling and modeling analyses were conducted using R statistical software ver. 3.4.1 (R Core Team 2017) and R Stan ver. 2.14.1 (Stan Development Team 2016).

Results

1. Relationship between BGI and environmental factors

Figure 4 shows boxplots of BGI based on *fed* and the relationships between BGI and *stockD*, BW_i and *duration*. Mean (±SD) values of BGI in fed-none (*fed* = 0) and

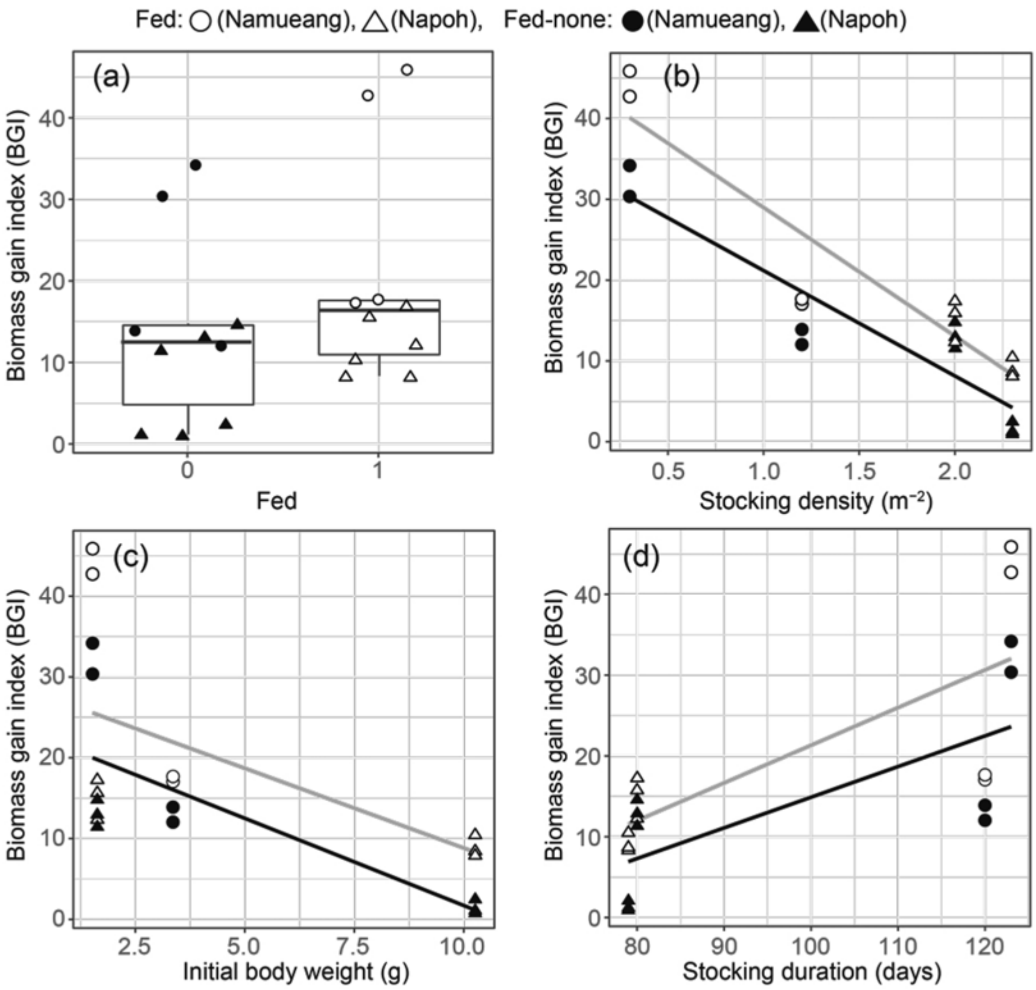

Fig. 4. Boxplots of the biomass gain index (BGI) for fed (a), and relationship between BGI and stocking density (b), initial body weight (c), and stocking duration (d)
[Symbols (open/solid circles and triangles) indicate each experimental site and feeding condition (fed or fed-none).]

fed (*fed* = 1) were 13.49 (±11.26) and 19.60 (±13.51), respectively. Although there was no significant difference ($P < 0.287$, *t*-test), BGI in the fed condition tended to be slightly higher than that of the fed-none condition. The following correlation coefficients (*r*) for BGI against the other parameters were: *stockD* in fed-none ($r = –0.918$, $P < 0.001$) and fed ($r = –0.933$, $P < 0.001$); *BWi* in fed-none ($r = –0.768$, $P < 0.01$) and fed ($r = –0.585$, $P = 0.07$); and *duration* in fed-none ($r = 0.731$, $P < 0.05$) and fed ($r = 0.749$, $P < 0.05$).

2. Linear equation from GLM

MLR models were developed by creating linear equations using variable selection based on the AIC. Table 3 summarizes the equations, regression coefficients, and accuracy between the observed and predicted values of BGI. Based on the AIC, model 5 was considered the best model with the smallest AIC value (99.94). The final model included two factors (*stockD* and *duration*) and *fed* as a fixed effect.

3. Predicted parameters with MCMC simulation

Table 4 shows the posterior means, standard deviation (SD), and 95% posterior probability intervals (PPIs) obtained via MCMC simulation. Figure 5 illustrates the boxplot of the 95% PPIs for each parameter. The R-hat values (data not shown), which are indicators of the convergence assessment, achieved 1.0 for all of the parameters, and the effective sample size was sufficient for MCMC sampling. The results of the posterior distribution computed by MCMC showed positive values for *fed* and negative values for *stockD* and *duration* (Table 4 and Fig. 5). The PPI for *stockD* (–32.75 to –23.36), *duration* (–0.70 to –0.36), and *fed* (3.49 to 8.54) did not include 0. This indicated that the largest negative posterior value of *stockD* was the primary factor for reducing fish productivity, while *fed* contributed to increasing fish productivity. Based on the smaller PPI

Table 4. Posterior means (Mean), standard deviations (SD), and quartiles (2.5, 50 and 97.5%) obtained from Markov Chain Monte Carlo (MCMC) simulation

Coefficient	Mean	SD	2.50%	50%	97.50%
a (intercept)	108.9	11.95	85.96	108.8	133.24
b_1 (stockD)	–27.97	2.36	–32.75	–27.98	–23.36
b_2 (duration)	–0.53	0.09	–0.70	–0.53	–0.36
b_3 (fed)	6.07	1.28	3.49	6.08	8.54
σ	2.8	0.55	1.96	2.72	4.09

The values from 2.5% to 97.5% indicate the 95% posterior probability intervals.

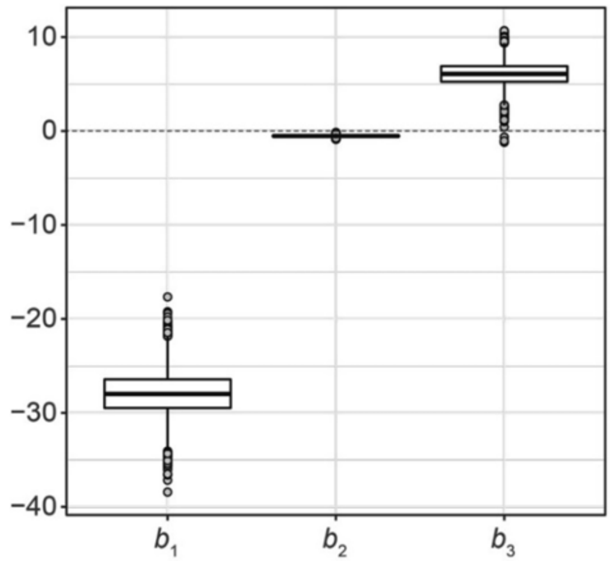

Fig. 5. Boxplot of the 95% credible intervals (CI) for parameters b_1, b_2 and b_3

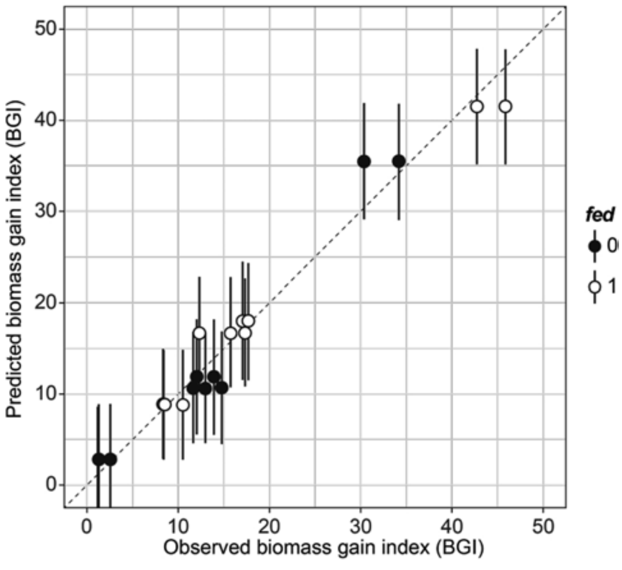

Fig. 6. Observed and predicted biomass gain index (BGI) values in feed treatments (0: *fed*, 1: *fed-none*) using Bayesian model
(Bars indicate the 95% credible interval.)

values of *duration*, it was considered to be less influential than *stockD* and *fed*, although it tended to negatively influence fish productivity.

Figure 6 shows the observed and predicted values of BGI in feeding treatments (0: *fed*, 1: *fed-none*) using the Bayesian model. The predicted values were the medians from the posterior distributions of BGI. In the validation plot, observed and predicted BGI values showed good correlation ($R^2 = 0.963$; y = 0.595), and the 95% confidence interval (CI) included the $y = x$ line. These results confirmed that fish productivity can be accurately predicted with using our model.

Discussion

In the present study, we tried to determine the influential factors [i.e., stocking densities, stocking durations, initial body weight of fingerling and feeding conditions (feeding or non-feeding)] for fish productivity in rice-cum-fish trials in Laos using multiple linear regression analyses with a Bayesian approach. Our results indicated that stocking density was a primary factor and that feeding also contributed to improving fish productivity; however, stocking duration was less influential compared with the other two factors. Initial body weight of fingerling was not selected in the final model. These findings suggest that a lower stocking density of fingerlings with feeding and a longer stocking duration are recommended for improving fish productivity in rice-cum-fish systems.

Most of the previous studies on rice-cum-fish used exotic/omnivorous fishes (e.g., tilapia *Oreochromis niloticus*, common carp *Cyprinus carpio*, silver carp *Hypophthalmichthys molitrix*) (Chapman & Fernando 1994, Rothuis 1998, Berg 2002), whereas this study used an indigenous/carnivorous fish (*Anabas testudineus*) in consideration of biodiversity conservation and the marketable value of the produced fish . Therefore, it is difficult to directly compare results, but the present study confirmed that rice-cum-fish systems are realizable, even with carnivorous fish at a low stocking density. In paddies with feeding, we used commercial pellet feed, but this is not economically feasible for many cases in deprived rural areas of Laos, although Rothuis et al. (1998) reported the importance of feeding to increase fish productivity in rice-cum-fish with which our results agreed (Tables 3 and 4, Figs. 3 and 4). Therefore, an applicable feeding regime with using costless/locally available materials is to be considered [e.g., insect larvae such as black soldier fly (*Hermetia illucens*)] (Nakamura et al. 2016). Meanwhile, in the 2013 trial, high productivity was observed even without feeding, illustrating that the natural prey items (e.g., aquatic insects, crustaceans) for carnivorous fish in paddies have the potential to provide high productivity when there is a low stocking density. Indeed, benthic insect larvae and small shrimps were often observed in the stomach of *A. testudineus* stocked in the paddies (S. Morioka unpublished data). This finding indicates that fish productivity using rice-cum-fish is promising, provided that there is a rich abundance of prey organisms in the rice paddy. The richness of aquatic organisms is generally supported by primary production that is probably influenced by soil fertility (Tezuka et al. 2004), suggesting that appropriate soil management leads to better fish productivity as well as improved rice productivity. Therefore, comparable examinations on soil fertility/rice productivity/fish productivity should be considered. In addition, applications of pesticide/herbicide are to be avoided to assure a sufficient presence of prey organisms (insects and weeds) in rice paddies, although these chemicals are not generally used for rice paddies in Laos. Other than the additional supply of fish to the farmers, Berg (2002) reported that fish stocking in paddies was efficient in improving rice productivity by pest reduction (Halwart 1998, Vromant et al. 1998), as was the fertilization effect of feeds through the digestion and excretion of fish.

Although the stocking duration was not very influential compared to stocking density and feeding treatment (Tables 3 and 4, Figs. 2 and 4), the stability of the amount of water, specifically the full-time ponding of a paddy, is an important condition for rice-cum-fish. The existing paddy areas throughout the country are estimated to total approximately 700,000 ha (MAF, 2014), but full-time ponding paddy areas are not known. However, the full-time ponding area in Nameuang Village was approximately 10 ha, out of a total paddy area of 81 ha (ca. 12%) (H. Ikeura unpublished data). Applying this proportion to the entire country, full-time ponding areas can be estimated to total approximately 84,000 ha. Using such areas for rice-cum-fish with 0.5-1.0 fish/m^2 and a harvested weight of 50 g/fish, approximately 21,000-42,000t of additional fish production could be expected annually.

As described above, we found several positive aspects of rice-cum-fish (i.e., additional fish production, rice productivity improvement, pest control) that do not entail large-scale artificial input/investment (e.g., pond construction) negative to the surrounding environment. However, there was an increase in methane emission from the bottom soil of paddies due to fish perturbation in stirring the bottom soil and consequently releasing soil-entrapped methane into the water (Frei & Becker 2005, Frei et al. 2007). This information suggests the need for countermeasures against methane emission by re-entrapping such methane.

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