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Disclaimers

Acknowledgments

Cover photo

Women and children learn about the nutrition benefits of fish in the Stung Treng province of Cambodia, where fish are a key food system component and where AquaFish Innovation Lab has conducted research on how nutrient-dense fish can contribute to household nutrition. Photo courtesy of AquaFish Innovation Lab.

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This research is still ongoing and will be included in a future Technical Report.
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Production of Nutrient-Rich Small Fish *Mola* and Freshwater Prawn Using Integrated Cage-Pond/Carp Polyculture for the Northwest Bangladesh

Production System Design and Best Management Alternatives/Experiment/13BMA03NC

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ABSTRACT

The research aims to increase aquaculture production and household earnings and nutrition of rural farmers by incorporating additional crops (mola fish and freshwater prawn in cages) into current carp pond farming practices in Northwest Bangaldesh. This impoverished region currently lacks crop diversification and could substantially benefit from incorporating both high-value (prawns) and highly nutritious (mola) aqua-foods into traditional carp farming. A small baseline survey assessed the socioeconomic and food consumption patterns of farmers in the region. Almost 45% of income (average of 13,145 Tk or $164/mo) is spent on food. Approximately 30.26% of farmers worried about having enough food, 24% were not able to eat the kinds of foods preferred because of a lack of resources, 76% household members have to eat a limited variety of foods due to lack of resources and 83% of household members have to eat fewer meals in a day because there was not enough food. Households would benefit with additional production of crops, particularly high-value prawns and enhanced production of nutrient rich mola. To this end, an on-farm study was conducted in the Nandigram Upazilla of Bogra District, the Northwest part of Bangladesh to asses the production of nutrient rich small fish mola (*Amblypharyngodon mola*) stocked at different densities with the pond culture of Indian major carps rohu (*Labeo rohita*), catla (*Catla catla)*, mrigal (*Chirninus cirrhosis)* and cage culture of prawns (*Macrobrachium rosenbergii)*. The study evaluated the feasibility of cage-prawn culture, a novel method of culturing prawn. Carp ponds in North Bangladesh are deeper than the Southwest region and often result in significant mortalities. The experiment had three treatments: T_1, T_2 and T_3, where mola were stocked 2.5 m^2, 5.0 m^2, and 10.0 m^2, respectively with five replications for each. The stocking densities of the Indian major carps and prawn were the same in all treatments as each species of the Indian major carps was 0.33 m^{-2} and prawn 15 m^{-2}. Fifteen farmers' ponds with an average of 909 m^2 and an average depth of 1.5 m each were selected randomly for this research. Fertilizers were applied at a rate of 28 kg ha^{-1} and 7kg ha^{-1}, respectively in each pond bi-weekly throughout the study period. Commercial feed (containing 30% crude protein) was used only for prawns at 5% body weight during the study period. Water quality parameters did not vary significantly ($p>0.05$) among the treatments, except transparency and chlorophyll *a*. The individual harvesting weight and individual weight gain of mola were significantly ($p<0.05)$ higher in T_1 treatment compared to T_2 and T_3 treatments, respectively but the gross and net yields of mola were significantly ($p<0.05)$ higher in T_3 than T_2 and T_1, respectively. A significantly ($p<0.05)$ higher individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of rohu were also obtained in T_1 treatment than T_2 and T_3 treatments, respectively. However, individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of *Catla*, mrigal and prawn did not differ significantly ($p>0.05$) among treatments. The combined gross yield of mola, prawn and the Indian major carps was significantly ($p<0.05)$ higher in T_1 and T_2 than T_3 treatment but the net yield of

the same species was significantly higher ($p<0.05$) in T_1 than T_2 and T_3, respectively. It was found that the stocking density of mola had significant ($p<0.05$) effect on growth and production of the Indian major carps and prawn. The study revealed that mola, Indian major carps and prawn with a stocking density at 2.5 m^{-2}, 0.33 m^{-2} and 15 m^{-2}, respectively was found to provide an higher net production in the mola, prawn integrated cage-pond/carp polyculture system.

INTRODUCTION

Fish are the largest source of animal protein for Bangladeshis. Among fish, small-indigenous species (SIS) are particularly high in minerals or micronutrients. Mola *(Amblypharyngodon mola)* is a SIS is found in the rivers, canals, ponds and ditches, etc., of Bangladesh (Rahman 1989). It is recommended as a potential species in polyculture with carps (Akhteruzzaman et al. 1997, Kohinoor et al. 1998). It is rich in micronutrients and vitamin-content (Thilsted et al. 1997) and can meet the need of family nutrition if grown in the household ponds. Malnutrition of children is a major health problem in Bangladesh. Up to 38% of all pre-school children have vitamin A deficiency, with up to 55% exhibiting signs of iron-deficient anemia (Micronutrient Initiative/UNICEF 2004, West 2002). These effects may be alleviated through the consumption of SIS especially mola which is rich in vitamin A (Ahmed 1981). Four medium size mola, if eaten daily may provide more than 1500 IU of vitamin A, sufficient to save a child from night blindness caused by vitamin A deficiency.

The freshwater prawn (*Macrobrachium rosenbergii)* is a high-value export commodity traditionally cultured in the modified rice fields (ghers) of the Southern Bangladesh (Wahab et al. 2012). Freshwater prawn farming is a major aquaculture industry in many Asian countries because with over 98% of the global production coming from the region (Asaduzzaman et al. 2009a, b). The global production of all freshwater prawns increased from 82,089 to 458,564 tons between 1998 and 2007 (FAO 2009). It is a very popular species in Bangladesh for its attractive look, good taste and growth (Uddin 2007). The increasing demand and steadily rising price in international markets, particularly in the USA, Europe and Japan, and high price in local markets has stimulated development of freshwater prawn farming in Bangladesh (Asaduzzaman et al. 2009a, b; Ahmed et al. 2007), particularly in the southwest coastal areas (DoF 2007). It is contributing to 30% of the total shrimp export, valued at $135 million (Khondoker 2009). Therefore, it is currently one of the most important sectors of the national economy of Bangladesh, and its development has attracted considerable attention during the last two decades because of its high export potential (Ahmed et al. 2010a).

Polyculture is one of the most important and efficient methods for increasing fish production. It encompasses the stocking of different species with complementary feeding habits for better utilization of natural food niches within ponds, often leading to increased fish production (Wahab et al. *2001*). Polyculture of carps in ponds is widely practiced in Bangladesh (DoF 2002). There there is little information on integrated cage-pond/carp polyculture practice with mola and prawns in Bangladesh. Introduction of prawn to the Northern regions of Bangladesh has not been successful, as many carp ponds are too deep for prawn culture. This investigation will employ a novel method of cage prawn culture in Bangladesh. This study will determine the feasibility of cage-prawn culture, in the deeper ponds of North Bangladesh, with both mola and Indian Major Carps. Culture of mola and Indian major carps with high-value prawns will promote greater dietary nutrition with higher earnings among the rural households.

- Conduct a small survey to determine the socioeconomic and food consumption pattern of farmers in the Bogra region;
- To determine the stocking densities of mola and prawns for integrated cage/pond aquaculture with Indian carps; and
- To identify the performances of this integrated system in terms of yields for all species, and effects on water quality.

MATERIALS AND METHODS

Study 1 — Socioeconomic and food consumption pattern of farmers in the Bogra region of Northwest Bangladesh. A survey was conducted using field survey method where the primary data were collected from the respondents. The study area was confined to Nandigram upazila in Bogra district, where the cultivation of indigenous fish is concentrated in family ponds and where AquaFish Innovation Lab on-farm trials are being done for Study 2. Purposive sampling technique was used for selecting the sample. Total sample size of the study was 15. All of the samples were collected from fish farmers. Simple statistical tools were applied for the data analysis in this survey report.

Study 2 — Evaluation of mola stocking density in prawn-cage carp polyculture, and its effect on production yield, market return, and environmental water quality

Experimental design
On-farm research was conducted using a completely randomized block design with three treatments, namely T_1, T_2, and T_3, according to stocking density of mola 2.5 m^2, 5.0 m^2, and 10.0 m^2, respectively, with five replications for each to asses the production of nutrient rich small fish mola (*A. mola)* with the pond culture of Indian major carps rohu (*Labeo rohita*), Catla (*Catla catla*), mrigal (*Chirninus cirrhosis*) and cage culture of prawns (*Macrobrachium rosenbergii*). The stocking densities of the Indian major carps and prawn were same in all treatments as each species of the Indian major carps was 0.33 m^{-2} and prawn 15 m^{-2}.

Experimental site and pond preparation
The experiment was conducted at the Nandigram Upazila of Bogra District, the northwest part of Bangladesh. Fifteen farmer ponds with an average of 909 m^2 and an average depth of 1.5 m each were selected randomly for this research. All aquatic weeds were cleaned manually and all unwanted fishes were eradicated by repeated netting. Lime ($CaCO_3$) was applied at all ponds at the rate of 1 kg per decimal according to the existing farming system before stocking. Fertilizers like Urea and TSP were applied at a rate of 28 kg ha^{-1} and 7kg ha^{-1}, respectively in each pond. Ponds were left for seven days to allow plankton development in water column and subsequently stocked with prawn, mola and Indian major carps.

Stocking and post stocking management
Juveniles of freshwater prawn and fry of mola and the Indian major carps were procured from a nearby commercial hatchery, which was stocked in all ponds according to the experimental design. The prawn juveniles were stocked separately in an experimental 15 m^3 cages (L× W ×H: 5m × 3m× 1m; nylon netting) in all ponds following the experimental design. Commercial feed containing (30% crude protein) was used only for prawns at 5% body weight during the study period. After stocking fertilizers such as urea and TSP were applied at the same rate before stocking in each pond throughout the experimental period.

Determination of water quality parameters
The water quality parameters were recorded at biweekly interval throughout the experimental period. Water quality measurement and sample collection were made between 9:00 and 10:00 h on each sampling day. The physico-chemical parameters such as temperature (°C), dissolved oxygen (surface and bottom, mgL^{-1}), pH, transparency (secchi-disk, cm), ammonia-nitrogen (mgL^{-1}), nitrate-nitrogen (mgL^{-1}), phosphate-phosphorous (mgL^{-1}) and chlorophyll a (mgL^{-1}) were measured using HQ40d Portable Multi Parameter meter during the study period.

Feeding and growth sampling of prawn and fishes
Individual weights of minimum 10% of initially stocked prawn in numbers were recorded monthly to estimate the biomass and adjust the feeding rate. A seine net (1.0 cm mesh size) was used to observe the growth and health condition of prawn and other fishes and even to make some rough assessment of their growth trends. Weight of prawn and fishes in each sampling was measured by using a portable balance (OHAUS, model No.CT-1200-S). General pond conditions and health condition of prawn and fishes were also monitored frequently throughout the culture period. Care was taken to handle the sampled prawn and fishes due to their susceptibility to handling stress.

Partial harvesting of mola
Partial harvesting of larger mola by using the same seine net (1.0 cm mesh size) in all treatments was started on day 75 and continued with 15-day intervals until final harvest, because mola bred during 60 to 70 days of stocking in all ponds. The weights of partially harvested mola were recorded.

Harvesting of prawn and fishes
At the end of the experiment (after 110 days culture period), all cages were lifted up from each of the ponds manually. Partial harvesting of fishes was done by repeated netting with a fine meshed net towing over the pond. Afterward, water was completely drained out from each pond separately using shallow pump. The rest of the fishes were then harvested from each pond separately. All prawn and fishes were then counted, measured and weighted individually for each pond to assess the survival rate and production. mola of each pond were batch weighed. Survival rate, specific growth rate (SGR) and individual weight of this species were not considered for calculation as mola were self recruiting and bred in all ponds during culture period. The following equations were used to determine the growth parameters.

Weight gain (g) = Mean final weight − Mean initial weight

SGR (% bw d^{-1}) = [{Ln (final weight) – Ln (initial weight)}/Culture period in days] x 100.

Survival (%) = (No of fish harvested / No fish stocked) x 100

Gross production = No of fish harvested x Final weight of fish

Net production = No of fish harvest x Weight gain of fish

Statistical analysis
One-way analysis of variance (ANOVA) was performed for comparing growth and production of prawn and tilapias among the treatments. Survival and percent data were analyzed using arcsine-transformed data but percent values were reported. The assumptions of normal distribution and homogeneity of variances were checked before analysis. All statistical tests were carried out at a 5% level of significance using SPSS (Statistical Package for Social Science) version 16.0.

Study 1 — Socioeconomic and food consumption pattern of farmers in the Bogra region of Northwest Bangladesh. In the study area, the average age of farmers engaged in the fish farming activities is 40 years. All of the fish farmers have on average five household members. Among the farmers, 46.67% possess their own land, 33.33% have a small business, 6.67% are students and 13.33% are engaged with other services.

Around 26.67% of fish farmers have signature level qualification, 13.33% have PSC level (grade 5) and 13.33% SSC level (grade 10) of educational qualification, 33.33% have JSC level (grade 12) qualification and 13.33% are illiterate.

Average monthly income of farmers is 13,145 Tk, with 43.481% of income spent on food, 15.943% for repayment of loan to NGOs and banks, 10.246% for education, 11.295% for transportation, 3.442% for garment requirements, 3.899% for medicine, 8.296% for fuel (cooking + lights), and 0.850% for nonfood household requirements and 1.050% spend for others activities in their life (Table 1).

It was found that in the prior four weeks, 30.26% of farmers worried about having enough food, 23.81% were not able to eat the kinds of foods preferred because of a lack of resources, 76.19% household members have to eat a limited variety of foods due to lack of resources, 67.29% have to eat some foods that they really did not want to eat because of a lack of resources in obtaining other types of food, 75.97% of household members felt they had to eat a smaller meal because there was not enough food, and 82.87% of household members have to eat fewer meals in a day because there was not enough food. Also, 17.35% of household members went to sleep feeling hungry because there was not enough food

Table 1. Expenditures Cost Of the household members.
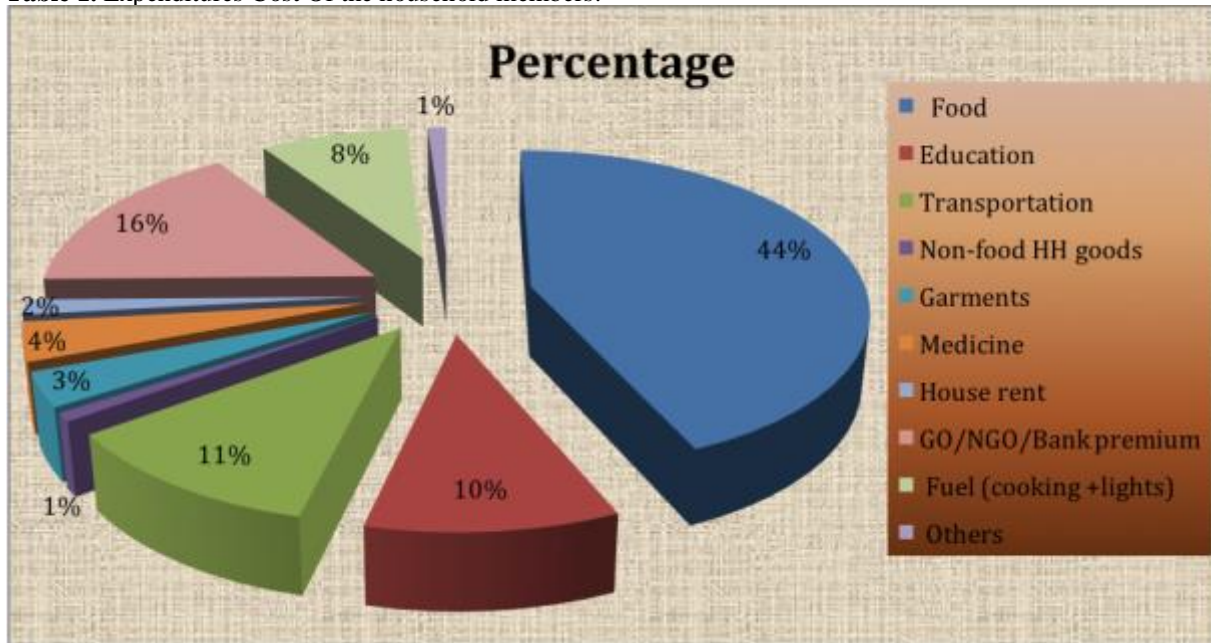
Expenditures Item	Cost Tk	Percentage
Food	87000	43.481
Education	20500	10.246
Transportation	22600	11.295
Non-food HH goods	1700	0.850
Garments	6886	3.442
Medicine	7801	3.899
House rent	3000	1.499
GO/NGO/Bank premium	31900	15.943
Fuel (cooking +lights)	16600	8.296
Others	2100	1.050
Total	200087	100.00

Over the prior six months, 76.67% of farmers produced some kind of vegetable. Around 25.67% of farmers consumed 25% of vegetables produced, 27.67% around 50% produced, 15.67 % farmers consumed 75%, and 17.67% farmers consumed all of the vegetables produced. In the past six months, farmers produced 67.67% vegetables on pond dyke.

In the prior six months, 48% of farmers that produced vegetables on their own land provided them to their children < 5 years of age, 17.67% farmers purchased vegetable from the market for their children and 34.23% farmers did not provide vegetables in the selected study areas.

In the prior six months, 42.5% of farmers fruit consumption came from fruits produced on their own land, 21.33% purchased fruits from the market and 11.33% purchased fruits from other sources. Around 15.33% of farmer households consumed a quarter, 37% a half, 22.33% three quarters, and 14.33% consumed all of the fruits produced. Seventy eight percent farmers could provide fruits for their children < 5 years of age and 22.00% farmers did not provide fruits to their children.

Around 73.67% of farmers cultivated fish in their own pond and 26.33% cultivated fish in rented ponds. Around 40.33% of farmers cultured carp fishes and 27.67% cultured small fish. Around 65% of farmers consumed fish each week with 46.33% of farmers consuming carp fish and 53.67% consuming small fish.

The baseline survey indicates there is wide range of scope for the development of mola fish and freshwater prawn.

Study 2 — Evaluation of mola stocking density in prawn-cage carp polyculture, and its effect on production yield, market return, and environmental water quality

Water quality
Water-quality parameters were analyzed in this experiment to observe any appreciable variation among the treatments and mean values and outcomes of one-way ANOVA in the different treatments are presented in Table 2. Water quality parameters did not vary significantly ($p>0.05$) among the treatments, except transparency and chlorophyll *a*. A significant ($p<0.05$) variation on different sampling dates (temporal effects) was also observed on the water quality parameters. No specific trends were observed for temperature, pH, dissolved oxygen, NH_3-N, NO_2-N, NO_3-N and PO_4-P but an increasing trend of transparency and a decreasing trend of chlorophyll *a* were observed in all treatments over the experimental period (Figure 1). Temperature varied from 25.10 to 30.9°C in all the treatments during the study period. The highest temperature was recorded (30.9 °C) in the 3rd week of August (August – December study period) in T_1 while the lowest temperature was observed the same week in T_3. DO

content was found higher (8.44 mgL-1) in the first week of September in T_2 and lowest was recorded (5.26 mgL-1) in the 3rd week of September in T_1. The value of pH was found higher in T_3 (8.44) and lowest was in T_2 (6.81). The recorded value of transparency was found higher in T_3 than T_2 and T_1. The highest value of transparency (41.20 cm) was found at the first week of December in T_3 and the lowest was recorded before starting of the experiment in the same treatment. A more or less increasing trend of water transparency was observed in all treatments during the experimental period (Fig. 1). The total alkalinity content was observed higher in T_3 and lowest was in T_1. The highest content of NH_3-N was found in T_1 and lowest was in T_2. The level NO_3-N was recorded maximum in T_3 and minimum was in T_1. During the study period, the extreme level of NO_2-N was found in T_1 and lowest level was in T_2. The value of PO_4-P was observed higher in T_3 and lower was in T_2. The chlorophyll a concentration was higher in T_1 than T_2 and T_3. The highest value of chlorophyll a (204.15 µg L^{-1}) was found in T_1 before commencing the experiment and the lowest (74.0 µg L^{-1}) was recorded in T_3 at the end of the experiment. A decreasing trend of chlorophyll-content was found in all treatments during the experimental period (Figure 1).

Table 2. Mean values of water quality parameter (± SD) in different treatments.

Variables	Treatments			Significance
	T_1	T_2	T_3	
Temperature (ºC)	28.73 ± 2.05	28.57 ± 2.14	28.36 ± 2.49	NS
Transparency (cm)	33.40±3.50^b	35.08±3.45^{ab}	37.33±5.4^a	*
pH range	7.60 ± 0.48	7.37 ± 0.63	7.49 ± 0.69	NS
Dissolved oxygen (mg L^{-1})	6.53 ± 1.01	6.41 ± 1.26	6.53 ± 1.06	NS
Total Alkalinity	74.80 ± 14.74	70.73 ± 17.17	78.30 ± 16.31	NS
Total NH$_3$-N (mg L^{-1})	0.19 ± 0.06	0.16 ± 0.04	0.19 ± 0.05	NS
NO$_3$-N (mgL^{-1})	0.07 ± 0.06	0.06 ± 0.05	0.06 ± 0.07	NS
NO$_2$-N (mg L^{-1})	0.041 ± 0.04	0.026 ± 0.04	0.026 ± 0.02	NS
PO$_4$-P (mgL^{-1})	0.17 ± 0.04	0.15 ± 0.07	0.23 ± 0.28	NS
Chlorophyll a (µg L^{-1})	141.88 ±45.55^a	128.83 ±36.12^{ab}	117.56 ±38.64^b	*

NS, Values are not significantly different ($p> 0.05$)
*Values with different superscript letters in the same row indicate a significant difference at 5% significance level based on one-way ANOVA followed by Tukey's test.

The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. Water quality in pond aquaculture are strongly dependent on pond management including combinations of cultured species, stocking densities, and the quantity and quality of the nutrient inputs (Milestein 1993, Diana et al. 1997). In the present study, all of the water quality parameters were within the acceptable range for aquaculture. The observed value of water temperature, dissolved oxygen concentration and pH were within the suitable range for freshwater prawn and tropical fish culture (Boyd and Zimmermann 2000; New 2002; Kunda et al. 2008; Asaduzzaman et al. 2008; Rahman et al. 2010b). Water transparencies, indicating sestonic food abundance, were found to fluctuate from 27–41cm in different treatments, exceeded the upper limit of the recommended range (15–40 cm) suggested by Boyd (1992), but were comparable to the values of Bangladeshi ponds (Wahab et al. 1995, Kohinoor 2000, Uddin 2002, Huq et al. 2004, Asaduzzaman et al. 2009a). Wahab et al. (1995) suggested that the transparency of productive water should be 40 cm or less. The transparency was lower at the beginning of the experiment, but it increased gradually over time in all treatments. These might be attributed to the higher grazing pressure of the cultured herbivorous organisms with the increasing biomass over time, which might be also evidence of the decreasing trends of chlorophyll a. It is reported that the transparency of water was affected by many factors such as silt, microscopic organisms, suspended organic matter,

season of the year, latitude and intensity of light, application of manure, grazing pressure of fishes or prawns and rainfall (Boyd 1990).

The observed mean values of total alkalinity with the ranges in all treatments were approximately identical to those suggested by Boyd (1990). He showed that alkalinity below 30 mg L^{-1} as $CaCO_3$ limits primary production in well-fertilized ponds, while in unfertilized ponds alkalinity below 120 mg L^{-1} can reduce primary production. It is also reported that 40- 100 mg L^{-1} $CaCO_3$ are optimum for growth of *M. rosenbegii* (New and Singholka 1985), while 20 to 200 mg L^{-1} is optimal (Vasquez et al. 1989). However, the mean values with the ranges of total alkalinity are also comparable to the findings of other authors in this region in prawn polyculture system (Kohinoor 2000, Kunda 2008, Kunda et al. 2008, Asaduzzaman et al. 2010; Rahman et al. 2010a). In the present study, the mean concentrations of all nitrogenous compounds with the ranges were approximately identical suggested by Boyd (1998) in pond aquaculture, and were comparable to the values found by some researchers in prawn polyculture systems in Bangladesh (Asaduzzaman et al. 2008, 2009a, b, 2010; Rahman et al. 2010b). The recorded values of PO_4-P concentrations in different treatments were more or less similar to the Bangladeshi ponds (Wahab et al. 1995, Alim 2005, Khan 2009). Chlorophyll *a* in water body is widely used as an indicator of productivity. The mean values of chlorophyll *a* in all treatments were comparable to the findings of some authors in this region and elsewhere (Kohinoor 2000; Kadir et al. 2006; Milstein et al. 2006; Kunda et al. 2008; Asaduzzaman et al. 2008, 2009a, b, 2010; Rahman et al. 2010b). The observed decreasing trends of chlorophyll *a* in all treatments might be due to higher grazing pressure on phytoplankton by the cultured herbivorus fish over time, which might explain increasing trends of transparency in all treatments over the experimental period. The significantly lower chlorophyll *a* in T_3 followed by T_2 and T_1 treatments might be due to higher grazing pressure associated with greater density of mola. Khatrai (1984) reported a positive relationship between phytoplankton and chlorophyll *a*, whereas Rahman et al. (2010b) found a negative co-relation between Chlorophyll *a* and transparency in their all-male freshwater prawn-finfish polyculture research.

Growth and yield parameters of mola, rohu, Catla, mrigal and prawn

Growth and yield parameters of mola, rui, *Catla*, mrigal and freshwater prawn are shown in Table 3. The individual harvesting weight and individual weight gain of mola were significantly ($p<0.05)$ higher in T_1 treatment compared to T_2 and T_3 treatments, respectively but the gross and net yields of mola were significantly ($p<0.05)$ higher in T_3 than T_2 and T_1, respectively. A significantly ($p<0.05)$ higher individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of Rohu were also obtained in T_1 treatment than T_2 and T_3 treatments, respectively. However, Individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of Catla, Mrigal and prawn did not differ significantly among treatments ($p>0.05)$. The combined gross yield of mola, prawn and the Indian major carps was significantly ($p<0.05)$ higher in T_1 and T_2 than T_3 treatment but the net yield of the same species was significantly higher ($p<0.05)$ in T_1 than T_2 and T_3, respectively.

Growth and yield of fish depends on the stocking size, stocking density, species combination, inclusion of shelter, management practices, and other factors. The higher individual harvesting weight and individual weight gain of mola in T_1 compared to T_2 and T_3 treatments might be due to lower stocking density of mola resulting in less intra-species competition for natural food and space. Mola is a phytoplankton feeder (Mamun et al. 2004) and density affects the amounts of natural food available per fish, and the level of supplementary feeding required (Moore 1986, Hepher 1988). It was noted that mola was generally a surface feeder and the food of the adult fish consisted of unicellular and filamentous algae, protozoan and rotifers (Mustafa 1991). However, the individual size of mola at harvest (3.80-5.3 g) in the present study was encouragingly higher than the size reported in other experiments in earthen ponds (Chowdhury et al. 2000 and Das 2002, Kadir et al. 2007, Kunda et al. 2008). The higher gross and net yields of mola in T_3 than T_2 and T_1, respectively, is likely due to the higher number of fish stocked rather than harvesting weight, which was lowest among the groups.

The significantly ($p<0.05$) higher individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of Rohu in T_1 treatment compared to T_2 and T_3 treatments might be due to less inter-species completion between Rohu and mola as both are phytoplankton feeder. It has been reported that Rohu feed on zooplankton in fry stage but juveniles and adults show a strong positive selection for phytoplankton, vegetable debris and aquatic plants and a negative selection for all zooplanktonic organisms (Chondar 1999). The individual harvesting weight, individual weight gain, specific growth rate, survival (%), gross and net yields of Catla, Mrigal and prawn did not differ among treatments. This might be due to their preferences for different food niches resulting in less competition. Catla is the fastest growing fish and a suitable species for polyculture (Rahman et al 2010). It is surface feeder and planktophagus, and the main item of its diet is zooplankton (Jhingran 1991). Khan and Siddique (1973) also stated that the food of the adult C. catla was chiefly composed of zooplankton and some phytoplankton. They also reported that the main food of adult C. cirrhosus was decayed organic matter, sand and mud supplemented by plankton. It is known that prawns feed on benthic organisms (Tidewell et al. 1995), detritus (Valenti 1993), and feces of other fishes (Zimmermann and New 2000). Prawn also preferred forage on animals like trichopterans, chironomids, oligochaetes, nematodes, gastropods and zooplankton. In addition, commercial feed was given only for prawn in cages; therefore, there is little opportunity to compete for feed with other species. Consequently, the production of prawn was same in all treatments. The combined net yield of mola, prawn and the Indian major carps was significantly ($p<0.05$) higher in T_1 than T_2 and T_3, respectively which likely reflects the higher production of rohu as well contribution to net yield. However, the combined production was higher than Rahman et al (2010) and lower than Haque (2014). This might be due to difference on culture techniques, stocking density, stocking size, location, duration and so on.

Table 3. Growth and yield parameters (mean ± SD) of mola, *Catla*, rohu, mrigal and prawn in different treatments during a 110-day culture period.

Variables	Treatments			Significance
	T_1	T_2	T_3	
Mola (*A. mola*)				
Individual stocking weight (g)	1.5±0.07	1.4 ± 0.11	1.4 ± 0.01	NS
Individual harvesting weight (g)	5.16±0.11[a]	4.70±0.26[b]	3.98±0.18[c]	*
Gross yield (kg ha^{-1} 110 d^{-1})	75.02±3.61[c]	103.86±11.89[b]	123.23±8.33[a]	*
Net yield (kg ha^{-1} 110 d^{-1})	105.86±5.96[c]	149.88±13.55[b]	190.16±7.91[a]	*
Catla (*C. catla*)				
Individual stocking weight (g)	42.20±2.95	43.80±1.79	43.00±1.87	NS
Individual harvesting weight (g)	284.88±6.09	281.70±10.57	281.82±4.97	NS
Individual weight gain (g)	242.68±7.65	237.90±10.90	238.82±6.37	NS
Specific growth rate (% bw d^{-1})	1.59±0.08	1.54±0.06	1.56±0.06	NS
Survival (%)	80.49±10.76	79.20±5.34	73.56±3.40	NS
Gross yield (kg ha^{-1} 110 d^{-1})	755.39±98.83	734.89±58.60	683.65±36.39	NS
Net yield (kg ha^{-1} 110 d^{-1})	642.70±76.59	620.76±54.59	579.28±31.60	NS
Rohu (*L. rohita*)				
Individual stocking weight (g)	43.60±1.82	45.00±1.58	45.00±1.87	NS
Individual harvesting weight (g)	379.60±8.56[a]	342.20±11.26[b]	308.00±2.92[c]	*
Individual weight gain (g)	336.00±8.22[a]	297.20±10.47[b]	263.00±3.08[c]	*
Specific growth rate (% bw d^{-1})	1.86±0.04a	1.72±0.03b	1.61±0.04c	*
Survival (%)	82.26±2.36[a]	76.25±1.48[b]	71.06±2.51[c]	*
Gross yield (kg ha^{-1} 110 d^{-1})	1029.80±51.92[a]	862.03±42.89[b]	724.32±25.70[c]	*
Net yield (kg ha^{-1} 110 d^{-1})	911.55±46.93[a]	748.71±39.19[b]	618.55±24.24[c]	*

Mrigal (*C. cirrhosus*)

Individual stocking weight (g)	32.34±1.96	33.84±2.17	33.80±2.68	NS
Individual harvesting weight (g)	281.14±14.88	291.46±10.41	283.58±10.25	NS
Individual weight gain (g)	248.80±16.25	257.62±11.89	249.78±10.90	NS
Specific growth rate (% bw d $^{-1}$)	1.85±0.11	1.85±0.10	1.82±0.09	NS
Survival (%)	77.63±4.67	74.49±5.48	70.82±2.79	NS
Gross yield (kg ha^{-1} 110 d^{-1})	741.91±62.10	738.09±59.78	681.95±26.90	NS
Net yield (kg ha^{-1} 110 d^{-1})	656.59±60.44	652.28±54.15	600.56±24.38	NS
Freshwater prawn (*M. rosenbergii*)				
Individual stocking weight (g)	6.50±0.31	6.16±0.38	6.08±0.66	NS
Individual harvesting weight (g)	13.70±0.29	13.76±0.20	13.52±0.15	NS
Individual weight gain (g)	7.20±0.55	7.60±0.56	7.44±0.70	NS
Specific growth rate (% bw d $^{-1}$)	0.08±0.10	0.17±0.11	0.17±0.17	NS
Survival (%)	65.73±2.03	63.33±3.92	63.47±3.03	NS
Gross yield (kg ha^{-1} 110 d^{-1})	15.69±1.24	14.54±1.99	13.46±0.32	NS
Net yield (kg ha^{-1} 110 d^{-1})	8.25±0.86	8.07±1.59	7.41±0.73	NS
Combined				
Gross yield (kg ha^{-1} 110 d^{-1})	2648.70 ±112.60[a]	2499.40±119.32[a]	2293.50 ±57.80[b]	*
Net yield (kg ha^{-1} 110 d^{-1})	2294.10±84.51[a]	2133.70 ±109.99[b]	1929.00 ±48.63[c]	*
Contribution to yield				
Mola net yield (kg ha^{-1} 110 d^{-1})	75.03±3.61[c]	103.86±11.90[b]	123.23±8.33[a]	*
Catla net yield (kg ha^{-1} 110 d^{-1})	642.70±76.59	620.76±54.59	579.28±31.60	NS
Rohu net yield (kg ha^{-1} 110 d^{-1})	911.55±46.93[a]	748.71±39.19[b]	618.55±24.24[c]	*
Mrigal net yield (kg ha^{-1} 110 d^{-1})	656.59±60.44	652.28±54.15	600.56±24.38	NS
Prawn net yield (kg ha^{-1} 110 d^{-1})	8.25±0.86	8.07±1.59	7.41±0.73	NS

CONCLUSION

The net and gross production of mola, *Catla*, rohu, mrigal and prawn in T_1 was significantly higher than T_2 and T_3, which indicate that these are the suitable candidate species in integrated cage-pond polyculture system due to their synergistic effects. From this research, it was revealed that the culture of mola, Indian major carps and prawn with a stocking density at 2.5 m^{-2}, 0.33 m^{-2} and 15 m^{-2} may be an appropriate polyculture technology for rural poor, and it would allow simultaneous production of mola for family consumption and large carps, prawn as cash crop.

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The Culture Potential of *Pangasius* Catfish in Brackish (Hyposaline) Waters of the Greater Barishal Regions in Southern Bangladesh

Production System Design and Best Management Alternatives/Experiment/13BMA02NC

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ABSTRACT

The river catfish (*Pangasius hypophthalmus*) was introduced to Bangladesh in the 1990s from Thailand and has since become a thriving aquaculture industry with more 3 million tons produced annually. The aim of this investigation was to assess the potential for expanding the culture of *Pangasius* catfish to hyposaline waters in Southern Bangladesh. If *Pangasius* culture can be introduced to coastal regions of Bangladesh, it may significantly improve food security and the economic viability of its communities. Therefore, this investigation assesses the potential to culture *Pangasius* in hyposaline waters endemic to the coastal in-land regions of Southern Bangladesh (0.5–12 ppt), as well as other regions where seawater incursion is expected to increase and where the livelihood of coastal communities have relied on culture of freshwater fishes for their livelihoods (e.g., Lower Mekong Delta).

An on-farm experiment was conducted in 18 ponds of different salinity levels for a period of 160 days from May through October 2014 in the coastal Patuakhali district. The experiment consisted of three treatments with salinity ranges of 0–0.5 ppt, 5–7 ppt and 10–12 ppt each replicated in six ponds. The average size of ponds was 400 m^2 with an average water depth of 1.3 m. Prior to stocking ponds were prepared by liming and fertilization. Overwintered fingerlings of *Pangasius* (~6 g) were stocked in all ponds at a density of 2/m^2. Fish were fed with commercial feed (Mega floating feed, 28% CP, 7% fat) at an initial rate of 10% body weight (bw)/day down to 3% bw/day. Feed was provided twice daily at 09:00 and 14:00 h.

Water-quality parameters among the different treatments were within suitable ranges for *Pangasius* culture. Average salinities were 0, 6.5 and 10.8 in T_1, T_2 and T_3, respectively. No significant differences ($p>0.05$) were observed in survival rate, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), yield, and benefit cost ratio (BCR) among the treatments. The results indicate that *Pangasius* catfish show similar survival and growth in hyposaline waters up to 10-12 ppt as is seen in freshwater.

A second on-farm experiment was subsequently undertaken to assess the effect of increased stocking density and of a commercial and formulated feed on *Pangasius* production over six months in ponds with an average salinity of 10 ppt. Fish (12 ponds, 4 ponds/group) were stocked at a density of 2/m^2 and fed a formulated (28% CP, T1 group) and commercial feed (CP Mega floating feed, 28% CP; T2 group). In a third treatment (T3) fish were stocked at 3/m^2 and fed the commercial feed. Fish were fed at an initial rate of 10% body weight (bw)/day down to 3% bw/day.

No significant differences were observed in survival rate, weight gain, SGR, and feed conversion ratio (FCR) among the treatments. Significantly higher production was observed in T3 (23,264 kg/ha) followed by T1 (15,538 kg/ha) and T2 (15,622 kg/ha). Because formulated feed is substantially cheaper than commercial feed net profit was greater in T1 (US$11,438/ha) than in T2 (US$8,275/ha). Total cost was higher in T3 than in T2 due to higher stocking densities, but net profit was higher in T3 (US$12,104/ha) than in T2 (US$8,275/ha).

Collectively, the results indicate for the first time that *Pangasius* can be grown in salinities as high as 10 ppt. The adoption of formulated feeds rather than commercial pelleted diets or of a higher stocking density of 3 fish/m^2 can provide additional profits of as high as 40%. The work demonstrates brackish water culture of *Pangasius* can provide an alternative livelihood for coastal communities including those impacted by water salinization associated with global climate change.

INTRODUCTION

In Bangladesh, *Pangasius* catfish is considered as one of the most successful aquaculture species due to its relative ease in culture, high-market demand, and suitability to local climate conditions (Rahman et al. 2005, Rahman et al. 2012, Sarker 2000). The focus of this investigation is to assess the potential for expanding the culture of *Pangasius* to Southern regions containing significant amounts of brackish (hyposaline) waters, the areas that are severely impacted by overfishing and global climate change (e.g., seawater encroachment, storms) and are currently underutilized for fish production. Production of *Pangasius* could theoretically ease poverty reductions for millions of low-income people in Southern Bangladesh by creating better employment opportunities through development of activities with backward and forward linkages to the market chain. Additionally, we will assess if indigenous mullet species (striped and goldspot mullet) can be incorporated into *Pangasius* culture to achieve better production yields (therefore better income earnings), potential improvements in environmental water quality through more efficient nutrient utilization, and improving nutrition through greater diversification of food resources in the coastal regions (greater Barisal District).

The river catfish (*P. hypopthalamus*) was introduced to Bangladesh in 1990s, and since then it has become a thriving aquaculture industry with more 300,000 tons produced annually (Ali et al. 2011, Edward and Hossain 2010, Munir 2009). Currently, much of the *Pangasius* production comes from the North and Central regions of Bangladesh (e.g., greater Mymensingh). In these regions, *Pangasius* are cultured both intensively with commercial feeds, semi-intensively (with limited feed), and in extensive (no feed) polyculture with both tilapia and carp (Ahmed et al. 2010). High disease resistance, along with high stocking density with greater production rates (up to 120 fish /m^2, average 40 tons/ha; UNFAO 2010), make *Pangasius* an ideal cultivar for increasing aquaculture production in Bangladesh, particularly in regions unfamiliar with farming this species, as well as reducing the burden of population growth. The greater Barisal district is one such region, which has traditionally relied on fishing or aquaculture of marine species (*e.g*., shrimp) for their economic livelihoods. Through overfishing and the increasing frequency of natural calamities like cyclones (e.g. Sidr, Aila), this region is nearing depletion of wild fish stocks and currently over half a million fishermen have been suffering from severe poverty. Introducing *Pangasius* aquaculture to these coastal communities, whose water resources are largely underutilized, may significantly enhance the dietary consumption of protein for low-income families, as well as provide new sources of income and employment in an area.

Some studies suggest *Pangasius* sp. may be tolerant of salinity (David 1962). Recently, juveniles of *Pangasius* catfish are reported to tolerate salinities up to 13 ppt without significant mortality (Castaneda et al. 2010). Before significant resources can be allocated for promoting this industry in coastal regions, the growth performance of *Pangasius* in hyposaline waters must first be evaluated. Through increasing tidal (seawater) encroachment and storms, nearly 40 percent of the farmable water bodies in the greater Barisal district are now hyposaline (0.5–7.5 ppt), and this percentage is expected to increase in future

years. This has significantly impacted culture of traditional freshwater species in the area. If *Pangasius* culture can be achieved in greater Barisal and other coastal regions, the production levels of this fish could effectively double (600,000 metric tons), thus may significantly impact the diet and economic viability of coastal communities. As similar problems exist for the lower Mekong Delta in Vietnam (Halls and Johns 2013), a better understanding of growth performance and salinity tolerance can benefit aquaculture production throughout South-East and Central Asia. This investigation will focus on the salinity ranges endemic to coastal in-land regions of Southern Bangladesh (0–8 ppt) to assess the economic feasibility of *Pangasius* culture in these locales.

OBJECTIVES

- Evaluate whether freshwater *Pangasius* catfish can be successfully cultured in seawater-encroached hyposaline waters of coastal Southern region of Bangladesh;
- Assess effect of increased stocking density and formulated diet on *Pangasius* culture in hyposaline waters; and
- Evaluate potential economic impacts for *Pangasius* culture in hyposaline environments.

MATERIALS AND METHODS

Study 1 — Effect of short-term acclimation to different salinity ranges on *Pangasius* survival. This initial experiment (Table 1) was done for seven days in May 2014 at the Itbaria fish farm in Kalapara Upozila of the Patuakhali district in Bangladesh. Eighteen ponds were selected for this short-term experiment on the basis of salinity. The average size of ponds was 10 decimal (400 m^2) with an average water depth of 1.3 meter. There were six treatment having three replications of each.

Pond preparation. All ponds were completely dried, and all unwanted species were removed from pond prior to the start of experiment. Liming was done at a standard rate of 1 kg per decimal. Lime was mixed with water and kept overnight and distributed on the pond surface early in the morning. After three days of liming, the ponds were filled in with water from adjacent saline water lake and freshwater reservoir. Freshwater was mixed with saline water to prepare the water of different salinity.

Stocking. Fingerling *P. hypophthalmus* (Thai pangas or river catfish) of an average size 5.78 g were stocked at a density of 10/decimal (0.25 fish/m^2).

Feeding. Fish were fed a Mega floating feed (Spectra Hexa Feeds Ltd.) at the rate of 10% of body weight (bw)/day. Feed was applied twice daily at 09:00 and 16:00 h. The feeds were dispersed by hand broadcasting over the water.

Study 2 — Evaluate the growth performance of *Pangasius* catfish cultured in brackish (hyposaline) water of different salinities. This study assessed whether *Pangasius* catfish can be successfully cultured in the brackish water ponds of Southern Bangladesh (greater Barisal region). The experimental design (Table 2) encompassed salinity ranges found in surface water salinities in this farming region (2–12 ppt). Ponds were selected on the basis of salinity from participating farmers in Kuakata, Kalapara and Amtoli Upzila of Patuakhali and Borgona districts. WorldFish aided in selection of farmers and 13 of the owners have cooperated with the WorldFish Center in Bangladesh. Of the selected ponds, 55% were operated by women (Table 4). The study contrasted *Pangasius* production under three different salinity treatment ranges, each replicated in six ponds. The average size of ponds was 10 decimal (400 m^2) with an average water depth of 1.3 m. Ponds were selected based on initial salinity ranges of 0–0.5 ppt, 5–7 ppt and 10–12 ppt in T1, T2 and T3 groups, respectively. Ponds were stocked at 2 fish/m^2 and fed at a standard rate. The growout study was conducted for 160 days from May through October 2014.

Pond preparation. All ponds were completely dried, and all unwanted species were removed. Ponds were surrounded by a filter net to avoid the escape of fish during any potential natural disaster, namely flooding. Following drying, liming was done at a standard rate of 1 kg per decimal (2.5 g/m^2 CaCO$_3$). Lime was mixed with water and kept overnight and distributed on the pond surface the next morning. After liming ponds were filled with surface water from the adjacent river and lakes. All ponds were fertilized initially with urea and triple super phosphate (TSP) at the rates of 150 g urea/decimal (3.75 g/m^2) and 75 g TSP/decimal (1.875 g/m^2). After six days, fish were stocked into ponds.

Stocking. Fingerling *Pangasius* of an average size of 6 g were obtained from the Chanchal Hatchery, Bauphol, Patuakhali, Bangladesh and stocked at a density of 80/decimal (2.0 fish/m^2).

Feeding. *Pangasius* were fed a Mega floating feed (28% CP, 7% fat; Spectra Hexa Feeds Ltd.) at the rate of 10% of bw/day down to 3% bw/day. Feed was applied twice daily at 09:00 and 16:00 h. The feeds were dispersed by hand broadcasting over the water.

Sampling. Fortnightly subsampling of the experimental fish was done by using cast net to measure growth of experimental fish and to adjust feeding rate. After 160 days of the growth trial, a subsample of fish weights and lengths and the total number of fish harvested for each pond was determined.

Water quality. Parameters such as water temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite and salinity were recorded fortnightly. Water pH was measured by a direct reading digital pH meter, temperature and oxygen with portable digital probes, hardness and alkalinity by test kits (Hanna Instruments), and ammonia and nitrite by colorimetry.

Production parameters. Weight gain of experimental fish was calculated as final fish weight (g) – mean initial fish weight (g). Length gain of experimental fish was calculated as mean final fish length (cm) – mean initial fish length (cm). SGR is the instantaneous change in weight of fish calculated as the percentage increase in body weight per day over a given time interval. It was calculated as $[(\ln W_2 - \ln W_1)/(T_2 - T_1) \times 100]$, where W_2 is the weight at the end of the growth interval (harvest weight) and W_1 is the weight at the beginning of the growth interval (initial stocking weight), while $T_2–T_1$ represents the duration (days) of the growing interval.

FCR is calculated as the amount of dry feed fed to fish divided by fish weight gain. Percentage survival rate is calculated as the number of fish that survived at harvesting divided by the number of fish stocked multiplied by 100.

Statistical analysis. Treatment differences in variables measured was evaluated by ANOVA (SPSS 11.5 software) followed by Duncan's new multiple range test (Duncan 1955) Results are shown as mean ± SD and statistical significance was set at a level of $p \leq 0.05$.

Study 3 — Evaluate the effect of increased stocking density and of formulated diet on growth performance of *Pangasius* catfish in brackish (hyposaline) water. Results from Study 2 showed that *Pangasius* survival, growth and production in salinities ranging from 5–12 ppt is similar to that of fish raised in fresh water. We then assessed (Table 3) if increasing density by 50% and applying a formulated diet as feed might provide further cost savings or economical benefit to *Pangasius* culture in brackish (hyposaline) waters of 10–12 ppt.

Ponds were selected based on surface water salinities on participating farms in Kuakata, Patuakhali. Of the selected ponds 60% were operated by women (Table 4). The average size of ponds was ~10 decimal (~400 m^2) with an average water depth of 1.3 m. Ponds were selected based on an initial salinity range of 10–12 ppt. The grow-out study was conducted for 6 months from May through October 2015.

Pond preparation. Ponds were prepared as described under Study 2.

Stocking. Fingerling *Pangasius* of an average size of 65 g were stocked at a density of 80 fish/decimal (2.0 fish/m^2) in treatments 1 and 2 (T1 and T2) and at 120 fish/decimal for treatment 3 (T3).

Feeding. For the T1 group, *Pangasius* was fed a formulated diet containing 28% crude protein (CP). Ingredients include 30% fish meal, 20% mustard cake oil and rice bran, 15% wheat bran, 3% wheat flour, 2.5% molasses, and 0.5% vitamin mix (Table 5). The diet was produced at a small community mill receiving technical support through WorldFish (Figure 1). Ingredients were pelleted by a press. The feed was distributed to all the farmers. For the T2 and T3 groups, fish were fed a 28% CP commercial diet (Mega floating feed). Fish were fed formulated and commercial feeds at the rate of 10% of bw/day down to 3% bw/day. Feed was applied twice daily at 09:00 and 16:00 h. The feeds were dispersed by hand broadcasting over the water.

Sampling. Fortnightly subsampling of the experimental fish was done by using cast net to measure growth of experimental fish and to adjust feeding rate. After 160 days of the growth trial, a subsample of fish weights and lengths and the total number of fish harvested for each pond was determined.

Water quality. Parameters such as water temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite and salinity were recorded fortnightly. Water pH was measured by a direct reading digital pH meter, temperature and oxygen with portable digital probes, hardness and alkalinity by test kits (Hanna Instruments), and ammonia and nitrite by colorimetry.

Production parameters. Production parameters, including weight, length, weight gain, SGR, FCR and yield were measured as described in Study 2.

Statistical analysis. Treatment differences in variables measured was evaluated by ANOVA (SPSS 11.5 software) followed by Duncan's new multiple range test (Duncan, 1955) Results are shown as mean ± SD and statistical significance was set at a level of $p \leq 0.05$.

On-farm training. All farmers in this and the previous studies were trained on all aspects of the research by the Patuakhali University of Science and Technology PI, graduate students, and a WorldFish field supervisor. The training included workshop discussions and presentations to outline the research and goals, and courtyard and on-farm sessions on how to prepare and manage ponds, methods for stocking and feeding fish, water quality and harvesting. All farmers were provided record keeping books to document activities including the amount of feed applied to their ponds.

RESULTS AND DISCUSSION

Study 1 — Effect of short-term acclimation to different salinity ranges on *Pangasius* survival. The survival rate of *Pangasius* catfish fingerlings is presented in Figure 2. In T1 (0–5 ppt) and T2 (7–8 ppt), survival rate was 100%. In the T3 group, fingerlings were stocked after acclimatization in 6 ppt saline water survival rate was 100%, but in T4 where fingerlings were stocked directly in 10–12 ppt saline water, survival rate was 87% with mortalities occurring as early as 5 days. In fish acclimated to 12–15 ppt (T5 group), survival rate was 30%. In the highest salinity (T6 group), all fingerlings died within one day. Based on these results, *Pangasius* catfish can easily survive up to 12 ppt saline water when provided a prior 24-h acclimation to moderate salinities of 6–7 ppt. Based on this work, *Pangasius* growth and production was assessed during a full grow-out trial in three salinity ranges up to 12 ppt.

Study 2 — Evaluate the growth performance of *Pangasius* catfish cultured in brackish (hyposaline) water of different salinities. The ability to culture *Pangasius* in the low-salinity waters would allow production of an additional species in the low lying coastal region of Bangladesh, where significant

seawater incursion exists and communities are threatened by frequent calamities and declines in shrimp production. Preliminary studies in tanks suggest that *Pangasius* can survive in salinities as high as 13 ppt for up to 22 days (Castaneda et al. 2010). However, it is unknown whether this species can be grown for extended periods in hyposaline, brackishwater environments. Therefore this study contrasted *Pangasius* growout performance in freshwater and in ponds with salinities as high as 12 ppt. We found that *Pangasius* growth, production and yield in brackish water ponds, reflective of coastal surface waters, is similar to that of fish grown in fresh water.

The average salinity in the on-farm growth trials was 0 ppt, 6.5 ppt and 10.8 ppt in in T1, T2 and T3 groups, respectively (Table 6). In the T3 ponds, salinity fluctuated over the course of the experiment. It was initially at ~12 ppt in May and declined slowly to 7 ppt in August and then rose again to 9 ppt at termination of the experiment. These changes coincide with the monsoon rainy season (June to September) in the region. As expected, hardness and alkalinity increased with salinity, while ammonia and nitrite levels declined in ponds at the higher salinity, the latter likely due to the naturally greater tidal water exchange that occurs closer to the sea (Table 6). Collectively, all water quality parameters were well within the acceptable range for *Pangasius* culture and that of other fishes (Bhatnagar and Devi, 2013).

Production of *Pangasius* grown in different salinities is shown in Table 7. We found no significant mortalities in the experiment with survival of around 96% in all groups (Table 7). Growth performance and yield of *Pangasius* did not vary with salinity and was similar to that of fish grown in fresh water (Huq et al. 2004, Azad et al. 2004, Khan et al. 2009). No significant differences were observed in survival rate, weight gain, SGR, FCR, yield, and BCR among the treatments ($p > 0.05$). At the termination of the 160-day trial the average body weight (684–687 g), weight gain (677–680 g), and SGR (2.868–2.871 %/day) at higher salinities were remarkebly similar to that of freshwater cultured fish. Likewise the FCR of experimental groups ranged from 1.61–1.63. Total yield ranged from 12,998 kg/ha to 13,239 kg/ha. A marginal economic analyses also indicates the benefit cost ratio which ranged from 1.32 to 1.36 was similar across the different groups (Table 8). Collectively, the work demonstrates that *Pangasius* can be grown in salinities as high as 11 ppt (10–12 ppt) with no negative impacts on fish growth and yield or profits.

Study 3 — The effect of increased stocking density and a formulated diet on growth performance of Pangasius catfish in brackish (hyposaline) water. We then evaluated if increasing the density of fish by 25% and feeding less costly formulated diet might further improve profits for farming *Pangasius*. As with the previous study, the growout trial was done on local farms, but here we evaluated performance and economic benefits in fish cultured over six months in ponds with an average salinity of 10 ppt. Fish (12 ponds, four ponds/group) were stocked at a density of 2/m^2 and fed a formulated diet produced at a local, small-scale community feed mill (28% CP, T1 group) and commercial feed (CP Mega floating feed, 28% CP; T2 group). In a third treatment (T3), fish were stocked at 3/m^2 and fed the commercial feed. All fish were fed similarly at an initial rate of 10% body weight (bw)/day down to 3% bw/day.

Pond salinity among the three treatments was similar averaging 9.5, 9.7 and 9.8 ppt in T1, T2, and T3 groups, respectively (Table 9). There were no significant differences in water quality among groups with mean values in the range of 5.17–5.23 mg/L for dissolved oxygen, 7.75–7.82 for pH, and 153–159 mg/L and alkalinity (153–159 mg/L) (Table 9). Temperature averaged around 30°C which is close to the optimal for *Pangasius* (Baras et al. 2011). Ammonia levels were very low and well below the 4 mg/L threshold impacting fish growth (Stone and Thomforde 2004). Likewise nitrite levels were around 0.05 mg/L and again was similar among groups.

Growth parameters are presented in Table 10 and a marginal cost benefit analysis in Table 11. At the termination of the six-month grow-out trial the average body weight of *Pangasius* was 786.34, 790.62

and 784.89 g in T_1, T_2 and T_3 respectively. Neither weight gain nor SGR of Pangaius was affected by the higher density or usage of formulated feeds. Mean SGR was 2.871, 2.869 and 2.868 in T1, T2 and T3, respectively, values that were more or less similar with the findings of Azad et al. (2004) and others. Feed conversion ratio estimates for experimental fishes under T_1, T_2 and T_3 were 1.63, 1.62 and 1.64, respectively, which is similar to (Kader et al. 2003) or better (lower FCR) than that of various other studies using supplemental feeds for *Pangasius* culture in fresh water (Ahmed et al. 1996, Azimuddin 1998, Halder and Jahan 2001, Maniruzzaman 2001, Sayeed et al. 2008, Khan et al. 2009, Kader et al. 2011). This suggests that the formulated feed was of adequate quality as that of the more expensive commercial diet and raises the possiblity that culture in salinity isosmotic to the fish's internal milieau may have additional benefits on growth and feed efficiency.

Pangasius survival was ~95% and again no significant differences were found among treatments ($p >$ 0.05). Considering similar survivorship and growth, the production of *Pangasius* was proportionally higher in ponds stocked at a density of 3 fish/m^2 (23,264 kg/ha) versus those stocked at 2 fish/m^2 (T3, 15,538 kg/ha; T2, 15,622 kg/ha). These results suggests that *Pangasius* can be grown semi-intensively in brackish water ponds at a density of as high as 3 fish/m^2 with little impact on performance or survival. Whether higher densities can be employed requires further study.

We found with the higher stocking density net profit exceeded that of the lower stocking density for fish grown on commercial feeds. However, it costs substantially more for feeds in T3 relative to T2 group (Table 11), which is why the BCR did not differ between the groups. Therefore, the T3 strategy requires a higher level of investment compared to that of T2 with a relatively modest net profit. For small scale operations T2 might be recommended, whereas higher stocking density in T3 might be more appropriate for larger scale operations that have more capitol to invest and can accept the risk associated with that investment and for those who desrire to make a greater profit. Regardless, both stocking densities were profitable showing a 50% return on investment.

Use of formulated and commercial feed was compared under the same stocking density (2/m^2), in T1 and T2 groups, respectively. Total cost for *Pangasius* culture was higher in T2 ($15,782/ha) than in T1 ($12,490/ha) due to the higher price of commercial ($0.54/kg) than formulated feed ($0.41/kg), but total yield of fish was similar among the two groups. Hence a significantly higher net profit was found in T1 ($11,438/ha) than in T2 ($8,275/ha). The BCR was also significantly higher in fish fed the formulated (BCR = 1.91) versus the commercial diet (BCR = 1.53). Collectively, the results indicate that *Pangasius* grown on the formulated diet provides a a 38% increase in profit over fish grown on commercial feeds.

CONCLUSION

Through increased tidal (seawater) encroachment and frequency of storms that are linked to global climate change nearly 40 percent of the farmable water bodies in the greater Barisal region of Southern Bangladesh are now hyposaline (0.5 to 7.5 ppt). Coastal communities have also been impacted by overfishing, declines in shrimp farming, and frequent calamities. Farming and fishing communities need additional cultivars that can grow in low saline waters for income generation and household nutrition. The river catfish is a thriving aquaculture industry with over 300,000 tons produced annually in Bangladesh. It is the third largest finfish industry in the country. Because of its relatively low cost it is a seafood favorite among the poor. To date, *Pangasius* culture has been limited to freshwater systems. This study assessed the potential for expanding the culture of *Pangasius* catfish to hyposaline waters in Southern Bangladesh. Working with farmers in the Barisal region of Bangladesh we demonstrate that *Pangasius* can easily survive and can be profitably grown in salinities up to 10–12 ppt with similar efficiency as in freshwater. The studies also show that formulated feeds produced locally in small scale mills and at a substantially lower cost is as effective as commercial feeds in growout of *Pangasius* in 10 ppt hyposaline waters. This, along with increases in stocking density from 2–3 fish/m^2 can enhance profits by 40%. Adoption of *Pangasius* culture in brackish water regions in the Southwest and Southern districts of Bangladesh has

opened up the potential for mass scale culture and production of *Pangasius* catfish as an alternative livelihood and nutrition for communities. The new technology could effectively double *Pangasius* production in Bangladesh and other coastal regions affected by rising salinity (e.g., Mekong Delta). This work was demonstrated to the farming community in the most effective way, by conducting the research on their farms. AquaFish personnel provided extensive training to farmers through the day-to-day research activities, workshops and pond side discussions. Future work should assess if other fishes tolerant to low saline waters can be effectively cultured in brackish waters either alone or with *Pangasius*.

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TABLES AND FIGURES

Table 1. Study 1 Experimental Design.

Parameters	Salinity	Conditioning at 6 ppt
T1	0–5 ppt	No conditioning
T2	7–8 ppt	No conditioning
T3	10–12 ppt	Conditioning before stocking
T4	10–12 ppt	No conditioning
T5	12–15 ppt	Conditioning before stocking
T6	18–22 ppt	Conditioning before stocking

Table 2. Study 2 Experimental Design.

Parameter	Treatment 1	Treatment 2	Treatment 3
Pangasius stocking density	80/decimal (2.0/m^2)	80/decimal (2.0/m^2)	80/decimal (2.0/m^2)
Salinity range (ppt)	0–0.5	5–7	10–12
Feeding	commercial feed	commercial feed	commercial feed
Replicates (*n*)	6	6	6

Table 3. Study 3 Experimental Design.

Parameter	Treatment 1	Treatment 2	Treatment 3
Pangasius stocking density	80/decimal (2.0/m^2)	80/decimal (2.0/m^2)	120/decimal (3.0/m^2)
Salinity range (ppt)	10–12	10–12	10–12
Feeding	Formulated Diet	Commercial Feed	Commercial Feed
Replicates (*n*)	4	4	4

Table 4. Location and number of ponds for assessing salinity effects on growth of *Pangasius* in the coastal greater

Area	Pond distance from sea	Salinity	Pond Number	Women owners
Amtoli (Borgona District)	40 km	0 ppt	6	4
Kalapara (Patuakhali District)	23 km	5-7 ppt	6	1
Kuakata (Patuakhali District)	5 km	10-12 ppt	6	5
Total			18	10 (55%)

Barisal region of Southern Bangladesh.

Table 5. Ingredients of formulated feed used in Study 2 to compare growout of *Pangasius* in hyposaline waters with commercial feed of similar crude protein levels.

Ingredients	% in feed	Amount of protein (%) in the ingredient	Protein contribution in the feed
Fishmeal	30%	60%	18 %
Mustard oil cake	20%	32%	6.4%
Rice bran	20%	7.5%	1.50%
Wheat bran	15%	11%	1.65%
Wheat flower	3%	15%	0.45%
Molasses	2.5%	–	
Vitamin mineral premix	0.5%	–	
Total	**100%**		**28%**

Table 6. Water quality parameters of ponds where *Pangasius* were grown at different salinities (0, 6.5, and 10.8 ppt) over 160 days in earthen ponds (Study 2). (Mean ± SE)

Parameters	T1	T2	T3
Water Temperature (°C)	30.18 ± 0.95	30.10 ± 0.42	30.52 ± 0.41
Dissolved Oxygen (mg/L)	5.16 ±0.12	5.24 ± 0.13	5.98 ± 0.21
pH	7.77 ± 0.37	7.49 ± 0.26	7.67 ± 0.14
Alkalinity (mg/L)	90.5 ± 6.12	110 ± 6.61	130 ± 5.15
Ammonia (mg/L)	0.67 ± 0.02	0.64 ± 0.03	0.02 ± 0.03
Nitrite (NO_2) (mg/L)	0.37 ± 0.02	0.24 ± 0.23	0.00
Hardness (mg/L)	70 ± 3.12	240 ± 5.12	560 ± 4.12
Salinity (PPT)	0	6.5	10.8

Table 7. Production parameters of *Pangasius* grown at different salinities (0, 6.5, and 10.8 ppt) over 160 days in earthen ponds (Study 2).

Parameters	T1, 0 ppt	T2, 6.5 ppt	T3, 10.8 ppt
Mean initial weight (g)	6.56 ± 0.53	6.56 ± 0.53	6.56 ± 0.53
Initial length (cm)	9.18 ± 0.68	9.18 ± 0.68	9.18 ± 0.68
Mean final weight (g)	686.76 ± 50.26	684.67± 52.25	683.89±56.80
Final length (cm)	38.94 ±4.33	38.50±5.30	37.86±5.29
Survival Rate (%)	96.76±2.67	96.56±2.78	96.12±2.85
Mean weight gain (g)	680.2±2.41	678.11±2.64	677.33±2.21
SGR (% per day)	2.871±0.27	2.869±0.25	2.868±0.23
FCR	1.61±0.23	1.62±0.26	1.63±0.32
Yield (kg/ha)	13,139±1293	13072±1374	12998 ±1347

Table 8. Cost benefit analyses of *Pangasius* grown at different salinities (0, 6.5, and 10.8 ppt) over 160 days in earthen ponds (Study 1). Values are in Bangladesh currency (BDT). Benefit-cost ratio is calculated as gross income/total cost. Values shown reflect combined data of all replicates within each treatment. The higher value of fish from the 10 ppt group was attained because the *Pangasius* supply in markets closest to the sea is more limited than inland regions.

Investment Costs	T1, 0 ppt	T2, 6.5 ppt	T3, 10.8 ppt
Pond Preparation (lime, fertilizer)	14,500	14,500	14,500
Fingerling	38,400	38,400	38,400
Total feed used (kg)	5152	5152	5152
Feed cost @ 46 BDT/Kg	236,992	236,992	236,992
Total cost	289,892	289,892	289,892
Returns			
Total Fish production (kg)	3,189.64	3,173.36	3,155.39
Retail price of fish/kg	120	120	125
Income (from fish sales)	382,756	380,803	394,423
Net profit (return)	92,264	90,911	104,531
Benefit-Cost Ratio (BCR)	1.32	1.31	1.36

Table 9. Water-quality parameters in ponds from Study 3 that assessed the effect of increased stocking density and of formulated diet on growth performance of Pangasius catfish in brackish (hyposaline) water (mean ± SE).

Parameters	T1 Formulated Feed 2 fish/m^2	T2 Commercial Feed 2 fish/m^2	T3 Commercial Feed 2.5 fish/m^2
Water Temperature (°C)	29.50 ± 0.42	29.50 ± 0.42	29.50 ± 0.42
Dissolved Oxygen (mg/L)	5.17 ± 0.12	5.20 ± 0.13	5.23 ± 0.12
pH	7.81 ± 0.32	7.82 ± 0.29	7.75 ± 0.34
Alkalinity (mg/L)	152.5 ± 2.12	155.3 ± 2.61	158.7 ± 2.15
Ammonia (mg/L)	0.05 ± 0.02	0.04 ± 0.03	0.05 ± 0.03
Nitrite (NO$_2$) (mg/L)	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
Salinity (PPT)	9.6 ± 0.7	9.4 ± 0.6	9.7 ± 0.6

Table 10. Production parameters of *Pangasius* catfish in Study 3 (mean ± SE).

Parameters	T1 Formulated Feed 2 fish/m^2	T2 Commercial Feed 2 fish/m^2	T3 Commercial Feed 2.5 fish/m^2
Mean initial weight (g)	65.56 ± 4.53	65.56 ± 4.53	65.56 ± 4.53
Initial length (cm)	21.18 ± 1.68	21.18 ± 1.68	21.18 ± 1.68
Mean final weight (g)	786.34 ± 45.21 [a]	790.62 ± 45.21 [a]	784.89 ± 51.80 [a]
Final length (cm)	42.87 ± 5.31	43.10 ± 5.23	41.96 ± 6.22
Survival Rate (%)	95.71 ± 3.64 [a]	95.45 ± 3.12 [a]	95.12 ± 2.85 [a]
Weight gain (g)	720.78 ± 36.41 [a]	725.06 ± 35.64 [a]	719.33 ± 37.21 [a]
FCR	1.63 ± 0.26 [a]	1.62 ± 0.28 [a]	1.64 ± 0.39 [a]
Yield (kg/ha)	15,538 [a]	15,622 [a]	23,264 [b]

Table 11. Marginal cost-benefit analyses of Study 3. Values in US$. Different letters reflect significant difference among treatments. Values reflect means of 4 replicates/group and costs and returns are based on 1 hectare of fish production. Benefit-cost ratio is calculated as gross income/total cost.

Investment Costs (US$)	T1 Formulated Feed 2 fish/m^2	T2 Commercial Feed 2 fish/m^2	T3 Commercial Feed 2.5 fish/m^2
Pond reparation (lime, fertilizer)	80	80	80
Fingerling	2,026	2,026	3,040
Total feed used (kg)	25327	25327	38,152
Feed cost (commercial feed $0.54/kg; formulated feed $0.41/kg)	10,384	13,676	20,602
Total cost	12,490 [a]	15,782 [b]	23,722 [c]
Returns			
Total fish production	15,538 [a]	15,622 [a]	23,264 [b]
Retail price of fish/kg	1.54	1.54	1.54
Income (from fish sale)	23,928 [a]	24,057 [a]	35,826 [b]
Net profit	11,438 [a]	8,275 [b]	12,104 [c]
Benefit-Cost Ratio (BCR)	**1.91** [a]	**1.53** [b]	**1.51** [b]

Figure 1. Preparation of formulated feed at a small community mill in Patuakhali district in Southern Bangladesh. The feed was used in Study 2 to compare growout of *Pangasius* fed a less costly formulated feed $0.41/kg versus a commercial feed ($0.54/kg).
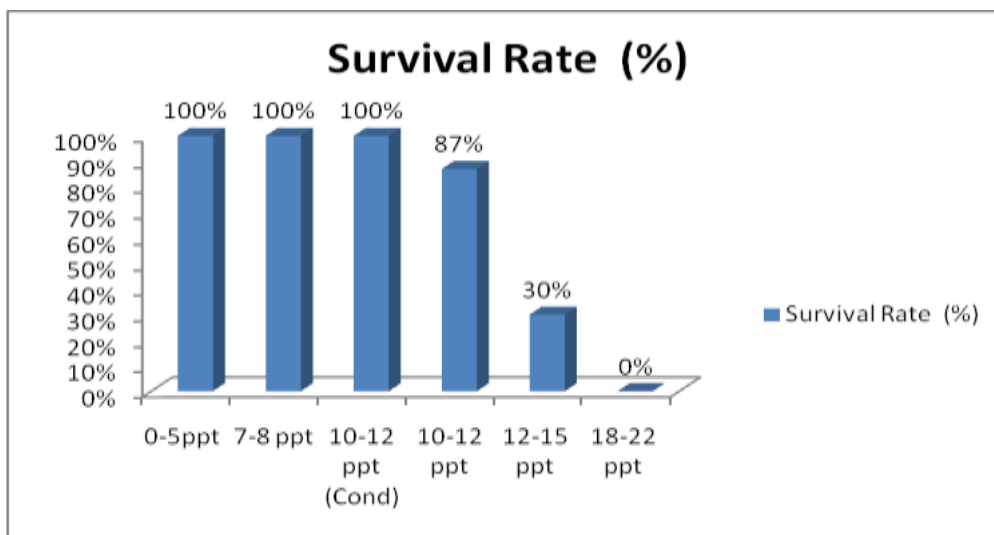
Figure 2. Survival rates of *Pangasius* following 7-day exposure to different salinity ranges in Study 1. For the 10–12 ppt (Cond), 12–15 ppt, and 18–22 ppt group fish were initially conditioned at 6 ppt for 24 h prior to exposure to their respective salinities.

Figure 2. Survival rates of *Pangasius* following 7-day exposure to different salinity ranges in Study 1. For the 10–12 ppt (Cond), 12–15 ppt, and 18–22 ppt group fish were initially conditioned at 6 ppt for 24 h prior to exposure to their respective salinities.

Coastal Women's Shellfish Aquaculture Development Workshop

Production System Design and Best Management Alternatives /Experiment/13BMA01PU

This research is still ongoing and will be included in a future Technical Report. See Implementation Plan 2013-2015, page 31.

Demonstrating the Value of Tilapia and Sahar Production in Polyculture Ponds Using Government Farm and On-Farm Trials

Production System Design and Best Management Alternatives/Experiment/13BMA06UM

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ABSTRACT

Carp polyculture is commonly practiced in Nepal but improving productivity of this aquaculture system is a major concern. Two trials were conducted to demonstrate the value of Nile tilapia and sahar in polyculture ponds. The first trial was conducted at the Fisheries Development Center, Bhairahawa, Nepal in nine earthen ponds of 200 m^2 for 240 days (9 August 2014 to 9 May 2015). The second trial was conducted in 12 farmer's earthen ponds (380–930 m^2) in Dayanagar-7, Rupandehi, Nepal for 165 days (10 July to 24 December 2015) to demonstrate the culture potential of sahar and tilapia to farmers. The first trial was conducted in a completely randomized design with three treatments in triplicate: a) Carps only or control (10,000 fish/ha) (T_1); b) Carps (10,000/ha) + tilapia (3000/ha) (T_2); and c) Carps (10,000/ha) + tilapia (3,000/ha) + sahar (1,000/ha) (T_3). Silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) of mean stocking size 6.7, 3.8, 7.3, 3.1, 1.9 and 2.0 g, respectively were stocked in all ponds at the ratio of 3.5:1:2.5:0.5:1.5:1. The mean stocking size of Nile tilapia (*Oreochromis niloticus*) and sahar (*Tor putitora*) were 4.4 and 7.2 g, respectively. The ponds were fertilized weekly with urea and di-ammonium phosphate at 4 g N and 1 g P m^{-2} day^{-1}. Fish were fed once daily with locally made mass feed (1:1 rice bran and mustard oil cake; 20% CP) at 2% body weight. At harvest, the combined net fish yield was significantly higher in T_3 (3.93 ± 0.15 t·ha^{-1}·yr^{-1}) compared to T_1 (3.05 ± 0.26 t·ha^{-1}·yr^{-1}) whereas there was no significant difference between T_2 and T_3. There were no significant differences in survival and water quality among treatments. The gross profit margin was significantly higher in T_3 ($2,357 \pm 211$ USD/ha) compared to T_1 ($1,300 \pm 316$ USD/ha) without any significant difference between T_2 and T_3.

The farmer's field trial was composed of two treatments with six replicates each: a) Carps only or control (10,000 fish/ha); and, b) Carps (10,000/ha) + tilapia (3,000/ha) + sahar (1,000/ha). Common carp, silver carp, bighead carp, grass carp, rohu, and mrigal of mean stocking size 5.2, 5.3, 24.0, 16.2, 4.5 and 5.1 g, respectively were stocked in all ponds. The mean stocking size of Nile tilapia and sahar were 3.8 and 10.1 g, respectively. The ponds were fertilized weekly with urea and di-ammonium phosphate at 4 g N and 1 g P m^{-2} day^{-1}. Fish were fed once daily with commercial mass feed of 20% CP bought from local feed industry at 2% body weight. Other culture practices were similar to the first trial. At harvest, the combined net fish yield was significantly higher in polyculture of carps, tilapia, and sahar (5.6 ± 0.8 t·ha^{-1}·yr^{-1}) compared to only carps polyculture (4.2 ± 0.2 t·ha^{-1}·yr^{-1}). There were no significant differences in water quality parameters between treatments. The gross margin was significantly higher in polyculture of carps, tilapia, and sahar treatment ($3,219 \pm 367$ USD/ha) compared to polyculture of only carps ($1,800 \pm 250$ USD/ha). This study concludes that the carp-tilapia-sahar polyculture system is a better practice than

presently used carp polyculture system to enhance pond productivity and economically viable aquaculture.

INTRODUCTION

Aquaculture and fisheries in Nepal contributes about 0.93% to national GDP and 2.61% in agriculture GDP. It is one of the fastest growing economic and food producing sectors with growth rate of 8.4% per annum (Mishra 2015a). Pond aquaculture is the major aquaculture system practiced in ponds contributing more than 95% of total aquaculture, and 70% of this is contributed by exotic carps (Mishra 2015b). Carp polyculture practiced in Nepal is the mixed culture of common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), rohu (*Labeo rohita*), naini/mrigal (*Cirrhinus mrigala*) and bhakur/catla (*Catla catla*). Though all seven species are recommended in polyculture in certain ratios with a combined stocking density of 7,000–10,000 fish/ha, fingerlings of all species are rarely available when needed for stocking (Pandey 2002). Nile tilapia (*Oreochromis niloticus*) has been considered as another suitable aquaculture species in tropical and sub-tropical regions. However, it creates a significant challenge to control excessive recruitment of mixed-sex tilapia in the culture system (Shrestha et al. 2011), although such tests have been successfully done (Wang and Lu 2015). Tilapia can also be successfully cultured with freshwater prawn (Uddin et al. 2007, Tidwell et al. 2010). Sahar (*Tor putiotora*) is an indigenous fish species of Nepal which can be cultured successfully with carps and mixed-sex tilapia (Jaiswal 2010, Shrestha et.al 2011). Though an omnivorous species, sahar also shows predatory habits and has been proven as a simple mechanism to control tilapia requirement in ponds (Acharya et al. 2007, Paudel et al. 2007, Shrestha 1997a, Yadav et al. 2007). Sahar is the largest riverine sport fish of Nepal (Rai et al. 1997), which is economically important and exists in the rivers and streams of lower and mid hills (Negi 1994). The population of this species is declining across much of its native range due to habitat loss, over fishing, ecological alterations, and physical changes in natural environment such as damming (Desai 1994, Shrestha 1997, Baidya et al. 2006). This has led to efforts to conserve, manage, and propagate the species (Shrestha 1997a). Among several ways of conservation, incorporation of this species into the existing carp polyculture system is one.

Success in artificial propagation of Sahar in recent years has provided additional enthusiasm towards the development of this species for commercial cultivation (Raiet et al. 2006). Moreover, growth of sahar was found better in southern warmer climate (20–30°C water temperature) of Nepal as compared to the mid hill's cooler areas (Shrestha et al. 2004). Being an omnivore in nature it feeds on filamentous algae, insect larvae, small mollusks, and algal deposits on the rocks and has been determined to be predatory on small fishes (Shrestha 1997b, Acharya et al. 2007, Paudel et al. 2007, Yadav et al. 2007).

OBJECTIVES
- To increase pond productivity through species diversification;
- To demonstrate a carp-tilapia-sahar polyculture system for outreach potential by government fisheries development program;
- To demonstrate the culture potential of sahar and tilapia to farmers; and
- To develop partial enterprise budgets of costs and value of fish crops among treatments.

MATERIALS AND METHODS
The first trial was conducted at the Fisheries Development Center, Bhairahawa, Nepal in nine earthen ponds of 200 m^2 for 240 days (9 August 2014 to 9 May 2015). The trial was conducted in a completely randomized design with three treatments in triplicate: a) Carps only or control (10,000 fish/ha) (T_1); b) Carps (10,000/ha) + tilapia (3,000/ha) (T_2); and c) Carps (10,000/ha) + tilapia (3,000/ha) + sahar (1,000/ha) (T_3). Silver carp, bighead carp, common carp, grass carp, rohu, and mrigal of mean stocking size 6.7, 3.8, 7.3, 3.1, 1.9 and 2.0 g, respectively were stocked in all ponds at the ratio of

3.5:1:2.5:0.5:1.5:1. All the experimental ponds were completely drained and treated with hydrated lime $(Ca(OH)_2)$ at the rate of 10.0 kg per 200 m^2 pond. The ponds were sun dried for two to three days and filled with ground water. Then, ponds were fertilized at 4 kg N and 1 kg P $m^{-2}.day^{-1}$ with di-ammonium phosphate (DAP) (18% N and 46% P_2O_5), urea (46% N) and farmyard manure (FYM). DAP and urea was used at 700 and 940 g, respectively and FYM at 60 kg for the 200 m^2 pond area. Fingerlings were stocked one week after pond fertilization.

Feeding was done with mass feed (20% CP) made from 1:1 mustard oil cake (28% CP): rice bran (12% CP) at 2% of total biomass per day in morning between 9:00 -10:00 am in feeding trays fixed in each pond. The quantity of feed was adjusted monthly based on fish sampling measurements. Fertilization with inorganic fertilizer was done at monthly after examining the Secchi disk measurements. Sampling of fish was done monthly from each pond starting from 30 days after stocking. During sampling about 10% of the stocked population of each species was weighed to calculate feed quantity for next month. For final harvest, all ponds were drained by pumping and all fish were harvested and weighed.

Partial budget analyses of all ponds were done using inputs and outputs. Inputs were calculated based on current market price of materials used in ponds. Similarly, outputs were calculated based on farm gate price of harvested fish. Average market price of carp fingerlings were USD 0.015, tilapia fingerlings was USD 0.03 and sahar fingerlings was USD 0.05 per piece. Similarly, market price of lime, urea, DAP and feed were 0.27, 0.30, 0.65 and 0.30 USD/kg, respectively. For output calculation farm gate price of silver and bighead carp was USD 1.5/kg, other carp species were USD 2.00/kg, Nile tilapia was USD 2.50/kg and sahar was USD 6.00/kg.

The second trial was conducted in 12 farmer's earthen ponds (380–930 m^2) in Dayanagar, Rupandehi, Nepal for 165 days (10 July to 24 December 2015) to demonstrate the culture potential of sahar and tilapia to farmers. The farmer's field trial was composed of two treatments with six replicates each: a) Carps only or control (10,000 fish/ha); and b) Carps (10,000/ha) + tilapia (3,000/ha) + sahar (1,000/ha). Common carp, silver carp, bighead carp, grass carp, rohu and mrigal of mean stocking size 5.2, 5.3, 24.0, 16.2, 4.5, and 5.1 g, respectively were stocked in all ponds at the ratio of 3.5:1:2.5:0.5:1.5:1. The mean stocking size of Nile tilapia and sahar were 3.8 and 10.1 g, respectively. The ponds were fertilized weekly with urea and di-ammonium phosphate at 4 g N and 1 g P $m^{-2}.day^{-1}$. Fish were fed once daily with commercial mass feed of 20% CP bought from local feed industry at 2% body weight. Other culture practices and economic parameters were similar to the pond trial at the Fisheries Development Center.

The data were analyzed using one-way ANOVA in SPSS (V 16.0). For the significant difference in growth parameters and gross margin analysis among different treatments LSD was used to compare the means. For testing different growth, production and economic parameters of tilapia, T-test was used.

RESULTS

Experiment 1: Pond trials at the Fisheries Development Center. The overall production, food conversion ratio, and survival of fishes in different treatments are presented in Table 1 and Figure 1. There was no significant difference (p>0.05) in extrapolated GFY of carps, overall FCR, and overall survival rate in different treatments. Similarly, there was no significant difference ($p>0.05$) in extrapolated GFY of tilapia in T_2 and T_3. However, combined extrapolated GFY of all species including tilapia recruits in T_3 was significantly higher ($p<0.05$) compared to T_1 but not with T_2. Similarly, the extrapolated NFY of combined species excluding tilapia recruit in T_3 was significantly higher ($p<0.05$) than T_1.

Table 1: Production parameters (mean ± SE) in different treatments. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatments		
	Carp (T_1)	Carp + Tilapia (T_2)	Carp + Tilapia + Sahar (T_3)
Extrapolated GFY (t·ha^{-1}·year^{-1})			
Carps	3.13±0.26[a]	3.02±0.15[a]	3.33±0.12[a]
Tilapia	-	0.49±0.05[a]	0.45±0.02[a]
Sahar	-	-	0.14±0.02
Combined	3.13±0.26[b]	3.51±0.20[ab]	3.93±0.16[a]
Including tilapia recruit		3.72±0.22[a]	4.04±0.15[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	3.05±0.26[b]	3.57±0.25[ab]	3.93±0.15[a]
FCR	2.53±0.24[a]	2.62±0.17[a]	2.41±0.11[a]
Overall survival	81.2±5.1[a]	76.0±3.0	80.5±1.3[a]

Growth and production parameters of different fish species are shown in Table 2. There were no significant differences in mean harvesting weight, total harvesting weight, mean daily weight gain (DWG), survival rate, extrapolated GFY, and extrapolated NFY of different carp species among treatments. However, total harvesting weight, extrapolated GFY and NFY of Nile tilapia in T_2 were significantly higher than in T_3.
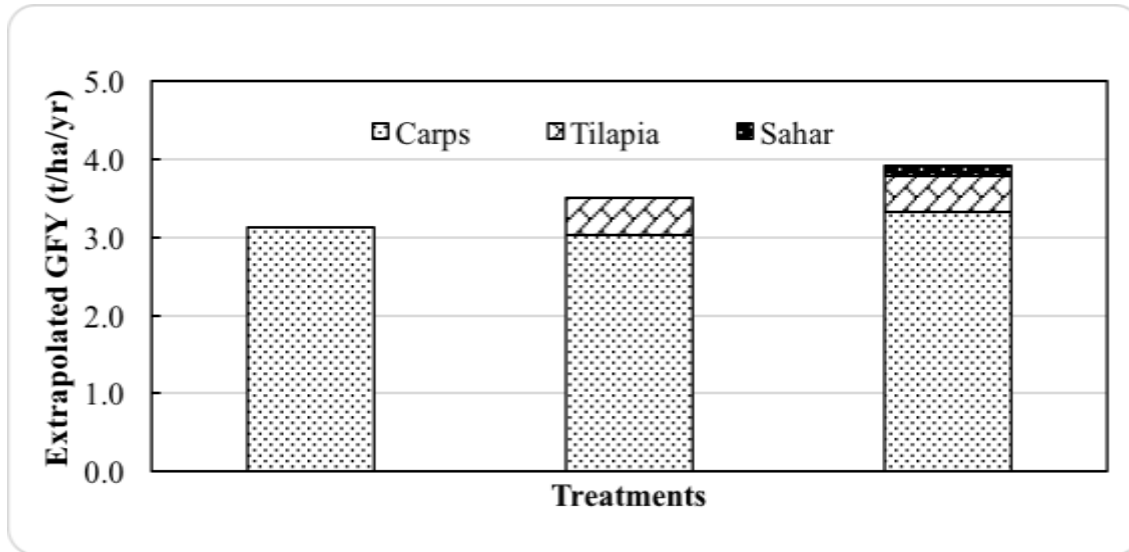

Figure 1: Extrapolated GFY of carps, tilapia and sahar in different treatments.

Table 2: Growth and production parameters (mean ±SE) in different treatments. Data based on 200 m^2 pond area. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatment		
	Carp (T$_1$)	Carp+ Tilapia (T$_2$)	Carp + Tilapia + Sahar (T$_3$)
Common Carp			
Mean Stocking Wt. (g)	7.26±0.09	7.22±0.06	7.27±0.06
Total Stocking Wt. (g)	363.1±4.45	361.47±3.23	363.93±3.12
Mean Harvesting Wt. (g)	285.74±46.77[a]	274.08±16.51[a]	299.91±41.33[a]
Total Harvesting Wt. (kg)	10.81±2.71[a]	10.80±1.66[a]	12.43±7.99[a]
Mean DWG (g·fish^{-1}·day^{-1})	1.16±0.19[a]	1.11±0.07[a]	1.22±0.17[a]
Survival (%)	73.33±8.67[a]	78.00±7.02[a]	84.67±6.36[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	0.82±0.21[a]	0.82±0.13[a]	0.95±0.06[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	0.79±0.21[a]	0.79±0.13[a]	0.91±0.06[a]
Grass Carp			
Mean Stocking Wt. (g)	3.05±0.06	3.13±0.00	3.18±0.12
Total Stocking Wt. (g)	30.46±0.55	31.38±0.04	31.87±1.15
Mean Harvesting Wt. (g)	204.76±32.67[a]	234.17±34.57[a]	212.88±42.84[a]
Total Harvesting Wt. (kg)	1.56±0.28[a]	1.46±0.24[a]	1.41±0.28[a]
Mean DWG (g·fish^{-1}·day^{-1})	0.84±0.14[a]	0.96±0.14[a]	0.87±0.18[a]
Survival (%)	76.67±8.82[a]	63.33±8.82[a]	66.67±3.33[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	0.12±0.02 [a]	0.11±0.01[a]	0.10±0.02[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	0.12±0.02[a]	0.11±0.01[a]	0.10±0.02[a]
Silver Carp			
Mean Stocking Wt. (g)	6.63±0.26	6.66±0.18	6.63±0.31
Total Stocking Wt. (g)	464.20±18.37	465.87±12.70	463.97±21.72
Mean Harvesting Wt. (g)	275.35±13.61[a]	278.07±8.79[a]	288.62±5.19[a]
Total Harvesting Wt. (kg)	16.91±0.95[a]	15.68±1.25[a]	17.39±0.46[a]
Mean DWG (g·fish^{-1}·day^{-1})	1.12±0.06[a]	1.13±0.04[a]	1.17±0.02[a]
Survival (%)	88.57±8.61[a]	80.48±5.49[a]	86.19±3.72[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	1.29±0.07[a]	1.19±0.10[a]	1.32±0.04[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	1.25±0.07[a]	1.16±0.09[a]	1.29±0.03[a]
Bighead Carp			
Mean Stocking Wt. (g)	3.86±0.01	4.07±0.02	3.61±0.15
Total Stocking Wt. (g)	77.2±0.26	81.47±0.39	72.1±2.92
Mean Harvesting Wt. (g)	322.97±33.78[a]	342.78±40.72[a]	284.13±6.65[a]

Parameters	Treatment		
	Carp (T_1)	Carp+ Tilapia (T_2)	Carp + Tilapia + Sahar (T_3)
Total Harvesting Wt. (kg)	5.79±0.49[a]	5.84±0.74[a]	5.21±0.22[a]
Mean DWG (g·fish^{-1}·day^{-1})	1.33±0.14[a]	1.41±0.17[a]	1.17±0.03[a]
Survival (%)	90.00±2.89[a]	85.00±2.89[a]	91.67±1.67[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	0.44±0.04[a]	0.44±0.06[a]	0.40±0.02[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	0.43±0.04[a]	0.44±0.06[a]	0.39±0.02[a]
Rohu			
Mean Stocking Wt. (g)	1.83±0.01	2.11±0.11	1.85±0.09
Total Stocking Wt. (g)	54.83±0.41	63.43±3.43	55.43±2.72
Mean Harvesting Wt. (g)	134.04±9.52[a]	136.36±35.26[a]	161.06±2.29[a]
Total Harvesting Wt. (kg)	3.24±0.38[a]	3.48±0.92[a]	4.19±0.20[a]
Mean DWG (g·fish^{-1}·day^{-1})	0.55±0.04[a]	0.56±0.15[a]	0.66±0.01[a]
Survival (%)	80.00±3.85[a]	84.44±2.94[a]	86.67±3.85[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	0.25±0.03[a]	0.26±0.07[a]	0.32±0.02[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	0.24±0.03[a]	0.26±0.07[a]	0.31±0.02[a]
Naini			
Mean Stocking Wt. (g)	1.93±0.15	2.16±0.14	2.01±0.09
Total Stocking Wt. (g)	38.6±2.92	43.27±2.83	40.10±1.70
Mean Harvesting Wt. (g)	206.67±5.91[a]	203.07±6.27[a]	200.62±15.19[a]
Total Harvesting Wt. (kg)	2.88±0.23[a]	2.42±0.81[a]	3.27±0.43[a]
Mean DWG (g·fish^{-1}·day^{-1})	0.85±0.03[a]	0.84±0.03[a]	0.83±0.06[a]
Survival (%)	70.00±7.64[a]	60.00±20.82[a]	81.67±10.14[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	0.22±0.02[a]	0.18±0.06[a]	0.25±0.03[a]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	0.22±0.02[a]	0.18±0.06[a]	0.25±0.03[a]
Nile tilapia			
Mean Stocking Wt. (g)	-	4.50±0.23	4.17±0.05
Total Stocking Wt. (g)	-	270.03±14.06	250.3±3.15
Mean Harvesting Wt. (g)	-	155.63±6.01[a]	156.17±5.41[a]
Total Harvesting Wt. (kg)	-	6.50±0.66[a]	5.94±0.29[b]
Mean DWG (g·fish^{-1}·day^{-1})	-	0.63±0.02[a]	0.63±0.02[a]
Survival (%)	-	69.44±5.47[a]	63.33±0.96[a]
Extrapolated GFY (t·ha^{-1}·yr^{-1})	-	0.65±0.09[a]	0.56±0.01[b]
Extrapolated NFY (t·ha^{-1}·yr^{-1})	-	0.63±0.09[a]	0.54±0.01[b]

Parameters	Treatment		
	Carp (T_1)	Carp+ Tilapia (T_2)	Carp + Tilapia + Sahar (T_3)
Sahar			
Mean Stocking Wt. (g)	-	-	7.22±0.32
Total Stocking Wt. (g)	-	-	144.57±6.37
Mean Harvesting Wt. (g)	-	-	104.61±9.49
Total Harvesting Wt. (kg)	-	-	1.81±0.20
Mean DWG (g·fish^{-1}·day^{-1})	-	-	0.41±0.04
Survival (%)	-	-	86.67±6.01
Extrapolated GFY (t·ha^{-1}·yr^{-1})	-	-	0.14±0.02
Extrapolated NFY (t·ha^{-1}·yr^{-1})	-	-	0.13±0.02

Total number and weight of small tilapia recruits in T_2 were higher than in T_3 while there were no medium tilapia recruit in T_3 (Table 3). However, total number and weight of large sized tilapia recruits were higher in T_3 compared to T_2.

Table 3: Tilapia recruitment in different treatments (weight in mean ± SE). Data based on 200 m^2 pond area.

Recruit Size	Parameters	Treatment	
		Carp+ Tilapia (T_2)	Carp+ Tilapia+ Sahar (T_3)
	Total count (no.)	2829	795
	Total wt. (kg)	4705	1205
Small (2-3 cm)	Mean wt. (g)	1.66±0.06	1.53±0.06
	Total count (no.)	32	
	Total wt. (kg)	455	
Medium (5-6 cm)	Mean wt. (g)	14.02±0.43	
	Total count (no.)	120	149
	Total wt. (kg)	3035	3290
Large (12-15 cm)	Mean wt. (g)	15.25±0.60	21.79±1.32

Water quality parameters of different treatments are shown in Table 4. There was no significant difference in average temperature or DO among treatments during the experimental period however, transparency was significantly higher in T_2 than in T_1 and T_3. Temperature decreased from September to January and later increased from January to May.

Table 4: Water quality parameters (mean ±SE with range in parentheses) of different treatments. Mean values in a row with same superscript are not significantly different (α = 0.05).

Parameters	Treatments		
	T_1	T_2	T_3
Temperature	23.03 ± 0.40^a	23.3 ± 0.20^a	23.48 ± 0.03^a
	(11.3-32.7)	(12.6-32.5)	(13.4-32.6)
pH	6.7	6.3	6.5
	(5.7-8.9)	(5.1-9.1)	(5.4-8.5)
DO	4.99 ± 0.82^a	5.70 ± 0.04^a	5.37 ± 0.34^a
	(2.1-7.2)	(4.9-6.6)	(4.0-6.5)
Transparency	33.38 ± 0.31^a	35.29 ± 0.49^b	32.14 ± 0.59^a
	(24-55)	(25-51)	(24-50)

The variable costs in all treatments consisted of seed, feed, feeding tray, lime, urea, DAP and FYM. Variable costs and outputs of different treatments are shown in Table 5. Cost of feed and total variable cost in T_1 was significantly lower than T_2 and T_3 ($p<0.05$). There was no significant difference in all other variable costs among different treatments ($p>0.05$). There was no significant difference in outputs from all fishes among different treatments ($p>0.05$). The gross profit margin was significantly higher in T_3 compared to T_1 without any significant difference between T_2 and T_3 (Table 5).

Table 5: Economic analysis (in USD) for each treatment. Data based on 200 m^2 pond area. Mean values in a row with the same superscript are not significantly different (α = 0.05).

Variables	Treatments		
	T_1	T_2	T_3
Seed	3.00	4.80	5.80
Feed	29.98 ± 0.21^b	36.67 ± 2.12^a	37.24 ± 0.58^a
Lime	2.43	2.43	2.43
Urea	1.57	1.57	1.57
DAP	0.97	0.97	0.97.
Feeding Tray	1.00	1.00	1.00
FYM	5.55	5.55	5.55
Total input	45.01 ± 0.21^b	53.50 ± 2.12^a	55.07 ± 0.58^a
Total output	71.00 ± 6.53^b	84.87 ± 5.76^{ab}	102.22 ± 4.80^a
Gross margin	24.90 ± 6.32^b	31.37 ± 3.54^{ab}	47.15 ± 4.22^a
Gross margin/ha	1299.50 ± 315.90^b	1568.65 ± 177.00^{ab}	2357.4 ± 210.8^a

Experiment 2: Pond trials in farmer's ponds. The addition of Nile tilapia and sahar into carp polyculture ponds did not affect the growth and production of all carp species (Table 6). All carp species showed better performance in all treatments with a daily weight gain of 0.8 to 3.0 g. The daily weight gain of Nile tilapia and sahar were 0.7 and 0.03 g, respectively.

Gross and net fish yield of common carp, silver carp and mrigal were significantly higher in polyculture of carps, tilapia and sahar compared to polyculture of only carps (P < 0.05). There were no significant differences in gross and net fish yield of bighead carp, grass carp and rohu between the treatments.

The combined gross and net fish yield was significantly higher in polyculture of carps, tilapia and sahar compared to polyculture of only carps ($p < 0.05$, Table 7). There was no significant difference in overall

food conversion ratio between the treatments. There was also no significant difference in water quality parameters between two treatments (Table 8). Economic analysis showed that the gross margin was significantly higher in polyculture of carps, tilapia and sahar compared to polyculture of only carps ($p <$ 0.05, Table 9).

Table 6. Individual performance of carps, Nile tilapia, and sahar (mean $\pm$ SE) in each treatment. Data based on 500 m^2 pond area. Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

Parameters	Treatments	
	Carp+tilapia+sahar	Carps only
Common carp		
Stock number	125	125
Total stock weight (kg)	0.6±0.0 [a]	0.6±0.0 [a]
Mean stock weight (g·fish[-1])	4.7±1.9 [a]	4.9±0.3 [a]
Harvest number	87.8±7.6 [a]	81.7±5.8 [a]
Total harvest weight (kg)	42.9±4.0 [a]	21.6±1.7[b]
Mean harvest weight (g·fish[-1])	489.5±18.4	265.0±13.8
Daily weight gain (g·fish[-1]·day^{-1})	3.0±0.1	1.6±0.1
Gross fish yield (t·ha^{-1}·crop[-1])	0.9±0.1 [a]	0.4±0.0[b]
Net fish yield (t·ha^{-1}·crop[-1])	0.8±0.1 [a]	0.4±0.0[b]
Survival (%)	70.3±6.1 [a]	65.4±4.7 [a]
Silver carp		
Stock number	175	133
Total stock weight (kg)	0.9±0.1[a]	0.7±0.1 [a]
Mean stock weight (g·fish[-1])	5.3±0.5 [a]	5.3±0.3 [a]
Harvest number	121.6±13.3 [a]	88.0±6.5[b]
Total harvest weight (kg)	39.8±4.3 [a]	28.1±2.5 [a]
Mean harvest weight (g·fish[-1])	317.9±49.8 [a]	328.7±33.9 [a]
Daily weight gain (g·fish[-1]·day^{-1})	1.9±0.3 [a]	2.0±0.2 [a]
Gross fish yield (t·ha^{-1}·crop[-1])	0.8±0.2 [a]	0.6±0.0[b]
Net fish yield (t·ha^{-1}·crop[-1])	0.8±0.2 [a]	0.5±0.0[b]
Survival (%)	69.5±7.9 [a]	66.0±0.0 [a]
Bighead carp		
Stock number	50	57
Total stock weight (kg)	1.2±0.0 [a]	1.4±0.2 [a]
Mean stock weight (g·fish[-1])	24.4±0.8	23.6±0.9
Harvest number	50.0±0.0 [a]	39.9±4.2[b]
Total harvst weight (kg)	16.5±2.6 [a]	13.0±1.6 [a]
Mean harvest weight (g·fish[-1])	329.8±52.5 [a]	322.5±14.5 [a]
Daily waight gain (g·fish[-1]·day^{-1})	2.0±0.3 [a]	2.0±0.1 [a]
Gross fish yield (t·ha^{-1}·crop[-1])	0.3±0.1 [a]	0.3±0.0 [a]
Net fish yield (t·ha^{-1}·crop[-1])	0.3±0.1 [a]	0.2±0.0 [a]
Survival (%)	100.0±0.0 [a]	70.0±0.0[b]
Grass carp		
Stock number	25	57
Total stock weight (kg)	0.4±0.0[b]	0.9±0.1 [a]
Mean stock weight (g·fish[-1])	15.8±0.6 [a]	16.5±0.0 [a]
Harvest number	24.9±0.1[b]	45.6±4.8 [a]
Total harvest weight (kg)	8.1±1.1[b]	15.2±2.7 [a]
Mean harvest weight (g·fish[-1])	324.7±44.1 [a]	320.5±38.8 [a]
Daily weight gain (g·fish[-1]·day^{-1})	2.0±0.3 [a]	1.9±0.2 [a]
Gross fish yield (t·ha^{-1}·crop[-1])	0.2±0.0 [a]	0.3±0.1 [a]
Net fish yield (t·ha^{-1}·crop[-1])	0.2±0.0 [a]	0.3±0.1 [a]

Survival (%)	99.6±0.4 [a]	80.0±0.0 [b]
Rohu		
Stock number	75	85
Total stock weight (kg)	0.3±0.0 [a]	0.4±0.0 [a]
Mean stock weight (g·fish^{-1})	4.5±0.1 [a]	4.5±0.1 [a]
Harvest number	75.0±0.0 [a]	68.4±6.3 [a]
Total harvest weight (kg)	9.5±1.3 [b]	17.4±2.9 [a]
Mean harvest weight (g·fish^{-1})	126.4±16.8 [a]	249.2±25.0 [a]
Daily weight gain (g·fish^{-1}·day^{-1})	0.8±0.1 [b]	1.5±0.2 [a]
Gross fish yield (t·ha^{-1}·crop^{-1})	0.2±0.0 [a]	0.3±0.1 [a]
Net fish yield (t·ha^{-1}·crop^{-1})	0.2±0.0 [a]	0.3±0.1 [a]
Survival (%)	100.0±0.0 [a]	80.0±0.0 [b]
Mrigal		
Stock number	50	63
Total stock weight (kg)	0.2±0.0 [b]	0.4±0.0 [a]
Mean stock weight (g·fish^{-1})	4.7±0.2 [a]	5.5±0.0 [a]
Harvest number	50.0±0.0 [a]	52.1±8.0 [a]
Total harvest weight (kg)	7.1±1.2 [b]	12.3±2.2 [a]
Mean harvest weight (g·fish^{-1})	142.0±25.4 [b]	234.4±9.3 [a]
Daily weight gain (g·fish^{-1}·day^{-1})	0.9±0.2 [b]	1.4±0.1 [a]
Gross fish yield (t·ha^{-1}·crop^{-1})	0.1±0.0 [b]	0.2±0.0 [a]
Net fish yield (t·ha^{-1}·crop^{-1})	0.1±0.0 [b]	0.2±0.0 [a]
Survival (%)	100.0±0.0 [a]	82.4±11.5 [a]
Tilapia		
Stock number	150.0	-
Total stock weight (kg)	0.6±0.0	-
Mean stock weight (g·fish^{-1})	3.8±0.1	-
Harvest number	150.0±0.0	-
Total harvest weight (kg)	18.4±2.9	-
Mean harvest weight (g·fish^{-1})	122.6±19.0	-
Daily weight gain (g·fish^{-1}·day^{-1})	0.7±0.1	-
Gross fish yield (t·ha^{-1}·crop^{-1})	0.4±0.1	-
Net fish yield (t·ha^{-1}·crop^{-1})	0.4±0.1	-
Survival (%)	100.0±0.0	-
Sahar		
Stock number	50	-
Total stock weight (kg)	0.5±0.0	-
Mean stock weight (g·fish^{-1})	10.1±0.2	-
Harvest number	50.0±0.0	-
Total harvest weight (kg)	1.3±0.3	-
Mean harvest weight (g·fish^{-1})	26.5±5.6	-
Daily weight gain (g·fish^{-1}·day^{-1})	0.03±0.01	-
Gross fish yield (t·ha^{-1}·crop^{-1})	0.02±0.01	-
Net fish yield (t·ha^{-1}·crop^{-1})	100.0±0.0	-
Survival (%)	100±0.0	-

Table 7. Combined performance of carps, Nile tilapia and sahar in each treatment. Data based on 500 m^2 pond area. Mean values with different superscripts in the same row were significantly different (P < 0.05).

Parameters	Treatments	
	Carp+Tilapia+Sahar	Carps only
Gross fish yield (t·ha^{-1}·crop^{-1})	2.9±0.4 [a]	2.2±0.1 [b]
Net fish yield (t·ha^{-1}·crop^{-1})	2.8±0.4 [a]	2.1±0.1 [b]
Gross fish yield (t·ha^{-1}·yr^{-1})	5.8±0.8 [a]	4.4±0.2 [b]
Net fish yield (t·ha^{-1}·yr^{-1})	5.6±0.8 [a]	4.2±0.2 [b]
Overall survival (%)	89.9±2.3 [a]	74.0±2.4 [b]
Apparent food conversion ratio	2.2±0.3 [a]	2.6±0.4 [a]

Table 8. Overall mean and range values of water quality parameters in each treatment.

Parameters	Treatments	
	Carp+Tilapia+Sahar	Carps only
Temperature (°C)	27.4±2.4 (21.3 – 35.1)	28.3±2.6 921.0 – 34.5)
Dissolved oxygen (mg·L^{-1})	3.4±0.2 (0.3 – 6.7)	3.4±0.2 (1.4 – 5.1)
pH	7.6 (7.1 – 9.0)	7.5 (7.0 – 8.3)

Table 9. Economic analysis (in USD) for each treatment. Data based on 500 m^2 pond area. Mean values in a row with the same superscript are not significantly different ($\alpha = 0.05$).

Parameters	Treatments	
	Carp+Tilapia+Sahar	Carps only
Seed	16.0±0.0	13.2±0.1
Feed	97.9±5.0	63.9±2.3
Lime	5.0±0.0	5.2±0.1
Fertilizer	31.0±0.1	29.2±0.8
Water filling, Feeding Tray	6.5±0.1	6.6±0.1
Total input	156.5±5.0	118.0±2.5
Total output	317.4±22.3	208.0±12.9
Gross margin	161.0±18.4 [a]	90.0±12.5 [b]
Gross margin/ha	3219±367 [a]	1800±250 [a]

DISCUSSION

This study was carried out to demonstrate the role of Nile tilapia and sahar in improving the productivity of carp polyculture. In both trials, addition of Nile tilapia and sahar did not have any adverse effect on growth and production of all carp species as well as in pond water quality. This result suggests that tilapia and sahar did not have competition for pond resources with any carp species.

The growth rates of all carp species were almost similar in both trials. However, the average growth rate of carp species in all treatments was lower than reported by previous studies in carp polyculture (da Silva et al. 2008, Rai et al. 2008, Jaiswal 2010). Lower growth rate of carps in the present study might be due to lower average temperature during experiment period. In the first trial, the daily weight gain of Nile tilapia and sahar were 0.6 and 0.4 g, respectively, whereas in the second trial, the daily weight gain of Nile tilapia and sahar were 0.7 and 0.03 g, respectively. The daily weight gain of Nile tilapia in both trials was higher than grass carp-tilapia polyculture system (0.2-0.5 g) (Pandit et al. 2004) and similar with tilapia-

sahar polyculture systems (0.6-0.9 g) (Shrestha et al. 2011), but lower than tilapia-sahar polyculture systems (1.15 g) (Acharya et al. 2007). Similarly, the daily weight gain of sahar in the first trial was higher than tilapia-sahar polyculture systems (0.3-0.4 g) (Shrestha et al. 2011) and almost similar to values (0.55-0.77 g) achieved in other systems (Islam 2002).

In the first trial, growth and production of original stock of Nile tilapia in T_2 (carp-tilapia) and T_3 (carp-tilapia-sahar) did not differ significantly suggesting that sahar did not affect the growth and production of Nile tilapia. However, production parameters like total harvested weight, extrapolated GFY and NFY were significantly affected by addition of sahar. This may be attributed to the fact that sahar consumes newly recruited tilapia. Total number and weight of small tilapia in carp-tilapia treatment (T_2) was significantly higher than that in carp-tilapia-sahar treatment (T_3). However, total number and weight of large tilapia recruits in T_2 and T_3 did not varied significantly. This may due to recruitment during nonpiscivorous stage of stocked sahar. Shrestha et al. (2011) also reported that there was significant lower average recruit number and weight of Nile tilapia in treatment with sahar than tilapia monoculture. Jaiswal (2010) also showed that average number and weight of tilapia recruits was lower in treatments with sahar than tilapia and carp only. Several other studies have also shown that number and weight of recruited Nile tilapia is lower in ponds with predator species (Yi et al. 2004, de Graaf et al. 2005, Acharya et al. 2007, Poudel et al. 2007, Jaiswal 2010, Shrestha et al. 2011).

In the farm trial, the combined gross fish yield, net fish yield and gross margin were significantly higher in polyculture of carps, tilapia and sahar treatment compared to polyculture of only carps.

Water quality was not significantly affected by stocking densities of fishes in species combination of carps, carp-tilapia, carp-tilapia-sahar polyculture in ponds, as water quality parameters did not differed significantly among treatments. Most water quality parameters in both trials were within acceptable ranges for fish culture (Boyd 1990).

CONCLUSION

Adding Nile tilapia and sahar did not affect the growth and production of carp species if included in carp polyculture system. However, adding these species can increase the total productivity in terms of GFY and NFY. Also, the problem of tilapia recruitment can be addressed by the introduction of sahar in polyculture. Thus, it can be concluded that Nile tilapia and sahar can be added in carp polyculture ponds without affecting the productivity of carp species.

QUANTIFIED ANTICIPATED BENEFITS

On-station experiment showed 25.5% increase in yield and in on-farm trial with six farmers showed 33.3% increase with this new production system.

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New Approaches to Inform, Motivate, and Advance Small and Mediums-scale Fish Farmers: Building Industry Capacity Through Cell Phone Networks, Training, and Market Participation

Production System Design and Best Management Alternatives/Study/13BMA04AU

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ABSTRACT

Mobile phones can improve aquaculture productivity by increasing access to technical guidance, extension services, product assembly, input coordination, and price discovery for small- and medium-scale fish farmers. Data were obtained in five focused group interviews held across Uganda. The findings indicate that the use of mobile phones is common among fish farmers in Uganda. Majority of the farmers reported that their mobile phones were primarily used for purposes that improve social development and livelihoods. Many fish farmers report using mobile phones to acquire technical guidance, contact family members, and communicate with those who provide agricultural inputs and market information, which results into increased income. The study also highlighted that access to agricultural information has been widespread, but support is needed for disseminating information on market prices and fish production. At present, most farmers depend on the word of mouth to get information from extension officers and intermediary fish farmers, who are not always available when needed. Farmers prioritized information on pond management, feed broodstock and water quality management, stocking and harvesting, and, most importantly, market prices. Although farmers were also interested in other categories of information, such as fish diseases, seed variety, fish species to be cultured, etc., only a small sample prioritized them.

INTRODUCTION

Aquaculture productivity in Uganda is limited, not from technical or genetic barriers, but from lack of compliance with known and standard methods for producing fish in earthen ponds and cages. Most small-scale fish farmers in Africa have limited access to reliable information about new and improved methods of farming. Most farmers do not attend agricultural fairs (where they exist), and aquaculture extension workers do not reach every farmer. In addition, extension workers who visit the farmers often give them discrepant information, leaving farmers confused (Mwangi 2008). Thus, farmers rely on traditional knowledge, experience and guesswork to make decisions for day-to-day activities, which has proved to be ineffective in managing a nontraditional enterprise like aquaculture.

Access to appropriate information, inputs, and technical support are significant determinants for maintaining a successful farming business (World Bank 2013). Farmers need to have access to agricultural information in order to improve their agricultural production (Adomi et al. 2003). Utilization of available information by farmers is very important because it justifies the efforts by research and related organizations to improve farmers' activities and output, among other factors (World Bank 2013). Information and communication technologies, such as mobile phones, could aid greatly in rural

development and poverty reduction within developing countries due to an increase in local people's ability to obtain information for sound decision-making (Hudson 2006). Mobile phones can improve aquaculture productivity by increasing access to technical guidance, extension services, product assembly, input coordination, and price discovery for small-scale fish farmers. Mobile phones have a rapid diffusion rate and facilitate farmers' access to information, helping increase their bargaining power, control over external events, develop new skills and grow revenues (Myhr and Nordstrom 2008). They can enable traders to reach more markets and establish wider contacts. Furthermore, mobile phones play a big role in providing information on market, weather, transport, and agricultural techniques through concerned agencies and departments (Aker 2011). For instance, in Tanzania the arrival of mobile phones, under the Vodafone Group, transformed agricultural business performance at all points by augmenting farmers' access to education and vital market information (Timuray 2014).

Mobile phones have the ability to provide information, and thus encourage greater production efficiency. Many dairy farmers in the Bugerere District in central Uganda were travelling approximately 75 miles to the main market in the capital (Kampala) blindly searching for buyers at the market. This often at times results in farmers having thousands of liters of unsold milk, which inevitably spoil and become worthless. However, after adopting the use of mobile phones, the farmers began using them to connect to Food Net, a service that supplies up-to-date price information for agricultural commodities, as well as contact details for interested buyers via text message (Karamagi and Nalumansi 2009).

The adoption of mobile phones by fishermen along the coast of India's Kerala State showed that the proportion of fishermen who travelled beyond their usual markets in Kerala to sell their fish jumped from 0% to about 35%. Furthermore, time wastage was eliminated completely, and the "law of one price" — the idea that in an efficient market, identical goods should cost the same — would come into effect (Jensen 2007) studied. Aker (2008) also reported similar results from her study on grain traders in Niger. Her study showed the primary mechanism by which mobile phones affect market-level outcomes appears to be a reduction in search costs; traders operating in markets with cell phone coverage search over and sell in a greater number of markets. The use of mobile phones by 134 younger agriculture-based entrepreneurs resulted in an expansion of their information network and faster information accessing speed that positively impacted their business profits (Shaffril et al. 2009).

Coupled with corresponding innovation in existing social and institutional arrangements, mobile phones have the potential to significantly increase the income of the small-scale fish farmers (Verheye 2000). As mobile phones converge with other devices, such as notebooks and tablets, opportunities will proliferate. Affordability will remain an issue, but cell phone capability and market penetration will grow. However, little is known about the use of mobile phones and the needs and interests of fish farmers in Uganda. There is a need to understand the use of mobile phones, and the needs and interests of fish farmers. Public agencies, nongovernmental organizations, and cellular service providers may be able to facilitate the use of cell phone as a means to guide, coordinate, and instruct fish farmers.

OBJECTIVES
- Assess fish farmer needs and expectations for cell phones as a source of information, technical guidance, and applications;
- Develop a program of technical collaboration among researchers, government technical staff, and cellular providers to advance aquacultural development; and
- Build on existing farmer-based institutions to use national trade shows, train-the-trainer, symposia and other events to stimulate value chain development and attention to proven production practices.

MATERIALS AND METHODS

Five focused group interviews were conducted in five districts of Uganda (Masaka, Mpigi, Bushenyi, Mukono, and Kalungu). A total of 48 small to medium scale fish farmers, comprising of 34 men and 14 women, participated in the interviews between the months of May and July 2014. Most fish farms in Uganda are owned by men. In order to include both genders in the discussion, one group was organized based on gender and at least one or two female fish farmers, out of the seven or eight total participants, were included. The size of each group was decided according to the proposition of Hennink (2014), who argues the number of participants in each 23 group should be six to eight for easy management, smooth interaction, rich details, and for equal opportunity to share insights. This research project received Auburn University Institutional Review Board approval, and consent from participants was obtained prior to the interview. A breakdown of the focus group is presented in Table 1 and a pictorial in Figure 1.

All the interviews were conducted in Luganda, and each was two hours in length and digitally recorded. This sample size was in line with Roscoe's (1975) rule of thumb that states a sample size between 30 and 500 is sufficient for a research study. Farmers were purposely selected for their voluntary participation through the help of Grameen Field Officers and Community Knowledge Workers (CKWs). All the participants were not remunerated, however, a light lunch and refreshments were given to them. All responses were transcribed verbatim and treated using thematic analysis. All responses were read multiple times, both to manually develop appropriate codes (Kelle 2004) and to uncover new or unique themes not identified in prior research. The data was systematically coded by writing names and a brief description of each code on a separate piece of paper to indicate potential patterns simultaneously categorized, summarized, and accounted for each theme in the data (Hennink 2014). Identified codes were then matched with data extracts to form a codebook from which themes emerged. Themes that emerged from the coded data (discussions) were identified and the name of each theme was finalized by writing a description to help communicate the meaning.

Table 1: Sites of focused group interviews with fish farmers, Uganda 2014

District	Date	Meeting place	Number of participants
Bushenyi	07.07.2014	Fish farmer home	9
Mpigi	07.18.2014	Fish farmer home	8
Masaka	07.15.2014	Hotel	8
Kalungu	08.05.2014	Sub-county office	9
Mukono	06.16.2014	Primary School	14

RESULTS

Use of mobile phones among fish farmers. Participants identified a number of roles that mobile phones play within the context of the aquaculture industry. These include marketing and coordination services, in particular, technical guidance, payment collection, and contacting family members. Extension services were discussed, but less emphasis was placed on it by participants in all the five focus group interviews. Mobile phones save time and reduce the distance between fish farmers and producers, as well as other fish farmers making the sharing of information and knowledge easier and more effective. Participants identified a number of roles that mobile phones play within the context of the aquaculture industry.

Coordination purposes. Farmers recognized the significance of mobile phones as a new form of technology not previously available to use. The farmers' responses to mobile phone usage and its efficiency were based on how mobile phones were used to make plans for procurement of fish farm inputs, such as seeds and feeds from fellow fish farmers, fisheries research centers, and non-governmental organizations (NGOs) that help to increase their income and productivity. Mobile phone use also included farmers receiving calls from their fellow fish farmers inviting them to attend group trainings on a village

level. They also indicated that intermediary farmers play an important role in providing technical guidance and information regarding fish farming.

"Without a phone, I would have been forced to walk and look for the market. That would have taken a lot of time." (Woman, about 55 years old)

For some of these farmers, a mobile phone represented the only appropriate and efficient means of communication. For many of the small farmers, the savings stemmed typically from avoiding local travel, with a cost range of 5,000–10,000 Ugandan Shillings per trip. The use of mobile phones also delivered convenience benefits to farmers who were starting to substitute some physical meetings with mobile phone conversations.

"… without mobile phones, we could spend a lot of money on travelling in order to get feeds and seeds without even contacting the service providers, but only to find that they are out of stock, I do not wish to live that kind of life anymore … It is good that I now own a mobile phone so I do not have to leave my fish ponds to get inputs. All I need to do is contact the service providers via my phone to know if the products are available" (Man, about 40 years old).

During harvesting, farmers use their mobile phones to call fellow farmers who have been in fish farming for some time, or call technical personnel from the Kajjansi Aquaculture Research Development Centre (KARDC) seeking advice on better ways of harvesting and handling fish during harvest and transportation.

Attain prices and market access. The long distance from farm to market has hindered the gathering of information about prices, but mobile phone use is efficiently fulfilling this gap by providing timely information about the market situation, transport, and agricultural prices. Farmers pointed out that the existence of mobile phones have made it easier for them to communicate with businessmen and middlemen by informing them of the availability of fish. Phones also enabled farmers to know the prevailing market prices of cultured fish in various markets, which enabled them to have bargaining power and sell fish at higher prices.

"… we talk with brokers by making a phone call and asking them about prices of fish per kilogram and also find out whether there is market for our fish, and if you have more than one ton of fish, they come directly to your farm and purchase them … they offer good prices and sometimes low prices … if you are not lucky enough on some days you can end up selling your fish at a zero profit. The government should set up standard market prices." (Man, about 40 years old)

Mobile banking and making payments. Farmers indicated that mobile payment systems gave them opportunity to access financial services and provided an inexpensive and secure way to transfer and save money using their mobile phones by incurring fewer charges. They allow small-scale farmers to save money, receive payments quickly in times of need, and pay for agricultural inputs via their phones.

Mobile payment systems replaced costly traditional bank transfer services and the need to travel long distances to collect funds from financial institutions. Before the introduction of mobile-money banking and transfer, farmers would spend too much time moving from financial institutions [such as Pride Micro Finance (PMF), Stanbic bank, Back of Africa, Barclays, Finance Trust bank, Orient bank, and Tropical bank] to save or receive money. Farmers would rather make use of mobile money services, and highlighted they no longer have to travel long distances to visit a bank, get funds, or make a transfer.

"… Mobile money helps us to save small amounts of money, receive payments quickly in times of need, pay for agricultural inputs, make mobile payments… replaces costly traditional transfer services and

reduce the need to travel long distances to collect funds….before the introduction of mobile-money, Warid pesa, Mpesa, we used to waste too much time moving to financial institutions to make payments, receive money or save money, and sometimes we could end up foregoing family activities." (Youth, about 26 years old)

Technical guidance. A number of farmers mentioned that they have tried to get technical guidance from their fellow fish farmers via their mobile phones. A few literate farmers have even tried to use the Google search engine on their phones to get information related to farming, but the information available is hard for them to follow and understand. In addition, farmers indicated that voice calls are more frequently used than SMS due language barriers and illiteracy.

"Farmers make voice calls more than sending and receiving SMS. The reason behind is that most farmers stopped is lower levels of education, they really find it hard to type and read a text message, and some think typing a message takes too much time; therefore, they prefer voice calls since they also have a good response rate than SMS." (Man, about 35 years old)

It was indicated that farmers usually have no one to contact in case of an emergency on their farm. Some of them have never had anyone give them any technical advice on how to go about fish farming. Farmers venture into fish farming without any fisheries background, and this has resulted into low productivity.

"We have more than 100 fish farmers in our district, but we have only one district Fisheries Officer to serve both fish farmers and fishermen –yet, farmers have diverse questions which an Officer may not handle even if he reached them since he is not a trained personnel." (Man, about age 48 old)

Contacting family members. Another main use of mobiles for the Ugandan fish farmers is to keep in touch with their relatives while in the field or while carrying out other businesses far away from home. This is a very good opportunity for them to make good progress in their daily fish farming activities without worries. Before the introduction of mobile phones, they were either prevented from getting in touch with relatives or had to forego farming activities in the case of family emergencies. Today, whenever the need arises, they are able to stand with their households while farming.

"…where I make my daily fish farming activities is far away from where my family and other relatives live. Through the use of my mobile phone, I can easily communicate with my family, getting to know how they are doing. Sometimes when there is an emergency, for example one of the family members is sick. When contacted, I immediately tell them to go ahead and take him or her to the hospital and then send money for covering the expenses through mobile money or Warid pesa." (Man, about 42 years old)

Challenges faced by fish farmers while using mobile phones. Mobile phones can act as a means of aquaculture information dissemination because of its wide reach and low cost of delivering critical information. Another benefit is greater flexibility since they enable information dissemination to the fish farmers through both voice and text messages. Despite this, there are certain factors that constrain the full utilization of the potential use of mobile phones by small-scale fish farmers in Uganda. Some fish farmers' perceptions of the dominant constraints of mobile phone use are outlined below.

Lack of access to electricity. Many farmers in rural communities of the country have no proper electric connections, and even where there is power, the challenge of power cuts is more recurrent than power accessibility. Some farmers indicated that their phone batteries do not hold a charge for a good period of time. Staying in areas where the power cuts are frequent and power availability is limited negatively impacts fish farmer's day-to-day activities.
"Weaker mobile battery systems that need to be always charged are a very serious issue yet we have no constant power supply." (Woman, about 45 years old)

Poor network connectivity. Mobile phones are accelerating ways in which farmers acquire, exchange, and maneuver information in developing countries, but around a million mobile users in rural communities of Uganda face unreliable networks. Therefore, more needs to be done to improve the network signal strength provided by mobile phones. It was mentioned that mobile phones are very useful mainly for communication purposes when faced with problems while on the farm, however, optimum use of mobile phone applications is prohibited by poor signals in villages, which limit its possibilities.

"Inadequate calling credit affects the ability to purchase important inputs and this also decreases the chances of getting the best price because of choice limitations on where we could sell ready fish and fingerlings. The middlemen dominate the supply chains and are the key price setters in the system. The farmers are often ignorant of how prices are set and end up taking whatever price they are offered." (Man about 30 years old)

"Sometimes you can have a problem with your water inlet, and outlet not working perfectly, your fish is not responding to feeding very well, the fish are swimming in a sluggish form, or pond is all covered with algae, and you need to contact one of the farmers or extension worker who can provide some guidance on how to handle such issues, but all to find poor network coverage and there is no way can keep in touch with anyone. We really get stuck when such cases occur." (Man, about 47 years old)

High maintenance costs. Many farmers said that it is expensive to maintain and afford the services provided by mobile phones. Lack of access to calling credit is a serious problem faced by the majority of fish farmers, since this hinders communication with customers and access to important information about fish farming. They stipulated that due to their inability to make calls no standard market prices have been set to be able to exploit price differences that exist between major and minor markets.

Lack of awareness and promotion. Most of the time, the farmers are not aware of important application services they can get through mobile phones. Sometimes, they do not know whom to call when they have problems with utilization of the few known services offered on their mobile phones. Therefore, most of them only make phone calls with their ordinary mobile phones. They lamented that poor promotion has prevented them from taking advantage of available mobile services for their farming activities.

"Though most of us use ordinary phones that do not have internet applications, less information has been provided about the use of smart phones and the important benefits they can provide; having inadequate knowledge on mobile phone applications has really affected achievements of our daily farm activities." (Woman, about 45 years old)

Interests and needs of fish farmers. Most fish farmers lack information on how to manage the different stages of fish production. This has partly hampered aquaculture development in most rural areas of Uganda. For this reason, potential farmers have not opted into fish farming and even others are becoming inactive because the usefulness of aquaculture has not been demonstrated to them. If the goal of reducing food insecurity is to be realized, practical actions must be taken to ensure that farmers receive the full package of technical support and guidance they need to benefit from fish farming.

Five focused group interviews with fish farmers indicated that there is great need for a wide range of varying information throughout the aquaculture production process. The broad categories of contextual information required were common to all the farmers, irrespective of their location and species cultured. These information categories were: pond construction, pond management, stocking and harvesting, feed management, brood stock management, water quality management, fingerling production, marketing information, and disease management.

Pond construction and management. Farmers acknowledged lack of knowledge on planning and constructing a pond, yet the most important aspect of pond management is deciding where and how to build the pond. Many farmers were broadly interested in knowing how to choose and prepare the site, construct a pond, locate a sustainable drainage area, determine the level of water a good pond can accommodate, locate a good water source depending on the fish species to be cultured, and finally, how to determine the best water control structure.

Stocking and harvesting. A major concern that was raised by most of the fish farmers was lack of technical knowhow on proper fish stocking and harvesting techniques. A few reported attending at least one or two fish farming trainings. Some indicated that fisheries scientists visited their farms and gave them some advice on how to stock and harvest fish. However, they noted discrepancies in the information given to them during the trainings or visits. Many farmers were disappointed by fish crops they harvested due to the varying information on stocking densities and sizes from the different training programs they had attended.

Feed management. Feed availability, quality of feeds, feeding rates, and acceptable food conversion ratios remain major constraints for small-scale farmers in Uganda. Farmers showed interest in knowing how to acquire good quality feeds, how much should be fed, when to feed, where to place the feeds, and how to make their own feeds. Most of the small-scale farmers mentioned this as a serious challenge to them simply because most of them venture into the business without any training, relying instead on peer information and guidance. This means that they do not have a firm idea on how to raise fish, or how to keep good feeding records. It was mentioned that when farmers buy fingerlings from prominent fish farmers close to their areas of residence, the sellers do not provide them with the necessary information on how to manage and feed the fish. Along these lines, fish farmers noted shortcomings in the quality of feeds sold to them by fellow farmers and other agricultural stores.

Brood stock management. Like any other farming sector, fish farmers require information on how to choose, breed, and manage their broodstock. Farmers showed interest in organizing hatcheries and producing their own fingerlings. They indicated a great interest in acquiring skills about selection of good brooders, fertilization, incubation and hatching, breeding, fish eggs and fish seed management, sex differentiation, suitable environmental conditions for breeding, stocking density of brooders per square meter, containers to be used, amount of water and oxygen needed, and recommended optimum temperature and light for mainly tilapia and catfish production.

Marketing information. Marketing has presented major challenges for many smallholder farmers, with almost all farmers in the five focused group interviews noting poor market infrastructure, unfair trading systems by middlemen, and poor prices as their major drawbacks to better income. Farmers were concerned about getting daily information updates on market prices since all business activities involved in the movement of fish from production to consumption is based on marketing. Market information enables farmers to make rational and relevant decisions. Farmers mentioned that market information such as prices, demand indicators, and logistical information should be made available in the form that is relevant to their decision-making. The market information needs of small scale fish farmers included: information on product planning, current prices, and group marketing.

Water-quality management. Managing water quality is one of the major challenges for fish farmers in Uganda that often limits the success of fish farming enterprises. The objective of pond management is to control water quality, so as to provide a relatively stress-free environment that meets the physical, chemical and biological standards for the fish's normal health and production performance. However, small-scale farmers in Uganda often are not aware of the appropriate environmental conditions for the fish species they raise. When asked what they use to know the quality of water on a daily basis, or before the introduction of seeds, most of them said they never take measurements. Few of them said they use

their hands by inserting them into the water to determine the temperature. This could be one of the reasons why some were not making good business progress, since it was indicated that some fish die off a few days after the stocking process.

CONCLUSION

This study indicates that the use of mobile phones is common among fish farmers in Uganda. Majority of the farmers reported that their mobile phones were primarily used for purposes that improve social development and livelihoods. Many fish farmers indicated that they use their mobile phones to acquire technical guidance, contact family members, and communicate with those who provide agricultural inputs and market information, which results into increased income. The study also highlighted that access to agricultural information has been widespread, but support is needed for disseminating information on market prices and fish production. At present, most farmers depend on the word of mouth to get information from extension officers and intermediary fish farmers, who are not always available when needed.

Farmers prioritized information on: pond management, feed management, broodstock and water quality management, stocking and harvesting, and most importantly, market prices. Although farmers were also interested in other categories of information, like fish diseases, seed variety, fish species to be cultured, etc., only a small sample prioritized them. There appears to be a great deal of potential for reaching smallholder fish farmers in Uganda, since all the fish farmers who participated in the focused group discussions have access to at least one mobile phone. It was also indicated that farmers enjoy the benefits of mobile phones because of the greater flexibility they offer through both voice and text messages. However, factors such as poor network coverage, frequent power cuts, lack of calling credit, awareness, and promotion has constrained the full utilization of the potential use of mobile phones.

In addition, it was found that farmers were excited about using mobile phones to access information on fish farming and market prices. This suggests that using cell phones, given fast growth and expanded connectivity in the country, could boost agricultural development in Uganda and best opportunities for use should be further explored by government, mobile phone service providers, and fisheries research institutions. In order to improve fisheries productivity in Uganda farmers must be able to access agricultural information and current market prices.

Additionally, we have been in discussion with developers of cell phone applications in the private sector and within NaFIRRI. Several efforts are being made to develop a program of technical collaboration among researchers, government technical staff, and cellular providers to advance aquacultural development, reflecting our Objective 2. We seek to identify sustainable business models that weave government expertise and private sector responsiveness to stimulate marketing, disease, and technical assistance applications that are readily accessible, technically current, and economical for producers.

We continue to work with WAFICOS and other fish farmer cooperative to build on existing farmer-based institutions to advance the development and use of cell phone applications by fish farmers (Objective 3). Presentations have been made to national trade shows, train-the-trainer events, and NaFIRRI symposia to stimulate the development of cell phone applications. Cell phones applications seem most immediately to advance value chain development by facilitating price discovery and product availability. These services also can reinforce proven production practices and help resolve management problems.

QUANTIFIED ECONOMIC BENEFITS

Identify target groups and direct and indirect benefits accruing from the research and outreach work. Benefits must be quantifiable.
- Availability of text-based fish market and fingerling supply information;
- New extension mechanism for reaching fish farmers on broad-scale; and
- Augmented value chain for tilapia and other species resulting in added farm-level income.

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TOPIC AREA:
SUSTAINABLE FEED TECHNOLOGY AND NUTRIENT INPUT SYSTEMS

❖

Assessment of Growth Performance of Monosex Nile Tilapia (Oreochromis niloticus) in Cages Using Low-Cost, Locally Produced Supplemental Feeds and Training Fish Farmers on Best Management Practices in Kenya

Sustainable Feed Technology and Nutrient Input Systems/SFT/13SFT06AU

This research is still ongoing and will be included in a future Technical Report.
See Implementation Plan 2013-2015, page 40.

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Formulation and Manufacture of Practical Feeds for Western Kenya

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT07AU

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ABSTRACT

Information about proximate composition of local feed ingredient for farm made fish feed is usually limited and not reliable. Farmers depend on the existing information about the feed composition given by different fish feeds manufacturers. Unbalanced dietary amino acid contents could result in an increased deamination and can increase ammonia levels released in water. This study intended to formulate on farm diets using locally available ingredients and balancing the Essential Amino Acids (EAA) to enhance both the physical quality and nutritive value for culture of *Oreochromis niloticus*. The study tested the four diets, comprising methionin+lysine and lysine supplemented diets at 24.5 g kg^{-1} to non-EEA supplemented and commercial tilapia diets, for 105 days in hapas installed in earthen ponds at the University of Eldoret Fish Farm from June to September 2015. The practical diets consisted of 48% wheat bran, 30% freshwater shrimp, 18% cotton seed meal, 2% fish oil, and 1% vitamin/mineral premix. The four diets were tested for growth performance of *O. niloticus* for 105 days in 1 m^3 hapas in three earthen ponds, each measuring 10 m by 15 m (150 m^2) in a complete randomized block design. Even though there were significant variations in temperature and pH, the values were still within suitable range for tilapia at 18°C to 20°C and 7.2 to 7.0. Dissolved oxygen (DO) did not vary significantly over the period (4.8 to 6.2 mg L^{-1}). All experimental test diets had 30% crude protein (CP) before EAA supplementation. The diets were estimated to provide about 17.17 MJ kg^{-1} with about 22.9% digestible CP level, 8.03% ash, and 90.7% dry matter (DM). Diet 2 with lysine supplement exhibited better growth than all the other diets with a Phi prime ($\varnothing'$) of 3.441, Body Weight Gain (BWG) of 289.8, Average Daily Weight Gain (DWG) of 1.92, Specific Growth Rate (SGR) of 5.4, Food Conversion Ratio (FCR) of 1.24, and Protein Efficiency Ratio (PER) of 2.68. The results show there is a high potential for on-farm fish feed formulation and processing, and the present knowledge will benefit more than 1000 fish farmers in formulation nutritionally balanced diets to improve growth and production of tilapia in Western Kenya. The present protocol will also be adopted in the new hatchery under construction at the University of Eldoret to provide quality fingerlings to more than 600 fish farmers in Uasin Gishu County.

INTRODUCTION

Tilapia farming poses a substantial challenge to fish farming in Sub-Sahara Africa, despite the huge technological leaps achieved worldwide on improved strains. According to Craig and Helfrich (2002), high cost of fish feeds, comprising of over 50% of the production costs limits tilapia production and sustenance. Sustainability and success of aquaculture depends on type of feed used and management. Success of intensive fish culture depends to a large extent on adequate information on nutrient requirements, especially dietary protein, which is the most expensive component in artificial diets (Tacon et al. 2009, 2011). Tilapia feed cost depends dietary protein level, the source, and type of ingredients derived from plant or animal sources.

Fish meal is very expensive thus increasing the cost of fish production. It competes with man for food and for use in aquaculture feeds. The demand and growth of tilapia farming has resulted in the expansion of

nutrient requirement data and improvements in feed formulations (NRC 1993). Selection of a protein ingredient is not limited to only assessing the crude protein levels of feed ingredients, but also involves in-depth knowledge in their amino acid profile and bioavailability. Commercially available ingredients have significant amounts of anti-nutritional factors and digestibility of the proteins is highly variable. It has been generally noted that nutrient are lost during feed manufacturing and improper storage of aqua feeds in low-technology production systems. Fish growth and survival can be compromised by poorly digested feed that can deteriorate water quality. Pellet type and manufacturing process also influence water quality and digestion efficiency of fish species under culture.

Standard nutrient requirement cannot be applied to practical feed formulation since it is not adequate for high density commercial rearing situations. Most of the research is actually done in aquaria and not ponds, therefore, the recommendations are for intensive systems. Many commercial feeds for tilapia contain lower protein levels (17% to 25%), which are considerably below recommended levels to reduce the cost of production (NRC1993). Unbalanced dietary amino acid contents could result in an increased de-amination and can increase ammonia levels released into the water (Hasan et al. 2007). Information about proximate composition of locally feed ingredient for farm made fish feed is usually limited and not reliable. Thus, farmers depends only on the existing information about the feed composition given by different fish feeds manufacturers. There have, however, been several attempts to profile the nutritional value of different feed ingredients from different agro-ecological and geographical locations in the world (Hasan et al. 2007, Tacon et al. 2009, 2011).

Fish diets can be improved considerably by inclusion of essential amino acids (EAAs) and EAA supplements whenever they become limiting for fish growth. It is also important to compose and process a balanced and biologically available levels of EAAs that meet the targeted species nutrient requirements (Nunes et al. 2014).

Appropriate dietary methionine and lysine levels improve the use of other Essential Amino Acids because they have the ability to reduce the oxidation rate of other amino acids (NRC 2011). Profiles on amino acid contents of fish feed ingredients provide valuable information necessary for formulating diets that support maximum growth of the fish under various cultural techniques. Commercial manufactures usually produce feeds in bulk, leaving small-scale fish farmers with the option of buying large quantities of expensive feed, which often goes to waste (Pandey 2013). Small quantities of fish feed required for experimental purposes can be easily made in the laboratory or on-farm, with particular ingredients of known nutritional quality, especially the EEAs.

Feed formulators are now adopting modern and environmentally sound formulation techniques based on nutrient value, on supplementation with crystalline EAAs and on animal nutrient requirements. Commercial feed formulation are intended to meet nutritional requirement with quality product at cheaper prices depending on type of fish species grown. In commercial aquaculture production feed costs can be reduced by developing proper feed management and husbandry strategies to improve fish growth. Best management practices (BMPs) in fish husbandry involve proper stocking densities, nutrient ratios, aeration, and water exchange to reduce metabolites that can deteriorate water quality. Plant proteins that are cheap and locally available are used to supplement animal protein at lower cost. Feeds consisting of soybean, wheat and corn meal, canola meal, and extruded pea seed meal, supplemented with methionine have been used for formulation of diets for carps, tilapia, and catfish without influencing growth performance (Tacon and Metian 2008).

Homemade feed is useful when specific diets are needed to improve fish growth performance. The nutritional requirements for protein, lipids and energy for optimum growth of specific fish species are necessary for formulating a balanced diet. Lysine and methionine are essential amino acids that cannot be synthesized by the body, but are obtained from the diet. Several studies have been conducted on the

Amino Acid Nutrition requirements, composition, supplementation and balanced ratios of protein in aqua feeds. (Ketola 1982, NRC 1993, Ahmadr 2008, NRC 2011, Munguti et al. 2012).

<div align="center">

OBJECTIVES

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- Develop low-cost, improved quality feeds using rice bran and freshwater shrimps (*Caridina niloticus*) as fish meal replacement;
- Assess the costs and benefits of three different feeding regimes in cages;
- Transfer technologies on management of monosex tilapia in cages through training farmers and extension officers; and
- Compare work conducted in this investigation on the use of low-cost supplemental feeds with the accomplishments of 20 years of CRSP-related work in the area.

<div align="center">

MATERIALS AND METHODS

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Study area. The study was conducted at University of Eldoret fish farm for three and a half months from June 2005 to September 2015 in seven earthen ponds of 150 m^2 and in fifteen hapas of capacity 1m^3 suspended in the pond. The hapas for the experiment were made of foul resistant synthetic netting of mesh size 1.5 mm and were closed from all sides except the top. The study was conducted for 15 weeks (105) days from June to September 2015.

Source of experimental fish. Monosex male *O. niloticus* juveniles were obtained from Sagana National Aquaculture Research and Development Centre (NARDC) and transported to the study area under well oxygenated condition in plastic bags. The juveniles were acclimatized in holding tanks for two weeks prior the experimental stocking in hapas and ponds.

Study design. The first experiment was conducted in twelve hapas suspended in one pond while the second experiment was. *O. niloticus* juveniles were stocked at density of sixty fingerlings per hapa. Complete randomized block design was used for the experiment. Four diets were tested for the experiment in triplicate for each treatment. The experimental ponds 150 m^2 were limed at the rate of 2,500 kg ha^{-1} with CaCO$_3$ and fertilized at a rate of 20 kg N and 8 kg P ha^{-1} with urea and diammonium phosphate (DAP) to facilitate growth of phytoplankton and zooplankton.

Feed ingredients and acquisition. The feed ingredients consisted of freshwater shrimp (C. niloticus), cottonseed cake, wheat bran, fish oil, and vitamin/mineral premix. Diet 1 was supplemented with both lysine and methionine at 2.7 g kg^{-1} each and 5.1 g kg^{-1} respectively (Santiago and Lovell 1988, Furuya et al. 2006). Diet 2 supplemented with lysine only at 2.7 g kg$^{-1.}$ Diet 3 did not receive any EAA supplement. Diet 4 was a commercial fish feed with 32% crude protein content. The three experimental diets were formulated at 30% crude protein at the University of Eldoret fish farm of which Diet 3 was used as a control (Table 1). All the diets were tested for three and a half months on monosex Oreochromis niloticus stocking densities of 2 fish m^{-2} in duplicate for growth performance.

Feed preparation and proximate analysis. Feed ingredients were subjected to proximate analysis before diet formulation to determine the crude protein, moisture content, lipid, crude fibre, ash, and digestible carbohydrate. Experimental diets were formulated using Winfeed Ver. 2.8 software. Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. The ingredients were weighed and ground to small particle size (approximately 250 μm) and thoroughly mixed with water to obtain a 30% moisture level. Oil, vitamins, and minerals mixture were added to the diets. The diet was dried for 8 h in the open air and broken to appropriate size of crumbles.

Table 1: Ingredients and chemical composition of experimental diets used for feeding O. niloticus fingerlings before methionine and lysine supplements

Ingredient	Diet 1	Diet 2	Diet 3
Wheat bran	48.0	48.0	48.0
Caridina niloticus	30.0	30.0	30.0
Cotton seed meal	18.0	18.0	18.0
Fish oil	2.0	2.0	2.0
Trace mineral	1.0	1.0	1.0
Lysine%	1.96% + Supplement 2.7 g kg^{-1}	1.96% + Supplement 2.7 g kg^{-1}	No supplement
Methionine%	0.95% + Supplement 5.1 g kg^{-1}	No supplement	No supplement

Feed floatability and quality. The experimental diets were tested for water stability using procedure described by Fagbenro and Jauncey (1995) to determine if the pellet were maintaining its form for at least five minutes before conducting a feeding trial. Water stability was expressed as a percentage of immersed diet weight/initial sample weight (Orire et al. 2010), or as percent loss of dry matter (%LDM) calculated as percent difference in sample weight (minus the initial diet moisture) after re-weighing (Johnston and Johnston 2007), or as percentage of dry matter remaining (%DMR) calculated as % DMR = W_o X (1-M) – Wt /Wo X (1-M) X 100 where W_o = pellet weight as fed, W_t = weight after immersion and drying, and M=moisture content of diet as a proportion (Ruscoe et al. 2005).

Protein digestibility, determination of crude fat, dry matter and ash. The chemical compositions of the formulated diets were determined following AOAC (1990) procedures: dry matter, by drying in an oven at 105ºC for 8 hours; crude fat, by Soxhlet extraction with ether; crude ash, by incineration in a muffle furnace at 580ºC for 8 hour; crude protein (N× 6 .25), by the Kjeldahl method after acid digestion; non digestible proteins, by Kjeldahl method after enzymatic hydrolysis of the digestible protein with pepsin; finally, digestible proteins were obtained as the difference between the crude and nondigestible proteins.

Feeding the experimental fish. Feeds were offered daily in hapas and ponds by broadcasting method at 10:00 h and 16:00 h. Initial feeding was offered at 10% of body weight adjusted to 5% and to 3% body weight, respectively. Feeding rations were adjusted after every two weeks.

Sampling water quality parameters. Water quality parameters to be measured include Dissolved oxygen, temperature, conductivity, total dissolved solid (TDS), pH, and ammonia. Water quality was determined three times a week at the surface, in the middle and pond bottom. Dissolved oxygen was measured using model 57 oxygen meter, pH was measured with a glass electrode-pH meter while total transparency was measured using Secchi disk.

Growth parameters and nutrient utilization. Total length of fish sample was measured in cm while weight measurements was taken monthly using 0.01g sensitive weighing balance. At the end of the experiment, all fish from the ponds were harvested, weighed and counted. Growth parameters and nutrient utilization calculated using the following formulas:

i) *Body Weight Gain* $(BWG) = \dfrac{\left[(Final\ weight\ (g)\ -\ Initial\ weight\ (g)\right]}{Initial\ weight\ (g)}$

ii) *Daily Weight Gain* $(DWG) = \dfrac{Final\ weight\ (g)\ -\ Initial\ weight\ (g)}{Time\ interval\ in\ days\ (t)}$

iii) *Specific Growth Rate* (*SGR*) $= \dfrac{\left[Ln(Final\ weight\ (g)) - Ln(Initial\ weight\ (g))\right] \times 100}{Time\ interval\ in\ days\ (t)}$

iv) *Food Conversion Ratio* (*FCR*) $= \dfrac{Weight\ of\ dry\ feed\ (g)}{\left[Final\ weight\ (g)\right] - \left[Initial\ weight\ (g)\right]}$

v) *Protein Efficiency Ratio* (*PER*) $= \dfrac{Final\ weight\ (g) - Initial\ weight\ (g)}{Protein\ consumed\ (g)}$

vi) *Length-frequency Analysis*

Munro's method. This method, based on Munro (1982), uses growth increment data to estimate L_∞ and requires growth increment data file. The approach minimizes the coefficient of variation of:

Ratio $= [\ln (L_\infty\text{-}L_m)\text{-}\ln (L_\infty\text{-}L_r)]/ (t_r\text{-}t_m)$

Where L_m is the initial length, L_r is the length at time t_m and t_r the corresponding dates of length measurements. Each growth increment leads to an estimate of K, given L_∞, and the variance of this estimate is computed. The value of L_∞ is selected which minimizes that variance.

Growth performance index ($\varnothing'$). The growth performance index (phi prime) was computed according to the relationship:

$\varnothing' = \text{Log}_{10} (K) + 2 \bullet \text{Log}_{10} (L_\square)$ (Pauly 1984)

Statistical analysis. The water quality parameters were compared through the growth period using one-way analysis of variance (ANOVA) while the growth of the monosex O. niloticus was compared using Phi prime ($\varnothing'$). All the statistical tests were carried out at a=0.05 using Minitab Version 17 and Statgraphics Version 16.

RESULTS

For temperature, there were significant variations with time ($F_{0.5,15,80} = 4.27$, $p < 0.00005$). Since the p-value of the F-test is less than 0.05, there is a statistically significant difference between the mean temperatures from one level of Days to another at the 95.0% confidence level.

The dissolved oxygen concentration did not show any significance with time ($F_{0.5,15,80} = 1.31$, $p = 0.217$). Since the p-value of the F-test is greater than or equal to 0.05, there was no statistically significant difference between the mean DO from one level of time to another at the 95.0% confidence level.

The water pH was statistically significant in time ($F_{0.5,15,80} = 7.63$, $p < 0.00005$). Since the p-value of the F-test is less than 0.05, there is a statistically significant difference between the mean pH from one level of Days to another at the 95.0% confidence level.

Temperatures were low, but maintained at a range of 18.5–20ºC in all the sampling weeks. Dissolved oxygen levels were at arrange of 4.8–6 mg L^{-1}, while the pH was 7.2–7.6 throughout the study (Figure 1). The range of these critical water quality parameters were within suitable range for tilapia culture.
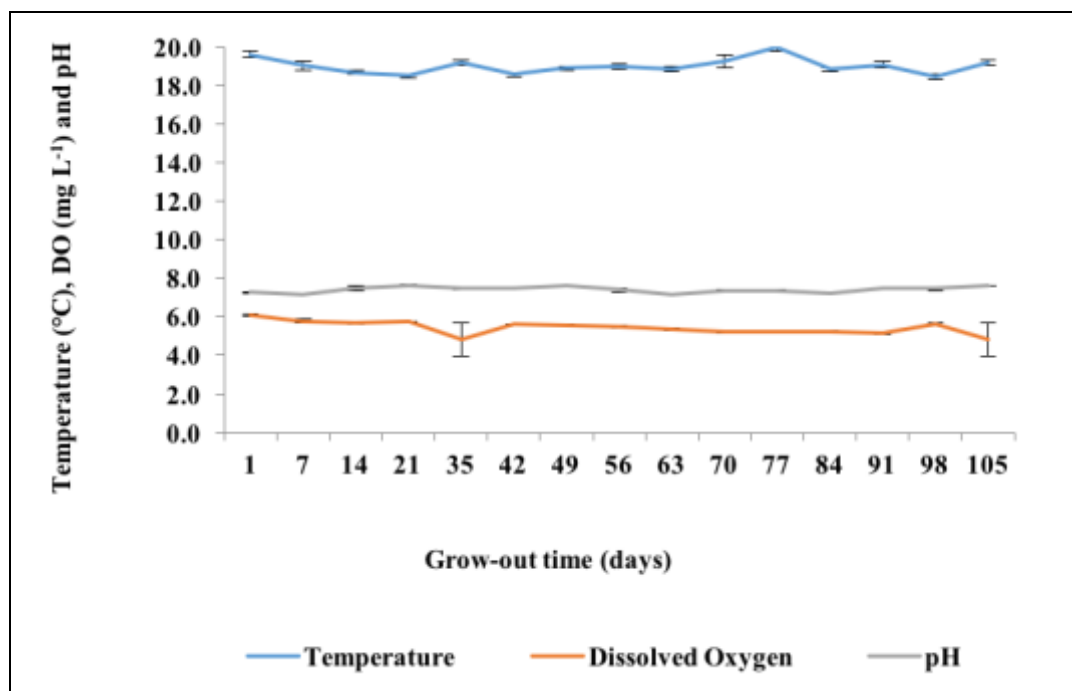
Figure 1: Water quality parameters of experimental pond at University of Eldoret fish farm.

Based on the diet formulation in this experiment, the following nutritional values were calculated based on the ingredient profiles from tropical areas on as-is basis of dry feedstuff (Table 2). The diet formulation provided a gross energy of 17.17 MJ kg^{-1} based on 5.65 kcal g^{-1} for protein 9.45 kcal g^{-1} for Fat, 4.1 kcal g^{-1} for Carbohydrate (Table 3). The conversion of kcal g^{-1} to kJ g^{-1} was carried out through the following relationship: 1 kcal to 4,184 kJ.

Table 2: Nutrient profiles estimates based on diet formulation in the study.

Parameter	Nutritive value	Parameter	Nutritive value
DM%	90.72	Cholesterol%	0.12
Ash%	8.03	Astaxanthin (mg kg^{-1})	804.05
GE MJ kg^{-1}	17.17	Arginine%	2.53
DE MJ kg^{-1}	9.67	Histidine%	0.81
CP%	30.15	Isoleucine%	1.04
Dig CP%	22.91	Leucine%	2.24
Lipid%	5.53	Lysine%	1.96
Fiber%	6.87	Methionine%	0.95
LOA (18:2n-6)%	0.72	M+C%	2.27
LNA (18:3n-3)%	0.03	Phenylalanine%	1.57
ARA (20:4n-6)%	0.02	P+T%	2.62
EPA (20:5n-3)%	0.64	Threonine%	1.21
DHA (22:6n-3)%	0.40	Tryptophan%	0.30
Total n-3%	1.07	Valine%	1.34
Total n-6%	0.75	Ca%	1.44
n3:n6	1.43	Available P%	1.14
Total phospholipids%	1.77		

The proximate analysis of all the diet composition before EAA supplementation for crude protein (CP%), Carbohydrates (%) and Fats (%) showed that the ingredients gave relatively consistent with nutrient profile for raising tilapia and were also close to the values used for formulation. Dry matter (DM) was 92%, CP was 29.6%, nondigestible CP was 9%, lipids was 6.3%, and ash was 10%.

The growth of monosex *O. niloticus* on the four test diets exhibited superior performance on Diet 2 which had lysine supplement, as compared to the commercial Diet 4 at the least end. The control and methionine/lysine test diets (Diet 1 and 3) had an average performance (Figure 2).
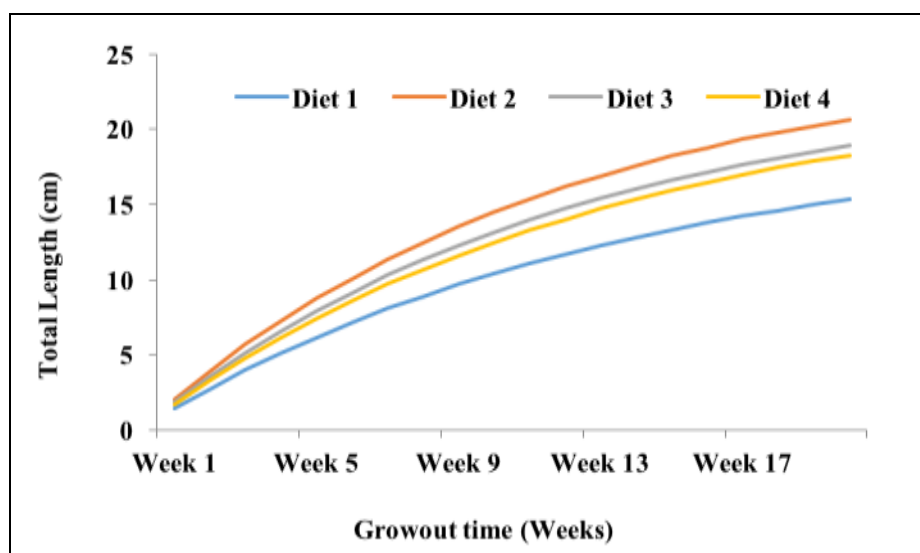

Figure 2: Growth of mono-sex O. niloticus over 105 days on the four test diets.

An index of growth performance, phi prime ($\varnothing'$) was calculated for each diet for the diets based on the asymptotic length (L_∞) and growth curvature (K), to show once again that lysine supplemented Diet 2 had better growth performance than the other diets (Figure 3).
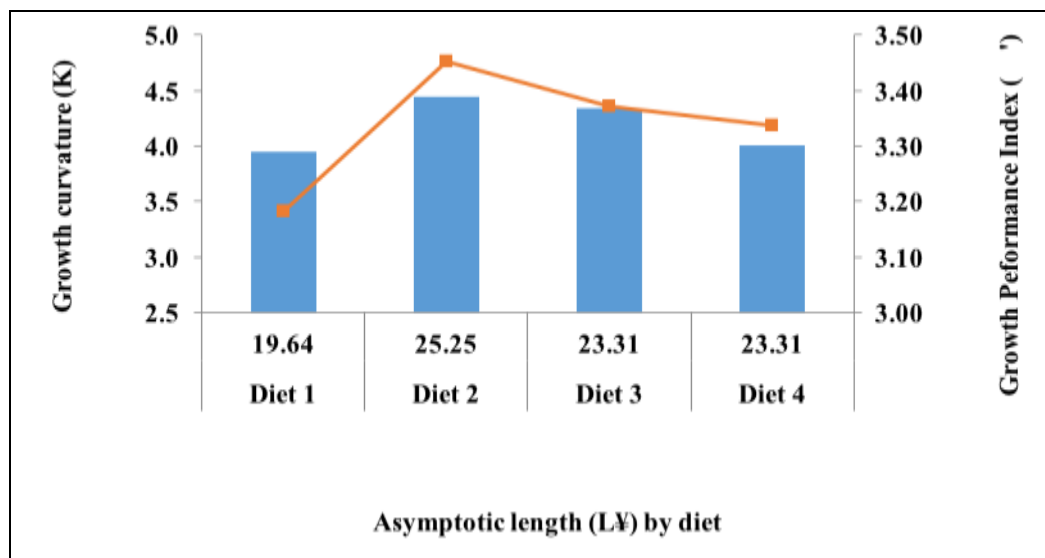

Figure 3: Growth performance, phi prime ($\varnothing$ 'of mono-sex O. niloticus on the four test diets.

The performance of the feeds also showed better final average weight for the lysine supplemented diet of 201.8 g, as compared to the methionine+lysine diet at 196 g and the non EAA supplemented diet at 182.2 g. The commercial diet performed least with the final average weight being 174.7 g. All the other performance indicators; BWG, DWG, SGR, FCR and PER showed that the lysine supplemented diet was better than all the other diets (Table 3).

Table 3: Performance indicators of test diets on monosex O. niloticus in hapas for 105 days.

Performance indicators	Diet 1	Diet 2	Diet 3	Diet 4
Initial average weight (g)	0.72	0.69	0.74	0.69
Final average weight (g)	196.00	201.81	182.19	174.66
Weight of dry feed (g)	250.00	250.00	250.00	250.00
BWG	271.22	289.79	245.53	253.60
DWG	1.86	1.92	1.73	1.66
SGR	5.34	5.40	5.25	5.28
FCR	1.28	1.24	1.38	1.44
PER	2.60	2.68	2.42	2.32

DISCUSSION

Most commercial tilapia feeds in East Africa region have achieved poor performance due to a number of reasons (Munguti et al. 2012) while there has been a worldwide increasing effort to supplement many fish diets with EAAs and other additives in order to improve diet quality and enhance growth (Ketola 1983, Ruscoe et al. 2005, Tacon and Metian 2008, Nunes et al. 2014). The cost, availability, and quality of tilapia fish feed still poses a challenge to many small-scale farmers in East Africa.

One possible solution to this feed challenge is for fish farmers to formulate their own feeds in the farm so as to guarantee the desired quality. However, on-farm formulated tilapia feeds may also not solve the feed scarcity and feed quality problems due to the nutritionally unbalanced composition. This experimental study has shown that the feed formulation, processing and nutritional balancing can be achieved through supplementing the diets with EAAs, as these are often the limiting factors in tilapia feed performance.

Our study has shown that nutritional balancing of tilapia fish feeds can provide up to 17.17 MJ kg^{-1} of energy to fish and at the same time produce high protein efficiency ratio of up to 2.7. The FCR values of 1.44 to 1.24 obtained in this study are close to the ones reported in the literature for extruded diets (1.88, 2.1, 1.5, and 1.7 kg feed for each kg gain in weight).

CONCLUSION

The study concludes that:

- Practical feeds for tilapia can be formulated using locally available protein sources at the farm level and supplementing the diets with EAA to produce a nutritionally balance diet for tilapia;
- Only limiting EAAs require supplementing in the on-farm diet formulations;
- Supplementing practical tilapia diets with lysine at a rate of 24.5 g per kg^{-1} improves the growth performance of monosex *O. niloticus* in hapas; and
- On-farm practical diets of tilapia can produce superior DWG, SGR, FCR, and PER as compared to commercial feeds in the market.

QUANTIFIED ECONOMIC BENEFITS

The experimentation and testing of the diets have shown that:

- The technology can be implemented by farmers at the farm level with minimal investment in a

hammer mill and pelletizing equipment;

- Farmers can now purchase EAA and supplement their formulated on-farm diets with recommended quantities;
- About 600 farmers in Uasin Gishu, 300 farmers in Vihiga County, and 400 farmers in Kakamega counties stand to benefit from this initiative through the University of Eldoret extension initiatives;
- Four undergraduate students and three postgraduate students are likely to undertake research projects on feed formulation, processing, and nutrition, with emphasis in EAA profiles; and
- This new knowledge was applied at the University of Eldoret hatchery which is under construction to produce and supply an estimated 1.2 million tilapia fingerlings to over 600 fish farmers in Uasin Gishu County.

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Alternative Feeds and Processing for Freshwater Aquaculture Species, Part I

SNAKEHEAD PROCESSING SND PRODUCTS

Enhanced Trade and Investment for Global Fishery Markets/Experiment/13SFT03UC

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ABSTRACT

Dried and fermented snakehead (*Channa striata*) fish production in An Giang province, Vietnam is growing, but product consumption is still mainly local with limited exports and it is difficult to finding outlets for the products. Most producers still base production on traditional fermentation processes that waste time, are laborious, and have low economic efficiency. Drying is a cheap, simple, and conventional method to preserve snakehead, and dried snakehead is an important protein source for Vietnamese. However, the quality and safety of these dried fish are not stable because improper processing, packaging, and preservation of dried snakehead are widespread, resulting in only short-term storage, as well as low economic returns for producers. Some immoral producers use harmful chemicals to produce snakehead products with the goals of good appearance and extending the products' shelf life.

We conducted surveys of processors, traders, and consumers to determine optimal practices for snakehead processing to meet consumer tastes. These results enabled us to tailor our research to developing certain types of processing and products. We then conducted 15 experiments to test several aspects of the drying and fermenting processes. Our experimental results showed that in the salty fermented snakehead processing, the application of mechanical treatment before mixing with salt helped shorten salting time from 30 days to 20 days, and adding crude bromelain at a rate of 3% fish weight shortened fermentation time from eight weeks to six weeks. The salty fermented snakehead product in treated treatments was similar in quality to control treatments that were fermented for eight weeks with no added bromelain. Overall, we have demonstrated processing techniques that allow safe consumption of snakehead for up to four weeks of storage, based on sensory, chemical, and microbiological parameters.

Finally, CTU researchers conducted a training course on processing of dried and fermented snakehead for women in An Giang province. Technical practices on processing of dried and fermented snakehead were introduced and a booklet describing processing of dried and fermented snakehead was released.

INTRODUCTION

Dried and fermented snakehead (*C. striata*) fish production in An Giang province, Vietnam is growing daily in the number of manufacturers and quantities produced. Previously, these products were primarily for family consumption or with a small amount to sell in small markets. Now, the production of dried and fermented fish is improved and is conducted at larger scale. Despite this growth, product consumption is still mainly local with limited exports and it is difficult to find outlets for the products. Therefore, we investigated the snakehead processing industry, types of snakehead products and market status, and consumer tastes.

In developing countries, traditional fermentation is one of the oldest food processing and preservation methods, helping not only to prolong preservation and usage time, but also to increase special flavor and taste (Anihouvi et al. 2006 and Nayeem et al. 2010) and nutritional quality of food (Stefánsson and Gudmundsdóttir, 1995; Dincer et al. 2010). Salty fermented fish and fish sauce are the two main products from fish fermentation process that are found in many countries. In Thailand, there are more than 16 different types of fermented fish (Saisithi et al. 1975). Fermented fish products are traditional foods in the Mekong Delta in particular and in Vietnam in general (Le et al. 2014). Vietnamese know how to process many types of fermented fish from many fish species such as snakehead, gourami and rohu, among which fermented snakehead is the most favored product because of its specific flavor and taste. Nowadays, fermented fish is not only a traditional food for daily meal but is also for exportation (Bui et al. 2014). In the Mekong Delta, the natural conditions are favorable for culturing snakehead (Le et al. 2014). Recently, some fermented fish producers have applied semi-industrial scale with more modern techniques and facilities. Although the producers have applied different recipes and techniques, most of them still base production on traditional fermentation processes that waste time, are laborious, and have low economic efficiency.

In the Mekong Delta, Vietnam, drying is a cheap, simple, and conventional method to preserve snakehead, and dried snakehead is an important protein source for Vietnamese. These days, there are two popular kinds of dried snakehead in the South of Vietnam including dried salted snakehead (without sucrose addition) and dried snakehead (with sucrose addition). The shelf life of dried salted snakehead depends entirely on moisture and salt contents. This product has traditionally been treated with high salt levels and drying well for long term preservation at room temperature, but consequently has a hard texture, dark color, salty taste. However, the quality and safety of these dried fish are not stable because improper processing, packaging, and preservation of dried snakehead are widespread, resulting in only short-term storage, as well as low economic returns for producers. While using less salt and not drying in a long period not only to satisfy the consumers but also to avoid weight loss for getting high economic returns, some immoral producers use harmful chemicals to produce snakehead products with the goals of good appearance and extending the products' shelf life. The use of these chemicals has led to toxicity to consumers (Khanh, 2014; Van, 2014). Therefore, microbial and chemical analyses for dried fish are necessary guarantees of food safety (Saritha et al. 2013). We wanted to enhance the sensory properties and safety of dried snakehead, with and without added sucrose, by improving the production processes based on evaluations of chemical, microbial, and sensory attributes. The consumer trend of choosing less salty dried fish for their diet is currently increasing to prevent illness, especially in old people, and sucrose addition to dried fish makes the flesh less salty, more flexible, and golden. As a result, there has been a remarkable price increase for dried snakehead with sucrose, which is a popular choice of smart customers now.

Women make up more than 50% of the population in the Lower Mekong Basin (LMB). Our previous studies showed that male labor was dominant in fish farming practices (78.4% of farmers), but the participation of women in farming snakehead species was high (21.6% of farmers) in comparison with other cultured fish species in Vietnam (often less than 10%) (AquaFish CRSP project 2010). The role of women in the value chain of snakehead fish in Vietnam is more important in trading and processing activities of snakehead products. AquaFish CRSP (2010) reported that in the LMB of Vietnam 26.7% of snakehead traders were female while the figures were much higher in the cases of processing and retailing activities with 90.9% and 93.3%, respectively. In addition, low educational level has been considered one of the constraints for improvement of the value chain of snakehead fish. About 10.1% of fish farmers were illiterate while the respective numbers for processors and retailers were 9.1% and 10.5% (AquaFish CRSP project 2010).

- Conduct surveys to find the optimal products from snakehead, as well as the best processes to increase outlets and meet export requirements in order to increase the incomes of farm owners.
- Investigate new rapid production techniques for fermented snakehead that maintains the quality seen in traditional techniques.
- Improve processing without use of harmful chemicals based adjustments to the traditional method.
- Train women to produce dried and fermented snakehead using the new techniques.

MATERIALS AND METHODS

The survey study was conducted from December 2013 to April 2014 and consisted of:
- A survey of a representative sample of 21 processors about their technological processes and quality of products from snakehead;
- A trader survey of a representative sample of 32 traders about types of products and trade status of products from snakehead; and
- A consumer survey of a representative sample of 110 consumers about customer tastes for the products from snakehead. Surveys were conducted at Long Xuyen, Chau Doc and Cho Moi districts, An Giang province, Vietnam.

A total of 15 experiments were conducted for this study: two to determine a) the effects of mechanical treatment and salting time, and b) effects of bromelain enzyme supplementation and fermentation time on product quality; nine to determine optimal processes for dried salted snakehead with sucrose; and four to determine optimal processes for dried salted snakehead without sucrose.

The general procedure for processing fermented snakehead according to the methods of Nguyen (2011) and Bui et al. (2013) is shown in Figure 1.

Snakehead (700-800 g/fish) purchased in An Thoi local market was mechanically treated for 10 minutes (using a 1.5 kg bundle of reeds per 10 kg fish contained in a 30 L bucket). After that, fish heads, fins, scales, and viscera were removed; fish were then washed with 5% salt solution and split open along the back and abdomen. After being washed, fish were left for three minutes to remove excess surface water and then mixed with salt at a rate of 30% fish weight for two days, fish were weighted with stones at a rate of 20% of fish weight. The salting times were according to experiment one (see below). After being mixed with salt, the mixture was divided into two parts: salted fish and brine. Brine was boiled for 10 minutes, filtered, allowed to cool for 20 minutes, and crude bromelain enzyme was added at rate mentioned in experiment two (see below) and placed in vat containing salted fish mixed with 9% roasted rice powder and 6% salt. Mixture was weighed with stones a second time and gradually added fish sauce at a rate of 20% fish weight onto the surface of the mixture. After a fermentation time as mentioned in experiment two (see below), fermented fish was covered with sugar solution at a rate of 20% fish weight and kept for two weeks to become a final product.

Experiment 1: Effects of mechanical treatment and salting period on quality of salted snakehead.
After mechanical treatment for 10 minutes, fish was mixed with salt following the general procedure given above. Fish was mixed with salt at a rate of 30% fish weight for different periods of time (five, 10, 15, 20, 25, and 30 days). In the control group, snakehead was not mechanically treated, but was mixed with salt at a rate of 30% fish weight for 30 days. The experiment was replicated three times. Fish weight after removal of head, fin, scales, and viscera was two kg. Analyzed parameters were texture, plus salt, moisture, and crude protein contents.

Experiment 2: Effects of crude bromelain supplement and fermentation time on quality of salty fermented snakehead product. Salted snakehead was selected from the optimal treatment of experiment 1 and fermented following general procedure given above. Bromelain was added to salted snakehead at 2%, 3%, 4% and 5% fish weight and fermented for either two, four, six, or eight weeks. In the control group, no bromelain was added and fermentation was for eight weeks. The experiment was replicated three times. Fish weight after removal of head, fin, scales, viscera was two kg. Analyzed parameters were sensory evaluation, texture, amino nitrogen content, plus salt, moisture, and crude protein contents.

Dried salted snakehead with and without sucrose. Snakehead with mass 800-900 g/ fish were purchased at Tan An local market (Ninh Kieu District, Can Tho city, Vietnam). Raw fish samples were washed, heads removed, carcasses split and gutted, deboned, and descaled. After pretreatment, weight of the fish was determined and the fish were salted in containers. Salting methods and time were determined by doing experiments. Fish were stored in cool air (8-10°C) for five hours to absorb the salt well, then mixed with sugar, MSG, fish sauce, sticky rice wine, glycerol, chili pepper, and pure garlic in cool air (8-10°C) for six hours. Pepper (1% w/w) was used to cover the fish surface before drying. Fish were dried by an air dryer, or by air dryer plus tent dryer to obtain dried products. Dried fish was packed in polyethylene bags and frozen at -20°C for long-term storage. Dried fish was taken out and heated under the sun for two hours. Then, dried fish was packed in polyamide (PA) bags under vacuum condition and stored refrigerated at 4-6°C. After that, sampling was done as indicated below.

Nine experiments were carried out to examine the process of dried snakehead with sucrose.

Experiment 3: Salting method trials for dried snakehead. We used a Complete Randomized Design with three treatments and three replicates. Treatments were dry salting 5% (w/w), and brine salting (5%; 10% solution). Analyzed parameters were moisture content, salt content, water activity, a_w, and sensory evaluation.

Experiment 4: Salting time trials for dried snakehead. Needling was performed on the skin side of fish flesh. We used a Complete Randomized Design with five treatments, one controlled sample (no needling) and three replicates. Treatments were salting time of 20, 25, 30, 35, and 40 minutes. Control was salted for 30 minutes with no needling. Analyzed parameters were moisture content, salt content, a_w and sensory evaluation.

Experiment 5: Quality assessment of dried snakehead with different percentage of sucrose, fish sauce, and MSG. We used a Complete Randomized Design with three treatments and three replicates. Treatments were 1) 4% sucrose: 2% MSG: 1% fish sauce; 2) 5% sucrose: 1.5% MSG: 2% fish sauce; and 3) 6% sucrose: 1% MSG: 3% fish sauce. Analyzed parameters were moisture content, a_w and sensory evaluation.

Experiment 6: Quality assessment of dried snakehead corresponding to amount of mixing time with herbs and spices. We used a Complete Randomized Design with three treatments and three replicates. Treatments were mixing times of three, six, or nine hours. Analyzed parameters were moisture content, a_w and sensory evaluation.

Experiment 7: Quality assessment of dried snakehead with different percentage of sticky rice wine (30%). We used a Complete Randomized Design with three treatments and three replicates. Treatments were different percentages of 1, 2, and 3% (w/w) of wine. Parameters, measured over two weeks, were moisture content, a_w, TVB_N values, peroxide values, Total Plate Count, and sensory evaluation.

Experiment 8: Quality assessment of dried snakehead corresponding to methods of garlic addition. We used a Complete Randomized Design with three treatments and three replicates. Treatments were no

garlic, pure garlic (2% w/w), and diluted garlic juice (50% water and 2% w/w) adding to fish flesh. Parameters, measured over two weeks, were moisture content, a_w, TVB_N values, peroxide values, Total Plate Count, and sensory evaluation.

Experiment 9: Quality assessment of dried snakehead with different percentages of glycerol. We used a Complete Randomized Design with four treatments and three replicates. Treatments were 0%, 1%, 2%, or 3% glycerol. Parameters, measured at week zero and week two were moisture content, a_w, TVB_N values, peroxide values, Total Plate Count, and sensory evaluation.

Experiment 10: Drying method trials for dried snakehead. We used a Complete Randomized Design with six treatments and three replicates. Treatments were drying in Air Dryer (AD) only at different temperatures or in both Air Dryer and Tent Dryer (TD). (AD)_65^{o}C: 28 hours; (AD)_60^{o}C: 31 hours; (AD)_65^{o}C: 15 hours and (AD)_60^{o}C: 13 hours; (AD)_65^{o}C: 22 hours and (TD): 6 hours; (AD)_65^{o}C: 18.5 hours and (TD): 9.5 hours; (AD)_65^{o}C: 14 hours and (TD): 14 hours. Analyzed parameters, measured at week zero and week two were moisture content, a_w, TVB_N values, peroxide values, and sensory evaluation.

Experiment 11: Storage methods for dried snakehead. Two treatments and three replicates were used. Treatments were storing dried fish at either chilled temperatures (4-6°C) or room temperatures (27-30°C), for four weeks. Analyzed parameters were moisture content, a_w, TVB_N values, peroxide values, and sensory evaluation.

Four experiments were conducted on dried salted snakehead without sucrose.

Experiment 12: Salting method trials for dried salted snakehead. Needling was performed on the skin side of the fish. Dry salting was used with salt levels of eight, 10, or 12% (w/w)) for either 20, 30, or 40 min. The experiment was in triplicate. Analyzed parameters were moisture content, a_w TVB_N values, peroxide values, and sensory evaluation.

Experiment 13: Quality assessment of dried salted snakehead with different percentages of fish sauce and MSG. We used a Complete Randomized Design with four treatments and three replicates were used. Treatments were 0% MSG: 0% fish sauce; 0% MSG: 2% fish sauce; 1% MSG: 0% fish sauce; and 1% MSG: 2% fish sauce. Analyzed parameters were moisture content, a_w, and sensory evaluation.

Experiment 14: Quality assessment of dried salted snakehead with different percentages of sorbitol. We used a Complete Randomized Design with four treatments and three replicates. Treatments were 0%, 1%, 2%, or 3% of sorbitol. Analyzed parameters, measured during four weeks were moisture content, a_w, TVB_N values, peroxide values, Total Plate Count, and sensory evaluation.

Experiment 15: Drying method trials for dried salted snakehead. We used a Complete Randomized Design with six treatments and three replicates. Treatments were drying snakehead in Tent Dryer vs. open-air drying at drying times of 33, 36, or 39 hours. Analyzed parameters were moisture content, a_w, TVB_N values, peroxide values, and sensory evaluation.

Analytical methods. Methods for chemical, microbiological, and sensory evaluations are given in Table 1.

Statistical analysis. Difference in means among treatments were statistically analyzed by one-way ANOVA and two-way ANOVA followed by Tukey test at $p < 0.05$, and t-test using SPSS 18.0.

Survey results. Results of the fermented snakehead processor survey (Table 2) indicate that the majority of the participating processors had a capacity range of three to five tons product/year (55.6%), followed capacity ranges of one to two tons product/year (22.2%), 5–10 tons product/year (11.1%) and >10 tons product/year (11.1%). Their markets included local markets (100%) and export (11.1%). The quality of raw fish, salting, and fermenting steps had effects on the quality of fermented fish, so the input materials were selected very carefully. Raw material for companies of three to five tons product/year and >5 tons product/year must be bought in areas with well-controlled antibiotics, metals, and chemicals (85.7% of processors) while only 50% of processors with the capacity of manufactory of one to two tons product/year had access to those sources. In addition, in companies with three to five tons product/year and >5 tons product/year, fish was mixed with salt at 28-30% of fish weight (100% of processors), the production time (salting and fermenting) was around six months and storage time ranged from six to 12 months. In companies with capacity of one to two tons product/year, fish was mixed with salt at only 20-22% of fish weight (50% of processors), the production time (salting and fermenting) was around three to four months and storage time was six to nine months.

Results from the dried snakehead processor survey (Table 3) indicated that the majority of the participating processors had production capacity of one to two tons product/year (58.3%), followed <1 ton product/year (25%) and >2 tons product/year (16.7%). Their markets included local markets (100%) and export (0%). The quality of raw fish, drying and storing steps had effects on the quality of dried fish. Raw fish for companies of one to two tons and >2 tons product/year must be bought in areas with well-controlled antibiotics, metals, chemicals (77.8% of processors) while that was true for only 33.3% of processors with capacity of one to two tons product/year. Dried snakehead was produced from cultured snakehead (100% of processors). In addition, in companies with capacity of one to two tons product/year and >2 tons/year, drying time was three to four days (eight to nine hours/day) (88.9% of processors) while only 11.1% of processors produced dried snakehead in one to two days (eight to nine hours/day). In companies with <1 tons/year, drying time was three to four days (eight to nine hours/day) (66.7% of processors) while 33.3% of processors produced dried snakehead in one to two days (eight to nine hours/day). The storage time was five to six months in freezer (77.8% of processors) while only 22.2% of processors produced dried snakehead with storing time was two to three months without freezing.

Results from the fermented and dried snakehead consumer survey (demographic information in Table four; other results in Figures two and three indicate that most of the participants were in the age range of 30–40 years old (63.6%), followed by those 20–30 years old (27.3%) and 40–50 years old (7.3%). The lowest percentage of participants (1.8%) was 50–60 years old. The percentage of female (63.6%) was higher than male (36.4%). For fermented snakehead, consumers often bought from the local market (87.3%), whereas others bought from the producer (64.5%) or from supermarket (10.0%). Consumers bought fermented snakehead according to the quality attributes of brand (44.5%), followed by its flavor (28.1%) and price (10.9%), respectively, they did not pay much attention on appearance, taste, or texture (Figure 2). For dried snakehead, consumers bought from the local market (86.4%), but also from producers (70.0%) and from supermarket (24.5%). Consumers bought dried snakehead based on quality attributes of brand (38.2%), texture (34.5%) and taste (15.5%), respectively; they did not pay much attention to appearance, taste, or flavor (Figure 3).

Experiments on processing techniques on snakehead quality

Experiment 1. Salt content and hardness of salted snakehead significantly increased (from 0.15% and 19273 g force to 20.62% and 20091 g force, respectively), whereas decrease in moisture and crude protein contents significantly decreased (from 77.53% and 18.94% to 55.53% and 18.94%, respectively) when salting time increased from 0 days to 20 days (Table 5). However, when salting time increased from 20 days to 30 days, the differences of salt, moisture and crude protein contents among treatments were not statistically significant (P>0.05) whereas hardness slightly decreased (Table 5).

Experiment 2. Fermentation time and level of crude bromelain significantly (p≤0.05) affected moisture, crude protein, amino nitrogen contents and hardness of fermented snakeheads, but not salt content (Table 6). As fermentation time increased from two to six weeks, moisture content increased and crude protein content decreased significantly at all levels of crude bromelain. As crude bromelain increased from 2% to 4%, moisture content was not significantly different, with figures rising at 5% crude bromelain whereas crude protein content decreased slightly when rate of crude bromelain increased from 2% to 5% at all fermentation times. In addition, there was a significant increase in amino nitrogen content, but a significant decrease in hardness (P<0.05) and no significant difference (P>0.05) in salt content during eight weeks of fermentation at all fermentation times and all levels of crude bromelain. For fermentation using treatment M7 (added 3% crude bromelain and fermented for six weeks), the salty fermented snakehead had the highest salt and amino nitrogen content (20.67% and 8.02 mg N/100 g, respectively), hardness, moisture, and crude protein contents (16607 g force, 56.46% and 19.79%, respectively), but the differences had no statistical significance (p≥0.05) compared with control treatments. Fermentation time and bromelain levels significantly (p≤0.05) affected the sensory scores of fermented snakeheads (Table 7). When fermentation time increased from two weeks to six weeks and bromelain increased from 2% to 3%, sensory scores of color, flavor, taste, and overall increased. However, when fermentation time increased from six weeks to eight weeks and bromelain increased from 3% to 5%, sensory scores of color, flavor, taste and overall decreased. In treatment M7 (3% crude bromelain and six weeks fermentation), the final product had the highest sensory scores of color, flavor, taste, and overall parameters (6.13; 6.07; 6.00; and 6.20, respectively), but the differences had no statistical significance (p≥0.05) compared to the control.

In conclusion, applying mechanical treatment on snakehead fish can shorten the period for soaking them in salt from 30 days to 20 days and supplementation of 3% bromelain enzyme can shorten the period for fermentation from eight weeks to six weeks. These techniques can shorten the processing period of the salty fermented fish product from commercial snakehead fish and ensures nutritional quality and sensory properties compared with traditional methods.

Experiments 3–6. For these experiments, besides dry salting method with 5% (w/w) of clean dried salt in 30 minutes, the combination of 5% sucrose, 1.5% MSG, and 2% fish sauce were chosen for the mixing time of six hours, based not only on the significantly highest points of overall acceptability (P<0.05), but also the low water activity and moisture (Table 8).

Experiment 7. Wine is able to enhance the sensory properties of dried fish due to reduce fishy odors by wine aroma. Besides, based on the alcohol levels, wine addition is used to inhibit bacteria growth as protein denaturation and lipid dilution (Mcdonnell and Denver, 1999). One percent of wine was too low to decrease the free water content and slow down microbial activity of dried snakehead, leading to limited ability to prolong the shelf life, as compared to 2 and 3% of wine adding (Table 9). Therefore, parameters of water activity, moisture content, TVB-N, peroxide values and total plate count of dried fish samples with 1% wine added were higher than those with 2 or 3% wine added at day zero and the second week (P<0.05). The product qualities of the 2% vs. 3% additions were not different. More importantly, dried fish samples with 2% wine yielded the lowest chemical parameters and total plate count at day zero and the fourth week due to the most appropriate supplement to combine free water present in fish flesh to decrease high water activity and moisture content. In addition, 2% wine is sufficient to induce a stressful environment for bacteria because ethanol is a toxic compound that impairs the integrity of bacterial (Hallsworth, 1998). Therefore, the growth of bacteria was restrained and the breakdown of proteins and lipids was slowed, resulting in a decrease in spoilage compounds (NH_3, H_2S, ketones, esters, CH_3SH, $(CH_3)_2S$). As a result, the levels of TVB-N, peroxide values and total plate count of the treatment of 2% of wine adding were low at day zero and the second week. Regarding organoleptic evaluation (P<0.05), overall acceptability of 2% of wine (6.17 and 6.04) had significantly higher scores because of the typical flavor, aroma, color and texture of dried snakehead, as well as almost no changes in appearance and taste

after two weeks. At the same time of sampling, smell of wine for the samples of 3% of wine were obvious, whereas those of 1% were changed most in two weeks of preservation.

Experiment 8. The recent trend of preservative supplements is toward natural products and garlic is an outstanding plant for inhibition of bacteria. In fact, allicin in garlic has not only antibacterial, antifungal, but also antiparasite activities (Ankri and Mirelman, 1999). Compared to the case of no garlic, pure garlic, and diluted garlic juice supplements made water activity of fish flesh be stable (Table 10). The fish sample with added pure garlic showed its important role in reducing the TVBN and peroxide values and the amount of aerobic bacteria for two weeks. Based on the highest scores of overall acceptability, pure garlic addition to snakehead was the chosen treatment for the next experiments.

Experiment 9. Glycerol (E422), a humectant, with three hydroxyl groups has the function of binding free water and controlling water activity in order to enhance food stability (U.S Food and Drug Administration, International Food Information Council- IFIC). The dried fish product qualities of four dried fish groups treated by 0, 1, 2, 3% of glycerol in four-week preservation indicated significant differences based on chemical, microbiological, and organoleptic analyses (P<0.05) (Table 11). In fact, moisture content, water activity, TVB-N, peroxide values, and total plate count of the samples with 2 and 3% of glycerol addition were lower than the remaining treatments from the first week to the fourth week. This resulted from retarding of bacteria growth, protein, degradation, and lipid oxidation over a month when adding the suitable amount of glycerol. Therefore, glycerol addition could be used in dried snakehead processing to obtain better product properties. Furthermore, the differences between dried fish samples with 2 and 3% added glycerol could not be discerned in chemical and microbial analysis. However, for sensory evaluation, samples with 2% added glycerol showed higher organoleptic points than those of 3% through one-month storage because of the typical flavor, aroma, color, and texture of dried snakehead. Adding 3% of glycerol made dried fish flesh sweeter and decreased the dried fish taste in comparison with 2% addition. In addition, because all glycerol was not absorbed, excess glycerol caused a thin layer covering the fish flesh making the dried fish less dry. The color of dried fish was also darker due to Maillard reactions (Figure 4). Hence, 2% of glycerol was the most suitable preservative not only to maintain dried fish qualities but also for long-term storage.

Experiment 10. The significant difference in chemical and sensory properties among samples treated with various drying method were due to temperature, time, and equipment used during drying periods (P<0.05) (Table 12). The temperatures, time and equipment selected for the next experiment mainly relied on the low chemical parameters analyzed and the high points of sensory evaluation of the eight panelists. For air dryer, chosen temperatures and drying time were 60°C and 31 hours. In case of combination between drying in the dehydrator and tent dryer, in order to obtain the best product, temperatures and time for the former were 65°C and 18.5 h while drying time for the latter were 9.5 h. These two dried snakehead products had an attractive appearance (Figure 5).

Experiment 11. The relatively constant sensory characteristics, chemical indices determination and total plate count were recorded for refrigerated storage (4-6°C) over four weeks. In contrast to low temperature preservation, product stored at room temperature showed a significant reduction in chemical and microbial quality, starting in the second week (Table 13), although sensory properties remained the same (Table 14). The parameters of water activity, moisture content, TVB-N, peroxide values, and total plate count of dried fish samples at room temperature storage were more accelerated than chilled storage from the second week to the fourth week. However, both storage conditions had the levels of water activity, moisture content, TVB-N, peroxide values, and total plate count in the range of acceptability limits of dried fish over a period of one month. Therefore, the safe consumption of dried snakehead products was illustrated over four weeks for both stored cases. The significant difference in sensory evaluation between the two storage conditions in terms of color and overall acceptability appeared in the third week and one

week later the additional change of aroma could be recognized. Refrigeration is the proper storing method to increase the shelf life of vacuum-packaged dried snakehead.

Experiment 12. Salting is a method of treating fish with salt to reduce water activity in fish flesh and to elevate flavor. In general, the salt penetration depends on amount of salt and salting time. Increased salt addition and extension of salting time raised the sodium chloride content in dried fish flesh, but lowered water activity and moisture content (Table 15). Fish samples treated with 10% salt for 30 minutes was optimal because this product had the highest points for overall acceptability as well as the low moisture content and water activity. Specifically, the water activity of this product (0.634) met the Vietnam Standard (TCVN, 2014) which was lower than 0.75. This value also satisfied the acceptable limit of The Codex Alimentarius Commission (2012) for smoke-dried fish which could minimize the mold or pathogen growth. In addition, the sodium chloride content in dried fish (9.42%) did not exceed 15%, meeting the requirement of dried fish products according to Vietnam Standard (TCVN, 2014).

Experiment 13. For dried and salted snakehead, monosodium glutamate (MSG) proved to enhance taste more than fish sauce. The salty taste could be decreased by MSG use whereas adding fish sauce into fish flesh was not able to be recognized by consumers. As a result, overall acceptability of sample with only 1% of MSG supplement got the highest points, significantly different from the other treatments ($P<0.05$). Water activity and moisture content of this sample were also the lowest among these treatments. Therefore, this dried fish product was chosen for the next experiments.

Experiment 14. Sorbitol is widely used as a sweetener, humectant, and texturing agent, and is currently permitted as a safe food additive in many countries with E number E420. More importantly, although it is approximately 60% as sweet as sucrose, it provides about one-third fewer calories, making it beneficial for people with diabetes. According to The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA), no limits for sorbitol's use are proposed. However, the U.S Food and Drug Administration's regulation states that sorbitol-food that may lead to daily consumption of 50 g of sorbitol must be labeled with the statement to prevent a laxative effect. Sorbitol addition played an important role in maintaining not only the sensory quality but also the safety of the dried products throughout the storage, indicated by the significantly unstable characters of fish sample without sorbitol in terms of microbial, sensorial, and chemical aspects as opposed to dried fish with sorbitol ($P<0.05$) (Table 16). The chemical and sensory properties of dried fish products belonging to the two groups with 2% and 3% of sorbitol showed significant difference compared to the remaining groups ($P<0.005$). For instance, moisture content, water activity, TVB-N, peroxide values, and total plate count of the samples with 2% and 3% of sorbitol addition were lower than the remaining two from the first week to the fourth week. The difference in chemical and microbial quality between dried fish samples with 2 and 3% sorbitol could not be distinguished. Nevertheless, for sensory evaluation, samples with 2% sorbitol showed better organoleptic points than those of 3% through one-month storage in terms of the typical flavor, aroma, color and texture of dried snakehead (Figure 6). Supplying 3% of sorbitol made dried fish flesh moist and sweeter to reduce the dried salted fish taste in comparison with 2% addition. Therefore, 2% addition of sorbitol was optimal preservative from this four-group comparison not only to maintain dried fish qualities but also for long-term storage.

Experiment 15. In general, with the longer dehydrating time both of sun drying and tent dryer, the peroxide values and TVBN of these treatments tended to increase (Table 17). The moisture loss was also recorded with longer drying time, resulting in lower moisture content and water activity. Moreover, total plate count data (Table 18), tent drying reduced bacterial contamination. Drying time of 36 h for dried salted snakehead was applied for both tent dryer or dehydrating in sun directly. Chemical and microbiological parameters between these two applications showed no difference, but the eight panelists scored higher points for the fish sample produced by tent dryer ($P<0.05$). Dried salted snakehead dehydrated by tent dryer for 36 h also significantly lowered peroxide and TPC values in contrast to other

treated methods (P<0.05). For production of dried salted snakehead with high quality and safety, dehydrating in the tent for 36 h ranked first followed by drying in open air with the same time. This study was carried out in the rainy season, so it was difficult to recognize the drying time difference between tent dryer and sun drying directly. Appearances of these two dried snakehead products are shown in the Figure 7.

CONCLUSION

Products from snakehead have traditionally been processed as dried snakehead and salty fermented snakehead, done mainly by traditional methods in fermented snakehead processing factories (3 to 5 tons/year) and dried snakehead factories (1 to 2 tons/year). Customers purchase fermented snakehead product based on company brand (44.5%), flavor (28.1%) and price (10.9%), but based on company brand (38.2%), texture (34.5%), and taste (15.5%) for dried snakehead products.

Our experimental results showed that in the salty fermented snakehead processing, the application of mechanical treatment before mixing with salt helped shorten salting time from 30 days to 20 days, and adding crude bromelain at a rate of 3% fish weight shortened fermentation time from eight weeks to six weeks. The salty fermented snakehead product in treated treatments was similar in quality to control treatments that were fermented for eight weeks with no added bromelain.

Overall, we have demonstrated processing techniques that allow safe consumption of snakehead for up to four weeks of storage, based on sensory, chemical, and microbiological parameters. For a dried snakehead (with sucrose) production line, the optimal processing parameters are illustrated in Figure 8 and for dried salted snakehead production the parameters are illustrated in the Figure 9.

Finally, CTU researchers conducted a training course on processing of dried and fermented snakehead for women in An Giang province (Figure 10). Technical practices on processing of dried and fermented snakehead were introduced and a booklet describing processing of dried and fermented snakehead was released.

QUANTIFIED ANTICIPATED BENEFITS

This investigation has led to development of a manual of the processes of dried and fermented snakehead (*C. striata*). It supported dissertations of four undergraduate students (three female and one male). Three faculty members (female) at CTU and two local staff (female) participated in this project. Thirty women were trained on the processes of dried and fermented snakehead. One-hundred manuals of the processes of dried and fermented snakehead (in Vietnamese) were delivered to farmers.

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TABLES AMD FIGURES

Table 1. Parameters and methods for analyzing and evaluating samples for the various experiments.

Parameters	Methods
Crude protein content	Kjeldahl method as described by AOAC (2000)
Moisture content	Moisture content was determined using AOAC (2000)
Salt content	Salt content as described by Zeng et al. (2013)
Amino nitrogen content	Formol titration method as described Besas and Dizon (2012)
Lipid content	Soxhlet method (AOAC, 2000)
Ash	Baking at 560ºC overnight (AOAC, 2000)
Water activity	Physic method (AOAC, 2000)
Peroxide values	Titration method (TCVN 6121: 1996)
Texture (Hardness)	Hardness was measured by using a Texture Analyser TA-XT.Plus with a double compression test. Eight samples of each treatment were allowed to 40% of original height, test speed 0.8 mm/s and time of 3 s was allowed to elapse between the two compression cycles.
Sensory evaluation	Sensory quality was evaluated on 7 point descriptive scale in hedonic scale scoring method as described Petrus et al. (2013)

Table 2. Results of the fermented snakehead processor survey (n = 9).

Criteria	Percentage (%)
Hired labors	
Hired labors	66.7
Family resources	23.3
Capacity	
< 1 tons/year	0
1-2 tons/year	22.2
3-5 tons/year	55.6
5-10 tons/year	11.1
>10 tons/year	11.1
Raw material	
Well-controlled antibiotics, metals, chemicals	88.9
Without controlled antibiotics, metals, chemicals	11.1
Market for product consumption	
Local market	100
Supermarket	11.1
Export	11.1

Table 3. Results of the dried snakehead processor survey (n=12).

	Percentage (%)
Hired labor	
Hired labor	41.7
Family resources	58.3
Capacity	
< 1 tons/year	25
1-2 tons/year	58.3
> 2 tons/year	16.7
Raw material	
Well-controlled antibiotics, metals, chemicals	66.7
Without controlled antibiotics, metals, chemicals	33.3
Market for product consumption	
Local market	100
Supermarket	25
Export	0

Table 4. Results of the fermented and dried snakehead consumer survey (n=110).

Criteria	Percentage
Gender	
Male	36.4
Female	63.6
Age	
20-30	27.3
30-40	63.6
40-50	7.3
50-60	1.8
Occupation	
Student	6.4
Housewife	59.1
Employee	31.8
Entrepreneur	2.7
Income (USD/month)	
< 50	9.1
50-150	60.9
150-250	27.3
250-350	0.9
>350	1.8

Table 5. Hardness, salt, moisture and crude protein contents of salted snakeheads in experiment 1. Values (mean ± SD; n = 3) followed by the same letter in a column are not significantly different (P<0.05). RM: Raw material; TM: treatment.

TM	Salting time (Days)	Salt (%)	Moisture (%)	Crude protein (%)	Hardness (g force)
RM	0	0.15	77.53	18.94	19273
M0	**30**	**19.08±0.26^{b*}**	**58.60±0.14^{ab}**	**19.01±0.51^{c}**	**14568±42.6^{e}**
M1	5	16.11±0.11^{d}	59.01±0.42^{a}	21.45±0.22^{a}	18936±38.9^{d}
M2	10	18.01±0.29^{c}	58.02±0.19^{bc}	20.09±0.18^{b}	19789±41.0^{c}
M3	15	19.06±0.23^{b}	57.73±0.18^{c}	19.07±0.09^{c}	19924±39.4^{b}
M4	**20**	**20.62±0.17^{a}**	**55.53±0.59^{d}**	**18.94±0.21^{c}**	**20091±43.9^{a}**
M5	25	20.28±0.33^{a}	56.08±0.65^{d}	19.03±0.55^{c}	19941±46.4^{b}
M6	30	20.67±0.33^{a}	55.42±0.65^{d}	18.79±0.46^{c}	18961±10.3^{d}

Table 6. Hardness, salt, moisture, crude protein and amino nitrogen contents of salty fermented snakeheads in experiment 2. Values (mean ± SD; n = 3) with the same letter in a column are not significantly different (P<0.05). TM: treatment.

TM	Crude bromelain (%)	Time (weeks)	Salt (%)	Moisture (%)	Crude protein (%)	Amino nitrogen content (mgN/100g)	Hardness (g force)
M0	0	8	**19.11±0.33^{c}**	**56.55±0.59^{abc}**	**19.19±0.43^{ef}**	**8.07±0.32^{b}**	**16565±17.9^{h}**
M1	2	2	20.39±0.12^{b}	55.64±0.54^{efg}	21.97±0.41^{a}	5.00±0.16^{f}	18285±7.09^{a}
M2	2	4	20.43±0.19^{b}	55.43±0.53^{g}	21.07±0.16^{b}	5.88±0.00^{e}	17639±40.2^{d}
M3	2	6	20.54±0.11^{b}	56.10±0.48^{cdef}	20.08±0.24^{c}	7.28±0.37^{c}	17797±36.3^{c}
M4	2	8	20.53±0.11^{b}	56.21±0.14^{cde}	19.71±0.24^{cd}	7.84±0.14^{b}	16914±41.5^{g}
M5	3	2	20.44±0.19^{b}	55.53±0.24^{fg}	20.88±0.20^{b}	5.02±0.07^{f}	17890±35.6^{b}
M6	3	4	20.45±0.00^{b}	55.42±0.34^{g}	20.69±0.13^{b}	6.58±0.14^{d}	17125±33.9^{f}
M7	3	6	**20.67±0.19^{ab}**	**56.45±0.17^{bcd}**	**19.79±0.36^{cd}**	**8.02±0.08^{b}**	**16607±10.1^{h}**
M8	3	8	20.67±0.22^{ab}	56.47±0.22^{bcd}	19.46±0.41^{de}	8.16±0.08^{ab}	15953±32.7^{i}
M9	4	2	20.46±0.00^{b}	56.06±0.13^{cdefg}	20.08±0.13^{c}	5.32±0.42^{f}	17571±40.4^{e}
M10	4	4	20.47±0.19^{b}	55.83±0.33^{defg}	19.90±0.24^{cd}	6.95±0.4^{cd}	17082±31.5^{f}
M11	4	6	20.64±0.20^{ab}	56.70±0.25^{abc}	19.57±0.01^{de}	8.07±0.21^{b}	15884±22.6^{j}
M12	4	8	20.66±0.39^{ab}	56.58±0.65^{abc}	19.39±0.26^{de}	8.21±0.42^{ab}	14920±47.2^{l}
M13	5	2	20.47±0.19^{b}	56.38±0.31^{cd}	19.56±0.41^{de}	4.93±0.11^{f}	15872±35.7^{j}
M14	5	4	20.48±0.20^{b}	56.41±0.07^{cd}	19.07±0.20^{efg}	7.04±0.21^{c}	15691±43.6^{k}
M15	5	6	20.67±0.33^{ab}	57.08±0.04^{ab}	18.78±0.23^{fg}	8.16±0.16^{ab}	14055±47.1^{m}
M16	5	8	20.71±0.58^{a}	57.12±0.20^{a}	18.62±0.10^{g}	8.58±0.29^{a}	13381±24.4^{n}

Table 7. Sensory evaluation of salty fermented snakeheads in Experiment 2.

TM	Crude bromelain (%)	Time (weeks)	Color	Flavor	Taste	Overall
M0	0	8	5.80±0.00[abc]	5.87±0.11[ab]	5.80±0.00[ab]	5.93±0.11[ab]
M1	2	2	5.33±0.11[def]	4.93±0.12[h]	5.47±0.23[bc]	5.40±0.20[cde]
M2	2	4	5.47±0.12[cdefg]	5.07±0.23[h]	5.60±0.20[bc]	5.53±0.11[cde]
M3	2	6	5.53±0.23[bcdef]	5.20±0.20[fgh]	5.67±0.11[abc]	5.67±0.30[bcd]
M4	2	8	5.60±0.20[bcde]	5.40±0.2[efg]	5.73±0.12[ab]	5.73±0.23[bc]
M5	3	2	5.60±0.20[bcde]	5.47±0.23[def]	5.60±0.20[bc]	5.67±0.11[bcd]
M6	3	4	5.67±0.30[bcd]	5.53±0.23[cde]	5.60±0.20[bc]	5.73±0.23[bc]
M7	3	6	6.13±0.11[a]	6.07±0.11[a]	6.00±0.00[a]	6.20±0.20[a]
M8	3	8	5.87±0.23[ab]	5.80±0.20[abc]	5.80±0.20[ab]	5.73±0.23[bc]
M9	4	2	5.13±0.12[gh]	5.20±0.20[fgh]	5.47±0.23[bc]	5.40±0.00[cde]
M10	4	4	5.20±0.20[fgh]	5.53±0.11[cde]	5.60±0.00[bc]	5.53±0.30[cde]
M11	4	6	5.40±0.20[defg]	5.87±0.12[ab]	5.73±0.23[ab]	5.73±0.11[bc]
M12	4	8	5.60±0.34[bcde]	5.73±0.11[bcd]	5.80±0.20[ab]	5.73±0.12[bc]
M13	5	2	4.93±0.11[h]	5.13±0.11[gh]	5.33±0.11[c]	5.27±0.11[e]
M14	5	4	5.13±0.12[gh]	5.47±0.12[def]	5.60±0.20[bc]	5.33±0.23[de]
M15	5	6	5.20±0.20[fgh]	5.60±0.20[bcde]	5.67±0.23[abc]	5.40±0.20[cde]
M16	5	8	5.27±0.11[efgh]	5.67±0.11[bcde]	5.67±0.11[abc]	5.47±0.11[cde]

Table 8. Effect of salting methods on moisture content, salt content and a_w of dried snakehead. Values (mean ± SD) with the same letter within a column are not significantly different ($p < 0.05$). a_w: water activity.

Treatment	Moisture (%)	Salt (%)	a_w	Overall Acceptability
Dry salting (5% salt w/w)	31.3[b] ± 0.29	6.4[a] ± 0.29	0.772[b] ± 0.00	5.96 ± 0.07[a]
Brine salting (5% solution)	34.2[a] ± 0.29	2.6[b] ± 0.29	0.791[a] ± 0.00	5.32 ± 0.17[c]
Brine salting (10% solution)	31.3[b] ± 0.41	6.7[a] ± 0.29	0.764[b] ± 0.00	5.65 ± 0.09[b]

Table 9. Effect of differing percentage of sticky rice wine (30%) on moisture content, a_w, TVB_N values, peroxide values and Total Plate Count (TPC) of dried snakehead. Values (mean ± SD) with the same letter within a column are not significantly different ($p < 0.05$). a_w: water activity, TPC: Total plate count, TVB-N: Total volatile base nitrogen.

Parameters	Treatment	Wine 30%– 1% (w/w)	Wine 30% - 2% (w/w)	Wine 30% - 3% (w/w)
Moisture (%)	Week 0	30.54± 0.15[a]	30.12 ± 0.04[b]	30.11 ± 0.14[b]
	Week 2	32.23 ± 0.16[a]	31.16 ± 0.09[b]	31.26 ± 0.05[b]
a_w	Week 0	0.696± 0.01[a]	0.690 ± 0.01[b]	0.687± 0.01[b]
	Week 2	0.719 ± 0.01[a]	0.703 ± 0.01[b]	0.705 ± 0.01[b]
TVBN (mgN/100g)	Week 0	30.70 ± 0.40[a]	29.86 ± 0.24[b]	29.92 ± 0.25[b]
	Week 2	32.85 ± 0.46[a]	30.81 ± 0.31[b]	30.85 ± 0.24[b]
Peroxide (m.eq/Kg oil)	Week 0	1.50 ± 0.03[a]	1.41 ± 0.02[b]	1.41 ± 0.02[b]
	Week 2	2.99 ± 0.05[a]	2.74 ± 0.06[b]	2.79 ± 0.01[b]
TPC (log/CFUg)	Week 0	4.5 ±0.08[a]	4.2 ± 0.06[b]	4.3 ± 0.07[ab]
	Week 2	5.3 ± 0.01[a]	4.8 ± 0.01[b]	4.8 ± 0.01[b]
Overall Acceptability	Week 0	5.88 ± 0.65[c]	6.17 ± 0.70[ab]	5.92 ± 0.77[bc]
	Week 2	5.25 ± 0.68[b]	6.04 ± 0.69[a]	5.58 ± 0.50[b]

Table 10. Effect of methods of garlic addition on moisture content, salt content and water activity of dried snakehead. Values (mean ± SD) with the same letter within a column are not significantly different ($p < 0.05$). a_w: water activity, TPC: Total plate count, TVB-N: Total volatile base nitrogen.

Parameter	Treatment	No Garlic	Garlic	Garlic juice: Water (1:1)
Moisture (%)	Week 0	29.87± 0.02[b]	30.1 ± 0.08[ab]	30.35± 0.28[a]
	Week 2	32.31 ± 0.10[a]	31.15 ± 0.33[b]	31.92 ± 0.22[a]
a_w	Week 0	0.678 ± 0.01[b]	0.681 ± 0.01[b]	0.686 ± 0.01[a]
	Week 2	0.725± 0.01[a]	0.697 ± 0.01[b]	0.700 ± 0.01[b]
TVBN (mgN/100g)	Week 0	26.99 ± 0.4[a]	25.03 ± 0.5[b]	26.03 ± 0.4[ab]
	Week 2	33.08 ± 0.86[a]	30.55 ± 1.14[b]	32.61 ± 0.48[a]
Peroxide (m.eq/Kg oil)	Week 0	1.55 ± 0.19[a]	1.39 ± 0.02[ab]	1.41 ± 0.02[b]
	Week 2	3.21 ± 0.19[a]	2.66 ± 0.19[b]	2.79 ± 0.10[b]
TPC (log/CFUg)	Week 0	4.7 ± 0.14[a]	4.3 ± 0.07[b]	4.4 ± 0.08[b]
	Week 2	5.3 ± 0.02[a]	4.6 ± 0.20[b]	4.8 ± 0.18[b]
Overall Acceptability	Week 0	4.58 ± 0.65[c]	6.22 ± 0.59[a]	5.88 ± 0.74[b]
	Week 2	4.33 ± 0.64[c]	6.08 ± 0.65[a]	5.63 ± 0.74[b]

Table 11. Effect of differing percentage of glycerol on moisture content, a_w, TVB_N values, peroxide values and Total Plate Count (TPC) of dried snakehead. Values(mean $\pm$ SD) with the same letter within a column are not significantly different ($p < 0.05$). NS: not significant. a_w: water activity, TPC: Total plate count, TVB-N: Total volatile base nitrogen.

Parameter	Treatment	Glycerol 0% (w/w)	Glycerol 1% (w/w)	Glycerol 2% (w/w)	Glycerol 3% (w/w)
Moisture (%)	Week 0	30.13 ± 0.18^{NS}	29.98 ± 0.10^{NS}	29.37 ± 0.56^{NS}	30.02 ± 0.37^{NS}
	Week 4	33.70 ± 0.06^a	32.77 ± 0.40^b	30.85 ± 0.17^c	32.10 ± 0.38^{cb}
a_w	Week 0	0.681 ± 0.01^{NS}	0.675 ± 0.01^{NS}	0.673 ± 0.01^{NS}	0.669 ± 0.01^{NS}
	Week 4	$0.745a \pm 0.01^a$	0.710 ± 0.01^b	0.692 ± 0.01^c	0.700 ± 0.01^c
TVBN (mgN/100g)	Week 0	29.91 ± 0.03^a	28.06 ± 0.60^b	27.77 ± 0.67^b	26.4 ± 0.30^c
	Week 4	31.90 ± 0.04^a	30.40 ± 0.19^b	29.44 ± 0.01^c	29.26 ± 0.19^c
Peroxide (m.eq/Kg oil)	Week 0	1.38 ± 0.04^a	1.33 ± 0.06^a	1.20 ± 0.02^b	1.11 ± 0.05^b
	Week 4	4.19 ± 0.09^a	3.81 ± 0.29^{ab}	3.24 ± 0.13^c	3.40 ± 0.14^{bc}
TPC (log/CFUg)	Week 0	4.3 ± 0.33^{NS}	4.2 ± 0.28^{NS}	4.1 ± 0.13^{NS}	4.0 ± 0.05^{NS}
	Week 4	5.8 ± 0.01^a	5.5 ± 003^b	5.0 ± 0.13^c	5.2 ± 0.13^c
Overall Acceptability	Week 0	4.79 ± 0.72^c	5.71 ± 0.69^b	6.25 ± 0.44^a	4.88 ± 0.74^c
	Week 4	3.58 ± 0.50^d	4.67 ± 0.48^b	5.75 ± 0.44^a	4.08 ± 0.65^c

Table 12. Effect of the drying methods on moisture content, a_w, TVB_N values and peroxide values of dried snakehead. Values (mean $\pm$ SD) with the same letter within a column are not significantly different ($p < 0.05$). NS: not significant. a_w: water activity.

Treatment	Moisture (%)	aw	TVB_N (mg/100g)	Peroxide (m.eq/kg oil)	Overall Acceptability
(AD)_65°C: 28hours	$29.4^c \pm 0.56$	$0.673^{cd} \pm 0.00$	$29.15^{NS} \pm 1.33$	$1.2^b \pm 0.02$	5.46 ± 0.55^b
(AD)_65°C:15 h+ (AD)_60°C: 13h	$32.1^a \pm 0.15$	$0.691^a \pm 0.00$	$29.38^{NS} \pm 0.02$	$1.2^b \pm 0.05$	4.96 ± 0.20^c
(AD)_60°C: 31h	$29.1^c \pm 0.49$	$0.670^d \pm 0.00$	$29.43^{NS} \pm 0.25$	$1.2^b \pm 0.05$	6.33 ± 0.48^a
(AD)_65°C: 22 h+ (TD): 6 h	$31.7^{ab} \pm 0.26$	$0.683^{ab} \pm 0.00$	$29.69^{NS} \pm 0.09$	$1.3^{ab} \pm 0.04$	5.42 ± 0.50^b
(AD)_65°C: 18.5 h + (TD): 9.5 h	$30.9^{ab} \pm 0.41$	$0.673^{cd} \pm 0.00$	$29.72^{NS} \pm 0.07$	$1.3^{ab} \pm 0.04$	6.63 ± 0.49^a
(AD)_65°C: 14 h + (TD): 14 h	$31.19^b \pm 0.32$	$0.679^{bc} \pm 0.00$	$29.77^{NS} \pm 0.30$	$1.3^a \pm 0.04$	4.42 ± 0.50^c

Table 13. Effect of the storage temperatures on moisture content, a_w, TVB_N values and peroxide values of dried snakehead. Values (mean $\pm$ SD) with the same letter within a column are not significantly different ($p < 0.05$). NS: not significant. a_w: water activity, TPC: Total plate count, TVB-N: Total volatile base nitrogen.

Treatment	Parameter	Moisture (%)	a_w	TVBN (mgN/100g)	Peroxide (m.eq/Kg oil)	TPC (log/CFUg)
Week 0	Room temp.	29.41 ± 0.09^{NS}	0.673 ± 0.00^{NS}	28.1 ± 0.59^{NS}	1.22 ± 0.04^{NS}	4.1 ± 0.10^{NS}
	4ºC – 6ºC	29.40 ± 0.53^{NS}	0.671 ± 0.00^{NS}	28.1 ± 0.72^{NS}	1.20 ± 0.03^{NS}	4.0 ± 0.08^{NS}
Week 4	Room temp.	30.54 ± 0.20^a	0.691 ± 0.00^a	32.4 ± 0.56^a	2.35 ± 0.04^a	5.0 ± 0.05^a
	4ºC – 6ºC	30.14 ± 0.04^b	0.682 ± 0.00^b	30.7 ± 0.33^b	2.12 ± 0.09^b	4.7 ± 0.05^b

Table 14. Sensory evaluation of dried snakehead. The values are based on a 7-point scale of 8-person panel response to each attribute. Means with the same letter within column are not significantly different ($p < 0.05$). NS: not significant.

Treatment	Parameter	Color	Texture	Aroma	Flavor	Overall Acceptability
Week 0	Room temperature	3.92 ± 0.65^{NS}	3.92 ± 0.41^{NS}	3.92 ± 0.65^{NS}	3.88 ± 0.68^{NS}	6.25 ± 0.53^{NS}
	4ºC – 6ºC	3.96 ± 0.55^{NS}	4.04 ± 0.55^{NS}	3.96 ± 0.36^{NS}	3.92 ± 0.65^{NS}	6.29 ± 0.55^{NS}
Week 4	Room temperature	5.00 ± 0.30^a	3.33 ± 0.48^{NS}	4.62 ± 0.50^a	4.46 ± 0.51^{NS}	5.62 ± 0.50^b
	4ºC – 6ºC	4.62 ± 0.50^b	3.54 ± 0.51^{NS}	4.29 ± 0.46^b	5.29 ± 0.46^{NS}	6.08 ± 0.50^a

Table 15. Effect of salting methods on moisture content, salt content and a_w of dried snakehead. Values (mean $\pm$ SD) with the same letter within a column are not significantly different ($p < 0.05$). NS: not significant. a_w: water activity.

% Salt	Time Treatment	Moisture (%)	a_w	Sodium chloride content(%)	Overall Acceptability
8%	20 minutes	30.83 ± 0.06^a	0.677 ± 0.01^a	7.28 ± 0.06^g	4.67 ± 0.56^c
8%	30 minutes	30.61 ± 0.06^{ab}	0.669 ± 0.01^a	7.91 ± 0.06^f	4.75 ± 0.68^c
8%	40 minutes	30.41 ± 0.07^{bc}	0.662 ± 0.01^a	8.39 ± 0.07^e	4.92 ± 0.58^{bc}
10%	20 minutes	30.27 ± 0.09^c	0.657 ± 0.01^a	8.41 ± 0.08^e	5.33 ± 0.70^b
10%	30 minutes	29.82 ± 0.02^d	0.634 ± 0.00^b	9.42 ± 0.02^d	6.25 ± 0.61^a
10%	40 minutes	29.51 ± 0.06^e	0.612 ± 0.00^{cd}	10.51 ± 0.06^c	4.71 ± 0.55^c
12%	20 minutes	29.58 ± 0.14^{de}	0.625 ± 0.00^{bc}	9.58 ± 0.12^d	4.42 ± 0.58^c
12%	30 minutes	28.92 ± 0.08^f	0.603 ± 0.00^{de}	10.92 ± 0.07^b	3.21 ± 0.72^d
12%	40 minutes	27.67 ± 0.13^g	0.584 ± 0.01^e	11.58 ± 0.12^a	2.83 ± 0.64^d

Table 16. Effect of different percentages of sorbitol on moisture content, a_w, TVB_N values, peroxide values and Total Plate Count (TPC) of dried snakehead. Values (mean ± SD) with the same letter within row are not significant different ($p < 0.05$). a_w: water activity, TPC: Total Plate Count, CFU: Colony Forming Unit.

Parameter	Treatment	Sorbitol 0% (w/w)	Sorbitol 1% (w/w)	**Sorbitol 2% (w/w)**	Sorbitol 3% (w/w)
Moisture (%)	Week 0	28.77 ± 0.48^b	29.10 ± 0.14^b	29.70 ± 0.01^a	29.92 ± 0.09^a
	Week 4	30.73 ± 0.17^a	30.26 ± 0.15^b	30.08 ± 0.11^b	30.30 ± 0.12^b
a_w	Week 0	0.630 ± 0.00^a	0.626 ± 0.00^a	0.614 ± 0.00^b	0.610 ± 0.01^b
	Week 4	0.658 ± 0.00^a	0.646 ± 0.00^b	0.628 ± 0.00^c	0.628 ± 0.00^c
TVBN (mgN/100g)	Week 0	28.95 ± 0.12^a	28.29 ± 0.06^{ab}	27.18 ± 0.04^{bc}	27.03 ± 0.09^c
	Week 4	31.66 ± 0.06^a	30.72 ± 0.11^a	28.99 ± 0.09^b	29.13 ± 0.02^b
Peroxyde (m.eq/Kg oil)	Week 0	1.27 ± 0.03^a	1.21 ± 0.02^b	1.14 ± 0.01^c	1.12 ± 0.02^c
	Week 4	1.82 ± 0.04^a	1.68 ± 0.03^b	1.47 ± 0.04^c	1.49 ± 0.01^c
TPC (log/CFUg)	Week 0	4.3 ± 0.04^a	4.1 ± 0.01^b	4.0 ± 0.01^c	3.9 ± 0.01^c
	Week 4	5.4 ± 0.00^a	5.2 ± 0.07^b	4.5 ± 0.05^c	4.6 ± 0.01^c
Overall Acceptability	Week 0	5.25 ± 0.44^c	5.71 ± 0.69^b	6.25 ± 0.44^a	5.38 ± 0.50^{bc}
	Week 4	4.83 ± 0.38^c	5.29 ± 0.46^b	5.96 ± 0.20^a	5.04 ± 0.20^{bc}

Table 17. Effect of the drying methods on moisture content, a_w, TVB_N values and peroxide values of dried snakehead. Values (mean ± SD) with the same letter within a column are not significantly different ($p < 0.05$). NS: not significant. a_w: water activity.

| Drying time	Moisture (%)	a_w	TVBN (mgN/100g)	Peroxyde (m.eq/Kg oil)	TPC (log/CFUg)	Overall Aceptalility
33 h (open air drying)	31.25 ± 0.87^a	0.633 ± 0.00^a	26.25 ± 0.04^{bc}	1.18 ± 0.04^c	5.3 ± 0.07^a	4.67 ± 0.57^c
36 h (open air drying)	29.50 ± 0.15^b	0.622 ± 0.00^{cd}	27.02 ± 0.06^b	1.33 ± 0.04^b	5.1 ± 0.02^b	6.25 ± 0.44^b
39 h (open air drying)	28.06 ± 0.34^c	0.616 ± 0.00^d	30.17 ± 0.06^a	1.64 ± 0.06^a	5.1 ± 0.06^b	5.92 ± 0.72^b
33 h (tent dryer)	31.92 ± 0.38^a	0.636 ± 0.00^{ab}	25.78 ± 0.06^c	1.12 ± 0.06^c	5.2 ± 0.05^a	4.75 ± 0.44^c
36 h (tent dryer)	29.91 ± 0.37^b	0.626 ± 0.00^{bc}	26.72 ± 0.07^{bc}	1.20 ± 0.04^c	4.9 ± 0.03^c	6.71 ± 0.46^a
39 h (tent dryer)	28.89 ± 0.17^{bc}	0.618 ± 0.00^d	29.95 ± 0.03^a	1.60 ± 0.03^a	5.1 ± 0.03^b	6.08 ± 0.50^b
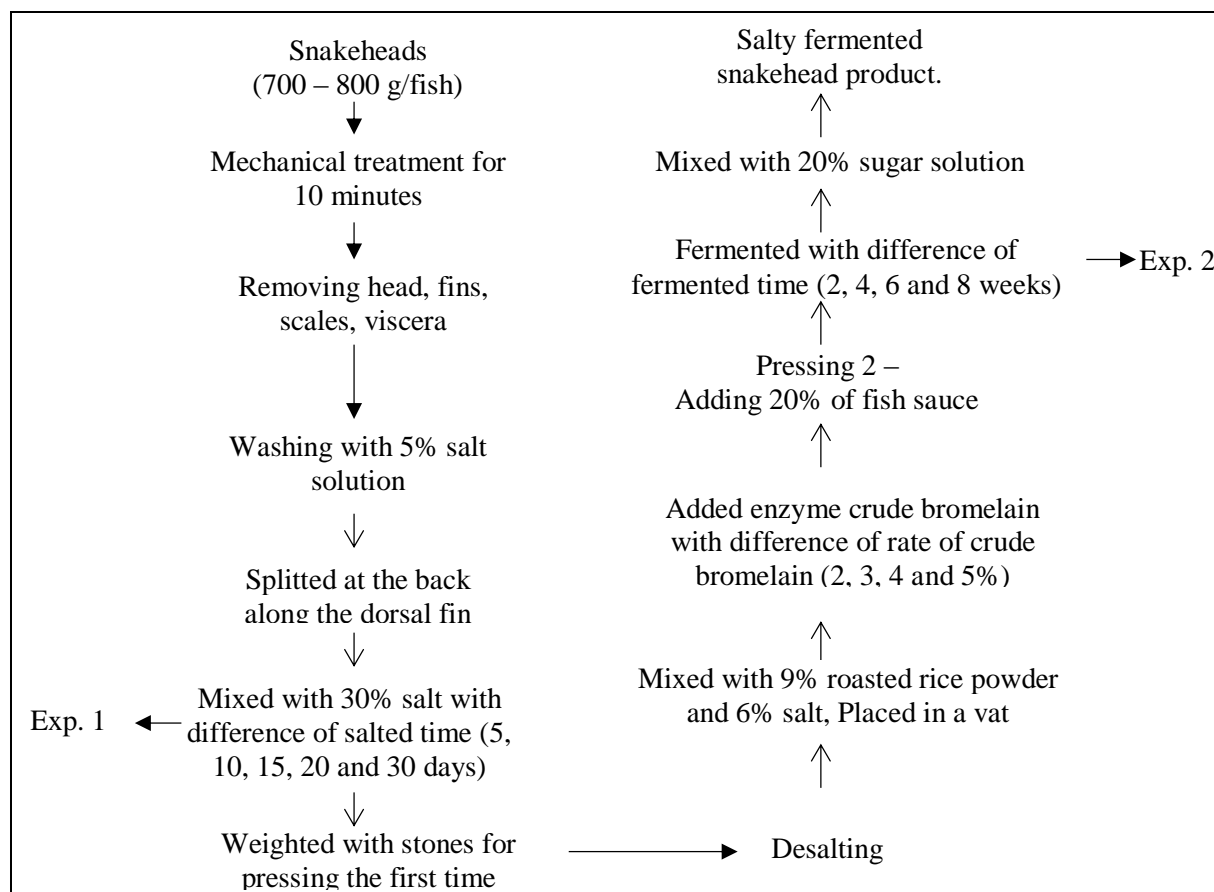
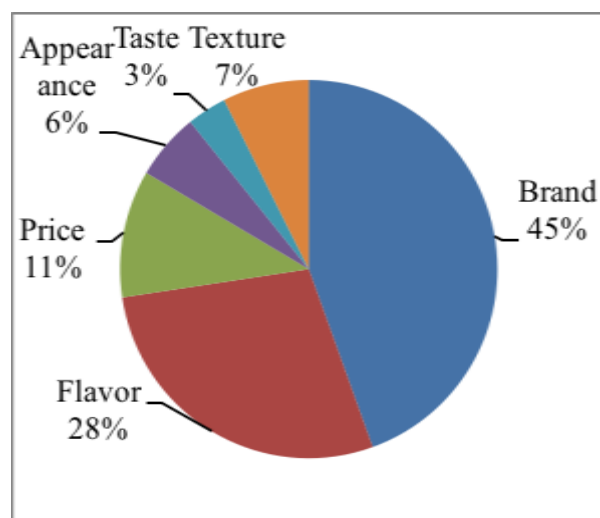Figure 1. General procedure for processing fermented snakehead.

Figure 2. Quality of fermented snakehead influencing purchased intent.
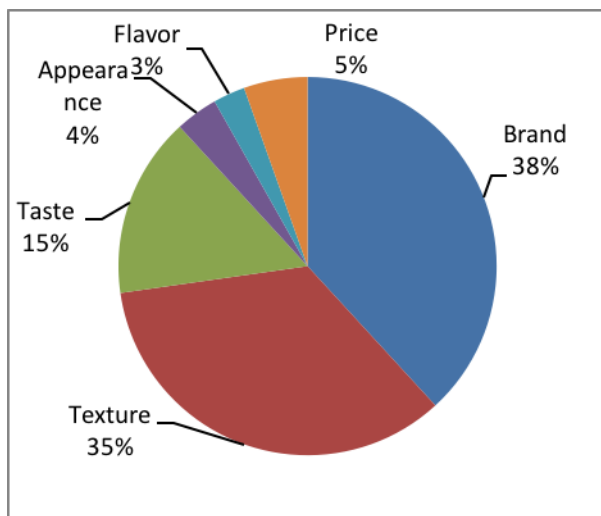
Figure 3. Quality of dried snakehead influencing purchased intent.

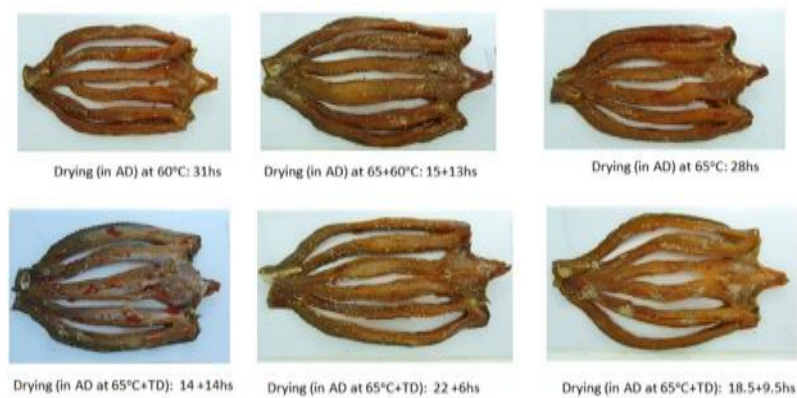**Figure 4.** Colors of dried snakehead with different percentages of glycerol at the initial week

Drying (in AD) at 60°C: 31hs   Drying (in AD) at 65+60°C: 15+13hs   Drying (in AD) at 65°C: 28hs

Drying (in AD at 65°C+TD): 14 +14hs   Drying (in AD at 65°C+TD): 22 +6hs   Drying (in AD at 65°C+TD): 18.5+9.5hs

Figure 5. Colors of dried snakehead with different drying methods.

Sorbitol 0%   Sorbitol 1%   Sorbitol 2%   Sorbitol 3%
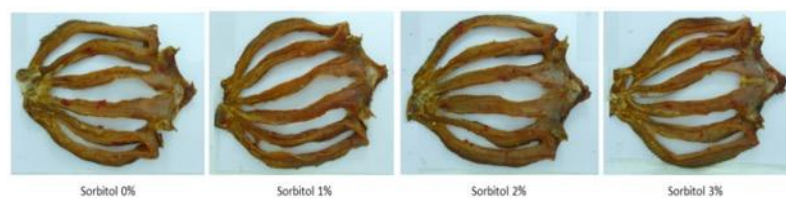
Figure 6. Appearances of dried snakehead with differing percentage of sorbitol at the initial week.
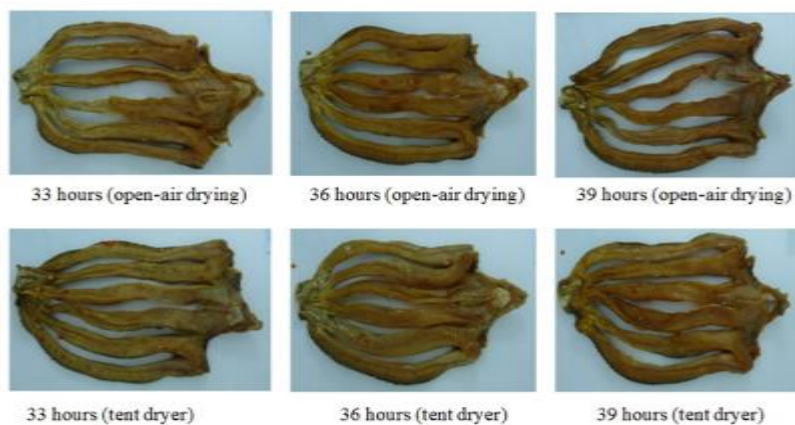
Figure 7. Appearances of dried snakehead with different drying methods.
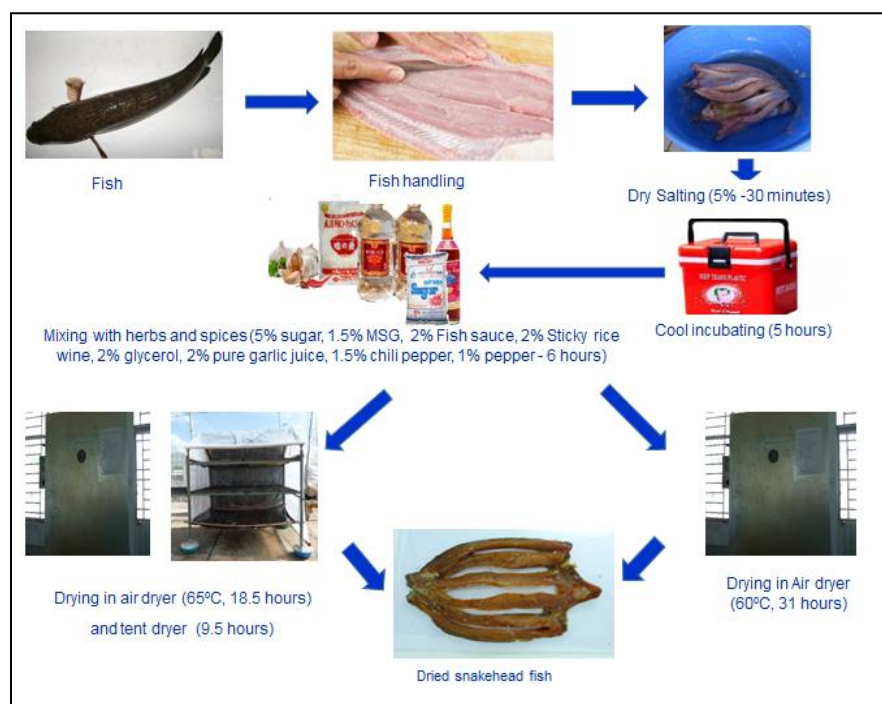
Figure 8. Process of dried snakehead with added sucrose.
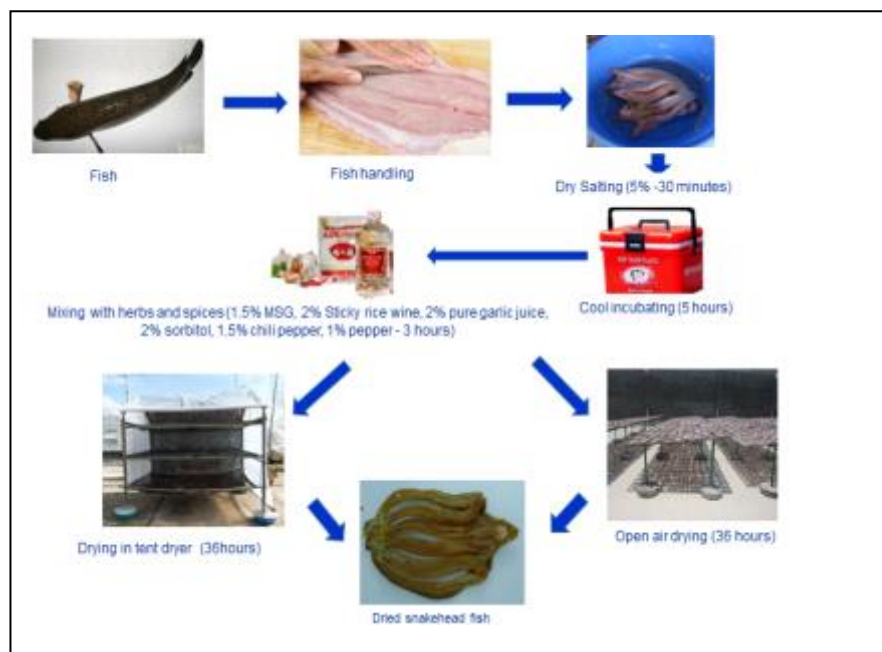
Figure 9. Process of dried salted snakehead without sucrose.

Figure 10. Training course on snakehead aquaculture for women in An Giang province.

Alternative Feeds and Processing for Freshwater Aquaculture Species, Part II

USE OF SOY PRODUCTS IN SNAKEHEAD DIET

Enhanced Trade and Investment for Global Fishery Markets/Experiment/13SFT03UC

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ABSTRACT

Soybean meal-based formulated feeds have recently become available for snakehead culture in Vietnam. The first part of this study was conducted to determine the appropriate replacement of fish meal (FM) protein by soy protein concentrate (SPC) in snakehead (*Channa striata*) diet. The study included five iso-nitrogenous (45%crude protein) and iso-caloric (19KJ/g) practical diets that were formulated to replace 0% (control), 40%, 60%, 80% and 100% of protein FM by protein SPC (100% FM, 40% SPC, 60% SPC, 80% SPC, and 100% SPC, respectively). Moreover, a digestibility experiment was also followed with the same formulated diet with addition of 1% of chromium oxide. Results showed that fish fed 100% FM and 40% SPC diets had significantly better growth rate (weight gain and daily weight gain) and survival rate compared to other treatments. Feed intake, feed conversion ratio, protein efficacy ratio, and net protein utilization, trypsin and chymotrypsin activities of experimental fish fed 100% FM and 40% SPC diets was significantly higher than those fed other diets. The apparent digestibility coefficient (ADC) of diet (ADC diet), ADC protein and ADC lipid of fish fed diet 40% SPC and 100% FM treatment was significantly higher than those of other treatments. The feed production cost in diet 100% FM and 40% SPC was much lower compared to other treatments. The increase level of SPC in diet above 40% significantly affected on the fish growth, economic efficiency, digestibility, and trypsin and chymotrypsin activities though fish chemical composition unlikely affected.

The second objective of this study was to evaluate the effectiveness on growth performance and immune responses of mannan oligosaccharide (MO) supplementation in both soybean meal (SBM) and soy protein concentrate (SPC) formulated feeds for snakehead (Channa striata). The experiment included three feed groups, one using fishmeal (FM) as the only protein source, the second replacing 40% of the FM with soybean meal (SBM), and the third replacing 40% of FM with SPC. Each feed group was then divided into three feed treatments which added 0%, 0.2%, and 0.4% MO. The diets were 44.3-45% protein and 19KJ/g energy. Eighty snakehead fingerlings (7.05 ± 0.08 g/fish) were assigned randomly to each of twenty-seven 500-L composite tanks with continuous aeration and 30% daily water exchange. MO supplementation of diets based solely on FM versus diets in which 40% of FM had been replaced by either SBM or SPC, both final weight (Wf) and weight gain (Wg) were significantly affected by diet and MO supplementation, as well as the interaction between the two. In general (with some exceptions), growth performance of fish was significantly better when they were fed SPC than when they were fed SBM or FM, and MO supplementation generally improved growth of the fish. FCR, PER, and survival of fish in this experiment was significantly affected by diet, but only survival was significantly affected by MO supplementation and in no case were the interactions significant. FCR was significantly improved

(i.e. lower) when fish were fed the SPC diet compared to the SBM diet, but neither was significantly different from fish fed the FM diet. PER for fish fed the FM and SPC diets was significantly greater than that for fish fed the SBM diet.

Survival of fish fed SPC diet was significantly lower than that of fish fed the FM and SBM diets, but supplementation with MO, especially at the level of 0.2%, significantly improved survival. Red blood cell (RBC) counts were not significantly affected by either diet, MO supplementation, or the interaction of the two, but white blood cell (WBC) counts were significantly affected by both diet and MO supplementation (although not the interaction). Fish fed the SPC diet had significantly higher WBC counts than fish fed the FM diet, but neither group was significantly different from fish fed the SBM diet. MO supplementation at both 0.2% and 0.4% levels significantly increased WBC counts compared to the unsupplemented diets. Immunoglobulin (Ig) levels were significantly increased by MO supplementation and the interaction of MO and diet, but diet did not affect Ig levels. At the end of the feeding trial but prior to the bacterial challenge, lysozyme levels were significantly affected by diet, MO supplementation and the interaction between the two. For each diet, the greater the level of MO supplementation, the greater the level of lysozymes is. Reduction of fish production costs per kg fish produced, compared to fish fed the FM unsupplemented diet as the standard, ranged from 8.7 − 15.1% for the various other diets tested. Following a 15-d bacterial challenge with Aeromonas hydrophila, fish lysozyme levels were significantly increased by MO supplementation and the interaction between MO and diet, but not by the diets themselves. Again, the greater the level of MO supplementation, the greater the lysozyme level is. After the 15-day challenge, cumulative mortality was lower for fish given MO supplementation than it was for fish fed the unsupplemented diets. In general, fish fed the SBM diet, supplemented or not, had lower cumulative mortality than fish fed the FM or SPC diets.

Women in An Giang province were trained to feed formulated feeds with MO to snakehead and they carried out demonstration projects to show that snakehead can be reared in hapas or tanks to increase household income by US$200–400.

INTRODUCTION

Snakehead is popularly cultured in the Mekong Delta of Vietnam in a variety of farming systerns such as ponds, hapas, cages, and lined tanks (Sinh and Chung, 2009). Over the past few years, snakehead culture increased rapidly; in 2010, total production of snakehead in the Mekong Delta was about 40,000 tons (Sinh et al. 2011). However, most snakehead farmers at that time cultured by the traditional method, using fishmeal and trash fish, meaning high production costs. According to Sinh and Pomeroy (2010) to harvest 30,000 tons of cultured snakehead fish, about 50,000 tons of small freshwater fish and 75,000 tons of small marine fish respectively are required per year. Wild fish supplies are reduced due to overfishing, so finding other sources of protein, especially plants, to replace fish meal (FM) is necessary and urgent. Feed costs account for 80% of the total snakehead production cost (Hien et al. 2011). Snakehead is a carnivorous species with high protein requirement, up to 40% (Samantary and Mohanty, 1997; Be and Hien, 2010). In aquaculture feed, FM is the main protein source due to high digestibility, vitamin, mineral, and high unsaturated fatty acid which is essential for aquatic animals. However, FM production is being reduced and prices have increased, so alternative protein sources for aquatic animals are important. Soybean meal (SBM) is becoming more widely used. Research on the replacement of FM by SBM has been conducted on many species, e.g. cobia (Rachycentron canadum) (Chou et al. 2004), Korean rockfish (Sebastes schlegeli) (Lim et al. 2004), Asian seabass (Lates calcarifer) (Tantikitti et al. 2005), Atlantic cod (Walker et al. 2010), snakehead (Channa striata) (Be and Hien, 2010), giant snakehead (Hien et al. 2010), and knife fish (Dan et al. 2013). However, SBM contains anti-nutritional factors like trypsin inhibitor, hemagglutinins, phytate, soyantigens, and lacking of methionine and cysteine (O'Keefe and Newman, 2011).

Many soybean products are used in aquafeed, e.g. SBM, soy protein concentrate (SPC), and fermented soybean. FM can be replaced by SBM up to 30% in diets for knife fish (Dan et al. 2013), but only at 20% inclusion in diets for spotted rose snapper (Lutjanus guttatus) (Silva-Carrillo et al. 2012). SPC (65- 67% crude protein) has had anti-nutritional factors removed by alcohol extraction (Dersjant-Li, 2002). FM can be replaced by SPC from 40 - 100% in diets for rainbow trout (Médale et al. 1998), juvenile cobia (Salze et al. 2010), and Atlantic cod (Walker et al. 2010). Moreover, the supply of soy products is more stable and economical than the supply of FM (Hertrampf and Piedad-Pascual, 2000). Since Hien et al. (2014, 2015, submitted, in preparation) found that replacing 40% of FM by SBM does not affect the survival and growth rate of snakehead (C. striata) use of commercial feeds containing SBM in snakehead farming has increased rapidly. The first aim of this study was to determine the appropriate replacement level of FM by SPC in diets for C. striata fingerlings.

Ward et al. (2016) showed that SBM-based diets likely contain immunostimulants for summer flounder (*Paralichthys dentatus*), whereas SPC-based diets do not. Furthermore, Ward (2014) and Ward et al. (in preparation) showed that the immunostimulants were most likely the oligosaccharides stachyose and raffinose. We had wanted determine whether those compounds would stimulate immune response in snakehead as well. Unfortunately, purified forms of those substances are prohibitively expensive for commercial usage in snakehead diets. We therefore chose to test commercial immunostimulant products (see below).

Intensive farming of snakehead often leads to disease development, with pathogens such as bacteria (*Aeromonas hydrophila* and *A. sobria*) and fungi (*Aphanomyces invandans*) causing economic losses. In intensive systems, the fish are sometimes cultured at stocking densities up to 120-160 fish/m^2 (Sinh et al. 2011) and fish easily get stressed and develop infectious diseases, especially epizootic ulcerative syndrome (EUS) (Miles et al. 2001). Additionally, Duc et al. (2012) demonstrated that parasites, fungi, and bacteria infected cultured snakehead in An Giang and Dong Thap provinces.

Successful supplementation with immunostimulants to diets for aquatic animals can increase the resistance to pathogens. Some of these immunostimulants derive from *Saccharomyces cerevisiae*, a fraction of the cell wall of which is rich in mannan oligosaccharides (MO). MO is effectively used as an immunostimulant in many species. For example, in striped catfish (*Pangasianodon hypophthalmus*) addition of 0.12% MO (Actigen, Alltech, USA) significantly increased the survival rate, lysozymes, and leukocytes during bacterial challenges with *Edwardsiella ictaluri* (Hung et al. 2008). Supplementation of 0.2% MO in the diets for salmon (*Oncorhynchus mykiss*), led to an absolute growth rate 5.59% higher than that of the control and survival rates of fish tended to rise (Sara et al. 2011). In tilapia (*Oreochromis noloticus*), adding 0.2% MO significantly improved the FCR, lysozyme, bactericidal activity compared to control and reduced by 20% the cost of feed to produce one kilogram of fish (Ahmad et al. 2013).

The second aim of this study was therefore to determine whether MO supplementation in diets containing 40% replacement of FM with SBM or SPC improves the growth and immune response of snakehead (*C. striata*). The study included both feeding trials and bacterial challenge experiments. If MO does improve growth and immune response, then a suitable concentration of MO might be used to improve commercial snakehead farming and increase economic efficiency.

Finally, scenarios of climate change and/or damming of the Mekong River could greatly impact the availability of fish-based products for inclusion in diets for snakehead. Soy-based products would still be available however. Furthermore, from the international commodity perspective, FM is both very expensive and subject to variable availability that causes price spikes. SPC is less expensive and not subject to such variability. For these reasons, development of snakehead diets with maximum inclusion of SPC and added immunostimulants should represent a more sustainable future for snakehead aquaculture. Women make up more than 50% of the population in the Lower Mekong Basin (LMB). Our previous

studies showed that male labor was dominant in fish farming practices (78.4% of farmers), but the participation of women in farming snakehead species was high (21.6% of farmers) in comparison with other cultured fish species in Vietnam (often less than 10%) (AquaFish-CRSP project, 2010). In flooding season, the changes in the hydrological regime (water levels, duration of flooding, timing of flooding) affects aquaculture in the LMB. From 2010, Can Tho University has developed small-scale aquaculture for flooding areas, especially small-scale farming of snakeheads in hapas and plastic linned tanks. These models were judged to be very effective for flooding seasons and women participants (more than 70% women participants). However, small-scale farmers of snakeheads still use small fish for feeding. So, developing the small-scale farming of snakeheads using formulated feed is very necessary for environmental control and aquatic resources management. On-farm trials in An Giang province aimed to apply the optimal formulated feed for snakehead culture from the CTU trials under actual farm conditions with owners.

OBJECTIVE

To continue the development of cost-effective alternative feeds for carnivorous freshwater species for small-scale farming of snakeheads by women during the flooding season; and to improve the processing activities for added value of cultured snakehead products, particularly for women.

MATERIALS AND METHODS

Replacement of FM by SPC, growth experiment. The study included five iso-nitrogenous (45% protein) and iso-caloric (19 KJ/g) practical diets that were formulated to replace 0% (control), 40%, 60%, 80%, and 100% of protein FM by protein SPC (100% FM, 40% SPC, 60% SPC, 80% SPC, and 100% SPC, respectively) (Table 1). Experimental fish (10.0 ± 0.5g in initial weight) were transferred to CTU from a nursery in An Giang province and acclimated in tanks (four m^3) for two weeks. Fish were then randomly assigned at stocking density of 30 fish/tank to 15 composite tanks (500L/tank) with aeration supplied. Each treatment was triplicated. Water was exchanged every two days at a rate of 50%. Experimental period was 42 days. Fish were fed twice a day (8:00 and 16:00) to satiation. Amount of feed was recorded very day and excess feed was removed and recorded. Dead fish were collected and weighed. Temperature, pH and dissolved oxygen were measured twice a day by YSI 556 (USA); NO_2^- and NH_3 were recorded every week by test kit SERA (Germany). Temperature was $28.6 - 31.2°C$ (morning and afternoon). Dissolved oxygen was above 5 mg/L. pH was $7.70 - 8.05$. NO_2^- ranged from $0.63 - 0.70$ mg/L and NH_3 was below 0.1 mg/L.

Initial fish weight (Wi) and final fish weight (Wf) were determined before and after the experiment. We determined Survival Rate (SR, %), Daily Weight Gain (DWG; g/d), Feed Intake (FI; %/fish/d), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Net Protein Utilization (NPU) as follows (where t = time in days):

SR = (number of initial fish/number of fish at the end of experiment) x 100
$DWG = (W_f - W_i)/t$
$FI = $ consumed feed$/(W_f \times W_i)^{0,5}/t$
FCR = amount of consumed feed in dry matter (g)/ weight gain (g)
$PER = (W_t - W_o)/$ protein intake
NPU = (protein intake-protein waste)*100/protein intake

Initial fish (six fish/tank), final fish (six fish/tank) and feed (100 g) were collected, minced and stored at -20°C until analysis. Chemical composition of fish and feed were analyzed following methods of AOAC (2000). Fish at the end of experiment (final fish, three fish/tank) were collected after one day of starvation for digestive enzyme analysis (trypsin and chymotrypsin) following methods described by Tseng et al. (1982) and Worthing (1982).

Replacement of FM by SPC, digestibility experiment. Experimental feeds were formulated in the same composition as the growth experiment, except chromic oxide (Cr_2O_3) was added to all diets at a level of 1%. The experimental system for feces collection (Figure 1) consisted of a series of 250-L composite tanks with aeration. Fish (10 g) were stocked at 20 fish/tank and fed twice a day (8:00 and 16:00) to satiation for the first ten days. Excess feed and dead fish were removed. Feces collection began after 10 days of feeding and continued for 20 days, when the amount of feces (about 10 g dry matter) was sufficient for analysis. For feces collection, fish were fed once at 8:00 and the remaining feed was removed after two hours, tanks were cleaned, and feces collection chambers were installed. Feces were collected overnight in a collection bottle stored in the chamber on ice. Feces were collected every day, dried at 60°C and stored at -20°C until analysis. Temperature, pH and dissolved oxygen of water were measured twice a day by YSI 556 (USA); NO_2^- and NH_3 were recorded every week by test kit SERA (Germany). Temperature was 27.0 – 29.3°C (morning and afternoon), dissolved oxygen was above five mg/L, pH was 7.60 – 8.20, NO^{2-} ranged from 0.12 – 0.17 mg/L and NH_3 was below 0.1 mg/L.

Feces and experimental feed were analyzed for crude protein, crude lipid, moisture and ash following AOAC (2000). Chromic oxide (Cr_2O_3 was analyzed following the method of Furukawa and Tsukahara (1966). Calculations included:

Apparent Digestibility Coefficient of diet (ADCdiet) $ADC_{diet} = 1 - \dfrac{\%A}{\%B}$

Apparent Digestibility Coefficient of nutrient in diet (ADCNu-Diet)

$ADC_{Nu-Diet} = 1 - \dfrac{\%A}{\%B} \times \dfrac{\%B'}{\%A'}$ where A is % Cr_2O_3 in feed, B is % Cr_2O_3 in feces, A' is % nutrient in feed, and B' is % nutrient in feces.

Feeding and bacterial challenge trials with mannan oligosaccharides. A feeding trial was conducted to evaluate survival, growth, feed efficiency, and immune response parameters. The experiment included three feed groups, one using only FM as the protein source and the second and third using SBM and SPC, respectively, to replace 40% of the FM. Each feed group was subdivided into three feed treatments to which were added either 0.0% (control), 0.2%, and 0.4% MO (Alltech, USA). Thus, the experiment consisted of nine treatments with three replicates. The nine experimental diets were formulated to be 45% protein, 9% lipid, and 4.2 Kcal/g energy (Table 2). All ingredients were mixed mechanically with water for 30 minutes and the resulting dough was then passed through an extruder to obtain pellets of 2-mm diameter. The diets were dried in direct sunlight for six hours, then allowed to cool at room temperature for 0.5 hours, and finally stored in airtight plastic bags until use. Proximate composition of the diets was analyzed using AOAC (2000).

Prior to the experiment, fingerlings were transferred from a hatchery in An Giang province to CTU, acclimated in a 2,000-L circular tank, and fed on the control (FM) diet. The average initial weight per fish was 7.05 g for each experiment. To start the experiment, fingerlings were randomly distributed into 27 composite tanks (500-L capacity, filled with 300 L of water) at a stocking density of 80 fish/tank. Each experimental tank was provided with continuous aeration and flow through water supply with 30% water exchange/day. Fish were fed two times/day (9:00 am, 3:00 pm) to satiation. The amount of consumed feed and uneaten feed in each tank was recorded daily (the amount of uneaten feed was siphoned out after 30 minutes, dried and weighed). At the end of the experiment, all of the fish in each tank were counted and weighed for calculation of growth rate and survival rate. Any fish mortality was recorded daily and dead fish were removed and weighed immediately. The experimental period was eight weeks. Temperature ranged from 27.5-30.1ºC, dissolved oxygen from 5.22 to 5.42 mg/L, pH from 7.53 to 8.01, NO_2^- from 0.62 to 0.69 mg/L and NH_3 <0.1 mg/L. Therefore, the water quality parameters in all treatments were a suitable range for the normal growth and development of fish.

After eight weeks, three fish from each tank were randomly collected and blood withdrawn for analysis of erythrocytes, leukocytes, lysozymes, and total immunoglobulin (Ig). The remainder of the fish were then tranferred to the bacterial challenge experiment. Data on FCR and PER were calculated as indicated above for the earlier feeding experiment. Red blood cells were counted by the usual method using the Neubauer chamber and Natt – Hedrick solution (Natt and Hedrick, 1952). White blood cells were counted on lame that was stained by Wright's & Giemsa solution (Hang et al. 2013). Lysozyme was analyzed by the method of Ellis et al. (1990). Total Ig was analyzed by the method of Siwicki and Anderson (1993), modified by Milla et al. (2010).

A bacterial challenge was conducted after the growth trial to determine the snakehead's immune response to *Aeromonas hydrophila*. Fish from each growth trial treatment were divided into two groups. Thus, the experiment consisted of 18 treatments: 9 treatments (3 from the FM group, 3 from the SB group, and 3 from the SPC group) were injected with 0.2 ml physiological saline (0.85%) and the remaining nine treatments (three from the FM group, three from the SB group, and three from the SPC group) were injected with 2.32×10^5 CFU/fish of bacterial strain CL1403 *Aeromonas hydrophila* based on the lethal dose determined by Duc et al. (2013). Each treatment was triplicated. Experimental fish had average weights of 49.87-50.73 g and were randomly assigned to 100-L plastic tanks at a density of 15 individuals/tank. The experimental period was 15 days, during which time fish were fed their respective diets and dead fish were recorded daily. For moribund fish, clinical signs were observed by gross inspection, and the lesions were sampled directly for bacteria. Re-isolation and re-identification of bacteria were carried out according to methods of Barrow and Feltham (1993). Water exchange was 20%/day. After 15 days, three fish were randomly collected to withdraw blood and analyze lysozyme as feeding trial.

On-farm trials with women farmers. Women snakehead farmers in An Giang province participated in a training course on snakehead culture using pelleted feed led by CTU researchers. Six of the women who operated small-scale snakehead farms were selected for demonstration projects using formulated feed. Three farms culture snakehead in hapas (24 m^2) and the others use plastic-lined tanks (15 m^2). Formulated feed was provided based on previous results with added 0.2% MO. Snakehead fingerlings were stocked at 80 fish/m^2 in hapas and 100 fish/m^2 in tanks. Culture period was 5-7 months, until snakehead reached market size. Information on total cost (fingerlings, feed, chemical and etc.) and income were recorded and calculated for economic benefits. Calculation methods were as described above for the laboratory studies. Dissolved oxygen, pH, nitrite, and ammonia were monitored during the rearing trial and were considered suitable for snakehead survival and growth.

Statistical analysis. Results of the first growth experiment and the digestibility study were compared by one-way ANOVA followed by Duncan's multiple range test (SPSS 16.0, USA) at significant level of 95%. Results of the second feeding trial and the bacterial challenge experiment were analyzed by two-way ANOVA followed by Duncan's multiple range test.t. at significant level of 95%.

<div align="center">RESULTS</div>

Replacement of FM by SPC, growth experiment. Survival rate did not differ significantly between treatments 100% FM and 40% SPC ($p>0.05$), which were both significantly greater than the other treatments ($p<0.05$) (Figure 2). Fish fed 100% FM and 40% SPC diets did not show significant differences in growth, but both grew significantly more than fish fed 60% SPC and 80% SPC diets ($p>0.05$), which in turn did not differ, and the smallest growth performance was found in fish fed 100% SPC diets (Table 3). There was no significant difference in the fish growth performance between and also in between 100% FM and 40% SPC diets. Feed intake (FI), feed conversion ratio (FCR), protein efficacy ratio (PER) and net protein utilization (NPU) of experimental fish fed 100% FM and 40% SPC diets was significantly higher than those fed other treatments ($p<0.05$; Table 4). Fish were classified into four size groups such as 10 – 20g, 20 – 40g, 40 – 60g and 60 – 75g. The fish size distribution was highly varied

between treatments (Figure 3). Fish fed 60% SPC, 80% SPC and 100% SPC diets were mostly in sizes of 10 – 20g and 20 – 40g. However, in treatments 100% FM and 40% SPC, harvested fish were mostly in sizes of 40 – 60 g and 60 – 75g, whereas only 2 – 4% were in the size range of 10 – 20g.

Chemical composition of experimental fish between treatments showed little variation (Table 5). Fish moisture in fish of treatment 100% SPC was significantly lower than that of 100% FM treatment ($p<0.05$). Fish protein content showed no significant differences between treatments ($p>0.05$). Lipid content of fish fed 100% FM (3.45%) was significantly higher than those of fish fed 60%, 80 and 100% SPC. Ash content of fish fed the 40% SPC diet was significantly lower than that of fish in all other treatments except 80% SPC, and ash content of fish fed the 60% SPC diet was significantly higher than that of all other treatments except 100% FM. Trypsin and chymotrypsin activities in fish fed diet 100% FM and 40% SPC, which did not differ, were significantly higher than those of fish fed 60, 80 and 100% SPC ($p<0.05$) (Fig. 4).

Feed production cost in diets 100% FM and 40% SPC was much lower compared to other treatments (Table 6). Although feed costs were quite similar between diets during replacement of FM by SPC, the significantly lower FCR in treatments 100% FM and 40% SPC appears to have lowered feed production cost.

Replacement of FM by SPC, digestibility experiment. The apparent digestibility coefficient of diet (ADC_{diet}) of fish fed diet 40% SPC was significantly higher than those of other treatments while ADC diet of 100% FM treatment was significantly higher than those of 60% SPC and 80% SPC treatments ($p<0.05$) (Table 7). No data are available for fish fed the 100% SPC diet due to failure of feces collection caused by limited feed consumption of these fish. $ADC_{protein}$ and ADC_{lipid} in treatments fed 100% FM and 40% SPC were significantly higher than those of 60% SPC and 80% SPC treatments ($p<0.05$).

Feeding and bacterial challenge trials with mannan oligosaccharides. In the feeding trial testing MO supplementation of diets based solely on FM versus diets in which 40% of FM had been replaced by either SBM or SPC, both final weight (Wf) and weight gain (Wg) were significantly affected by diet and MO supplementation, as well as the interaction between the two (Table 8). In general (with some exceptions), growth performance of fish was significantly better when they were fed SPC than when they were fed SBM or FM, and MO supplementation generally improved growth of the fish (Table 8). FCR, PER, and survival of fish in this experiment was significantly affected by diet, but only survival was significantly affected by MO supplementation and in no case were the interactions significant (Table 9). FCR was significantly improved (i.e., lower) when fish were fed the SPC diet compared to the SB diet, but neither was significantly different from fish fed the FM diet (Table 9). PER for fish fed the FM and SPC diets was significantly greater than that for fish fed the SBM diet (Table 9). Survival of fish fed SPC diet was significantly lower than that of fish fed the FM and SBM diets, but supplementation with MO, especially at the level of 0.2%, significantly improved survival (Table 9). Red blood cell (RBC) counts were not significantly affected by either diet, MO supplementation, or the interaction of the two, but white blood cell (WBC) counts were significantly affected by both diet and MO supplementation (although not the interaction) (Table 10). Fish fed the SPC diet had significantly higher WBC counts than fish fed the FM diet, but neither group was significantly different from fish fed the SBM diet (Table 10). MO supplementation at both 0.2% and 0.4% levels significantly increased WBC counts compared to the unsupplemented diets (Table 10). Immunoglobulin (Ig) levels were significantly increased by MO supplementation and the interaction of MO and diet, but diet did not affect Ig levels (Table 11). At the end of the feeding trial but prior to the bacterial challenge (i.e. "pre-challenge"), lysozyme levels were significantly affected by diet, MO supplementation and the interaction between the two (Table 11). For each diet, the greater the level of MO supplementation, the greater the level of lysozymes is (Table 11). Reduction of fish production costs per kg fish produced, compared to fish fed the FM unsupplemented diet as the standard, ranged from 8.7 – 15.1% for the various other diets tested (Table 12).

Following the 15-d bacterial challenge with *A. hydrophila*, fish lysozyme levels (i.e. "post-challenge") were significantly increased by MO supplementation and the interaction between MO and diet, but not by the diets themselves (Table 11). Again, the greater the level of MO supplementation, the greater the lysozyme level (Table 11). After the 15-day challenge, cumulative mortality was lower for fish given MO supplementation than it was for fish fed the unsupplemented diets (Figure 5). In general, fish fed the SBM diet, supplemented or not, had lower cumulative mortality than fish fed the FM or SPC diets (Figure 5).

On-farm trials. Women who completed the training course are shown in Figure 6. Fingerling snakehead had been trained to feed on pellets at the hatchery before stocking into tanks and hapas (Figures 7-9). Diets used during the trial are indicated in Table 13. Fish survival rate was relatively high in the on-farm trial, ranged from 72.4 to 76.2% in tank rearing and 62.5 to 80.0% in hapa rearing (Table 14). In both tanks and hapas, fish growth was similar, with FCR in tank models ranging from 1.15 to 1.34 and in hapas 1.00 to 1.34 (Table 15). In hapa farm 1 the FCR was 1.0 during a shorter culture period, four months compared to six months in the other farms. The early harvest at this farm was determined by the woman owner who saw the high price in the market during the rearing period. In tank farm three, lower fish survival led to lower production and yield (Table 15). Production in tanks was lower than in the hapas but its profit was higher (Tables 15 and 16). Farmers reared snakehead in tanks selected big size fish for selling in the time when the market price was high. Discussions with farmers indicated that they saw less disease during the rearing period compared to rearing snakehead with trash fish.

DISCUSSION

Our results show that FM can be replaced by SPC at levels up to 40% and that MO supplementation improves growth, physiological variables, and survival in a bacterial challenge. Salze et al. (2010) concluded that replacement of herring meal by SPC up to 75% did not effect on cobia survival. In Atlantic cod, replacement of FM by SPC up to 50% did not affect growth and survival (Walker et al. 2014). SPC can be used up to 40% in diet for different fish species e.g. Japanese flounder (Deng et al. 2006), gilthead seabream (Kissil et al. 2000), Atlantic salmon (Restie et al. 1998). FM can be replaced by SBM up to 30% in diet for carnivorous fish like snakehead (Be and Hien, 2010), red tail catfish (Lam et al. 2012), and knife fish (Dan et al. 2013). FM replacement by SB can be done at 20% inclusion in diet for spotted rose snapper, but inclusion at 40 and 60% resulted in lower growth and reduced protein and lipid utilization (Silva-Carrillo et al. 2012). For Diplodus puntazzo, 60% FM could be replaced by SB (Hernandez et al. 2007) and replacement of 40% of FM by SBM did not affect growth of Oncorhynchus mykiss (Jalili et al. 2013). In our experiment the significantly lower fish growth found in treatments 60% SPC, 80% SPC, and 100% SPC can be explained by limited feed intake, suggesting that SPC levels above 40% reduced palatability of the feed.

Our findings on the chemical composition of the fish agreed with previous studies in which replacement of FM by SPC did not affect on fish protein content (Cheng et al. 2003; Dan et al. 2013), but did lead to decreased fish lipid content (Dan et al. 2013; Tantikiti et al. 2015). Trypsin and chymotrypsin inhibitors limit the use of SB in diets for carnivorous fish (Baeverfjord and Krogdagl, 1996; O'Keefe and Newman, 2011). Eshel et al. (1993) reported that trypsin contributed to 40 – 50% protein digestibility process in carnivorous fish. Replacing FM by SBM in diets for rainbow trout, Krogdahl et al. (1994) concluded that trypsin activity was reduced due to proteinase inhibitors and reduced enzyme activities were observed with increase levels of SBM in diet. Hart et al. (2010) also concluded that SBM levels above 40% reduced digestibility due to the presence of trypsin inhibitor. Protein and lipid digestibility of SPC in snakehead in our study was relatively high and similar to those for sharp snout sea bream (Hernandez et al. 2007) and rainbow trout (Mambrini et al. 1999). Protein and lipid digestibility were reduced when SBM levels were increased in diets for gilthead sea bream (Venou et al. 2006) and rainbow trout (Mambrini et al. 1999).

Staykvo et al. (2007) demonstrated that growth of rainbow trout given MO was 10% higher than that of fish fed control diets. Sara et al. (2011) showed that growth rate increased by 5.23 % when feed of rainbow trout was supplemented with 0.2% MO. FCR and PER of Diplodus puntazzo were not affected when up to 0.8% MO was added to feed in which 40% SB flour replaced by FM. MO significantly reduced FCR and increased PER in O. mykiss (Staykov et al. 2007), Sparus aurata (Gultepe et al. 2011) and Oreochromis niloticus (Ahmad et al. 2013). However, in our experiment, MO did not affect FCR or PER, a result also seen in Dicentrarchus labrax (Torrecillas et al. 2007), Ictalurus punctatus (Perterson et al. 2010), Piaratus mesopotamicus (Sado et al. 2013) and Channa striata (Talpur et al. 2014).

Increase in number of WBC in fish may serve as protection against pathogenic infection. According to Huong and Tu (2010), leukocytes greatly change under physiological and nutritional conditions of fish and the number of leukocytes can reflect the health status of fish. Lysozyme plays an important role in the innate immune response, it widely distributed in vertebrate and invertebrate (Magnadóttir et al. 2005). Lysozyme is an enzyme that is effective against gram-positive bacteria (Ellis, 1999) and has the ability to resolve gram-negative bacteria, parasites and fungi (Dalmo et al. 1997; Saurabh and Sahoo, 2008). Serum and mucus lysozyme are an important part of non-specific immune system (Kiczka, 1994; Bayne and Gerwick, 2001). Lysozymes kill gram-negative bacteria after the outer cell wall of bacteria is disrupted (Ahmad et al. 2014). Ahmad et al. (2014) indicated that adding 0.2% MO significantly increased lysozyme and bactericidal activity of fish. Talpur et al. (2014) demonstrated that adding 0.2% MO significantly increased lysozyme of C. striata). Oanh and Phuong (2007) stated that globulin antibodies in the blood serum of animals have are able to link to specific antigens. Jalili et al. (2013) demonstrated no difference in lysozyme and Ig compare to the control when plant-based protein (mainly from SBM) replaced 40, 70 or 100% of FM in diets for O. mykiss. Talpur et al. (2014) showed that Bio-Mos at 0.2% in feed for 8 weeks increased Ig and lysozyme but did not statistically improve survival in a bacterial challenge with A. hydrophila. IgM in serum of experimental fish increased as probiotics (yeast cell wall) were added to the feed of Sparus aurata (Cuesta et al. 2004) and Salmo couhensis (Can et al. 2012).

MO supplementation appears to improve survival in bacterial challenges for many species. Torrecillas et al. (2007) fed seabass Dicentrarchus labrax MO at 0.4% of diet to improve survival and Ahmad et al. (2014) demonstrated that the mortality of tilapia (Oreochromis niloticus) is 0% when fed diets supplemented 0.05; 0.1, 0.2% MO. Samrongpan et al. (2008) showed that mortality of tilapia (Oreochromis niloticus) fingerlings decreased with MO supplementation and saw 0% mortality at the level of 0.4 and 0.6%. Addition of 0.2 MO in diet for C. striata significantly reduced mortality in a challenge with A. hydrophila (Talpur et al. 2014).

CONCLUSIONS

The replacement of fishmeal by soy protein concentrate can be done at level of 40% in diet for snakehead. The increase level of SPC in diet above 40% significantly affected on the fish growth, economic efficiency, digestibility, and trypsin and chymotrypsin activities though fish chemical composition unlikely affected. Follow up study should focus on supplementation of other nutrients which can enhance the use of SPC in diet for snake head. Incorporation of MO from 0.2% to 0.4% level in snakehead diets yields better growth performance results and higher immune response. In short, FM based diets with 0.2 MO could be used for snakehead farming, which has more chance to gain revenue. Moreover, SPC based diets with 0.2 MO should be used not only to reduce fish caught to produce fishmeal but also to ensure the sustainable development. This test should be carried out in ponds or lined tanks. Women are now trained for, and active in, rearing snakehead in their spare time in ponds and hapas, increasing income for their households by 200-400 USD. They confidently feed fish with commercial pellets with less disease occurrence, likely due to addition of mannan oligosaccharide to the diets.

QUANTIFIABLE ANTICIPATED BENEFITS

The project has developed and refined a formulated, pelleted snakehead feed that reduces the use of small-scale fish (SSF) and FM without decreasing growth performance and marketability. Results of the research were disseminated directly to feed manufacturers and more than ten aquaculture fish feed manufacturers in the Mekong Delta now make pellet diets containing a mixture of fish meal and soybean meal. In 2015, more than 90% of snakehead farmers (who produce 99% of the total production of snakehead) in 13 provinces the southern region of Vietnam, including the Mekong Delta, were using these diets instead of SSF, thereby reducing fishing pressure on the SSF in the Lower Mekong Delta. In An Giang, Dong Thap and Tra Vinh provinces, mainly snakehead culture provinces, about 2500 farmers now use pelleted feed.

This investigation supported research activities of one female PhD student, theses of four Master students (two female and two male), and dissertations of 10 undergraduate students (six female and four male). Nine faculty members (six female and three male) in CTU and three local staff members (two female and one male) participated in this project. Eighty-seven women were trained in small-scale snakehead culture with pelleted feed. Five-hundred copies of a handbook of snakehead (*C. striata*) culture using pelleted feed (in Vietnamese) was published and delivered to farmers.

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Table 1. Formulation and chemical composition (% dry matter) of diets based completely on fish meal (FM) versus diets in which FM was replaced at varying levels with soy protein concentrate (SPC).

Ingredients (%)	100%FM	40%SPC	60%SPC	80%SPC	100%SPC
Kien Giang fish meal	60.7	36.2	23.9	11.6	0.00
Soy protein concentrate (SPC)	0.00	24.1	36.1	48.2	59.6
Rice bran	10.0	10.0	10.0	10.0	10.0
Wheat flour	23.8	20.7	19.1	17.6	16.1
Premix and Vitamin C[1]	2.00	2.00	2.00	2.00	2.00
Oil	2.69	3.38	3.72	4.07	4.39
Carboxymethylcellulose	0.82	3.40	4.70	5.99	7.22
Lysine	0.00	0.06	0.36	0.11	0.13
Methionine	0.00	0.24	0.08	0.47	0.59
Total	100	100	100	100	100
Chemical composition (% in dry matter)					
Crude protein	44.40	43.22	43.22	44.65	44.47
Crude lipid	8.28	8.53	8.61	8.48	8.44
NFE	29.0	29.04	29.04	29.04	29.04
Ash	16.2	12.77	11.15	9.46	8.35
Fibre	2.05	6.39	7.96	8.35	9.68
Energy (KJ/g)	18.9	18.7	18.7	19.0	19.0

[1]Mineral mixture and vitamin (unit/Kg) (Vemedim. Can Tho. Vietnam): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6 g; Vitamin B_1. 800 mg; Vitamin B_2. 800mg; Vitamin B_{12}. 2mg; Calcium D. Panthotenate. 2g; Folic acid. 160mg; Vitamin C. 15 g; Cholin Chloride. 100 g; Ferous (Fe^{2+}). 1 g; Zinc (Zn^{2+}). 3 g; Manganese (Mn^{2+}). 2g; Copper (Cu^{2+}). 100mg; Iodine (I^-). 20mg; Cobalt (Co^{2+}). 10mg. Kien Giang fishmeal were obtained from local provider (Minh Tam company. Can Tho. Viet Nam). Soy protein concentrate (SPC) was provided from Yihai (Fangchenggang) soybeans industries Co. Ltd. Oil including vegetable oil (Simply. Vietnam) and squid oil (Vemedim. Vietnam) was supplied at ratio of 1:1. Carboxylmethyl cellulose (CMC) was provided by Xilong Chemical Industry Incorporated Co.. Ltd (China).

Table 2. Ingredients and proximate chemical composition of experimental diets primarily made of fish meal (FM). soybean meal (SBM) or soy protein concentrate (SPC) with or without supplementation with mannan oligosaccharides (MO).

Ingredients (%)	FM	FM 0.2MO	FM 0.4MO	SBM	SBM 0.2MO	SBM 0.4MO	SPC	SPC 0.2MO	SPC 0.4MO
Kien Giang fishmeal	60.7	60.7	60.7	35.8	35.8	35.8	36.2	36.2	36.2
Defatted soybean meal	-	-	-	33.4	33.4	33.4	-	-	-
SPC	-	-	-	-	-	-	24.07	24.07	24.07
Cassava	23.8	23.6	23.4	8.26	8.06	7.86	20.68	20.48	20.28
Rice bran	10.0	10.0	10.0	15.0	15.0	15.0	10.0	10.0	10.0
Premix mineral and vitamins	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Oil	2.69	2.69	2.69	3.08	3.08	3.08	3.38	3.38	3.38
Carboxymethyl cellulose	0.82	0.82	0.82	0.40	0.40	0.40	3.40	3.40	3.40
Lysine	-	-	-	0.40	0.40	0.40	0.06	0.06	0.06
Methionine	-	-	-	0.28	0.28	0.28	0.24	0.24	0.24
Fish solution	-	-	-	1.50	1.50	1.50	-	-	-
Phytase	-	-	-	0.02	0.02	0.02	-	-	-
Mannan oligosaccharides	0	0.20	0.40	0	0.20	0.40	0	0.20	0.40
Total	100	100	100	100	100	100	100	100	100
The chemical composition of feed (%)									
Crude protein	44.5	44.3	44.8	45.0	44.3	44.3	45.5	44.7	44.2
Crude lipid	8.56	8.62	8.69	8.91	8.45	8.76	8.76	8.83	8.90
Ash	15.4	15.2	15.3	12.5	12.3	12.5	12.18	12.18	12.17
Fiber	1.55	1.61	1.50	2.15	2.16	2.15	4.79	4.75	4.81
NFE	29.99	30.27	29.71	31.4.8	32.7	32.2.9	28.81	29.58	29.95
Energy (KJ/g)	19.17	19.20	19.25	19.69	19.58	19.61	19.27	19.25	19.22

Premix mineral and vitamin (unit/Kg): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6g; Vitamin B_1. 800mg; Vitamin B_2. 800mg; Vitamin B_{12}. 2mg; Calcium D. Pantothenate. 2g; Folic acid. 160mg; Vitamin C. 15g; Choline Chloride. 100g; Iron (Fe^{2+}). 1g; Zinc (Zn^{2+}). 3g; Manganese (Mn^{2+}). 2g; Copper (Cu^{2+}). 100mg; Iodine (I^-). 20mg; Cobalt (Co^{2+}). 10mg. Mannan oligosaccharides were the products of Alltech. USA. Fishmeal was from Kien Giang. SPC was a product of Taiwan. Cassava and rice bran were local products. CMC. methionine and lysine were products of Evonik

Table 3. Initial weight (Wi). final weight (Wf). weight gain (WG) and daily weight gain (DWG) of *Channa striata* fed diets in which the indicated percentage levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

Treatment	Wi (g)	Wf (g)	WG (g)	DWG (g/day)
100% FM	10.0±0.13[a]	44.4±1.42[a]	34.8±1.55[a]	0.83±0.04[a]
40% SPC	9.87±0.12[a]	42.7±2.44[a]	33.1±2.27[a]	0.79±0.05[a]
60% SPC	10.0±0.18[a]	19.3±0.98[b]	9.50±1.07[b]	0.23±0.03[b]
80% SPC	10.0±0.07[a]	18.6±0.54[b]	8.93±0.64[b]	0.21±0.02[b]
100% SPC	9.98±0.20[a]	13.4±0.26[c]	3.73±0.07[c]	0.09±0.00[c]

Table 4. Feed intake (FI). feed conversion ratio (FCR). Protein efficacy ratio (PER) and net protein utilization (NPU) of fish fed diets in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

Treatment	FI (%/fish/day)	FCR	PER (%)	NPU (%)
100% FM	3.36±0.13[a]	0.84±0.10c	2.76±0.34[a]	46.9±4.25[a]
40% SPC	3.12±0.32[a]	0.83±0.05[c]	3.17±0.82[a]	50.2±1.70[a]
60% SPC	1.96±0.14[b]	1.20±0.03[b]	1.93±0.05[b]	21.3±1.78[b]
80% SPC	1.84±0.10[b]	1.19±0.03[b]	1.89±0.05[b]	19.4±1.17[b]
100% SPC	1.31±0.08[c]	1.70±0.09[a]	1.32±0.07[b]	12.6±2.83[c]

Table 5. Chemical compositions of fish prior to (Initial fish) and after (Final fish) an experiment in which fish were fed diets in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

Treatment	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Initial fish	75.2	16.2	2.71	1.31
Final fish				
100% FM	72.0±0.81[b]	18.2±0.20[a]	3.45±0.27[a]	5.15±0.24[ab]
40% SPC	73.2±0.45[ab]	17.9±0.44 [a]	3.23±0.06[ab]	4.48±0.22[c]
60% SPC	73.4±1.31[ab]	17.7±0.71 [a]	2.26±0.25[d]	5.62±0.05[a]
80% SPC	73.6±1.01[ab]	17.4±0.61 [a]	2.81±0.51[bc]	4.89±0.45[bc]
100% SPC	73.8±0.32[a]	17.5±0.46 [a]	2.47±0.12[cd]	5.07±0.12[b]

Table 6. Comparision of feed cost (FC) and fish production cost (FPC) among treatments in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC).

Treatments	FC (USD/kg feed)	FPC (USD/kg fish gain)
100% FM	1.131	0.950
40% SPC	1.096	0.910
60% SPC	1.079	1.294
80% SPC	1.061	1.263
100% SPC	1.045	1.776

Table 7. Apparent digestibility coefficient (ADC) of diets (ADC_{diet}). $ADC_{protein}$ and ADC_{lipid} in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean $\pm$ SD) in a column followed by the same superscript letter are not significantly different.

Treatments	ADC_{diet} (%)	$ADC_{protein}$ (%)	ADC_{lipid} (%)
100% FM	78.1±0.26[b]	89.9±0.24[a]	92.9±1.08[a]
40% SPC	78.9±0.08[a]	89.9 ±0.16[a]	92.2±1.41[a]
60% SPC	77.4±0.13[c]	84.0±0.11[b]	82.1±0.20[b]
80% SPC	77.5±0.29[c]	82.0±0.29[c]	76.5±0.50[c]

Table 8. Growth performance of *Channa striata* fed for 8 weeks on diets supplemented with mannan oligosaccharides and a control diet. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p>0.05$).

Treatment	Wi (g)	Wf (g)	WG (g)
FM	7.00±0.14[a]	47.7±0.6[d]	40.7±0.5[f]
FM 0.2MO	7.05±0.03[a]	52.5±1.2[b]	45.5±1.2[b]
FM 0.4MO	7.01±0.05[a]	52.1±0.2[b]	45.0±0.2[b]
SBM	7.08±0.11[a]	50.5±2.1[bc]	43.4±2.0[bd]
SBM 0.2MO	7.08±0.10[a]	57.7±1.7[a]	50.6±1.6[a]
SBM 0.4MO	7.05±0.06[a]	57.4±1.5[a]	50.3±1.5[a]
SPC	7.23±0.36[a]	49.4±0.6[cd]	42.2±0.4[cf]
SPC 0.2MO	7.00±0.03[a]	51.3±1.2[bc]	44.3±1.3[bc]
SPC 0.4MO	7.06±0.05[a]	48.9±1.8[cd]	41.9±1.8[df]
P values			
Diets	-	*0.000*	*0.000*
MO	-	*0.000*	*0.000*
*Diets*MO*	-	*0.003*	*0.003*

Table 9. Feed convertion ratio (FCR). protein efficiency ratio (PER)and survival rate (SR) of *Channa striata* fed for 8 weeks on different protein diets and supplemented with different mannan oligosaccharide levels. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p>0.05$). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

Treatment	Growth parameters		
	FCR	PER (%)	SR (%)
Diet sources			
FM	0.97 ± 0.09^{ab}	2.56 ± 0.28^{a}	76.2 ± 3.3^{a}
SBM	1.05 ± 0.08^{a}	2.20 ± 0.24^{b}	78.1 ± 3.9^{a}
SPC	0.93 ± 0.09^{b}	2.67 ± 0.24^{a}	66.6 ± 5.3^{b}
MOS levels (%)			
0	1.03 ± 0.20^{a}	2.35 ± 0.57^{b}	67.9 ± 12.4^{b}
0.20	0.94 ± 0.23^{a}	2.53 ± 0.66^{a}	78.8 ± 0.2^{a}
0.40	0.98 ± 0.35^{a}	2.54 ± 0.52^{a}	74.2 ± 9.9^{ab}
P values			
Diets	*0.037*	*0.004*	*0.000*
MO	*0.167*	*0.260*	*0.000*
*Diets*MO*	*0.843*	*0.800*	*0.217*

Table 10. Total red blood cell and white blood cell in serum of *Channa striata* fed different protein diets and different mannan oligosaccharide levels at the end of the growth trial. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p>0.05$). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

Treatment	Blood parameters	
	RBC (10^6 cells/mm^3)	WBC (10^3 cells/mm^3)
Diet sources		
FM	2.17 ± 0.18^{a}	60.9 ± 5.9^{b}
SBM	2.18 ± 0.33^{a}	66.3 ± 7.1^{ab}
SPC	2.20 ± 0.17^{a}	74.6 ± 3.4^{a}
MOS levels (%)		
0	2.17 ± 0.87^{a}	58.6 ± 2.7^{b}
0.20	2.26 ± 0.66^{a}	71.8 ± 1.8^{a}
0.40	2.14 ± 0.52^{a}	71.4 ± 19.8^{a}
P values		
Diets	*0.973*	*0.001*
MO	*0.567*	*0.000*
*Diets*MO*	*0.273*	*0.081*

Table 11. Immunoglobulin (Ig). pre-challenge lysozyme and post-challenge lysozyme levels of *Channa striata* fed supplemented with and without mannan oligosaccharides. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (*p*>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and iteraction effects.

Treatment	Ig (mg/ml)	Pre-challenge lysozyme (µg/ml)	Post-challenge lysozyme (µg/ml)
FM	9.09±0.85[c]	263±6[df]	459±3[df]
FM 0.2MO	10.10±0.57[bc]	276±13[d]	503±11[bc]
FM 0.4MO	12.90±0.83[a]	346±15[b]	536±27[a]
SBM	9.08±0.49[c]	248±23[f]	479±6[cd]
SBM 0.2MO	9.42±0.50[c]	283±3[d]	485±7[c]
SBM 0.4MO	12.00±0.69[a]	308±14[c]	529±24[a]
SPC	8.75±1.36[c]	271±14[df]	443±4[f]
SPC 0.2MO	11.60±0.69[ab]	323±12[bc]	524±7[ab]
SPC 0.4MO	10.20±1.27[bc]	371±11[a]	487±3[c]
P values			
Diets	*0.383*	*0.000*	*0.069*
MO	*0.000*	*0.000*	*0.000*
*Diets*MO*	*0.000*	*0.023*	*0.003*

Table 12. Comparision of feed costs (FC). fish production costs (FPC). and reduction in fish production cost compared to control among a diet based completely on fish meal (FM) versus diets in which 40% of FM was replaced by soybean meal (SBM) or soy protein concentrate (SPC). with or without supplementation with mannan oligosaccharides (MO) (based on February. 2015 prices).

Treatments	FC (USD/kg feed)	FPC (USD/kg fish gain)	Reduction in fish production cost (%)
FM	1.063	1.117	0.0
FM 0.2MO	1.072	0.964	13.4
FM 0.4MO	1.085	1.018	8.7
SBM	0.933	1.013	8.9
SBM 0.2MO	0.946	0.955	14.4
SBM 0.4MO	0.955	1.009	9.1
SPC	1.027	0.978	12.3
SPC 0.2MO	1.040	0.946	15.1
SPC 0.4MO	1.049	0.987	11.4

Table 13. Feed formulas used for snakehead culture. During the first 2 months, fish were fed CTU - CRSP 1 (44% CP). In the third and fourth months, fish were fed CTU- CRSP 2 (41% CP) and CTU- CRSP 3 (38% CP) were used for the last months.

Ingredients	CTU - CRSP 1	CTU – CRSP 2	CTU – CRSP 3
Fish meal (Kien Giang)	34.5	31.8	29.1
Soybean meal	32.0	29.5	27.1
Rice bran	20.0	20.0	20.0
Cassava	6.35	11.3	16.3
Vitamin premix	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00
Fish oil	2.45	2.70	2.94
Binder	0.40	0.40	0.40
Lysine	0.20	0.21	0.23
Methionine	0.19	0.21	0.23
Phytase	0.02	0.02	0.02
Fish fluid	1.5	1.5	1.5
MO	0.2	0.2	0.2
Total	100	100	100

Table 14. Survival rate (SR). initial fish weight (Wi) and final fish weight Wf (g). daily weight gain (DWG) (g/day) and feed conversion ratio (FCR) of *C. striata* reared in plastic lined tanks and hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

Models	SR (%)	Wi (g)	Wf (g)	WG (g)	DWG (g/day)	FCR
Tank						
Farm 1	74.1	4.80	450	445	2.42	1.15
Farm 2	76.2	4.70	350	345	1.88	1.25
Farm 3	72.4	5.00	350	345	1.88	1.34
Mean	74.2±0.02	4.83±0.15	383±57.7	379+57.8	2.06±0.31	1.25±0.1
Hapa						
Farm 1	72.0	11.5	250	239	1.99	1.00
Farm 2	80.0	11.2	350	339	1.84	1.29
Farm 3	62.5	4.60	400	395	2.15	1.34
Mean	71.5±0.01	9.10±3.9	333±76.4	324±79.4	1.99±0.15	1.21±0.2

Table 15. Production and yield of *C. striata* reared in plastic-lined tanks and in hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

Models	Production (kg)	Yield (kg/m^2)
Tank		
Farm 1	500	33.0
Farm 2	400	26.3
Farm 3	380	25.0
Mean	427±64	28.1±4.29
Hapa		
Farm 1	360	14.3
Farm 2	560	22.6
Farm 3	500	20.6
Mean	473±102	19.2±4.31

Table 16. Financial efficacy analysis of *C. striata* reared in plastic-lined tanks and in hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

Models	Cost per kg fish (USD	Total Investing cost (USD)	Selling price USD/kg fish	Total income (USD)	Profit (USD)
Tank					
Farm 1	1.19	594	2.00	1000	406
Farm 2	1.32	530	1.70	682	152
Farm 3	1.36	516	1.67	636	120
Mean	1.29±0.09	548±41.6	1.79±0.18	773±198	226±157
Hapa					
Farm 1	1.05	378	1.73	623	245
Farm 2	1.24	697	1.61	901	205
Farm 3	1.30	649	1.55	773	124
Mean	1.20±0.13	575±172	1.63±0.09	766±139	191±61.6

Figure 1. Experimental system for feces collection in digestibility experiment.
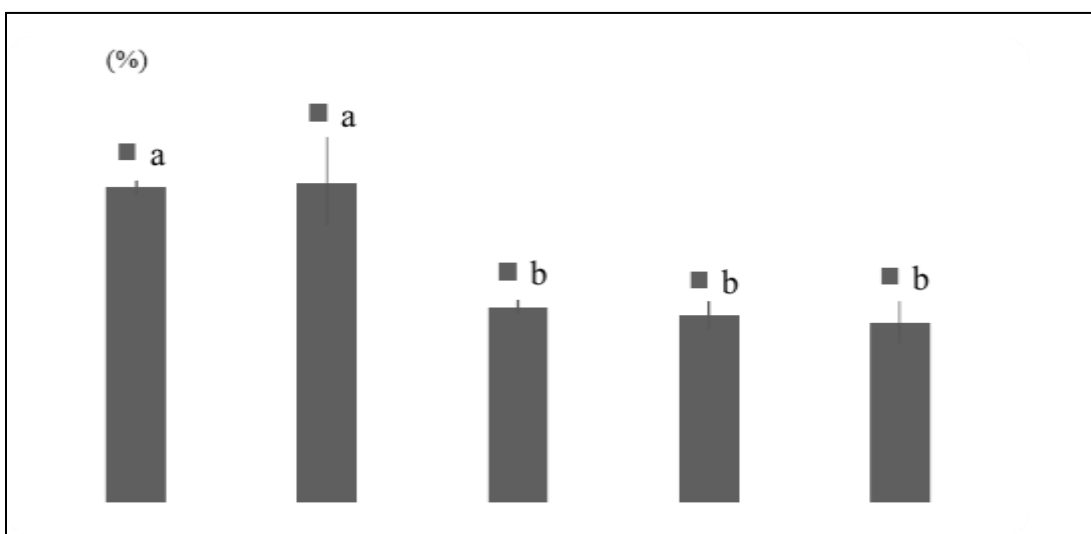
Figure 2. Survival of fish in treatments where fish meal (FM) was replaced by varying levels of soy protein concentrate (SPC). The different letters above the error bars indicated the significant difference between treatments ($p<0.05$).
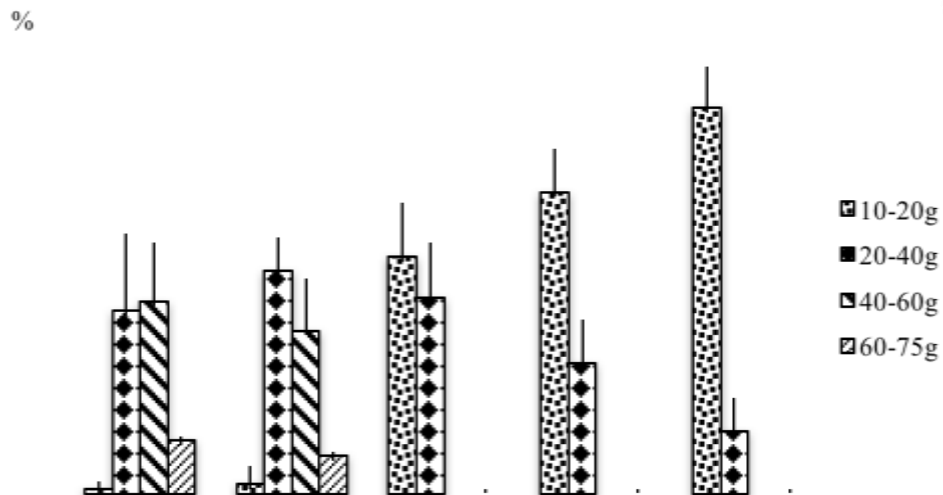
%

Figure 3. Size distribution of fish fed diets in which varying degrees of fish meal (FM) were replaced by soy protein concentrate (SPC).
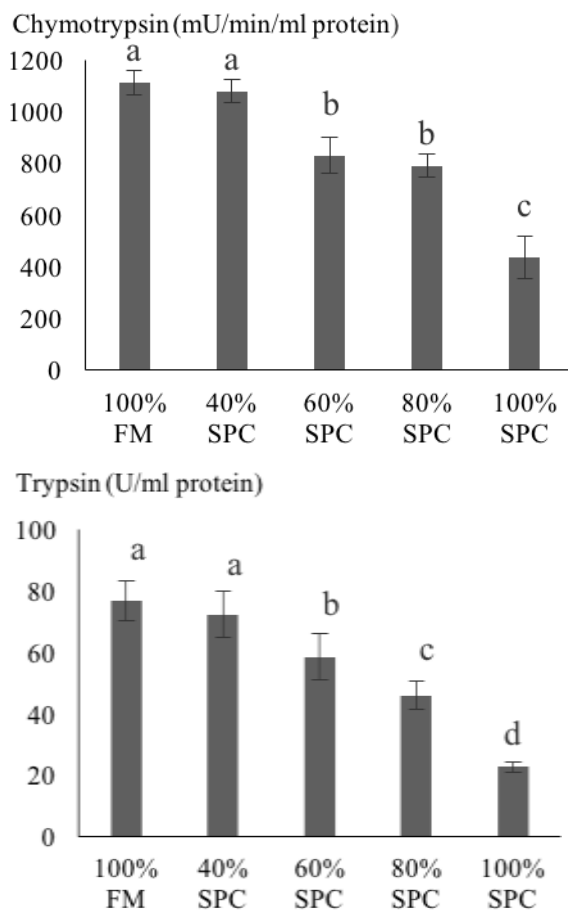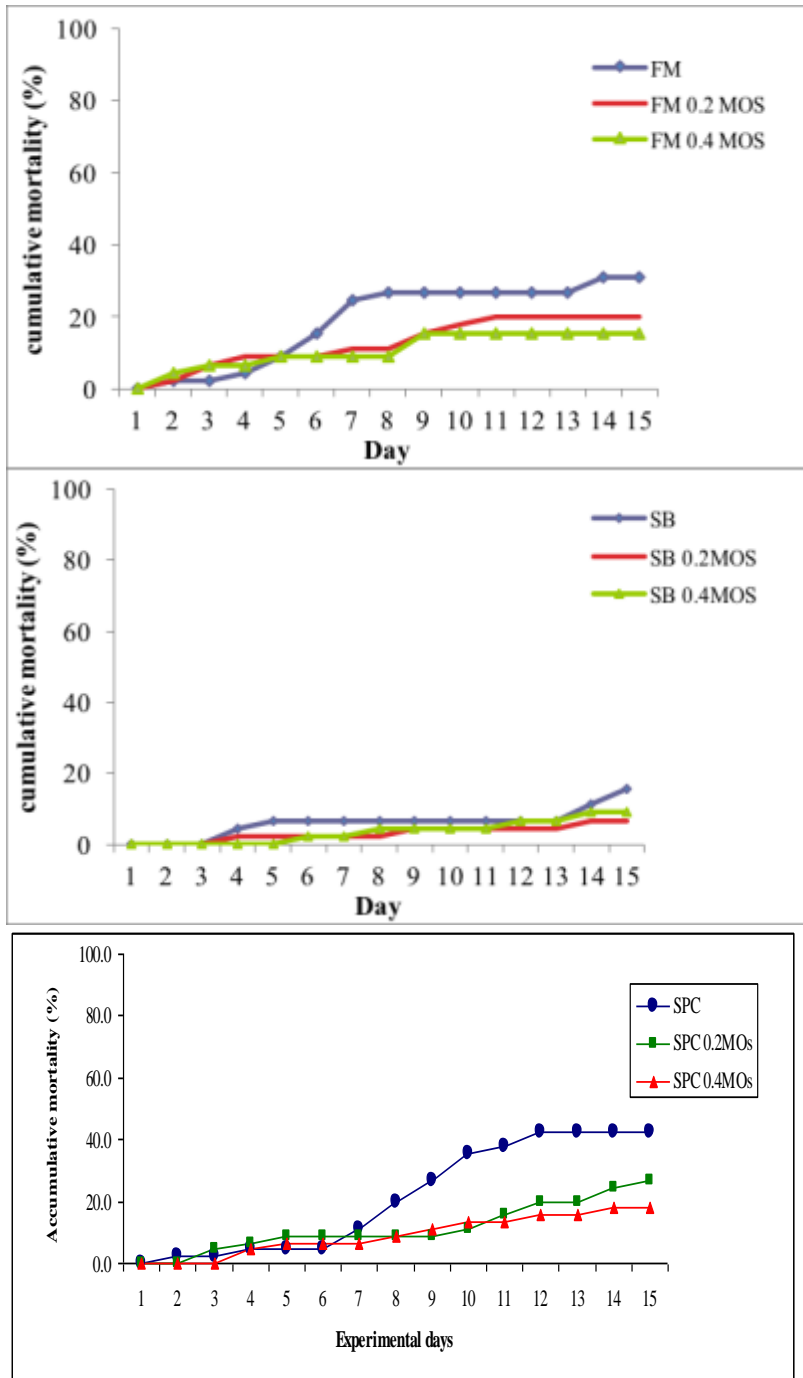

Figure 4. Trypsin and chymotrypsin activities of fish fed diets in which varying amounts of fish meal (FM) were replaced by soy protein concentrate (SPC). The different letters above the error bars indicated the significant difference between treatments ($p<0.05$).

Figure 5. Cumulative mortalities of *Channa striata* during 15-d bacterial challenges with *Aeromonas hydrophila* following a feeding trial in which they had been fed fish meal (FM) diet (top panel). a diet in which soybean meal (SBM) replaced 40% of FM (middle panel) or a diet in which soy protein concentrate (SPC) diet replaced 40% of FM (bottom panel) with or without mannan oligosaccharides (MO) supplementation at 0.2% or 0.4% of the diet (as indicated).

Figure 6. Training course on snakehead aquaculture for women in An Giang province.

Figure 7. Releasing fingerlings at a farm for the on-farm trials and project feed bag.

Figure 8. Snakehead culture in a plastic-lined tank during the on-farm trials.

Figure 9. Snakehead culture in a hapa placed in a pond during the on-farm trials

Economic and Environmental Benefits of Reduced Feed Inputs in The Polyculture of Tilapia and Major Indian Carps

Sustainable Seed Technology and Nutrient Input Systems/Experiment/13SFT04NC

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ABSTRACT

The aim of these studies was to determine if reductions in feed inputs and introduction of native Indian carp, rohu (*Labeo rohita*) and catla (*Catla catla)*, can increase economic benefits of tilapia culture in earthen ponds in Bangladesh. Two on-station pond trials were carried out for 150 days at the Fisheries Field Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. In the first study, ponds consisting of four treatments (T1, T2, T3, and T4) with four replications each were stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*, 5 fish/m^2) without (T1) or with (T2) addition of rohu (0.25 fish/m^2) and fed a full daily ration of feed (CP — 35% protein; 10%–3% body weight/day). Ponds were fertilized weekly (28 kg N and 5.6 kg P ha/week) in the other treatments and tilapia were grown in the absence (T3) or presence of rohu (T4) at half the daily feed ration as T1 and T2. Pond water temperature, transparency, dissolved oxygen, nitrate-nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), ammonia-nitrogen (NH_3–N), phosphate-phosphorus (PO_4–P) and chlorophyll-a did not vary among treatments, while pH was slightly lower in T1 than the other treatments, but well within the suitable range for tilapia growth. A total of 27 genera of phytoplankton and 12 genera of zooplankton were identified from the pond water samples. Total phytoplankton levels were highest in the T4 and T3 groups. The survival rates (%) of tilapia were 81.06 ± 1.03, 76.89 ± 1.28, 76.24 ± 2.06 and 75.29 ± 2.45 in T4, T3, T2, and T1 groups, respectively. The specific growth rate (% day^{-1}) of tilapia was higher in the T3 (1.87 ± 0.00) and T4 (1.85 ± 0.03) than the T2 (1.76 ± 0.05), and T1 (1.71 ± 0.06) groups ($p < 0.05$). Feed efficiency was significantly better in the T3 and T4 groups relative to those treatment fish fed the full ration ($p < 0.05$) with feed conversion ratios of 0.49 ± 0.03, 0.47 ± 0.03, 1.13 ± 0.11 and 1.23 ± 0.16 for the T4, T3, T2, and T1 groups, respectively. Gross production of tilapia was higher in the T4 ($5,385.23 \pm 276.98^a$ kg ha^{-1}) followed by T3 ($5,340.62 \pm 156.47$ kg/ha), T2 ($4,440.99 \pm 440.04$ kg/ha) and T1 ($4,089.83 \pm 518.46$ kg/ha) groups, respectively. Rohu gross production was similar among the T2 and T4 groups. A significantly higher net return (BDT 743,977/ha with benefit cost ratio of 2.92) was found in T3 followed by T4 (BDT 673,750/ha with benefit cost ratio of 2.72), T2 (BDT 286,469/ha with benefit cost ratio of 1.49) and T1 (BDT 226,675/ha with benefit cost ratio of 1.37) groups, respectively.

In the second study, ponds consisting of three treatments (T1, T2, and T3) with four replications each were stocked with sex-reversed Nile tilapia (5 fish/m^2) with rohu (0.625 fish/m^2; T1), with catla (0.625 fish/m^2; T2), or with both rohu and catla (0.32 fish/m^2 and 0.31 fish/m^2, respectively; T3). All ponds were fed a half daily ration of feed and ponds were fertilized weekly. Pond water temperature, transparency, dissolved oxygen, pH, nitrate-nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), ammonia-nitrogen (NH_3–N), phosphate-phosphorus (PO_4–P) and chlorophyll-a did not vary among treatments. A total of 30 genera of phytoplankton and 12 genera of zooplankton were identified from the pond water samples. Total phytoplankton levels were highest in the T1. The

specific growth rate (%/day) of tilapia was higher in the T2 (1.66 ± 0.05) than in T1 (1.60 ± 0.04), and T3 (1.54 ± 0.03) groups ($p < 0.05$). No difference in feed efficiency was found with feed conversion ratios of 1.47 ± 0.10, 1.41 ± 0.21, and 1.46 ± 0.27 for T1, T2, and T3, respectively. Gross production of tilapia was higher T2 ($7,737.78 \pm 646.51$ kg/ha) followed by T1 ($6,867.11 \pm 570.36$ kg/ha), and T3 ($6,272.23 \pm 183.44$ kg/ha), respectively. Rohu gross production was higher in T1 and catla production was higher in T2. There was no significant difference in net return or benefit cost ratio between treatments.

Based on the higher net return and benefit-cost ratio it may be concluded that pond fertilization with feeding at half ration is substantially more cost effective over standard full feeding for growout of tilapia. Addition of major Indian carps to tilapia culture may also provide further income benefits to farmers as net production of fishes is greater in polyculture than tilapia monoculture systems regardless of the feeding regimen applied. Since tilapia growth was little impacted by feeding at half ration and but tended to grow better when polycultured with either catla or rohu alone compared with rohu-catla combined, it might be preferential to either of them in polyculture with tilapia. Regardless, the results indicate profits can increase by 200% if tilapia are grown with native Indian carps and provided half the standard ration level typically used for tilapia monoculture.

INTRODUCTION

Pond production of fish constitutes almost 85% of total aquaculture output in Bangladesh, with 60% coming from indigenous Indian major carps, Catla (*Catla catla)* and rohu *(Labeo rohita)*, and 17% from exotic Chinese carps (Belton et al. 2011). About six to seven carp species are cultured together on an *ad hoc* basis, and the fish subsist primarily on primary production (pond fertilization) with occasional feed inputs of rice bran and oil cake. Because carps are omnivores, herbivores, planktivores, and/or filter feeders, these fish prefer natural food organisms enhanced by pond fertilization, fish waste, and feed inputs (Wahab et al. 2002). When cultured together with other fishes (polyculture), carps enhance dietary household consumption and income earnings through greater production yields and better nutrient efficiency (Azim et al. 2004). This constitutes a significant improvement for household dietary nutrition, as 66% of per capita animal protein intake in Bangladesh comes from fish (Hussain 2009, Belton et al. 2011).

Tilapia (*Oreochromis niloticus*) was introduced to Bangladesh more than 20 years ago and is now one of the fastest growing components of the aquaculture sector, ranking 2nd to carps in total finfish production. Significant work remains to develop better management practices for this cultivar. Current monoculture practices in Bangladesh require significant feed inputs (Dey et al. 2008, Belton et al. 2011) and high production costs, which limit participation of smaller homestead farmers. This practice also degrades environmental water quality through nutrient loading and pond eutrophication. This investigation sought to promote better management practices for this industry and greater inclusion by small farms through implementation of a feed reduction strategy, complimented with cheaper fertilizer application (semi-intensive management) thereby reducing the costs constraining participation. Also we integrated polyculture of major Indian carps with tilapia, providing additional sources of income for farming families throughout Bangladesh and increasing the overall efficiency of the water resources used to grow fish. We anticipated that tilapia-carp farming under reduced feeding would produce greater production yields with less cost, thereby significantly increasing economic profitability for this endeavor. Additionally we anticipated this refined strategy would improve environmental water quality and enhance dietary nutrition for rural farming families: first, by improving income earnings potential, which has been identified as a direct link towards improving household nutrition (FAO 2012), and secondly by generating a more diverse crop for consumption. Currently, little is known about the production parameters of tilapia cultured with Indian carps,

despite widespread appeal for these fish in Bangladesh, however tilapia has been previously cultured with Chinese carps (Abdelghany and Ahmad 2002, El-Sayed 2006), and this integrated polyculture produced better yields than for tilapia cultured alone (Khouraiba et al. 1991).

In aquaculture, feed is recognized as the dominant cost component of fish farming, representing 50%–70% of the total production costs for small-scale, rural farmers (ADB 2005). Because of this, any reductions in feed can significantly improve the earned incomes for farmers, and reduce negative environmental impacts of fish farming. Further, the promotion of semi-intensive tilapia farming to homesteads practicing extensive farming will be key to enhancing income earning potential, fish consumption and household nutrition in Bangladesh. It is estimated that even modest intensification can improve food security by four-fold (Dey et al. 2008, Belton et al. 2011). Previous CRSP work has demonstrated that tilapia grown with both feed and fertilizer is more efficient than using either input alone (Diana et al. 1994). Monocultured tilapia grown under reduced feed rations of up to 50% had little impact on production yields (kg), but improved water quality through reductions in nutrient loading (Diana et al. 1994, Lin and Yi 2003). Further, our CRSP on-farm trials in the Philippines show that reduced-feeding can improve feed conversion rates by up to 100% (Bolivar et al. 2010). The present investigation sought to integrate these strategies with mixed carp polyculture to gain improvements in both tilapia production efficiency and environmental water quality, while also promoting more sustainable, less intensive farming practices.

OBJECTIVES
- Evaluate production parameters and potential economic and environmental benefits of reducing feed inputs by half in tilapia-rohu carp polyculture in earthen ponds;
- Assess the benefit of adding a second major carp, catla, to tilapia-rohu carp polyculture; and
- Evaluate the potential benefits of tilapia–Indian carp polyculture and feed management technology to rural household farmers.

MATERIALS AND METHODS
Study 1 — Evaluate production parameters and potential economic and environmental benefits of reducing feed inputs with adoption of carp (rohu) into the growout of tilapia in earthen ponds. This study addressed the value of combining feed restriction and fertilization with polyculture of Indian carps into tilapia farming. It also addressed if the addition of carp under current practices might provide additional environmental and economic benefits to medium and small-scale farmers practicing monoculture. Twelve freshwater ponds (100 m^2) at Bangladesh Agricultural University's (BAU) Fisheries Field Laboratory (on-station) were stocked with sex-reversed Nile tilapia alone or with rohu at an 8:1 ratio.

Table 1. Experimental design for Study 1.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu	0	25 (0.625/ m^2)	0	25 (0.625/ m^2)
Tilapia	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)
Fertilization	0	0	4:1 (N: P)	4:1 (N: P)
Feeding	Full daily ration	Full daily ration	Half daily ration	Half daily ration
Replicates (*n*)	4	4	4	4

In the proposed design, T1 is a control group representing the current practice of tilapia farming. Although ancillary to our primary objective (semi-intensive farming), we also tested whether improvements in water quality can be achieved solely through addition of rohu carp (T2). Inclusion of this treatment group also balanced our experimental design. Treatment 3 (T3)

examined semi-intensive farming of tilapia through a 50% feed restriction/fertilization strategy, and whether growth, production yields, and water quality could be further improved with rohu (T4). Weekly pond fertilization (generally 14% the cost of feed) accompanied the feed reduction treatments (T3, T4) to promote utilization of pond primary production by both tilapia and carp. Ponds were dried and limed (2 kg $CaCO_3$) and prepared according to standard procedures. Treatment 1 and 2 ponds were fertilized initially using inorganic fertilizers, urea and triple superphosphate (TSP) at a rate of 28 kg N and 5.6 kg P ha/week (4:1 N:P). Treatment 3 and 4 were fertilized initially at a rate of 28 kg N and 5.6 kg P ha/week (4:1 N:P) and weekly thereafter at a similar rate. The fertilization levels and rate employed are those that were previously developed by CRSP research activities in numerous countries and encompass those ranges recommended for tilapia culture (Egna and Boyd 1997, Green and Duke 2006). Other studies have shown that fertilization every two weeks works as well as weekly at least when using fish at lower densities (see Egna and Boyd 1997). We fertilized weekly considering the higher density (5 fish/m^2) used here and to remain consistent with most studies (Egna and Boyd 1997, El-Sayad 2006, Green and Duke 2006). We also incorporated carp, which likely required additional primary productivity.

Fish were fed with a pelleted commercial feed (CP Bangladesh, 30% crude protein, 3% fat, 12% moisture, 8% fiber) initially at around 10% bw/day and titered down to ~3% bw/day during the final grow-out (10% bw/day for 5–10 g fish, 8% bw/day for 15–60 g fish, 5% bw/day for 60–100 g fish, 3% bw/day for 100–200 g fish). Animals were fed twice daily at the appropriate daily rate based on treatment groups. Tilapia were subsampled every two weeks by cast net for growth rate determinations and feeding rate adjustments. Animals were fed twice daily at the appropriate daily rate based on treatment groups. After 150 days of grow-out, all fish were harvested by seining and complete draining of the pond. The total weight of fish stocks were recorded at harvest and a subset of fish were measured for weight and length. Feed conversion ratio (FCR), feed inputs, specific growth rates, and total production biomass were calculated. A basic marginal cost-return analysis based on input costs (fertilizers/ feed/ fingerlings) and sales determined if the reduced feeding-fertilization strategy and/or incorporation of carp can provide cost savings or additional incomes relative to monocultured tilapia provided daily full feeding rates (Bolivar et al. 2006). We anticipate based on previous work that 50% reduction in feeding combined with fertilization would prove better than feeding alone at full satiation (Diana et al. 1994, Bolivar et al. 2010). It was anticipated that the incorporation of carps would also provided additional fish yield and profits to tilapia culture.

Standard water quality parameters such as water temperature, dissolved oxygen, pH, total alkalinity, phosphate, total phosphorus, nitrate, nitrite, ammonia, total nitrogen, chlorophyll *a*, algae, and zooplankton community were assessed on the spot using meters/sensors as appropriate or at the Water Quality and Pond Dynamics Laboratory at BAU. Most parameters were measured weekly and temperature, DO, pH and alkalinity were measured daily (APHA 2012). Treatment groups were tested for significant differences in growth performance, production yields, and water quality using two-way analysis of variance (feed regimen; addition of carp, interaction effects) followed by Tukey's HSD test.

Study 2 — To assess the benefit of addition of a second major carp catla with tilapia-rohu polyculture. This experiment further refined the reduced-feeding/fertilization and polyculture strategy outlined in Study 1. Based on the results of Study 1 we found tilapia production efficiency was higher with reduced-feeding regimes and addition of rohu alone. We attempted to further improve our technology by use of a second Indian carp (*C. catla*) alone or in combination with rohu. These two carps encompass discrete niches. Rohu feed primarily in the water column and bottom on algae and vegetation, while Catla are omnivorousm surface feeders preferring

zooplankton as adults, although these preferences may shift depending on their life history stage or whether artificial feeds are present (Rahman et al. 2006). Catla tend to grow faster than rohu, Therefore greater biomass (production yield) along with further improvements in water quality may be achieved using catla alone or the combination of both carps.

The experimental design is shown in Table 2. This experiment tested whether the reduced-feeding/fertilization strategy with rohu carp (T1) could be significantly improved by switching to polyculture with Catla (T2), or in mixed polyculture with both rohu and catla (T3). Twelve ponds at BAU were used for this second trial. Fish were grown out for 150 days. Feeding rates, production parameters, water quality, and cost-return analyses were assessed as outlined under Study 1.

Table 2. Experimental design for Study 2.

Parameter	Treatment 1	Treatment 2	Treatment 3
Rohu	25 (0.625/m^2)	0	13 (0.32/m^2)
Catla	0	25 (0.625/m^2)	12 (0.31/m^2)
Tilapia	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)
Feeding	Half daily ration	Half daily ration	Half daily ration
Fertilization	4:1 (N: P)	4:1 (N: P)	4:1 (N: P)
Replicates (n)	4	4	4

Activity 3 — Disseminate potential benefits of tilapia–Indian carp polyculture and feed management technology to rural farmer households. We developed and produced two extension leaflets outlining the advantages of reducing feed ration by half and in incorporating major Indian carps in culture of tilapia. We also organized a workshop for local farmers in the Mymensingh region to demonstrate the new feed reduction and polyculture technologies outlined under studies 1 and 2.

RESULTS AND DISCUSSION

Study 1 - Evaluate production parameters and potential economic and environmental benefits of reducing feed inputs with adoption of carp (rohu) into the growout of tilapia in earthen ponds. The water quality parameters recorded in treatments T_1, T_2, T_3 and T_4 were found to be within the suitable range of fish culture (Table 3). Water transparency among the four treatments ranged from 10.67 to 12.06 cm and did not vary significantly among groups ($p > 0.05$). Values found among the treatments is similar to that found in other studies (Asaduzzaman et al. 2006, Kundu et al. 2008). Chlorophyll a ranged from 100.28 to 143.32 µg/L with no significant differences among the five treatments and was similar to those recorded by Kohinoor (2000) and Rahman (2000). Chlorophyll a was highest in T_3 possibly due to a combination of higher primary productivity associated with fertilization and absence of carp feeding on phytoplankton. The lowest mean value was recorded in T_1 likely a result of grazing pressure from tilapia and the lack of carp and weekly fertilization with inorganic nitrogen or phosphorous.

Four different groups of organisms were identified in the benthos samples collected in this study (Table 4). These included chirnomid larvae, Oligocheta, mollusks, and unidentified organisms. Among the benthos communities, only chironomid larvae were found to be significantly different between treatments (highest in treatment fed full daily ration with no fertilization than in those fed half ration with pond fertilization; $p < 0.1$).

During the study period, 27 genera of phytoplankton belonging to six families, Bacillariophyceae (6), Chlorophyceae (11), Cyanophyceae (6), Euglenophyceae (2), Rhodophyceae (1), and Xanthophyceae (1), and 12 genera of zooplankton belonging to four groups, Cladocera (4),

Copepoda (2), Rotifera (5), and crustacean larvae (1), were identified in the experimental ponds (Table 5). Plankton populations were identified to the genus level (see Table 6). The total phytoplankton among four treatments ranged from 61.14 ± 7.21 to 85.94 ± 6.70 ($\times 10^3$ cells/L). The total zooplankton ranged from 15.28 ± 1.22 to 25.17 ± 3.68 ($\times 10^3$ cells/L) among the different treatments. Similar results have been previously reported (Dewan et al. 1991, Wahab et al. 1995).

For monitoring of growth parameters, weight of 50 tilapia and 10 rohu from each pond was collected at two-week intervals. In terms of growth and production, the mean stocking weight of tilapia was same among all four treatments at 8.34 ± 1.85 g (Table 7). The harvesting (final) weight and weight gain of tilapia was significantly higher for T_3 and T_4 (50% feed and fertilization: 137.69 ± 2.04 g and 132.57 ± 5.42 g, 129.35 ± 2.04 g and 124.23 ± 5.43 g) than for T_1 and T_2 (full feed and no fertilization: 108.96 ± 9.82 g and 116.99 ± 9.04 g, 100.62 ± 9.82 g and 108.64 ± 9.04 g) (Table 7). The highest mean weight gain of tilapia in T_3 and T_4 could have resulted from better utilization of natural foods boosted by pond fertilization and the use of commercial feed. Weight gain of monosex tilapia has been reported as 64.23 ± 2.35 g to 193.58 ± 3.59 g (Siddik et al. 2007), 250.55 ± 2.38 g to 315.92 g ± 1.11 g (Reza 2013), and 118.5 g to 124.0 g (Hossain et al. 2011) and variability might be related to differences in feed, fertilizer, and quality of fingerlings used in the studies.

In this experiment, the specific growth rate (SGR) of tilapia in T_1, T_2, T_3 and T_4 were $1.71 \pm 0.06\%$/day, $1.76 \pm 0.05\%$/day, $1.87 \pm 0.00\%$/day, and $1.85 \pm 0.03\%$/day, respectively, and was significantly higher in T_3 and T_4 (Table 7). Siddik et al. (2007) recorded that the specific growth rate of Nile tilapia ranged from $2.44 \pm 0.02\%$/day to $3.33 \pm 0.04\%$/day. The higher SGR observed in this study relative to that shown here is likely associated with lower stocking density (3.125 fish/m^2).

The feed conversion ratio (FCR) for tilapia was significantly lower for T_3 and T_4 (50% ration and fertilization; 0.47 ± 0.03 and 0.49 ± 0.03) than for T_1 and T_2 (100% ration and no fertilization; 1.23 ± 0.16, 1.13 ± 0.11) (Table 7, Figure 1). Siddik et al. (2007) and Reza (2013) recorded higher feed conversion ratios for Nile tilapia that varied from 1.61 ± 0.05 to 1.65 ± 0.06 and 1.41 to 1.59, respectively. The variations in FCR values might be due to differences in quality and amounts of feed given, quality of fingerlings, pond fertilization strategy, or water quality of the ponds.

The mean survival rates of tilapia were $75.29 \pm 2.45\%$, $76.24 \pm 2.06\%$, $76.89 \pm 1.28\%$, and $81.06 \pm 1.03\%$ in T_1, T_2, T_3, and T_4, respectively, and was significantly higher in T_4 than in the other three treatments (Table 7). Previous studies have shown that survival of sex-reversed Nile tilapia varied from $84.2 \pm 3.1\%$ to $89.3 \pm 5.3\%$ (Borski et al. 2011) and 94% to 95% in experimental ponds (Haque et al. 2010). The variation of survival rate (%) among different treatments might be related to natural mortality and other factors.

The highest production of tilapia were found in T_4 and T_4, likely due to the fish's capacity to utilize feeds containing high protein content and large quantity of natural foods available in the pond during the study period (Table 7, Figure 2). Borski et al. (2011) and Hossain et al. (2011) recorded that the production of Nile tilapia ranged from 3,062 to 3,080 kg/ha and 3,180 to 3,435 kg/ha, respectively, and is likely due to lower stocking densities. Here we found net production ranged from 3,777 kg/ha to 5,046 kg/ha with a stocking density of 5 tilapia/m^2.

The major input cost variable for culture of tilapia and other fishes is feed. Total expenditures were nearly twice as high for T_1 and T_2 with fish that were fed a full daily ration than in T_3 and T_4 fed at half ration (Table 8, Figure 3). Accordingly, the BCR was significantly higher in T_3 and T_4.

Carp production and survival was similar whether ponds were fertilized or not and when tilapia were fed either a full or half daily ration. Hence the addition of carp provided additional crop while having no impact on tilapia growth.

Collectively these results suggest that feeding at half ration combined with pond fertilization substantially increases the net return and benefit-cost ratio for growing tilapia whether in monoculture or in polyculture with rohu. The polyculture of carp with tilapia allows additional production of fish that can enhance incomes of small-scale farmers.

Study 2 — To assess the benefit of addition of a second major carp catla with tilapia-rohu polyculture. Water quality variables, e.g., pH, TDS, Temperature, dissolved oxygen, alkalinity, nitrate-nitrogen, nitrite-nitrogen, phosphate-phosphorus and chlorophyll *a* did not vary significantly among the three groups that had either rohu or catla alone or the two combined (Table 9). The water quality parameters recorded throughout this study were within the suitable range for fish culture.

Four different groups of benthic organisms were identified in this study: chirnomid larvae, Oligocheta, mollusks, and organisms that could not be identified (Table 10). There was no significant difference of benthos between the three treatments.

Plankton populations were identified into genus level (Table 11). During the study period, 30 genera of phytoplankton belonging to five families were identified: Bacillariophyceae (10), Chlorophyceae (11), Cyanophyceae (6), Euglenophyceae (2), and Rhodophyceae (1); and 14 genera of zooplankton belonging to four groups were identified: Cladocera (4), Copepoda (3), Rotifera (5) and Protozoa (1). Phytoplankton counts were significantly higher in T_1 (polyculture of rohu and tilapia together leading to lower grazing pressure) while zooplankton counts were significantly higher in T_2 (polyculture of catla and tilapia together). Overall, plankton counts were lowest for T_3 in ponds that had all three species grown together.

Growth parameters of 50 tilapia and 15 rohu and 15 catla from each pond in each treatment were collected at two-week intervals. The mean stocking weight of tilapia was the same among all three treatments (9.14 g). The harvesting (final) weight and weight gain of tilapia were significantly higher in T_2 (149.58 ± 11.32 g and 140.44 ± 11.32 g), followed by T_1 (133.50 ± 8.80 g and 124.36 ± 8.80 g), and T_3 (121.25 ± 7.02 g and 112.11 ± 7.02 g). The highest weight gain was observed in T_2 may have been due to greater uptake of commercial feed (30% CP) and plankton while the lower weight gain in T_3 may have been due to competition for available food resources among the three species. The specific growth rate (SGR) of tilapia found in T_1, T_2 and T_3 were 1.60 ± 0.04%/day, 1.66 ± 0.05%/day, and 1.54 ± 0.03%/day, respectively (Table 13). The variation of SGR was probably due to differences in competition for available food resources among the different species cultured together in each treatment. There was no difference in tilapia survival rate among any of the treatments (Table 13) and were similar to those found in other studies (Borski *et al.* 2011; Haque *et al.* 2010).

The feed conversion ratio (FCR) for tilapia in T_1, T_2 and T_3 were 1.47 ± 0.10, 1.41 ± 0.21, and 1.46 ± 0.27, respectively, but no significant difference was found (Table 13). Siddik *et al.* (2007) and Reza (2013) recorded that the feed conversion ratio for Nile tilapia varied from 1.61 ± 0.05 to 1.65 ± 0.06 and 1.41 to 1.59, respectively.

Net production of tilapia was highest in T_2 (7,181.09 ± 654.71 kg/ha) followed by T_1 (6,273.11 ± 567.89 kg/ha) and T_3 (5,689.10 ± 193.30 kg/ha) (Table 13, $p < 0.05$). The highest production found in T_2 may have been due to greater uptake of commercial feed and natural foods, while the lower weight gain in T_3 may have been due to competition for available food resources among the three species.

Overall, there were no significant differences in total input costs or return variables between any of the treatments (Table 14). Although the benefit-to-cost ratio (BCR) was highest for T_2, it did not significantly vary between the other two treatments.

Based on the presented results, a better production and net return may be achieved with tilapia/catla or tilapia/rohu polyculture when feed is provided at half the daily ration and pond are fertilized. Either of these polyculture systems leads to nominally higher benefit-to-cost ratios and is more economical than tilapia raised with rohu and catla together.

Activity 3 — Disseminate benefits of tilapia–Indian carp polyculture and feed management technology to rural farmer households. Two extension leaflets were produced demonstrating the advantages of reducing feed ration and of incorporating catla or rohu in culture of tilapia (see Addendum). The new "Tilapia-Carp Polyculture Technology" also was demonstrated to farmers through a Farmer's day workshop held at BAU. Thirty-seven farmers from the Mymensingh area attended the workshop where the advantages of reducing feed inputs and incorporating carps into tilapia culture was discussed. The procedure for undertaking the new polyculture-reduced feeding practice was provided to farmers. Farmers were also brought to the field station at BAU for pond-side training. Farmers were very excited about the research and many intend on incorporating the new culture management practice to their current tilapia monoculture production system.

<div align="center">

CONCLUSION
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Small-scale farmers often use extensive or improved extensive agricultural practices, where fertilizer is added to stimulate pond primary production but no additional fish feeds are used. The promotion of semi-intensive farming practices is a key target for increasing personal household income and fish consumption and greater food security for impoverished farmers in Bangladesh. A significant hurdle for the implementation of semi-intensive farming is the cost of feed, comprising up to 50%–70% of total cost. Further, as local feed formulations often have low protein content, farmers compensate by overfeeding their fish, leading to poor water quality. One solution to these issues are to reduce the amount of feed input into the system by providing half the daily ration along with fertilizing ponds to boost primary production of plankton. Also, polyculture of tilapia with other valuable fish species such as rohu and catla can help to reduce feed wastes and help in maintaining good water quality. The results of this investigation indicates that a higher net return and benefit-cost ratio for tilapia production can be achieved by feeding fish at 50% the typical daily ration level in fertilized ponds. Addition of the major Indian the carps, rohu and catla to tilapia culture may also provide further income benefits to farmers as net production of fishes is greater in polyculture than tilapia monoculture systems regardless of the feeding regimen applied. Since tilapia growth was little impacted by feeding at half ration but tended to grow better when polycultured with catla compared to rohu alone or rohu-catla combined, it might be preferential to use catla in polyculture with tilapia. Regardless, the results indicate profits can increase by 200% if tilapia are grown with native Indian carps and provided half the standard ration level typically used for tilapia monoculture. An extension fact-sheet describing these improved management strategies was produced and a Workshop was provided to disseminate the results to rural farmers in the Mymensingh region. This research has contributed to the training of six Masters of Science students who are in the process of finishing their degrees.

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Table 1. Experimental design for Study 1.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu	0	25 (0.625/ m^2)	0	25 (0.625/ m^2)
Tilapia	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)
Fertilization	0	0	4:1 (N: P)	4:1 (N: P)
Feeding	100% Satiation	100% Satiation	50% Satiation	50% Satiation
Replicates (n)	4	4	4	4

Table 2. Experimental design for Study 2.

Parameter	Treatment 1	Treatment 2	Treatment 3
Rohu	25 (0.625/m^2)	0	13 (0.32/m^2)
Catla	0	25 (0.625/m^2)	12 (0.31/m^2)
Tilapia	500 (5.0/ m^2)	500 (5.0/ m^2)	500 (5.0/ m^2)
Feeding	50% Satiation	50% Satiation	50% Satiation
Fertilization	4:1 (N: P)	4:1 (N: P)	4:1 (N: P)
Replicates (n)	4	4	4

Table 3. Water quality parameters from Study 1. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$).

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Dissolved Oxygen (mg/L)	6.31±0.40	6.49±0.28	6.73±0.42	6.53±0.46
Temperature (°C)	23.82±0.03	23.88±0.03	23.83±0.03	23.86±0.05
pH	8.04±0.11[b]	8.19±0.02[ab]	8.26±0.10[a]	8.27±0.10[a]
Transparency (cm)	12.06±2.33	11.44±0.58	10.67±0.96	10.94±1.32
Total Alkalinity (mg/L)	140.00±24.57	142.52±10.45	143.64±5.46	143.73±6.24
TDS (mg/L)	132.18±26.32	135.34±17.79	134.75±4.74	126.88±11.01
Chlorophyll-a (µg/L)	100.28±25.43	104.27±32.71	143.32±33.36	130.86±33.24
Ammonia (mg/L)	0.626±0.152	0.507±0.058	0.523±0.089	0.582±0.090
Phosphate (mg/L)	1.357±0.184	1.282±0.106	1.215±0.064	1.434±0.200
Nitrite (mg/L)	0.036±0.008	0.052±0.025	0.038±0.016	0.041±0.006
Nitrate (mg/L)	0.310±0.009	0.327±0.022	0.297±0.055	0.346±0.026

Table 4. Benthic organisms identified in Study 1. Values are mean abundance ($\times 10^3$ cells/L) ± SD. Values with different letters are significantly different ($p < 0.05$).

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Oligochaeta	217.28±45.80	206.58±36.14	195.06±42.03	188.89±39.28
Chironomid Larvae	386.83±77.06[a]	316.87±56.96[b]	257.61±50.24[c]	261.73±73.66[c]
Mollusks	69.68±27.76	56.79±14.70	70.78±20.49	53.91±26.13
Unidentified	22.50±15.24	21.81±12.76	17.70±18.41	9.05±9.09

Table 5. Plankton populations identified in Study 1. Values are mean abundance ($\times 10^3$ cells/L) $\pm$ SD. Values with different letters are significantly different ($p < 0.05$).

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Bacillariophyceae	21.14±4.36[b]	22.86±4.42[b]	28.72±5.94[a]	27.28±5.95[a]
Chlorophyceae	27.47±3.86[c]	29.17±3.63[b]	34.31±3.09[a]	33.17±2.98[a]
Cyanophyceae	5.58±0.88[c]	8.53±0.88[a]	6.97±1.17[b]	7.17±0.84[ab]
Euglenophyceae	6.72±1.62[b]	7.86±1.04[a]	6.53±0.85[b]	5.53±1.28[c]
Rhodophyceae	1.94±0.62[a]	1.39±0.33[b]	1.17±0.38[c]	1.44±0.30[b]
Total Phytoplankton	61.14±7.21[c]	64.47±4.52[c]	73.39±5.87[b]	85.94±6.70[a]
Copepoda	5.64±0.67	6.31±0.74	5.94±1.01	5.81±0.86
Rotifera	9.39±2.36	10.06±2.36	12.72±4.26	11.19±1.88
Cladocera	2.44±1.07	1.92±0.78	1.97±1.19	2.08±0.98
Protozoan	0.61±0.25[a]	0.28±0.15[c]	0.67±0.41[a]	0.39±0.28[b]
Total Zooplankton	15.28±1.22	16.58±0.86	20.39±2.32	25.17±3.68
Total Plankton	76.42±7.87	81.06±5.00	93.78±7.96	111.11±9.76

Table 6. Plankton identified in Study 1.

Family	Genus
Prokaryotes	
Bacillariophyceae	*Cyclotella*
	Surirella
	Cosmarium
	Nitzschia
	Navicula
Chlorophyceae	*Actinestrum*
	Ankistrodesmus
	Chlorella
	Closterium
	Volvox
	Pediastrum
	Scenedesmus
	Tetraedon
	Ulothrix
	Crucigenia
	Pleorococcus
	Stichococcus
Cyanophyceae	*Anabaena*
	Aphanizomenon
	Gomphosphaeria
	Microcystis
	Oscillatoria
Euglenophyceae	*Euglena*
	Phacus
Rhodophyceae	*Hildenbrandia*
Eukaryotes	
Rotifera	*Asplanchna*
	Brachionus
	Filinia
	Keratella
	Polyarthra
	Trichocerca
Cladocera	*Diaphanosoma*
	Sida
	Moina
Copepoda	*Cyclops*
	Diaptomus
Crustacea	*Nauplius*

Table 7. Growth performance outcomes for Study 1. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$). NA = not applicable.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Tilapia				
Stocking Weight (g)	8.34±1.85	8.34±1.85	8.34±1.85	8.34±1.85
Harvesting Weight(g)	108.96±9.82[b]	116.99±9.04[b]	137.69±2.04[a]	132.57±5.42[a]
Weight Gain (g)	100.62±9.82[b]	108.64±9.04[b]	129.35±2.04[a]	124.23±5.43[a]
Survival Rate (%)	75.29±2.45[b]	76.24±2.06[b]	76.89±1.28[b]	81.06±1.03[a]
Specific Growth Rate (SGR)	1.71±0.06[b]	1.76±0.05[b]	1.87±0.00[a]	1.85±0.03[a]
Gross Production (kg/ha)	4,089.83±518.46[b]	4,440.99±440.04[b]	5,340.62±156.47[a]	5,385.23±276.98[a]
Net Production (kg/ha)	3,777.52±52[b]	4,124.67±431.38[b]	5,017.16±151.46[a]	5,046.53±272.30[a]
Feed Conversion Ratio (FCR)	1.23±0.16[a]	1.13±0.11[a]	0.47±0.03[b]	0.49±0.03[b]
Rohu				
Stocking Weight (g)	NA	26.60±8.50	NA	26.60±8.50
Harvesting Weight(g)	NA	61.00±13.76	NA	59.93±2.04
Weight Gain (g)	NA	35.00±13.76	NA	33.33±2.04
Survival Rate (%)	NA	80.56±18.44	NA	77.29±22.94
Gross Production (kg/ha)	NA	126.502±52.63	NA	116.107±35.13
Net Production (kg/ha)	NA	73.12±42.14	NA	64.78±20.75
Specific Growth Rate (SGR)	NA	0.55±0.14	NA	0.54±0.02

Table 8. Economic analyses from Study 1. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$). NA = not applicable.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Fingerlings cost (Tk/ha)	99,505.71± 1,411.43[b]	111,943.93 ±1,587.86[a]	100,917.14±1,411.43[b]	112,737.86±1,833.50[a]
Feed cost (Tk/ha)	118,210.14±4,309.44[a]	118,210.14±4,309.44[a]	62,777.92± 1,074.71[b]	60,407.64± 981.23[b]
Lime Cost (Tk/ha)	1,885.58±935.91	1,389.38±1,273.01	1,896.61±1,784.02	2,172.28±1457.26
Fertilizers cost(Tk/ha)	NA	NA	6,661.74± 93.55[a]	6,614.97± 108.02[a]
Labor cost (Tk/ha)	42,664.53±568.91[a]	43,560.18±555.06[a]	26,948.17±431.08[b]	27,244.54±747.29[b]
Total Expenditure (Tk/ha)	611,524.94±8,154.32[a]	624,362.60±7,955.90[a]	386,257.17±6,178.87[b]	390,505.00±10,711.16[b]
Gross return (Tk/ha)	838,200.68±116,951.37[c]	932,932.67±77,451.22[bc]	1,130,234.07±86,477.25[a]	1,064,254.63±89,098.58[ab]
Net return (Tk/ha)	226,675.58±110,128.31[b]	286,469.43±95,884.41[b]	743,976.95±80,584.80[a]	673,749.64±79,583.97[a]
Benefit Cost Ratio (BCR)	1.37±0.17[b]	1.49±0.11[b]	2.92±0.18[a]	2.72±0.17[a]

Table 9. Water quality parameters from Study 2. Values are mean ± SD.

	Treatment 1	Treatment 2	Treatment 3
Temperature (^{0}C)	29.55±0.35	29.70±0.22	29.75±0.19
Transparency (cm)	10.58±0.96	13.58±1.67	11.83±0.58
DO (mg/L)	4.50±0.12	4.38±0.20	4.38±0.15
pH	7.89±0.24	7.79±0.10	7.77±0.22
Alkalinity (mg/L)	112.80±6.22	103.20±7.48	104.05±7.24
TDS (mg/L)	93.36±4.89	90.32±10.44	79.77±7.95
Nitrate (mg/L)	0.067±0.031	0.078±0.035	0.057±0.022
Nitrite (mg/L)	0.034±0.011	0.055±0.024	0.036±0.020
Phosphate (mg/L)	1.22±0.10	2.43±1.19	1.35±0.12
Chlorophyll *a* (µg/L)	208.80±20.52	169.91±13.75	189.32±13.53

Table 10. Benthic organisms identified in Study 2. Values are mean abundance ($\times 10^3$ cells/L) $\pm$ SD.

	Treatment 1	Treatment 2	Treatment 3
Oligochaeta	337.04±122.37	348.15±137.08	360.00±145.33
Chironomid Larvae	336.67±144.64	402.59±247.31	362.22±176.39
Mollusks	147.04±30.44	139.26±27.44	145.19±55.51
Unidentified	59.26±28.48	54.81±15.00	50.06±18.33

Table 11. Plankton populations identified in Study 2. Values are mean abundance ($\times 10^3$ cells/L) $\pm$ SD. Values with different letters are significantly different ($p < 0.05$).

	Treatment 1	Treatment 2	Treatment 3
Bacillariophyceae	8.90 ± 0.39^a	6.57 ± 0.39^b	5.38 ± 0.28^c
Chlorophyceae	10.03 ± 0.49^a	7.40 ± 0.49^b	6.64 ± 0.55^c
Cyanophyceae	3.88 ± 0.30^a	2.57 ± 0.31^b	1.96 ± 0.21^c
Euglenophyceae	3.22 ± 0.22^a	2.11 ± 0.22^b	1.96 ± 0.19^c
Rhodophyceae	0.82 ± 0.07^a	0.53 ± 0.12^b	0.27 ± 0.05^c
Total phytoplankton	26.86 ± 1.59^a	19.18 ± 1.03^b	16.20 ± 1.90^c
Copepoda	0.16 ± 0.33	0.18 ± 0.31	0.13 ± 0.23
Rotifera	3.58 ± 0.43^b	4.36 ± 0.39^a	2.52 ± 0.38^c
Cladocera	1.31 ± 0.14^a	1.46 ± 0.11^a	0.90 ± 0.11^b
Protozoan	0.24 ± 0.06	0.26 ± 0.05	0.11 ± 0.06
Total zooplankton	6.77 ± 0.61^b	7.90 ± 0.55^a	4.79 ± 0.55^c
Total plankton	33.62 ± 1.62^a	27.07 ± 1.47^b	20.99 ± 1.60^c

Table 12. Plankton identified in Study 2

Family	Genus
Prokaryotes	
Chlorophyceae	*Ankistrodesmus*
	Botryococcus
	Chlorella
	Closteridium
	Pleurococcus
	Pediastrum
	Scenedesmus
	Tetraeodon
	Ulothrix
	Zygnema
	Volvox
Cyanophyceae	*Anabaena*
	Aphanizomenon
	Mycrocystis
	Gomphosphaeria
	Spirulina
	Oscillatoria
Bacillariophyceae	*Asterionella*
	Cyclotella
	Cosinodiscus
	Diatoma
	Fragillaria
	Gomphonema
	Navicula
	Nitzchia
	Suirella
	Triceratium
Euglenophyceae	*Euglena*
	Phacus
Rhodophyceae	*Hildenbrandia*
Eukaryotes	
Rotifera	*Asplanchna*
	Brachionus
	Filinia
	Polyartha
	Trichocerca
Cladocera	*Daphnia*
	Moina
	Bosmina
	Sida
Copepoda	*Cyclops*
	Diaptomus
	Nauplius

Table 13. Growth performance outcomes for Study 2. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$). NA = not applicable.

	Treatment 1	Treatment 2	Treatment 3
Tilapia			
Stocking Weight (g)	9.14±0.00	9.14±0.00	9.14±0.00
Harvesting Weight (g)	133.50±8.80[b]	149.58±11.32[a]	121.25±7.02[c]
Weight Gain (g)	124.36±8.80[b]	140.44±11.32[a]	112.11±7.02[c]
Survival Rate (%)	92.96±2.91	97.41±1.50	94.80±2.59
Specific Growth Rate (SGR)	1.60±0.04[b]	1.66±0.05[a]	1.54±0.03[c]
Gross Production (kg/ha)	6,130.21±443.60	7,199.18±569.21	5,672.81±214.50
Net Production (kg/ha)	5,710.49±438.71[b]	6,759.36±566.85[a]	5,244.76±221.47[c]
Feed Conversion Ratio (FCR)	1.47±0.10	1.41±0.21	1.46±0.27
Gross Production (kg/ha)	6,867.11±570.36[b]	7,737.78±646.51[a]	6,272.23±183.44[c]
Net Production (kg/ha)	6,273.11±567.89[b]	7,181.09±654.71[a]	5,689.10±193.30[c]
Rohu			
Stocking Weight (g)	34.70±0.00	NA	34.70±0.00
Harvesting Weight (g)	146.67±43.96	NA	122.00±9.70
Weight Gain (g)	111.97±43.96	NA	87.30±9.70
Survival Rate (%)	81.11±2.30[b]	NA	88.68±2.11[a]
Gross Production (kg/ha)	736.89±221.29[a]	NA	345.14±22.31[b]
Net Production (kg/ha)	562.62±220.73[a]	NA	246.88±23.43[b]
Specific Growth Rate (SGR)	0.84±0.17	NA	0.75±0.05
Catla			
Stocking Weight (g)	NA	22.92±0.00	22.92±0.00
Harvesting Weight (g)	NA	108.17±32.41	102.88±9.72
Weight Gain (g)	NA	85.25±32.41	79.96±9.72
Survival Rate (%)	NA	82.34±8.82	80.64±13.99
Gross Production (kg/ha)	NA	538.59±100.36[a]	254.28±42.66[b]
Net Production (kg/ha)	NA	421.73±112.48[a]	197.46±34.62[b]
Specific Growth Rate (SGR)	NA	0.90±0.17	0.89±0.05

Table 14. Economic analyses from Study 2. Values are mean ± SD. NA = not applicable.

	Treatment 1	Treatment 2	Treatment 3
Financial Input			
Rotenone (Taka/ha)	8,398	8,398	8,398
Lime (Taka/ha)	2,964	2,964	2,964
Urea (Taka/ha)	9,296	9,296	9,296
TSP (Taka/ha)	7,382.58	7,382.58	7,382.58
Tilapia (Taka/ha)	98,800	98,800	98,800
Rohu (Taka/ha)	12,479.68	NA	6,422
Catla (Taka/ha)	NA	55575	28899
Feed (Taka/ha)	435,462.41	448,127.16	437,151.89
Labor and Others (Taka/ha)	10,000	10,000	10,000
Total Cost (Taka/ha)	584,782.67	640,542.74	609,313.47
Financial Return			
Tilapia (Taka/ha)	735,990.84	864,542.48	711,305.85
Rohu (Taka/ha)	73,569.67	NA	35,929
Catla (Taka/ha)	NA	55,080.16	29,907.22
Total Return (Taka/ha)	809,560.51	919,622.65	777,142.07
Net Return (Taka/ha)	224,777.85	279,079.91	167,828.59
Benefit Cost Ratio (BCR)	1.38	1.44	1.28
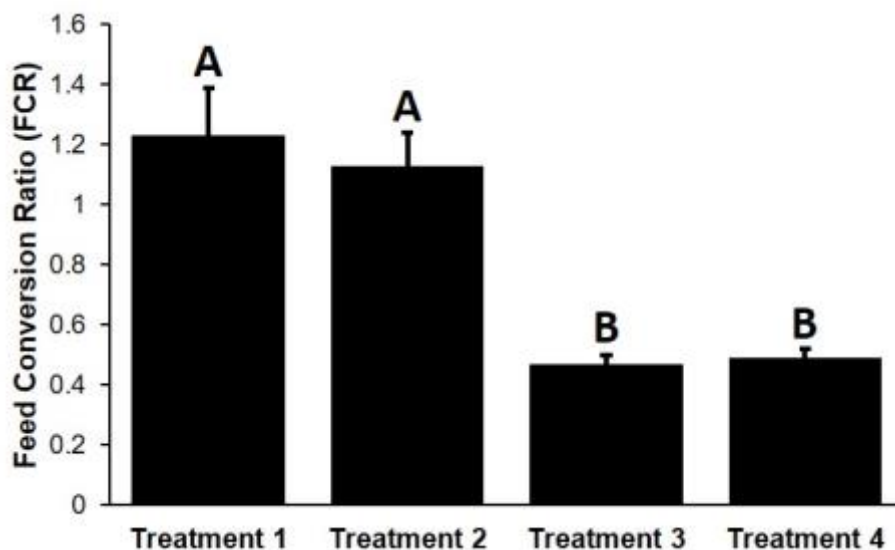
Figure 1. Feed conversion ratio (FCR) for tilapia (*O. niloticus*) in Study 1. The FCR for tilapia in treatments 1 and 2 were significantly higher than in treatments 3 and 4. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$).
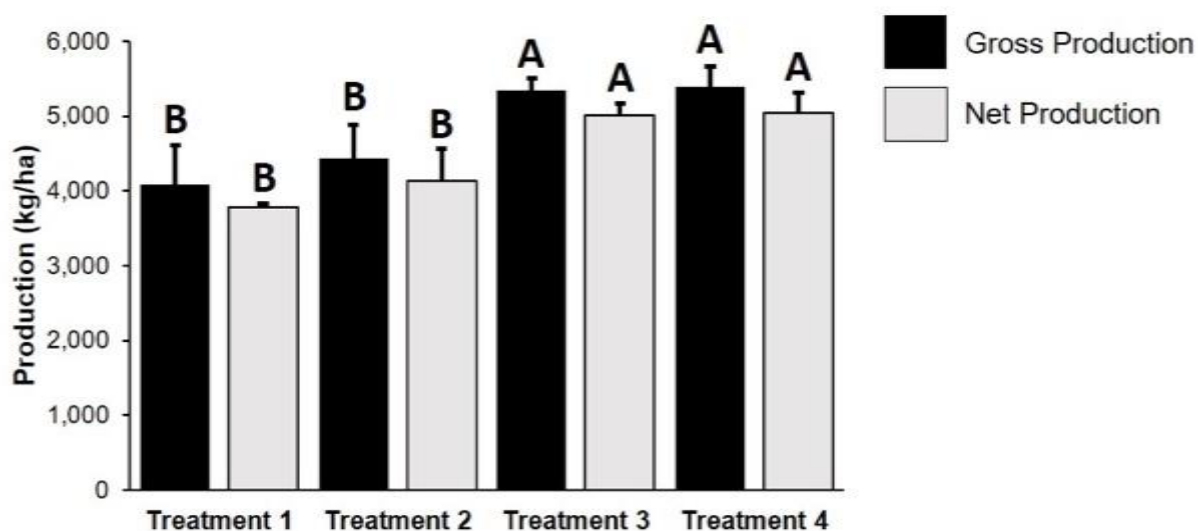
Figure 2. Gross and net production parameters for tilapia (*O. niloticus*) in Study 1. For both parameters, treatments 1 and 2 (100% daily ration without pond fertilization) were significantly lower than treatments 3 and 4 (50% daily ration with pond fertilization). Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$).
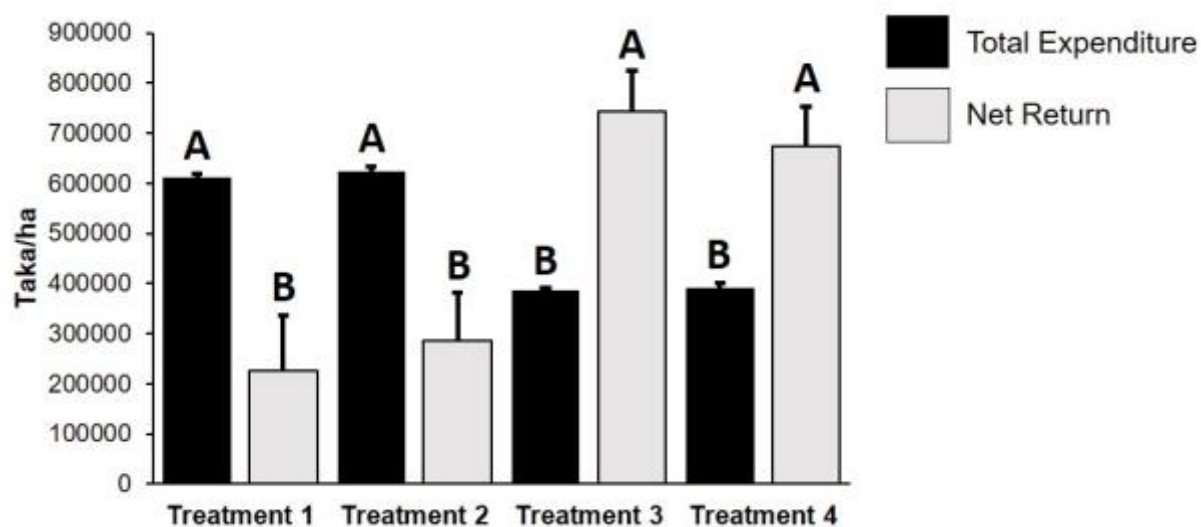
Figure 3. Combined total expenditure and net return for tilapia (*O. niloticus*) and rohu (*L. rohita*) in Study 1. The total expenditure was significantly higher in treatments 1 and 2 (100% daily feed rations) than in treatments 3 and 4 (50% daily feed rations). The net return for treatments 3 and 4 were significantly lower than treatments 1 and 2. Values are mean for treatments. Values with different letters are significantly different ($p < 0.05$).

Pulsed Feeding Strategies to Improve Growth Performance, Gastrointestinal Nutrient Absorption Efficiency, and Establishment of Beneficial Gut Flora for Tilapia Pond Culture

Sustainable Seed Technology and Nutrient Input Systems/Experiment/13SFT05NC

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ABSTRACT

Feed constitutes 60%−80% of total production costs of tilapia (*Oreochromis* spp.). Reductions in quantity of feed used for fish growout and in the cost of formulated feeds are two approaches to containing feed costs. We evaluated pulsed-feeding strategies for improving production efficiency of Nile tilapia (*Oreochromis niloticus*). In a 12-week pond trial, fish were fed daily, every other day, every third day, or not at all. In all groups ponds were fertilized weekly to enhance natural foods. In the other group fish were fed daily without weekly pond fertilization. Fish fed daily with or without pond fertilization and fish fed every other day along with pond fertilization had greater weight gain, higher specific growth rates and survivability, and better net production than the other two treatments. There was no difference in feed conversion ratio in fish from ponds fed daily whether the ponds were fertilized, but feed efficiency was substantially improved in fish fed every other day. The benefit to cost ratio was highest for treatments fed in a pulsatile manner (i.e. fed every other day or every third day) with fish fed on alternate days providing the best net return among all groups. To determine if pulsed-feeding regimes affected nutrient uptake in the gut, gene expression of key solute-linked nutrient carriers was analyzed that included those for amino acids, fatty acids and simple carbohydrates (sugars). There were no significant differences in gene expression of these nutrient transporters in any treatment that incorporated both feeding and pond fertilization. Expression of the transporters was higher in those fish that were not fed and grown in fertilized ponds only and lower in fish that were fed daily without pond fertilization. Fish on alternate day feeding had more moderate expression levels of certain transporters which may allow for a more balanced and efficient nutrient uptake. Metagenomic analyses identified 145 different families of prokaryotic (all bacteria) and 132 genera of eukaryotic organisms in the fecal material of tilapia. The highest diversity of prokaryotes was found in fish fed either daily or every other day in fertilized ponds and the highest diversity of eukaryotes was found in fish fed every other day. Taken together, these studies along with those previously shown in the Philippines indicates feeding Nile tilapia on alternate days along with weekly pond fertilization has no deleterious effects on growth, survivability, or production versus daily feeding regimes and produces the greatest net return on investments. By feeding on alternate days, feed costs can be reduced by half without any decrease in production of farmed Nile tilapia. Our studies also suggest for the first time that combined feeding and fertilization produces the greatest biodiversity of microbiomes in the intestine that could contribute to enhanced feed efficiency and overall health of tilapia, particularly those subjected to more moderate feeding strategies.

Global production of farmed Nile tilapia (*Oreochromis niloticus*) has increased exponentially since 1985, with more than 2.4 million metric tons consumed in 2010 (FAO 2013). In Bangladesh, Nile tilapia comprises a significant source of per capita caloric and protein intake, with production increasing 30-fold from 1999-2007 (Gupta et al. 1992, Hussain 2009). Currently, small-scale farmers often use extensive or improved extensive agricultural practices, a process where fertilizer is added (to stimulate pond primary production) but no feeds are used (Belton et al. 2011). As the addition of even modest amounts of feed (semi-intensive) can effectively quadruple production, the promotion of semi-intensive farming practices is a key target for increasing personal household income and fish consumption, and greater food security for impoverished farmers in Bangladesh (Belton et al. 2011, Dey et al. 2008). A significant hurdle for the implementation of semi-intensive farming is the cost of feed, comprising up to 50%–80% of total costs. Further, as local feed formulations often have low protein content, farmers compensate by overfeeding their fish, leading to poor water quality (Phillips 2013, USAID 2012). This study addressed these issues by demonstrating that equivalent production yields can be achieved with much less feed (50% reduction), through the implementation of pulsed feeding strategies, thereby reducing feed and labor costs and making the prospect of switching to semi-intensive culture more attractive to local farmers. Additionally, examined how alternate-day feeding strategies may enhance nutrient absorption by measuring nutrient transporter abundance and gut microbial diversity in response to different feeding regimes.

A better understanding of how finfish acquire and utilize nutrient inputs is requisite for future improvements in aquaculture production efficiency. Part of this investigation sought to further determine how intestinal nutrient absorption and gut microbial diversity change in response to the use of alternate feeding strategies, which have previously led to dramatic improvements in feed efficiency. Currently, the underlying mechanism explaining how alternate-day (pulsatile) feeding strategies can achieve equivalent production yields with less feed is poorly understood. Some evidence suggests that during periods of fasting, nutrient uptake efficiency in the intestine is intrinsically enhanced, leading to a more-efficient uptake of nutrients at the next feeding period. Thus, fish being fed a daily regime have lower uptake efficiency and do not receive maximal dietary benefit. A similar phenomenon has been postulated, in part, to explain the compensatory growth (CG) response observed in some aquaculture species (Farmanfarmaian and Sun 1999, Picha et al. 2006). Additionally, reduced feeding may promote foraging on primary production within the ponds, leading to a more diverse diet (e.g., algae, insect larvae, plankton), enhancing nutrient recycling within the ponds, also may also directly influence intestinal absorption by promoting increases in nutrient transporters not utilized by fish with a constant and predictable diet (Heikkinen et al. 2006, Sigiura et al. 2009). Using the alternate-day feeding experiment, we will evaluate the mRNA expression of key nutrient transporters (involved in protein, lipid, and phosphate uptake) in response to pulsed-feeding strategies. This analysis will further our understanding of how greater nutrient uptake efficiency may be achieved for greater optimization of feeding protocols in the future, generating potential benefits not only for rural farmers in Bangladesh, but could also improve both US and global tilapia farming practices. Testing reduced feeding frequency (every third day) could provide an additional level of cost savings beyond alternate-day feeding, a protocol that could be adapted to studies in Bangladesh and elsewhere.

The emerging field of metagenomics has substantial implications for sustainable aquaculture, as diet, feeding strategy, and other environmental factors strongly influence the diversity and constitutive abundance of intestinal microbiota in both humans and fish (Al-Harbi and Uddin 2004, 2005; De Filippo et al. 2010; Heikkinen et al. 2006). In aquacultured finfish, new research has shown that probiotic maintenance of beneficial gut flora can promote growth, greater nutrient availability, and better stock health (Nayak 2010, Welker and Lim 2011). Early studies in channel

catfish (*Ictalurus punctatus*) and carp (*Cyprinidae*) identified several limiting nutrients (e.g., biotin, pantothenic acid, vitamin B12), which are produced by intestinal microbes, but may be limiting in lesser-quality feeds (Robinson and Luvell 1978, Kashiwada and Teshima 1966). In tilapia, the bacterium *Virgibacillus pantothenticus* stimulates intestinal production of alkaline protease, an enzyme involved in the digestion of dietary protein (Thillaimaharani et al. 2012). Naturally occurring lactic acids strains (such as *Leuconostoc mesenteroides*), appear to inhibit colonization of known fish pathogens (*Vibrio* and *Mycobacterium* sp.) through stimulation of the immune system (Zappata 2013). Interestingly, proper intestinal flora in tilapia may also positively impact human health as natural flora innoculates could theoretically out-compete non-natural pathogenic microbes. In Nile tilapia cultured in Saudi Arabia, fecal coliform bacteria (*E. coli*) comprised up to 10% of gut microbiota, which could be passed on to consumers through improper storage and handling practices (Al-Harbi and Uddin 2003, Mandal et al. 2009). We will test whether the tilapia intestinal microbiome differs in composition with alternate-day feeding, and identify key microbial factors associated with increased nutrient uptake and utilization. Identification of beneficial microbes that improve nutrient uptake will benefit current research into the application of probiotic supplements for the further enhancement of nutrient uptake in finfish cultivars. These investigations will lay the framework toward development of probiotic supplements that can be incorporated into diet formulations for improving growth and nutrient absorption. Ultimately, this work may have significant effects on tilapia culture, whose global production exceeds 2.4 million metric tons. The intensive tilapia culture practiced in the U.S. may also benefit substantially from this work.

OBJECTIVES

- Evaluate the effectiveness of pulsed feeding on tilapia production yields in fertilized ponds;
- Identify changes in key amino acids, sugars and fatty acid transporters in the intestine that may be linked to improved nutrient uptake efficiency;
- Characterize changes in gut microbial communities in response to pulse-feeding strategies; and
- Identify changes in key microbial communities that may be associated with increased nutrient availability or production efficiency in tilapia and that could potentially be used as probiotic dietary supplements for enhancing nutrient uptake in fish.

MATERIALS AND METHODS

Study 1 — The effects of alternate feeding strategies on tilapia growth performance. This study design was composed of 5 treatment groups as shown in Table 1.

This investigation follows previous studies from the Philippines (Bolivar et al. 2006, Borski et al. 2011) where a 50% reduction in feeding frequency resulted in equivalent production yields. This study was reproduced at the Fisheries Field Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh with additional reductions in feeding (every third day) utilized. All-male sex reversed Nile tilapia (~3.5 g) were stocked at 5 fish/m^2 in 16 ponds (0.1 ha, 4 replicates per treatment), with weekly pond fertilization at a rate of 28 kg N and 7 kg P/ha/week for all treatment groups. Fish were fed with formulated feed (CP Bangladesh, 30% crude protein) initially at 10% and then down to 3% body weight/day based on a standard tilapia feed schedule.

Growth (length and weight) was monitored at 2-week intervals (sub-sampling) over a twelve-week growing period. Feed rates were adjusted accordingly based on this biweekly sampling. Samples of tilapia anterior intestinal tissue and fecal material from the colon were collected for further analysis at NCSU (see Studies 2 and 3). Water temperature (°C), transparency (cm), pH and dissolved oxygen (mg l–1) were measured weekly and total alkalinity (mg l–1), ammonia-

nitrogen (mg l–1), nitrate-nitrogen (mg l–1), nitrite-nitrogen (mg l–1), phosphate-phosphorus (mg l–1) and chlorophyll *a* (µg l–1) were measured biweekly. Production parameters (FCR, growth rate, yield) were determined at the end of study. A marginal cost-return analysis was also conducted. Significant differences between treatment groups were determined by two-way ANOVA analysis using JMP (SAS Institute, Cary, NC).

To collect tilapia gastrointestinal samples, tilapia from all 20 ponds were sampled using a seine net. Five fish were randomly collected from each pond, anesthetized, and decapitated. Samples for gene expression analyses was taken from a 1 cm section of the anterior intestine 5 cm posterior of the duodenal bulb (stomach sphincster where intestine meets the stomach) and placed in RNALater. Tilapia fecal material was collected from the posterior intestine (colon) and placed in a vial containing buffer and bullet homogenized with a portable homogenizer.

Study 2 — Assessment of tilapia nutrient uptake efficiency in response to alternate feed strategies. From data obtained from the publicly available tilapia genome assembly (http://cichlid.umd.edu), we identified 6 candidate transporters putatively involved in the digestive transport of amino acids, dietary sugars, and lipids across the intestinal epithelium: (1) facilitated glucose/fructose transporter (*slc2a5*), (2) facilitated glucose transporter (*slc2a6*), (3) long-chain fatty acid transporter (*slc27a4*), (4) Na^+-amino acid transporter 2 (*slc38a2*), (5) Na^+-amino acid transporter 4 (*slc38a4*), and (6) large neutral amino acid transporter subunit 3 (*slc43a1*). Samples (n=12; N=60) of tilapia intestine were collected at week 12 of the growth trial (see Study 1).

Tilapia gut transporter mRNA expression was quantified using real-time quantitative PCR (qPCR) performed as previously described with a few exceptions (Tipsmark et al. 2008). Briefly, total RNA was isolated from the anterior intestine using Tri Reagent (Invitrogen, Carlsbad, CA). One microgram of the total RNA was used to synthesize cDNA using a High Capacity cDNA Synthesis kit (Applied Biosystems, Carlsbad, CA) following treatment with Turbo DNA-free (Ambion, Foster City, CA). RNA was quantified and checked for quality at each step using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and agarose gel electrophoresis, respectively. Gene expression (mRNA) of the different transporter forms was measured in the tilapia cDNA using SYBR Green chemistry. Transporter gene-specific primers (Integrated DNA Technologies, Inc., Coralville, IA) were designed using the IDT PrimerQuest Tool (Integrated DNA Technologies, Inc.). Optimization for appropriate annealing temperature, primer concentrations, and cycling parameters was performed using pooled cDNA from the above reverse transcription reactions. One hundred ng of starting total RNA was used for qPCR analysis with Brilliant II QPCR Master Mix (Agilent Technologies Inc., Clara, CA) containing 1.5 µM gene-specific primers. No template controls and no reverse transcription controls were incorporated into the assay. Pooled cDNA was used to produce the cDNA for creating the standard curves. All qPCR assays were run in triplicate wells on a CFX384 real-time PCR system (BioRad Laboratories Inc., Hercules, CA). Cycling conditions were: 1 cycle, 50°C for 2 min; 1 cycle, 95°C for 10 min; 40 cycles, 95°C for 15 s, 60°C for 1 min. Melting curve analysis was performed to determine primer specificity. Data was normalized to the starting total RNA concentration. Gene copy number was predicted by comparing the mean cycle threshold (Ct) to the serially diluted cDNA standard curve ($R^2 = 0.98$). The gene expression data were then normalized to the expression of 18S ribosomal RNA, whose levels were found to be similar across treatment groups. Significant differences between treatment groups were determined by 2-way ANOVA analysis using JMP (SAS Institute, Cary, NC).

Study 3 — Characterize changes in gut microbial communities in response to pulse-feeding strategies for identifying microbes that may be associated with increased nutrient availability and production efficiency in tilapia. Samples of tilapia fecal material were collected from the Fisheries Field Laboratory, Bangladesh Agricultural University field study (see Study 1) and analyzed at North Carolina State University. Samples were collected from fish following 12 weeks of growout, with samples from 2 fish per pond pooled together from 2 sample replicates (total of 4 fish/pond). Samples were take from all 4 replicate ponds for all treatment group (n = 8 pooled samples per treatment; N = 40). A pooled sample design was used to offset potential variability of microbiota within individuals, instead focusing on common patterns, which may be more reflective of changes with treatment group among the population (pond) as a whole.

Ribosomal RNA extraction. Extraction of ribosomal RNA from tilapia fecal samples was performed using an XpeditionTM Soil/Fecal DNA MiniPrep Kit (Zymo Research, Corp., Irvine, CA) following the included protocol. Up to 0.25 g of fecal sample was placed into a ZR BashingBead Lysis Tube with 750 µL Xpedition Lysis/Stabilization Solution. The tube was secured in an Xpedition Sample Processor and processed for 30 s and stored at room temperature until extraction. The concentration and quality were determined by Nanodrop (Thermo Fisher Scientific Inc., Waltham, MA). The extracted rRNA was stored at -20°C for sequencing library preparations.

Prokaryotic 16S and eukaryotic 18S rRNA sequencing library preparation. Prokaryotic 16S and eukaryotic 18S rRNA gene amplicons were prepared following the 16S Metagenomic Sequencing Library Preparation protocol for the Illumina MiSeq system with some modifications. Primers were designed to amplify the V3 to V5 regions of 16S rRNA (Muyzer et al. 1993, Sim et al. 2012) and the V9 region of 18S rRNA (Amaral-Zettler et al. 2009, Chariton et al. 2010) with overhang adapter sequences compatible with the Illumina index and sequencing adapters and allowed for double indexing to increase the accuracy of the multiplexed reads. Amplicon PCR was used to amplify the region of interest from the gDNA extracted from the tilapia fecal material samples. A Nile tilapia-specific blocking primer was introduced during the 18S amplification step to reduce the amplification of tilapia 18S rRNA and increase the amplification of lower abundance eukaryotic rRNA present in the samples. The PCR was performed: 1 cycle of 95°C for 3 min; 25 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 1 cycle of 72°C for 5 min; and hold at 4°C. Clean-up of the PCR amplicon products to remove free primers and primer dimers was performed using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA). Fresh 80% ethanol was prepared prior to clean-up. Following amplicon clean-up, index PCR was performed to attach indexes to the amplicon PCR products. Dual-index primers were designed so that samples could be multiplexed in one MiSeq lane. Index PCR was performed as follows: 1 cycle, 95°C for 3 min; 8 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 1 cycle, 72°C for 5 min; and hold at 4°C. Clean-up of the PCR index products was performed as above. All indexed amplicon concentrations were normalized and amplicons pooled into a single tube. The pooled library was checked for quality and quantified using a High Sensitivity DNA chip on the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). The library was diluted and combined with a PhiX Control library (v3) (Illumina, San Diego, CA) at 10%. The library was sequenced on an Illumina v3 300PE MiSeq run, using standard sequencing protocols. Base calls were generated on-instrument during the sequencing run using the MiSeq Real Time Analysis (RTA 1.18.54) software and fastq generation; demultiplexing, adapter trimming, and quality filtering were performed by the MiSeq Reporter Software (2.4 and 2.5.1). The library was run on two lanes to increase the number of reads for each sample.

Sequence and statistical analyses. The resulting demultiplexed reads were processed using the QIIME 1.9.1 toolkit (Caporaso et al. 2010). Briefly, the paired end reads were joined together and uclust (Edgar 2010) was used to search against Greengenes 13_8 reference database (DeSantis et al 2006) for 16S (prokaryote) analysis and SILVA release 119 reference database (Pruesse et al 2007, Quast et al 2013, Yilmaz et al. 2014) for 18S (eukaryote) analysis both filtered at 97% identity. Reads not matching a reference sequence were removed from analysis. OTUs were assigned based on a database hit of 97% or greater sequence identity and taxonomy was assigned against the appropriate database. Core diversity analysis was used to perform α-diversity and rarefaction and β-diversity and rarefaction functions. Weighted and unweighted Unifrac distances (Lozupone and Knight 2005) were used to compute the between sample diversity which was visualized using principal coordinates analysis (PCoA) plots with Emperor (Vazquez-Baeza et al 2013).

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RESULTS AND DISCUSSION

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Study 1 — The effects of alternate feeding strategies on tilapia growth performance. Results suggest that the optimal benefit: cost ratio and net return for tilapia production occurs when fish are fed on alternate days in fertilized ponds. Studies also suggest that tilapia production is similar with fish fed daily in non-fertilized ponds to those fish fed daily in fertilized ponds, suggesting there is no added benefit of fertilizing ponds when providing a full daily ration of formulated feeds. In previous studies with lower stocking densities tilapia production was similar with half satiation and pond fertilization as feeding alone to full satiation (Diana et al. 1994).

Water quality parameters for each treatment are provided in Table 2. Ammonia and nitrites were highest in the feed-plus-fertilization group and lowest in the fertilization alone group likely a result of the relative amounts of nitrogen input provided from feeds and/or fertilizers in these relative to the other groups. Nonetheless, these and all other water parameters were well within the range for suitable growth of tilapia.

There was no significant difference in weight gained, specific growth rate, survival and production of Nile tilapia between groups fed daily with (Treatment 1) or without (Treatment 5) pond fertilization and those fed on alternate days with pond fertilization (Treatment 2, Figure 1). These treatments had the highest fish growth and net production followed by those fish fed every third day (Treatment 3, Figures 2 and 3). Fish in the Treatment 4 ponds showed the worst growth of all groups.

The fish produced with pond fertilization alone (Treatment 4) had the lowest survival relative to the other groups. The feed conversion ratio (FCR) was lowest for Treatment 3 (group fed every third day with pond fertilization), followed by Treatment 2 (Figure 2). The worst FCRs were found for Treatments 1 and 5, both of which had greater fish feed inputs, but had similar growth to Treatment 2.

Economic analyses from this study suggest feeding on alternate days along with weekly pond fertilization (Treatment 2) gives the best return on investment (Table 4). Feed is the most costly aspect of fish farming, representing the majority of the total production costs for tilapia. Here we show it constitutes almost 80% of total variable expenditures. This is noteworthy as both Treatments 1 and 5, which were fed daily, had the highest expenditures in our study while Treatment 4 with only pond fertilization had the lowest cost input. Net return values were highest for Treatment 2, followed by Treatment 3 (Figure 2). Overall, Treatment 2 had the highest benefit:cost ratio (BCR) but was not significantly different from Treatments 3 or 4; all three of these treatments had significantly higher BCRs than Treatments 1 and 5.

Although the BCR for Treatment 2 was not significantly different from that of Treatments 3 and 4, the fish showed much better growth and total production was highest. Based on these results we conclude that feeding tilapia on alternate days in fertilized ponds provides for the best return on investment and has little impact on growth of fish compared with fish fed daily. Feed efficiency is improved by 100% with no loss in production yield.

Small-scale tilapia farmers in Bangladesh can see marked reduction in production costs due to decreased feed costs by following the alternate-day feeding strategies. These findings support previous CRSP work done in the Philippines (Bolivar et al. 2010, Borski et al. 2011) and suggests that alternate day feeding is likely a cost-effective strategy that can be used for semi-intensive culture throughout the world.

Study 2 — Assessment of tilapia nutrient uptake efficiency in response to alternate feed strategies. Pulsatile feeding strategies can achieve equivalent production yields with less feed, however the mechanisms are poorly understood. This investigation assessed if differences in feed efficiency observed with feeding strategies might be assoicated with altered expression of nutrient transporters in the gastrointestinal tract of Nile tilapia. Absorption of nutrients from feed is facilitated through membrane bound transporters located within enterocytes of the gastrointestinal lumen (Broer 2008, Titus 1991). We evaluated how alternate-feeding strategies may change the gene expression of key solute-linked nutrient carriers, whose abundance may impact nutrient absorption efficiency (Broer 2008). The mRNA expression of six intestinal nutrient transporters was measured via real-time PCR in the intestine of fish from the growth trial. The nutrient transporters evaluated were:
- Facilitated glucose/fructose transporter (*slc2a5*);
- Facilitated glucose transporter (*slc2a6*);
- Long-chain fatty acid transporter (*slc27a4*);
- Na+-amino acid transporter 2 (*slc38a2*);
- Na+-amino acid transporter 4 (*slc38a4*); and
- Large neutral amino acid transporter subunit 3 (*slc43a1*).

The efficiency of all real-time PCR reactions were determined to be between 90%−110% and the standard curve correlations were greater than 0.97 for all assays performed. Our resulting gene expression values were normalized to 18S gene expression (no significant difference between treatments was observed in the 18S gene).

Results indicate no significant difference in gene expression of the six nutrient transporters analyzed between the treatments with both feeding and pond fertilization (Treatments 1, 2, and 3) regardless of the frequency of food applied (Figure 4). There was, however, a significant difference in gene expression between Treatments 4 and 5, fertilization only and daily feeding only, respectively, for the transporters *slc2a5*, *slc2a6*, *slc27a4*, and *slc38a2*. For these transporters, gene expression was significantly higher in fish from Treatment 4 than Treatment 5. This could signify that the fish in Treatment 4 that were not fed commercial diet are not getting the nutrients necessary to maintain growth and healthy nutrition from natural flora and fauna boosted by fertilization application alone, while the commercial diet provided the necessary nutrients for these purposes. Indeed, the upregulation of gene expression for nutrient transporters may be induced by a lack of certain nutrients present. This upregulation may have the effect of preparing the intestinal cells for rapid uptake of nutrients once they become present (Diamond and Karasov 1987). Nutrient transporters in tilapia that are grown in ponds that are fertilized, therefore, may be upregulated due to lack of nutrients that would otherwise be available with commercial diets. Interestingly, the nutrient transporters were most downregulated with fish that

were raised in unfertilized ponds on a pelleted diet, suggesting they had adequate nutrition. Fish grown in ponds that are both fed and fertilized had an intermediate expression of certain solute transporters suggesting they may be more efficient at nutrient uptake and utilization, particularly when commercial feeds are only periodically available.

Gene expression of *slc38a2* increases in response to an absence of amino acids. This increase leads to the translation of the gene to make the SNAT2 protein which has been shown to the recovery of cell volume following amino acid starvation or hypertonic stress (Franchi-Gazzola et al 2004, Franchi-Gazzola et al 2006, Gaccioli et al 2006, Nardi et al 2015). In addition, amino acid transporters may serve as sensors of nutrient supplies to regulate expression of the transporters and thus nutrient uptake by the cells (Taylor 2014). The results show that gene expression of this gene is upregulated in Treatment 4 with only pond fertilization and lower in fed treatments regardless of fertilization. This signifies that natural biota increased through fertilization of ponds may lack the full complement of amino acids necessary for the intestine to maintain cellular osmolality. In this case again the *slc38a2* would be upregulated to improve the efficiency of absorbing amino acids under limiting conditions.

The results show tilapia subsisting on endemic flora and fauna alone (fertilization only group) have increased gene expression of nutrient transporters. This may signify their intestine is conditioned for enhanced uptake in a nutrient limiting environment. By providing a diet of fish feed along with fertilization, which leads to an intermediate nutrient transporter gene expression, intestinal cells may be more efficient at nutrient uptake and utilization.

Study 3 — Characterize changes in gut microbial communities in response to pulse-feeding strategies for identifying microbes that may be associated with increased nutrient availability and production efficiency in tilapia. This investigation assessed how the gut microbial flora is altered by feeding/fertilization strategies that could potentially identify microbes beneficial to tilapia growth and health. The establishment of beneficial gut flora to increase nutrient absorption is an emerging research focus in human biology and aquaculture science (Welker and Lim 2011), and may serve to augment existing practices of sustainable feeding and reduction in environmental footprint.

A total of 715,725 reads (330,883 reads for 16S, 384,842 reads for 18S) were obtained following sequencing of the V3 to V5 regions of 16S rRNA (prokaryote) and the V9 region of 18S rRNA (eukaryote) after quality filtering of the reads.

Prokaryotic assessment. A total of 20 prokaryotic phyla, 43 classes, 92 orders, and 145 families were associated with the fecal material of tilapia used in this study (100% bacteria, no archea). The dominant phyla identified from these samples belonged to Fusobacteria (80.4%), Firmicutes (13.8%), Cyanobacteria (2.4%), Bacteroidetes (1.3%), and Proteobacteria (1.3%) (Figure 5a). The proportions of the identified microbes varied between treatments with an increase in the proportion of Fusobacteria (62.6% to 84.6%) and a decreasing proportion of Firmicutes (32.1% to 11.5%) with decreasing rates of feed along with fertilization (treatments 1 to 3). The fish fed a commercial diet only with no fertilization (treatment 5) had the highest proportion of Fusobacteria (90.8%) and lowest proportion of Firmicutes (6.5%) of all treatments. An increase in the relative abundance of Firmicutes is indicative of obesity in mammals and positively correlated with caloric intake in bony fishes (Jumpertz et al. 2011, Ley et al. 2006, Turnbaugh et al. 2009). The fact that we also saw a higher proportion of Firmicute bacteria in tilapia from treatments fed daily in fertilized ponds indicates that there may be an abundance of high-calorie food resources available to these fish.

Bacteria from the classes Fusobacteriaceae and Clostridiaceae were most abundant in the fecal material of Nile tilapia used in our study. These groups include many pathogenic strains that that they may cause disease with immunosuppression or injury to the gut epithelium (Olsen 2014, Stackebrandt 2014). *Cetobacterium somerae* (a Fusobacteriaceae) had the highest number of reads or abundance in our study with no apparent trend in regulation by feeding or fertilization (Figure 5b). This species has been isolated from the intestinal tract of cultured freshwater fish (Tsuchiya et al. 2008).

Alpha diversity measures are indices of the diversity within a community. The Chao 1 index is commonly used to estimate the total number of species within a community and is based on the number of rare OTUs in that community (Chao 1984). Alpha diversity and rarefaction curves were determined for all treatments evaluated. Treatment 1 had the highest Chao 1 diversity index of all treatments. The bacterial diversity in the fecal material in tilapia guts decreased with increasing times between feeding when paired with pond fertilization (Figure 6a). Although there was no significant difference in species richness between treatments due to sample variation within treatments (P = 0.2555), the trend was for those treatments given a greater variety (feed and fertilization) to have higher diversity. The Chao 1 index was highest for treatments 1 and 2 (994.6 ± 162.1 SEM and 942.3 ± 98.9 SEM, respectively). Treatments 3 (668.0 ± 136.6 SEM), 4 (685.4 ± 176.2 SEM), and 5 (692.9 ± 62.4 SEM) had similar diversity indices.

Beta diversity, as illustrated by Principle Coordinates Analyses (PCoA) plots, is a measure of the microbial diversity between treatments. Our results indicate that there are some community differences between the treatments in our study (Figure 7). Treatments 1 and 5 had the most similar profiles while treatment 4 was the most dissimilar.

There were 41 and 40 bacterial OTUs identified in treatment 1 that were not identified in treatments 4 (Table 5) and 5 (Table 6), respectively. In addition, there were 37 and 53 OTUs identified that were unique to Treatment 2 versus Treatments 4 (Table 7) and 5 (Table 8). As described in Study 1, Treatment 2, feeding on alternate days with pond fertilization, had the best benefit to cost ratio of all treatments. It can be assumed that those OTUs identified in Treatment 2 that were not identified in Treatment 4 (pond fertilization only) were due to the feed given to Treatment 2 and those OTUs identified in Treatment 2 that were not identified in Treatment 5 (feed only) were due to fertilization. Of the differences between Treatment 2 and Treatments 4 and 5 the bacterial strains *Methlyobacterium* sp. and *Methylobacterium hispanicum* were present in Treatment 2 but not Treatments 4 and 5. *Methylobacterium* sp. are found in a wide variety of environments including freshwater sources and can be associated with terrestrial and aquatic plants (Austin et al.1978, Yoshimura 1982, Corpe and Rheem 1989, Trotsenko et al. 2001, Lidstrom and Chistoserdova 2002). Other bacteria identified include common gut colonizers in many vertebrates, many of which can become pathogenic if there is an increase of that bacterial strain due to the animal having a depressed immune system. The identified unique species and strains of bacteria found in Treatments 1 and 2 (food from formulated diet and pond microbiota boosted by fertilization) versus Treatments 4 and 5 (fertilization only and fed only groups), may be promising candidates for isolation that may be beneficial to increased growth and feed efficiency and reduced mortality in tilapia. We are currently conducting deeper analyses to determine the function of these bacteria.

Eukaryotic assessment. A total of eight major eukaryotic groups and 132 genera were associated with the fecal material of tilapia used in this study. Treatment 2 (fed alternate days with pond fertilization) had the greatest number of reads for all groups (58,450) followed by Treatments 4 (fertilization only, 49,112), 1 (feed every day with fertilization, 38,378), 5 (feed every day only, 31,758), and 3 (feed every third day with fertilization, 25,752). The dominant groups identified

from these samples belonged to the Opisthokonta (Metazoans and Fungi) (34.8%), Archaeplastida (green plants and red algae) (34.2%), and SAR supergroup (Stramenopiles, Alveolates, and Rhizaria; Dinoflagellates, Diatoms, Oomycetes, etc.) (30.7%) (Figure 8a). There were no significant differences in abundance of particular eukaryotic groups among the treatments.

Class Chlorophyceae (green algae), Class Mediophycae (diatoms), Phylum Rotifera, and group Magnoliophyta (flowering plants) made up the highest proportion of identified eukaryotic organisms (Figure 8b). Treatments 1 and 3 had the highest proportions of rotifers (31% and 35%, respectively). The largest proportion of Magnoliophyta were in Treatments 2 (19%) and 3 (20%) and Chlorophycae were in Treatments 1 (27%) and 4 (24%), while Treatment 5 had the highest proportion of Mediophycae (21%) of any other treatments. However, there were no significant differences in abundance of eukaryotes among the treatment groups.

The most abundant identified groups overall include the rotifers (50,705 counts), green algae (34,755 counts), and flowering plants (19,951 counts). We used blocking primers to eliminate amplification of tilapia 18S that might come from the intestine and that would reduce our capacity to detect low abundant reads of other eukaryotes of interest in this studies. Previous studies have shown that without the use of blocking primers in the amplification step of the 18S DNA library preparation from eukaryotic sources, the source DNA overwhelms the amplification of lower abundant DNA present in the samples making analysis of overall eukaryotes impossible (Levine and Salger, not published). Teleost 18S made up a low proportion of identified OTUs (11%), in our samples, indicating the success of the blocking primers. The teleost 18S that was detected aligned with Atlantic Salmon (*Salmo salar*). Whether this was derived from the fishmeal in the diet fed to tilapia is uncertain.

Alpha and beta diversity measures were also determined for the 18S amplicons. Similar to the prokaryotic analyses there was no significant difference in the Chao 1 index between treatments ($p = 0.1298$). Although this was true, treatment 2 had the highest diversity (677.5 ± 93.8 SEM), followed by treatment 1 (593.7 ± 112.1 SEM), treatment 5 (483.7 ± 96.1 SEM), and treatment 4 (444.1 ± 88.0 SEM). Treatment 3 had the lowest diversity measure at 363.2 ± 43.3 SEM (Figure 6b). There was significant overlap in the PCoA plots between treatments. Treatments 2 and 4 were most different from all treatments showing that the eukaryotic communities of these two treatments were different from the other treatments (Figure 9).

CONCLUSION

It is estimated that 60-80% of total variable costs for growing tilapia is attributable to formulated feeds. This study demonstrates that alternate-day feeding reduces the costs of feeds for growout of tilapia by 50%, increases feed efficiency by almost 140%, and has little impact on growth, survival or yield of fish farmed in Bangladesh. These findings can be readily adopted by small-scale farmers in Bangladesh for enhancing growout of tilapia. Insofar as our work supports that of previous Collaborative Research Support Program research in the Philippines, the study suggests that the alternate-day feeding strategy can be practically applied anywhere in the world for improving the livelihood of tilapia farmers.

Our work also suggests that:
- Tilapia grown in fertilized ponds without supplemental feeds may be nutritionally impaired as key nutrient transporters in the gut are enhanced in preparation for increased uptake of solutes should food become available, a process that is mitigated when animals are provided supplemental feeds alone; and

- The intermediate expression of gut nutrient transporters in alternate-day fed tilapia may reflect a condition for most efficient uptake of nutrients from the gastrointestinal tract.

Finally, our work shows that fish on the alternate-day feeding strategy have the greatest diversity of intestinal microbiota that may function in promoting growth and nutrient assimilation in these fishes. The presence of unique species and strains of bacteria in tilapia on supplemental feeds may serve as promising candidates for isolation and development of probiotics beneficial to increase growth, feed efficiency and health in tilapia.

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Table 1. Experimental design of this study

Treatment / Factors	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Stocking Density	5 fish/ m^2	5 fish/ m^2	5 fish/ m^2	5 fish/ m^2	5 fish/ m^2
Feeding Strategy	daily feeding	feeding every other day	feeding every 3rd day	no feeding	daily feeding
Pond Fertilization	4:1 (N:P), weekly	4:1 (N:P), weekly	4:1 (N:P), weekly	4:1 (N:P), weekly	no fertilization
Replicates (n)	4	4	4	4	4

Table 2. Water quality parameters (mean ± SD) for all treatments. Values with different letters are significantly different ($P < 0.05$).

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Temperature (°C)	30.11±0.01	30.15±0.02	30.17±0.10	30.10±0.15	30.07±0.08
Transparency (cm)	16.31±2.13	14.49±0.88	20.51±7.78	17.92±6.76	15.18±1.28
TDS (mg/l)	119.34±11.13	113.97±8.43	95.42±7.39	106.23±8.56	103.90±12.06
Alkalinity (mg/l)	127.70±7.44[a]	128.95±9.61[a]	107.00±6.37[b]	119.55±4.10[ab]	116.55±14.72[ab]
pH	7.72±0.06[b]	7.84±0.04[ab]	7.75±0.10[b]	7.95±0.11[a]	7.82±0.11[ab]
Dissolved Oxygen (mg/l)	5.64±0.30[b]	5.96±0.13[ab]	6.10±0.17[a]	6.15±0.27[a]	5.60±0.28[b]
Nitrate (mg/l)	0.34±0.07[b]	0.31±0.01[b]	0.33±0.07[b]	0.25±0.05[b]	0.17±0.04[a]
Nitrite (mg/l)	0.13±0.03[ab]	0.13±0.04[ab]	0.14±0.05[ab]	0.16±0.05[a]	0.08±0.05[b]
Ammonia (mg/l)	0.83±0.36[a]	0.52±0.19[ab]	0.48±0.30[ab]	0.38±0.12[b]	0.48±0.12[ab]
Phosphate (mg/l)	1.12±0.18[b]	1.14±0.11[b]	1.11±0.23[b]	1.51±0.06[a]	0.82±0.19[c]
Chlorophyll *a* (µg/l)	123.74±22.24	116.20±36.21	109.62±62.39	91.49±28.28	129.40±21.42

Table 3. Growth and production performances of tilapia (*O. niloticus*). Values are mean±SD.
Values with different letters are significantly different ($P < 0.05$).

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Initial Weight (g)	3.55±0.90[a]	3.55±0.90[a]	3.55±0.90[a]	3.55±0.90[a]	3.55±0.90[a]
Final Weight (g)	127.63±2.75[a]	120.17±5.44[a]	85.10±11.13[b]	43.15±4.28[c]	129.53±8.59[a]
Weight Gain (g)	124.08±2.75[a]	116.62±5.44[a]	81.55±11.13[b]	39.60±4.28[c]	125.98±8.59[a]
SGR (%/day)	3.14±0.02[a]	3.09±0.04[a]	2.78±0.12[b]	2.19±0.09[c]	3.15±0.06[a]
FCR	1.64±0.10[c]	0.93±0.09[b]	0.68±0.15[a]	--	1.61±0.10[c]
Survival Rate (%)	93.44±6.26[a]	91.66±8.00[a]	90.70±9.74[ab]	76.79±2.68[b]	97.71±2.11[a]
Production (kg/pond)	92.98±10.29[ab]	86.40±9.33[b]	58.93±7.17[c]	27.50±4.57[d]	102.63±10.66[a]
Net Production (kg/ha)	6091.42±354.52[a]	5658.26±527.83[a]	4086.39±640.07[b]	1796.04±233.89[c]	6392.81±461.41[a]
Total Production (kg/ha)	6282.09±354.52[a]	5837.84±527.83[a]	4179.08±640.07[b]	1950.35±233.89[c]	6578.53±461.41[a]

Table 4. Economic analysis of tilapia (*O. niloticus*) production among five treatments. Leasing cost for pond is not included. Values are mean±SD. Values with different letters are significantly different ($P < 0.05$).

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Expenditure (Tk/pond)					
Fingerlings cost	925.00±50.00	925.00±50.00	881.25±177.22	881.25±177.22	975.00±50.00
Feed cost	8,783.57±555.26[b]	4,613.61±166.49[c]	2,303.69±588.95[d]	--	9,567.44±631.31[a]
Lime Cost	44.40±2.40	44.40±2.40	42.30±8.51	42.30±8.51	46.80±2.40
Fertilizers cost	354.76±19.18	378.41±20.45	360.51±72.50	360.51±72.50	--
Operational cost*	758.08±46.15[a]	447.11±17.42[b]	269.08±63.36[c]	96.30±19.37[d]	794.19±50.48[a]
Total Expenditure	10,865.81±661.50[a]	6,408.52±249.71[b]	3,856.83±908.16[c]	1,380.36±277.59[d]	11,383.44±723.59[a]
Income					
Gross return (Tk/pond)	11,157.00±1,234.60[ab]	10,368.00±1,120.01[b]	5,892.50±716.82[c]	1,925.00±319.76[d]	12,315.00±1,279.39[a]
Net return (Tk/pond)	291.19±691.31[c]	3,959.48±979.04[a]	2,035.67±629.63[b]	544.64±197.83[c]	931.56±763.10[c]
Gross return (Tk/ha)	752,270.83±42,542.75[ab]	700,291.67±63,339.67[b]	426,388.89±64,007.44[c]	138,147.92±16,372.06[d]	788,437.50±55,369.36[a]
Net return (Tk/ha)	18,041.18±43,978.87[c]	266,987.40±62763.26[a]	154,640.31±77,869.53[b]	40,249.82±16,372.06[c]	58,739.54±47,129.35[c]
BCR (Benefit Cost Ratio)	1.03±0.06[b]	1.62±0.14[a]	1.58±0.33[a]	1.41±0.17[a]	1.08±0.07[b]

*Operational cost is considered as 7.5% of total cost (ADCP, 1983).

Table 5. Prokaryotes (16S rRNA OTUs) identified in Treatment 1 but not Treatment 4.

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Rathayibacter;s__caricis
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Actinoplanes;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium;s__
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__[Amoebophilaceae];g__Candidatus Cardinium;s__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium;s__
k__Bacteria;p__Chloroflexi;c__SHA-26;o__;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__Chlamydomonadaceae;g__Chlamydomonas;s__reinhardtii
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__Trebouxiophyceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__Cylindrospermopsis;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;Other;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Oscillatoriales;f__Phormidiaceae;g__Microcoleus;s__
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;Other;Other;Other
k__Bacteria;p__Fibrobacteres;c__TG3;o__TG3-1;f__TSCOR003-O20;g__;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__02d06;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__intestinale
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Clostridium;s__ruminantium
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__;s__
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;Other;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__hispanicum

Table 5. Continued

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Pleomorphomonas;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Anaerospora;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Azospirillum;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Plesiomonas;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Plesiomonas;s__shigelloides
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;Other;Other
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;g__Legionella;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas;s__
k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__;g__;s__

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Table 6. Prokaryotes (16S rRNA OTUs) identified in Treatment 1 but not Treatment 5.

k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111;g__;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Actinoplanes;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium;Other
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__[Amoebophilaceae];g__Candidatus Cardinium;s__
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium;s__
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__;g__;s__
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__WCHB1-50;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__Chlamydomonadaceae;g__Chlamydomonas;s__reinhardtii
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__Cylindrospermopsis;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;Other;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;f__Cyanobacteriaceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;f__Gomphosphaeriaceae;Other;Other
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;f__Pseudanabaenaceae;g__Leptolyngbya;s__
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Synechococcales;f__Synechococcaceae;g__Paulinella;s__
k__Bacteria;p__Fibrobacteres;c__TG3;o__TG3-1;f__TSCOR003-O20;g__;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__flexus
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__02d06;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__intestinale
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;g__Clostridium;s__ruminantium
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__u114;s__
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__[Thermodesulfovibrionaceae];g__HB118;s__
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__;s__

Table 6. Continued

k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__hispanicum
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Pleomorphomonas;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rhodobacter;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Azospirillum;s__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other;Other
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__;f__;g__;s__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae;Other;Other
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas;s__

Table 7. Prokaryotes (16S rRNA OTUs) identified in Treatment 2 but not Treatment 4.

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Actinoplanes;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__;s__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;Other;Other
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Blvii28;s__
k__Bacteria;p__Cyanobacteria;c__;o__;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__Chlamydomonadaceae;g__Acutodesmus;s__obliquus
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Chlorophyta;f__Trebouxiophyceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__Cylindrospermopsis;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;Other;Other;Other;Other
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__02d06;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__intestinale
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__Anaerovorax;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__hispanicum
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Pleomorphomonas;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Anaerospora;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Wolbachia;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae;g__Desulfococcus;s__
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__;s__

Table 7. Continued

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Plesiomonas;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Plesiomonas;s__shigelloides
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Rickettsiella;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Grimontia;s__hollisae
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium;Other
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Photobacterium;s__rosenbergii
k__Bacteria;p__TM7;c__TM7-3;o__EW055;f__;g__;s__

Table 8. Prokaryotes (16S rRNA OTUs) identified in Treatment 2 but not Treatment 5.

k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other;Other;Other
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;g__;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Actinoplanes;s__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__;s__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;Other;Other
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;Other;Other
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Porphyromonas;s__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Blvii28;s__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1;g__;s__
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__VC21_Bac22;g__;s__
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__;g__;s__
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Rhabdochlamydiaceae;g__Candidatus Rhabdochlamydia;s__
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__;f__;g__;s__
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__A4b;g__;s__
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__WCHB1-50;f__;g__;s__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Nostocophycideae;o__Nostocales;f__Nostocaceae;g__Cylindrospermopsis;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;Other;Other;Other;Other
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;f__Cyanobacteriaceae;g__Cyanobacterium;s__
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae;o__Chroococcales;f__Gomphosphaeriaceae;Other;Other
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;f__Pseudanabaenaceae;g__;s__
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;f__Pseudanabaenaceae;g__Leptolyngbya;s__
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Synechococcales;f__Synechococcaceae;g__Paulinella;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__;g__;s__
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;Other
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__flexus
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__;s__
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__02d06;s__

Table 8. Continued

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__intestinale
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__u114;s__
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__[Thermodesulfovibrionaceae];g__HB118;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__hispanicum
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Pleomorphomonas;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rhodobacter;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Wolbachia;s__
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;Other;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__;g__;s__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__;f__;g__;s__
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter;s__
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__Rickettsiella;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae;g__;s__
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Grimontia;s__hollisae
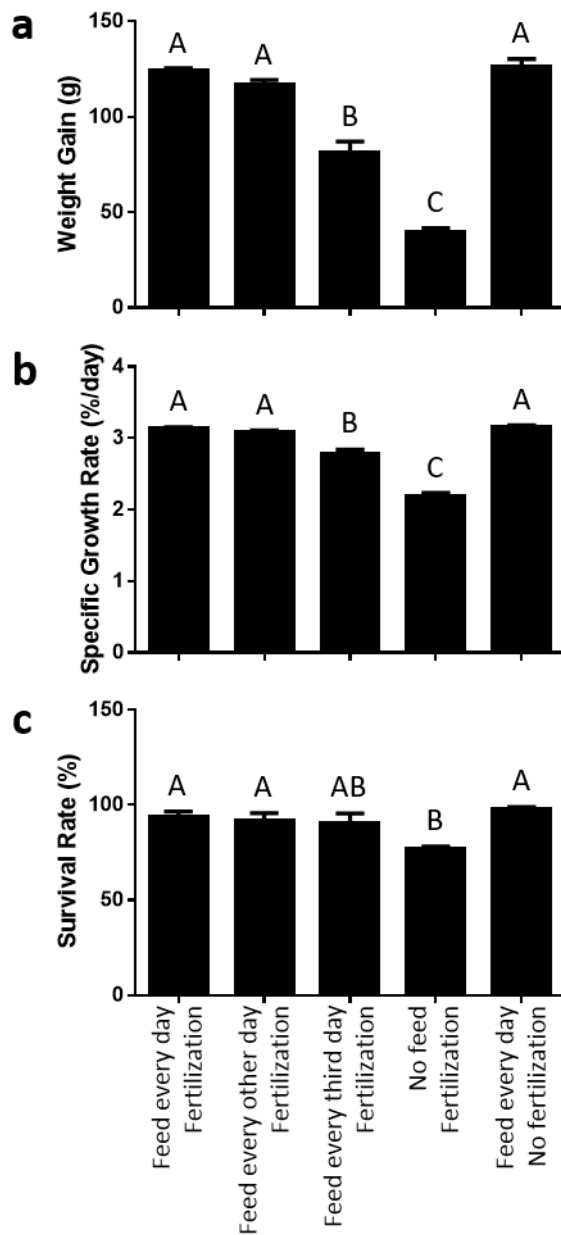
Figure 1. Differences in growth parameters of Nile tilapia in varying pulsed-feeding regimes (mean ± SEM). (a) Weight gain; (b) Specific growth rate (SGR); (c) Survival rate. Treatments with different letters are significantly different ($p < 0.05$).
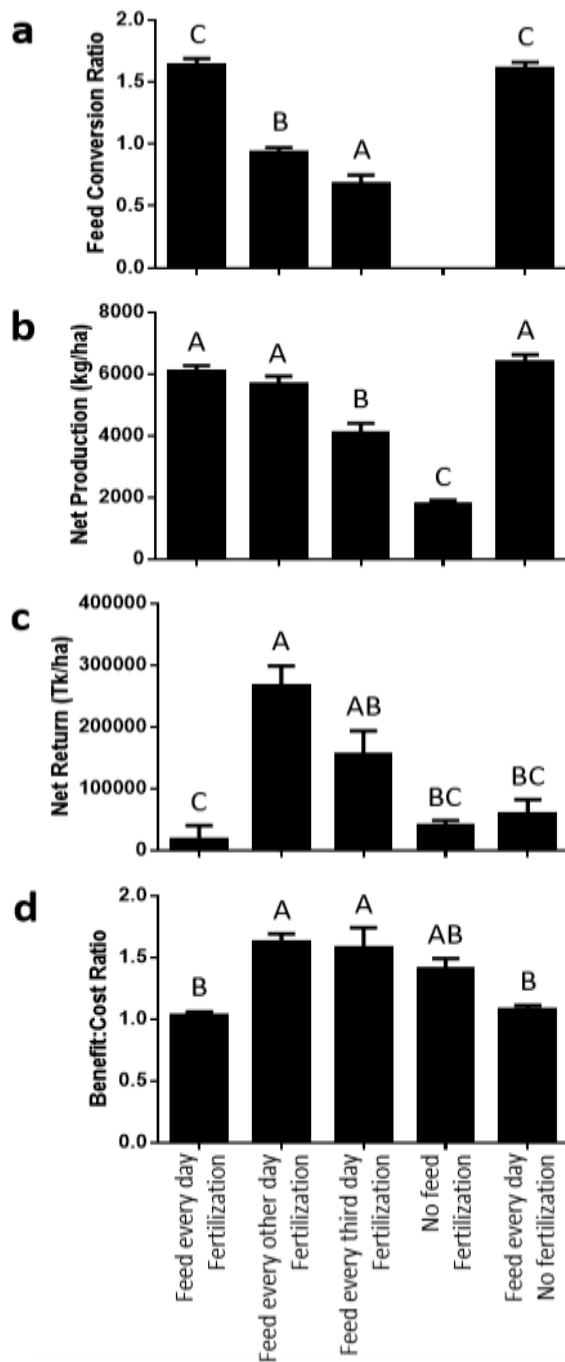
Figure 2. Differences in production parameters of Nile tilapia in varying pulsed-feeding regimes (mean ± SEM). (a) Feed conversion ratio (FCR); (b) Net production; (c) Net return; (d) Benefit to Cost ratio (BCR). Treatments with different letters are significantly different ($p < 0.05$).
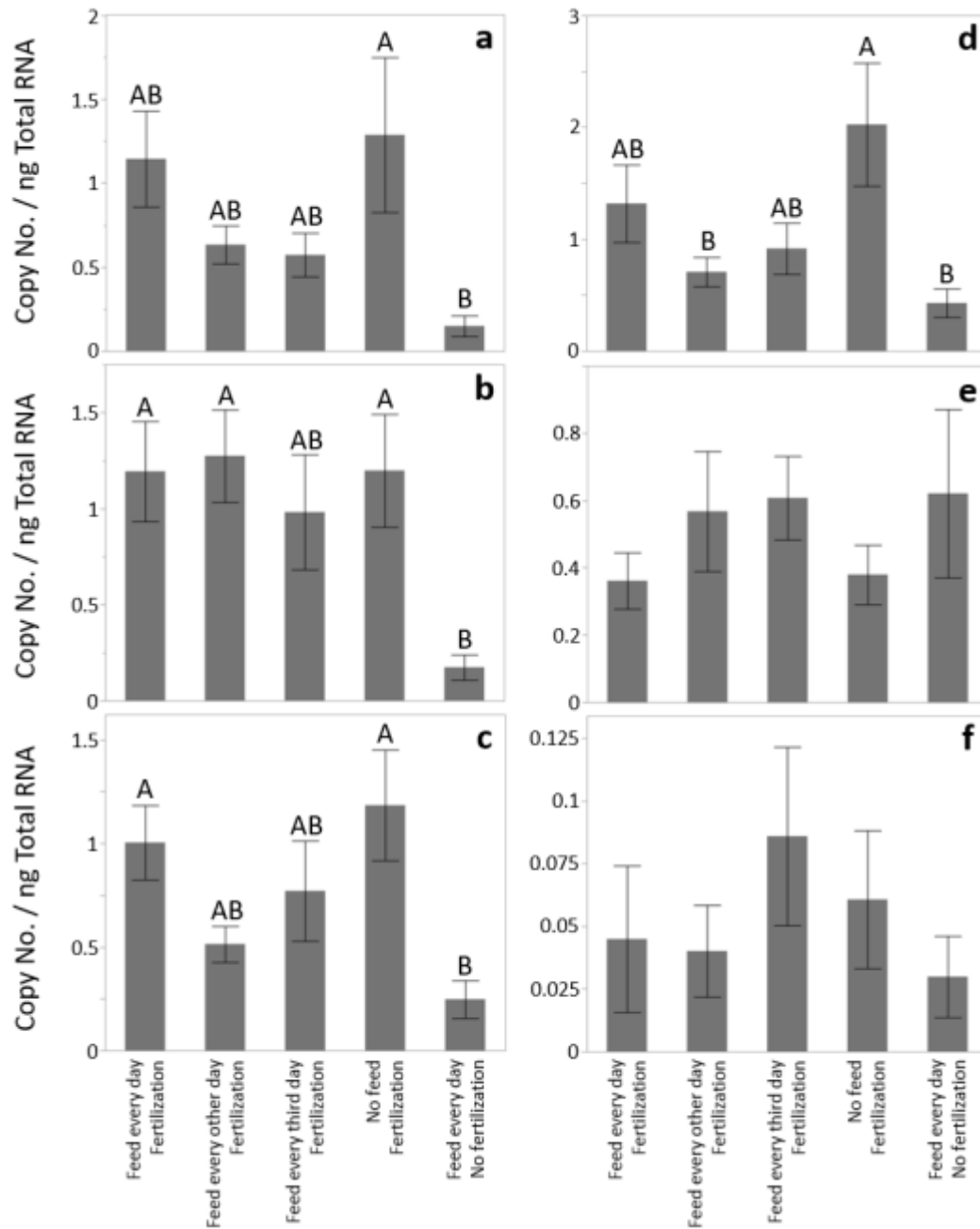
Figure 3. Change in weight (a) and length (b) of Nile tilapia in varying pulsed-feeding regimes throughout the study (mean ± SEM).
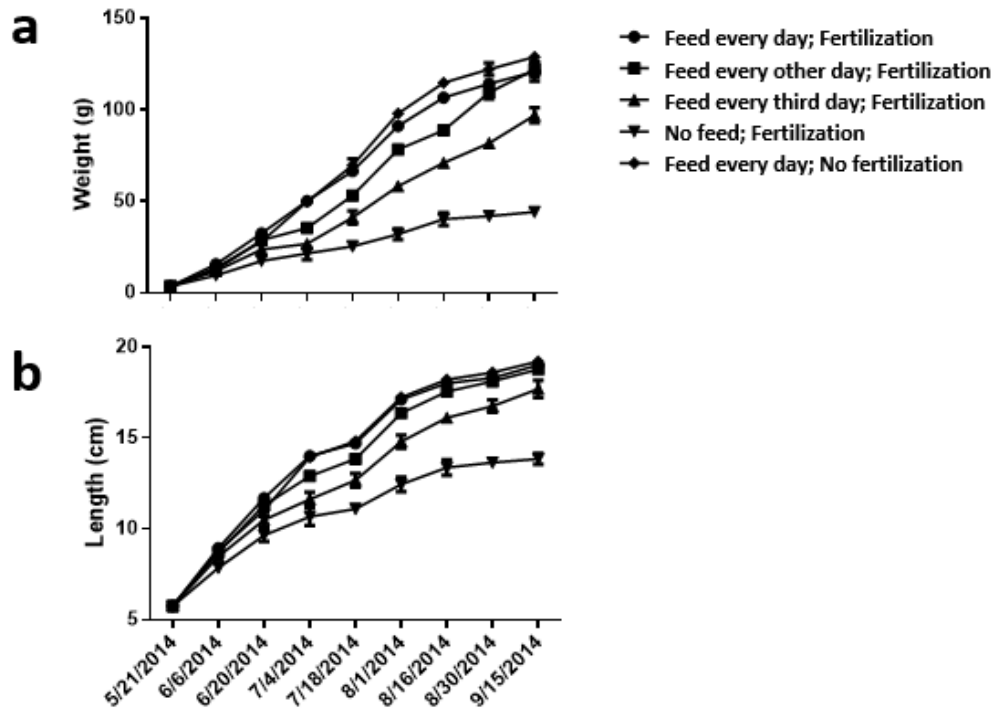
Figure 4. Gene expression of nutrient transporters in the intestine of Nile tilapia subjected to varying pulsed-feeding regimes (mean ± SEM). (a) *SLC2A5*; (b) *SLC2A6*; (c) *SLC27A4*; (d) *SLC38A2*; (e) *SLC38A4*; (f) *SLC43A1*. Treatments with different letters are significantly different ($p < 0.05$).
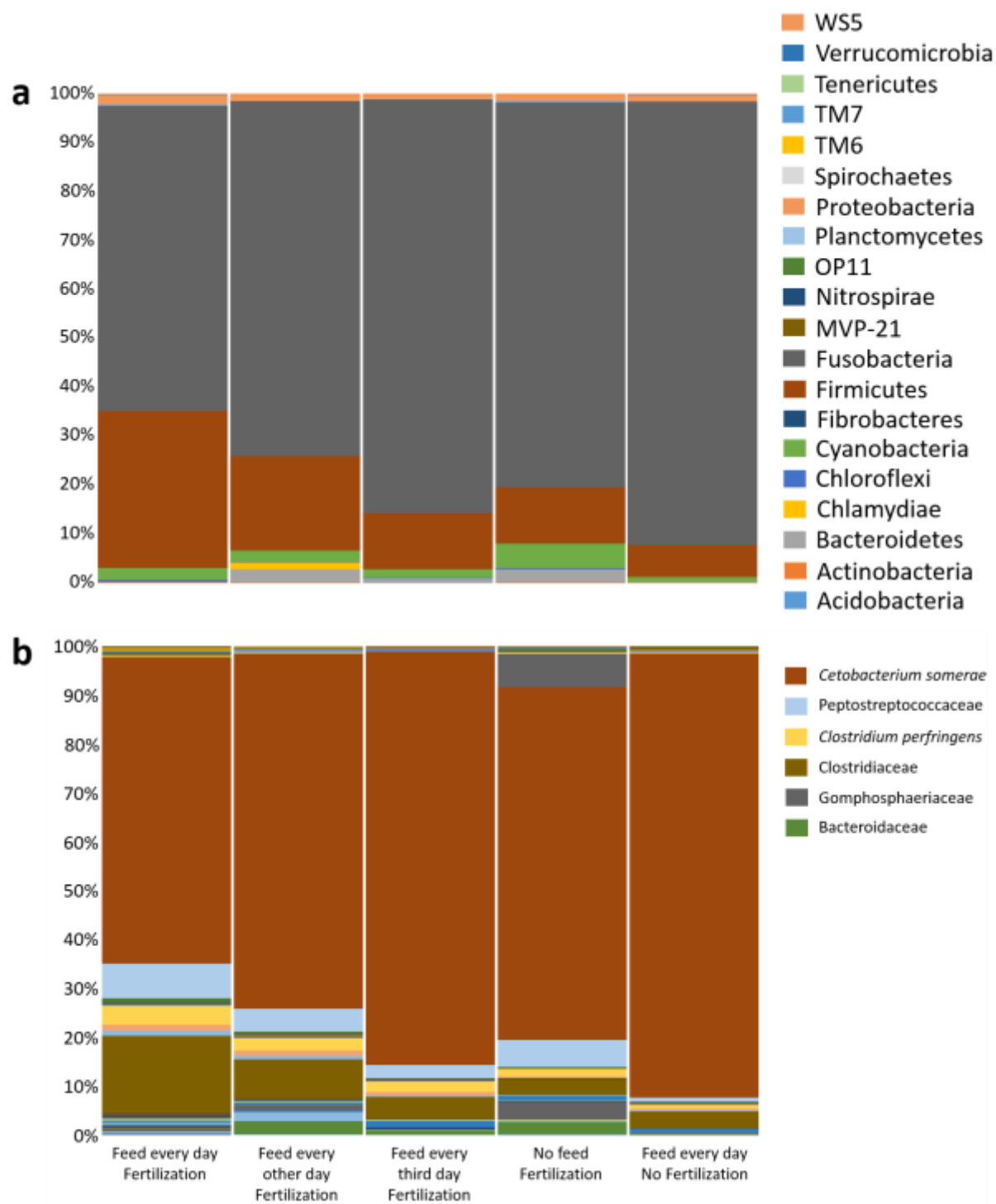
Figure 5. Relative abundance of bacteria in the fecal material of Nile tilapia subjected to varying pulsed-feeding regimes at the level of Phylum (a) and Family/Species (b).
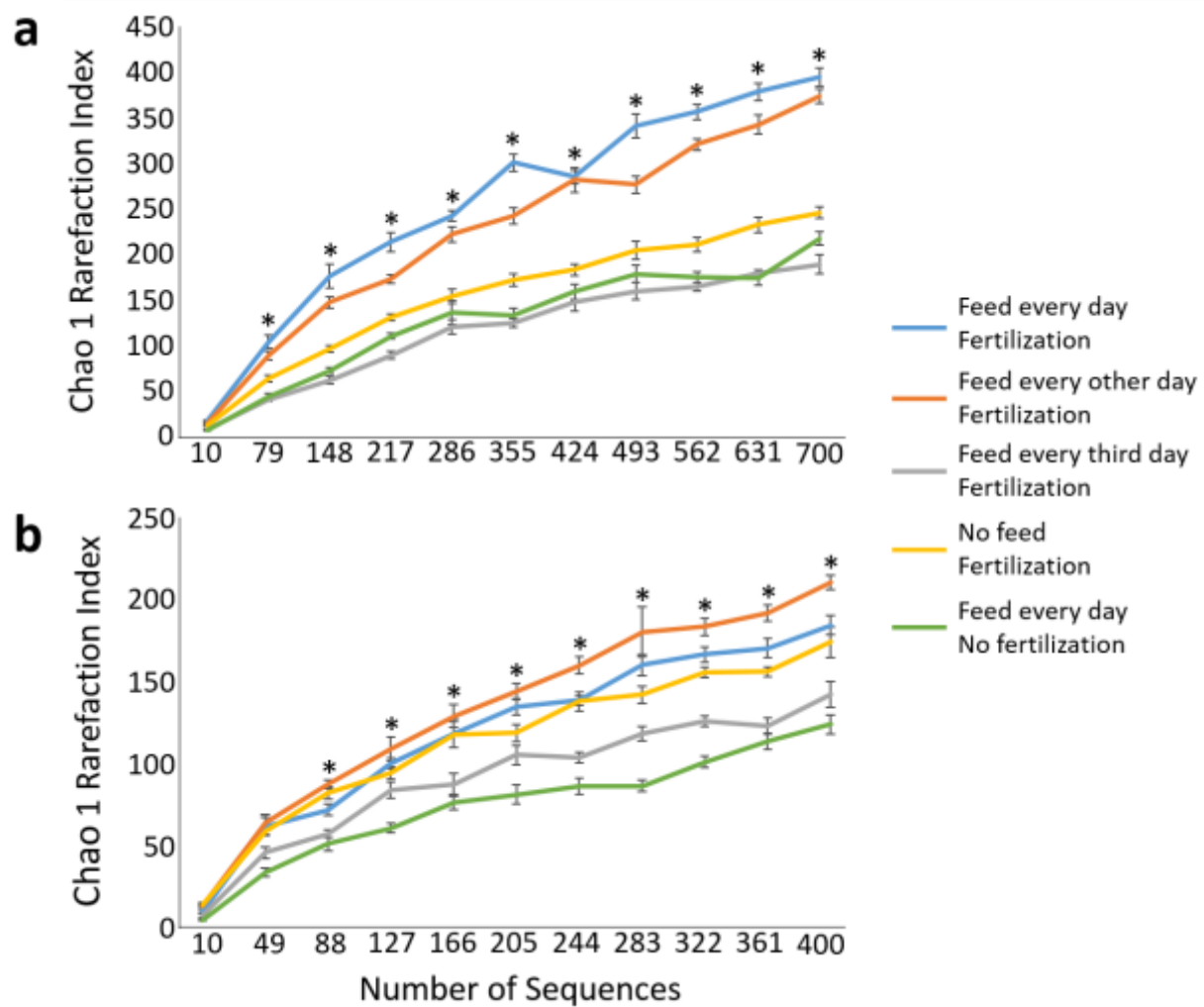
Figure 6. Diversity indices (Chao 1) of microbes found in the gut of Nile tilapia subjected to varying pulsed-feeding regimes (mean ± SEM). (a) 16S rRNA (prokaryote); (b) 18S rRNA (eurkaryotes). Asterisks indicate significant differences between treatments ($p < 0.05$).
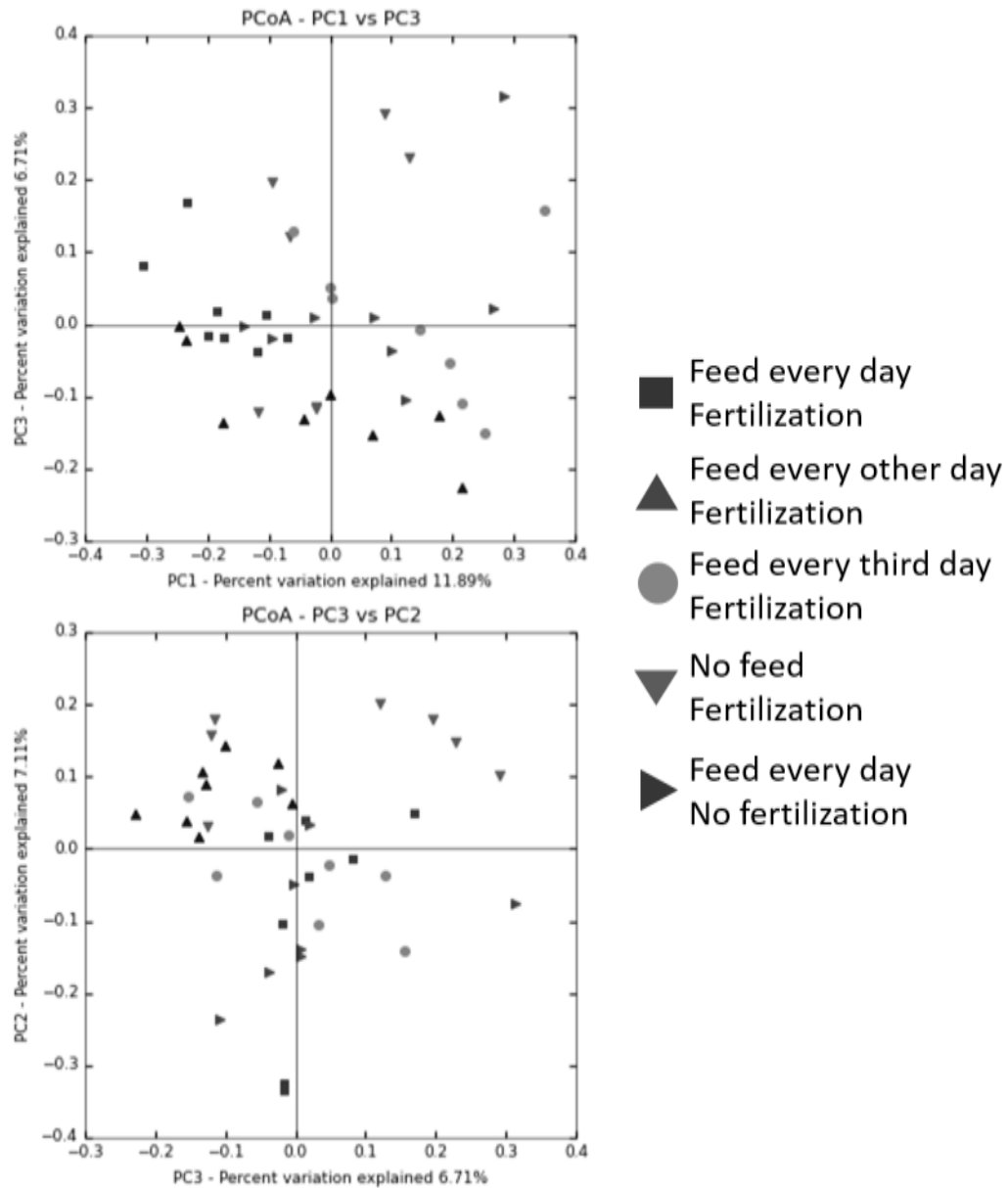
Figure 7. Principal coordinates analysis (PCoA) plots of bacterial communities found in the fecal material from Nile tilapia subjected to different pulsed-feeding regimens. Each replicate sample is represented in the plot.
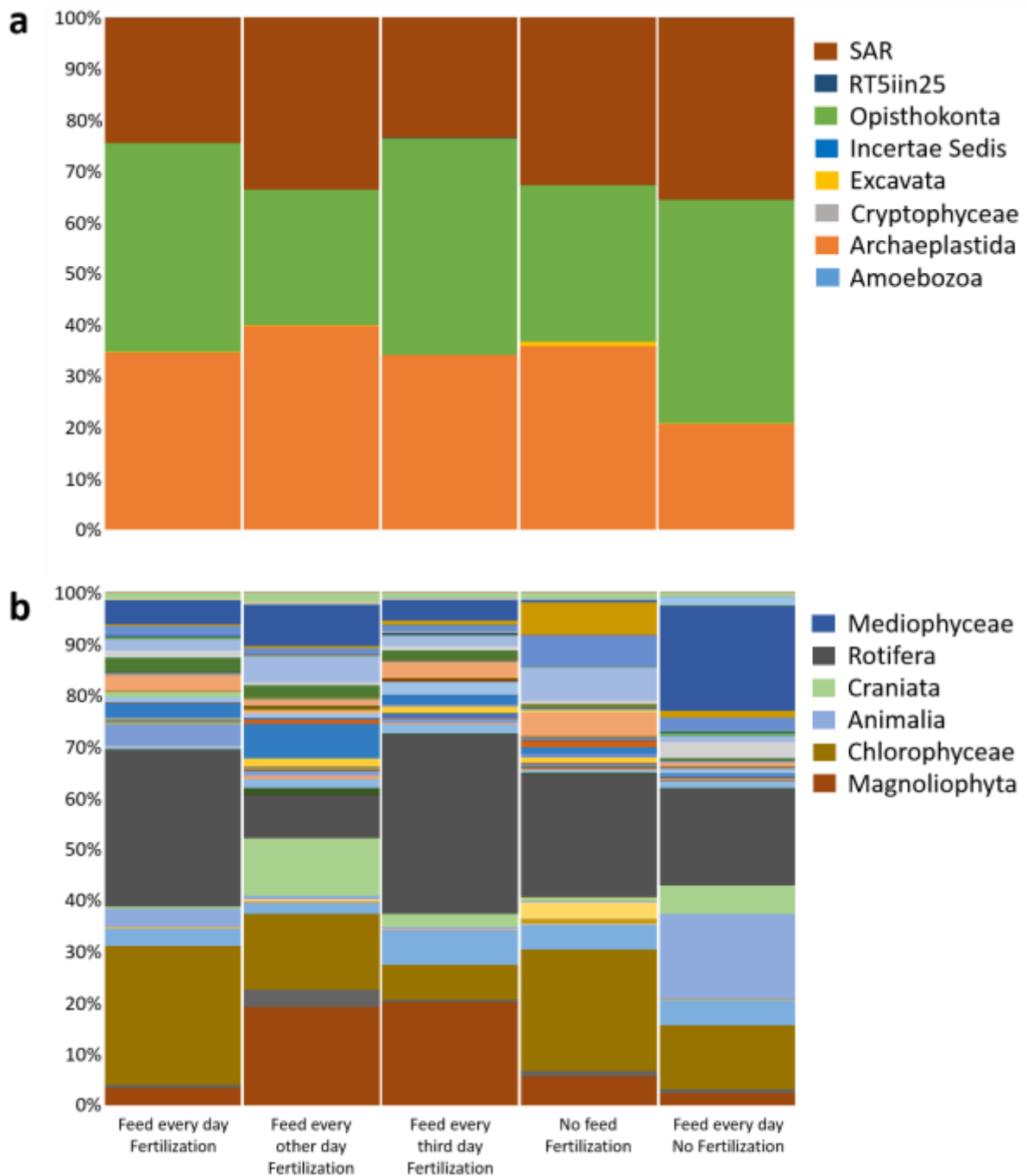
Figure 8. Relative abundance of phyla (a) and higher groups (b) of eukaryotes in the fecal material of Nile tilapia subjected to varying pulsed-feeding regimes.
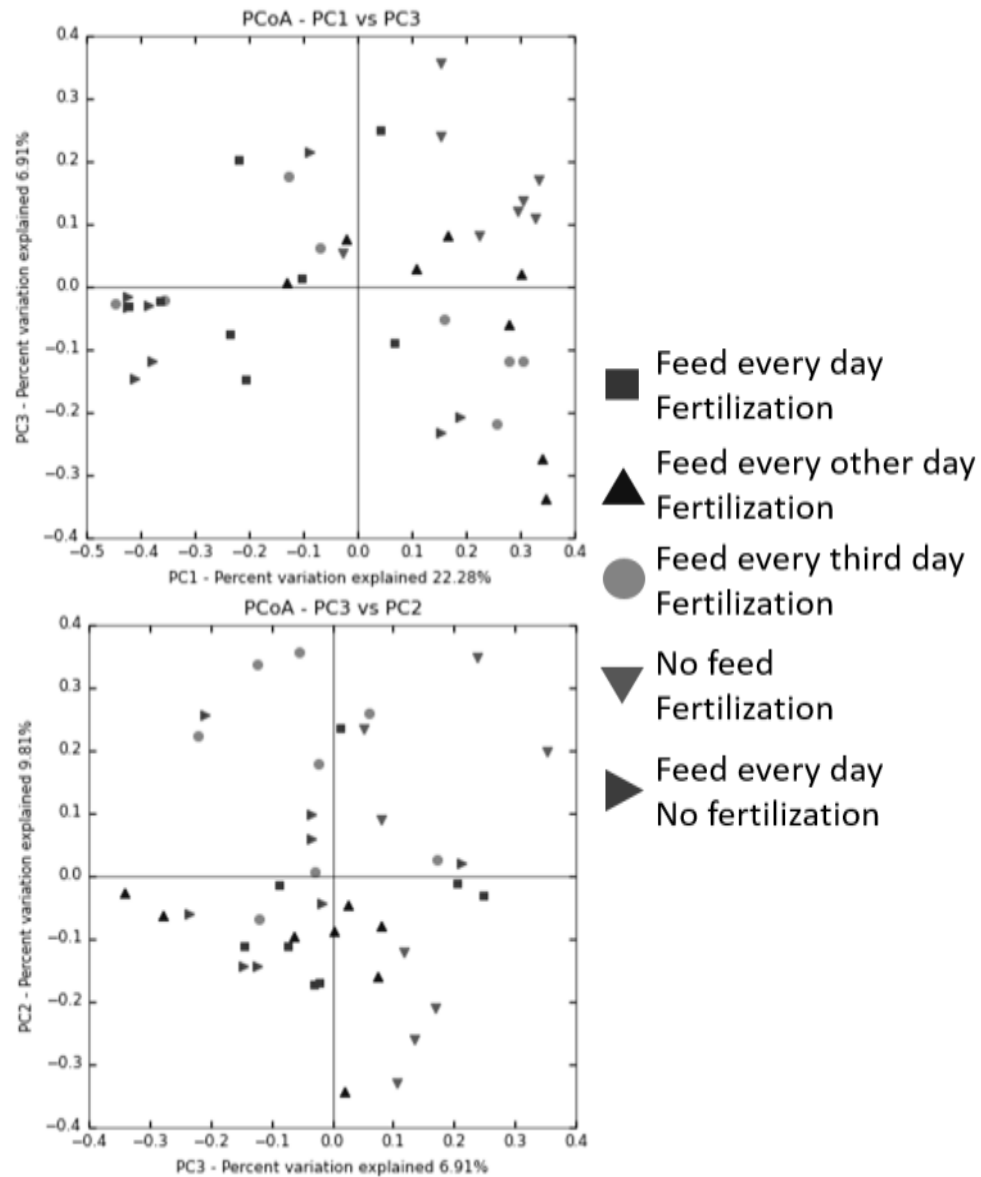
Figure 9. Principal coordinates analysis (PCoA) plots of eukaryotic communities found in the fecal material from Nile tilapia subjected to different pulsed-feeding regimens. Each replicate sample is represented in the plot.

Evaluation of Invertebrates as Protein Sources in Nile Tilapia (*Oreochromis niloticus*) Diets

Sustainable Seed Technology and Nutrient Input Systems/Experiment/13SFT01PU

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ABSTRACT

The study was conducted to approximate the composition of house fly maggots and earthworms from different substrates as well as to evaluate their suitability as protein sources in the Nile tilapia (*Oreochromis niloticus*) diets. Chicken manure, cattle manure and fermented maize were used as substrates for production household fly maggots (HFM), while chicken, cattle and rabbit manures were used as substrates for production of earthworm meal (EWM). HFMs and EWMs with the highest protein content were used to formulate practical isonitrogenous diets (30% crude protein) containing graded levels of HFM and EWM meals (25%, 30%, 35% and 40%). The diets were fed to juveniles with an average weight of 2.6g in a growth trial that lasted for eight weeks. There were significant ($p<0.05$) differences in the crude protein contents between the HFMs as well as EWMs raised on three culturing media. Chicken manure produced HFM with significantly high protein content, while cow manure did the same for EWM. Growth and feed utilization was significantly higher in fish fed diets HFM35 and EWM35. The same diets were more cost effective to produce a unit of fish. Therefore, it is recommended to include either HFM or EWM meals at 35% in practical diets containing 5% fishmeal and cotton seedcake or any similar plant protein.

INTRODUCTION

Globally, aquaculture is increasingly becoming an important source of fish protein as fish supply from capture fisheries dwindles. However, in Tanzania, aquaculture has remained mostly rural, secondary, and part-time activity taking place in small freshwater ponds within small farm holdings. Further growth has been limited by a number of factors including lack of affordable fish feeds. Feeds are expensive due to high cost of protein sources. Fish meal and oil seedcakes have high demand from terrestrial animal feed, making them scarce and unaffordable. Therefore, for aquaculture to grow, there is a need to search for affordable and locally available sources of protein. Among potential sources are invertebrates such as earthworms and housefly maggots, which are often abundantly available locally, palatable and relatively more affordable. They have short life cycle and high fecundity rate within a short period time (FAO 2012).

The study was therefore conducted to evaluate the suitability of earthworms and housefly maggots as protein sources in Nile tilapia (*O. niloticus*) diets. Specifically, the study sought to understand the following:
- Effect of different culture media on yield and proximate composition of housefly maggot and earthworm meals;
- Performance of *O. niloticus* juveniles fed diets containing different grade levels of housefly maggot and earthworm meals as protein sources; and
- Cost effectiveness of diets containing housefly maggot and earthworm meals.

MATERIALS AND METHODS

The study was conducted at the Aquaculture Research Facility belonging to the Department of Animal Science and Production at Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

Effect of substrate on yield and proximate composition of housefly maggot and earthworm meal.
Three substrates (fermented maize grain, cattle manure and poultry manure) in triplicates with abattoir and fish wastes as attractants were evaluated on their suitability to produce household fly maggots (HFM). Plastic buckets with a capacity of 10 L and mosquito nets were used in culturing the maggots indoors as suggested by Nzamujo (2001). The mature maggots were harvested according to Aniebo and Owen (2010). Harvested housefly maggots were blanched in 100^0C hot water and weighed to determine total weight per harvest. After completion of harvesting, the samples were oven dried at 65^0C for 48 hours and ground into a meal.

Figure 1. Culture of HFM.

Figure 2. Harvested HFM blanched in hot water.

For earthworm meal (EWM), three substrates (cattle, rabbit and poultry manures) were evaluated in triplicates. Plastic water basins with a capacity of 12 liters were used in the production of earthworms. Dried rice residues were added as bedding materials. The substrates were inoculated with adult earthworms collected from the wild and placed indoors as suggested by Nzamujo (1999). Each culture media was moistened with 1 liter of water once every week. Harvesting of earthworms was done in batches after every month when the reproduced young got matured. During harvesting, the screening method stated by El Boushy et al. (1985) and Sogbesan et al. (2006) was used, and then sorted, washed, weighed and stored at -5^0C until the required amount was obtained. Thereafter harvested earthworms were weighed, oven dried at 65^0C for 48 hours and then ground into powder.

Figure 3. Culture of earthworms.

Figure 4. Harvested earthworms.

Yields and proximate analysis of HFM and EWM were determined to establish most suitable culture media.

Growth trial to determine growth, feed utilization and cost effectiveness of diets containing of graded levels of housefly maggot and earthworm meals as protein sources in diets of *O. niloticus* juveniles. For each meal, five isonitrogenous (30% crude protein) practical diets with 5% fish meal and graded levels of HFM and EWM were formulated as shown in Tables 1 and 2.

Table 1. Percentage inclusion levels of the ingredients in HFM Based Diets (g/100g diet).

Ingredients	Diets				
	HFM0	HFM25	HFM30	HFM 35	HFM40
FM	5.0	5.0	5.0	5.0	5.0
HFM	0.0	12.0	24.5	40.0	48.8
CSM	50.0	39.0	25.0	9.0	0.0
MM	40.0	38.5	40.5	42.0	42.2
WM	2.0	2.0	2.0	2.0	2.0
SFO	1.0	1.5	1.0	0.0	0.0
Vit/Min	2.0	2.0	2.0	2.0	2.0
TOTAL	100	100	100	100	100

Table 2. Percentage inclusion levels of the ingredients in EW Based Diets (g/100g diet).

Ingredients	Diets				
	EWM0	EWM25	EWM30	EWM45	EWM40
FM	5.0	5.0	5.00	5.0	5.0
EWM	0.0	12.0	24.0	39.8	45.0
CSM	50.0	38.0	24.5	5.0	0.0
MM	40.0	38.0	39.5	42.7	42.0
WM	2.0	2.0	2.0	2.0	2.0
SFO	1.0	3.0	3.0	3.5	4.0
Vit/Min	2.0	2.0	2.0	2.0	2.0
Total	100	100	100	100	100

D1= Diet 1, D2 = Diet 2, D3 = Diet 3, D4 = Diet 4, D5 = Diet 5, D6 = Diet 6, D7 = Diet 7, D8 = Diet 8, D9 = Diet 9, FM = Fish Meal, EWM = Earthworm meal, CSM = Cotton seed meal, MM = Maize meal, WM = Wheat meal, SFO = Sunflower oil, Min/Vit. = Minerals/Vitamin premixes
**Vitamin A 25,500,000 IU, Vitamin D3 5, 000, 000 IU, Vitamin E 5,050 IU, Vitamin B2 mg 4,750, Vitamin B6mg 2,750, Vitamin B12 mcg 11, 750, Vitamin K3 mg 4,850, CAL PAN mg 5,750, Niacinamide mg 16, 500, Vitamin C 10, 000 mg, IRON 5,250 mg, MANGANESE 12, 760 mg, COPPER 13, 250 mg, ZINC 13, 250 mg, SODIUM CHLORIDE 48, 750 mg, MAGNESIUM 12, 750 mg, POTASSIUM ACETATE 73, 750 mg, LYSINE 15,000 mg, METHIONINE 12, 000 mg, antioxidant and anticaking qsf 1 kg.

An eight-week growth trial was conducted in a recirculation system using 40-liter buckets as culture units as shown in Figures 5 and 6. Dietary treatments in triplicates were assigned randomly to 40-liter tanks each containing 14 fingerlings with an average weight of 2.6 grams. The fish were fed up to 5% of their body weights twice daily throughout experimental period.

Figure 5. Fish culture tanks.

Figure 6. Water treatment tanks.

Feed intake, feed costs, body weight measurements were used to calculate growth, nutrient utilization and cost effectiveness as follows:

- Average daily gain (ADG)

$$ADG = \frac{Final\ body\ weight - Initial\ body\ weight(g)}{Time\ (day)}$$

- Metabolic Growth Rates (MGR)

$$MGR = \frac{(Final\ body\ weight - Initial\ body\ weight)}{[\{(Initial\ body\ weight/1000)^{0.8} + (Final\ body\ weight/1000)^{0.8}\}/2]/trial\ days}$$

- Percentage Survival (PS)

$$PS = \frac{Final\ number\ of\ fish\ at\ harvest \times 100}{Initial\ number\ at\ stocking}$$

- Protein Efficiency Ratio (PER)

$$PER = \frac{Body\ weight\ gain\ (g)}{Crude\ protein\ intake\ (g)}$$

- Feed Conversion Rate (FCR)

$$FCR = \frac{Feed\ supplied\ (g)}{Body\ weight\ gain\ (g)}$$

- Daily Feed Intake (DFI)

$$FI = \frac{Feed\ Supplied\ (g)}{Time\ (day)}$$

- Cost effectiveness

$$FI = FCR \times Price\ of\ feed\ per\ kg$$

The main hypothesis tested was "there is no significant difference between treatment means." One-way analysis of variance (ANOVA) was used to determine differences between treatment means which were deemed significant at $P<0.05$.

The model used was $Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Where,

        $i = 1, 2... t_i$

        $j = 1, 2... r_j$

        Y_{ij} = Observed value (yields, nutritional composition of experimental meals, fish growth performance and feed utilization)

        μ = General means (yields, growth performance and feed utilization)

        T_i = the effects of culturing media and dietary treatment

        ε_{ij} = Error factors

Tukey's honest significant difference test was used for mean separation where significant differences existed.

RESULTS

Effect of substrate on proximate composition of housefly maggot and earthworm meals. Housefly maggots from chicken manure had significantly high protein content while that from cow manure had significantly low protein content (Table 3).

Table 3. Proximate Composition of House Fly Maggots Produced from Different Substrates (Means±SE)

Item	Culturing media		
	Chicken Manure	**Fermented Maize**	**Cow Manure**
Dry Matter	97.52 ±0.47[a]	96.42±1.47 [a]	96.71±0.08 [a]
Crude Protein	48.55±0.81[a]	42.63±0.23[b]	40.43±0.21[c]
Crude Fibre	5.71±0.25[a]	5.02±0.25[a]	6.00±0.25[a]
Ether Extract	19.07±0.46[c]	20.40±0.42[c]	20.00±0.06[c]
Ash	11.13±0.23[a]	10.70±0.48[a]	18.47±0.19[b]

Means with different superscript letters within a row are significantly ($p <0.05$) different.

Protein content of earthworms produced from cow manure was significantly higher than either of chicken manure or rabbit manure (Table 4).

Table 4. Proximate Composition of Earthworms Produced from Different Substrates (Means±SE)

Items (%)	Culturing media		
	Cow Manure	**Chicken Manure**	**Rabbit Manure**
Dry Matter	95.02±0.96[a]	97.20±0.47[a]	95. 26±0.67[a]
Crude Protein	48.61±0.18[a]	40.83±0.43[b]	39.80±0.41[b]
Ether Extract	6.80±0.49[c]	5.60±0.22[c]	5.23±0.12[c]
Ash	28.60±0.11[b]	28.77±0.48[b]	29.77±0.10[b]

*Earthworm meal used in the formulation of fish diet

Means with different superscript letters within a row are significantly ($p <0.05$) different.

Proximate composition of other ingredients used in formulating experimental diets is shown in Table 5.

Table 5. Proximate Composition and Gross Energy Content of Other Feed Ingredients Used in Formulation of Treatment Diets.

	Ingredients			
Item	FM	MM	WM	CSM
Dry matter	98.96	88.01	96.9	97.50
Crude Protein	69.20	10.5	11.74	41.60
Ether Extract	10.28	3.60	1.80	8.5
Crude Fibre	1.0	2.3	-	14.37
Ash	22.76	1.30	1.91	6.70
NFE[1]	2.38	84.30	79.15	23.34
GE(Kcal/g)	19.99	17.93	17.10	18.88

[1] NFE=Nitrogen free extract + fiber, (NFE) = 100 - (% protein + % fat + % ash).

The different inclusion levels of HFM and EWM had effect on proximate composition of the diets as shown in Tables 6 and 7, respectively. For instance, crude fiber decreased as inclusion levels of HFM and EWM increased in the diet. On the other hand, ash content increased with increasing inclusion levels of HFM and EWM.

Table 6. Proximate Composition and Gross Energy of Diets Containing Graded Levels of HFM Fed to *Oreochromis niloticus* Juveniles.

	Diets				
Item	HFM0	HFM25	HFM30	HFM35	HFM40
Dry Matter	93.55	92.71	91.48	90.02	93.07
Crude Protein	30.39	30.19	30.18	30.20	30.25
Ether Extract	8.49	10.42	10.50	10.80	11.90
Crude Fibre	8.17	7.20	5.89	4.45	4.43
Ash	5.03	6.52	7.95	9.80	11.12
Nitrogen Free Extract	46.86	43.99	43.41	42.19	38.84
Gross Energy	18.35	18.07	18.34	18.77	18.85

Table 7. Proximate Composition and Gross Energy of Diets Containing Graded Levels of EWM Fed to *Oreochromis niloticus* Juveniles.

	Diets				
Item	EWM0	EWM25	EWM30	EWM35	EWM40
Dry Matter	93.55	95.79	95.79	92.5	91.9
Crude Protein	29.59	30.31	30.31	29.58	29.80
Ether Extract	8.49	10.34	9.34	9.53	9.84
Crude Fibre	8.17	7.76	5.76	4.69	4.22
Ash	5.03	7.90	7.90	11.80	12.96
Nitrogen Free Extract	46.86	41.61	41.61	40.32	39.10
Gross Energy	18.35	17.21	17.21	16.30	16.07

Nile tilapia fed the different diets gained weights throughout the experiment period as shown in Figures 7 and 8. Fish fed diets containing 25%–35% HFM gained more weight compared to those fed diet control and 40% HFM diets. The same trend was observed in fish fed diets containing EWM but with much lower weight gain in fish fed the control diet and 40% EWM diets.
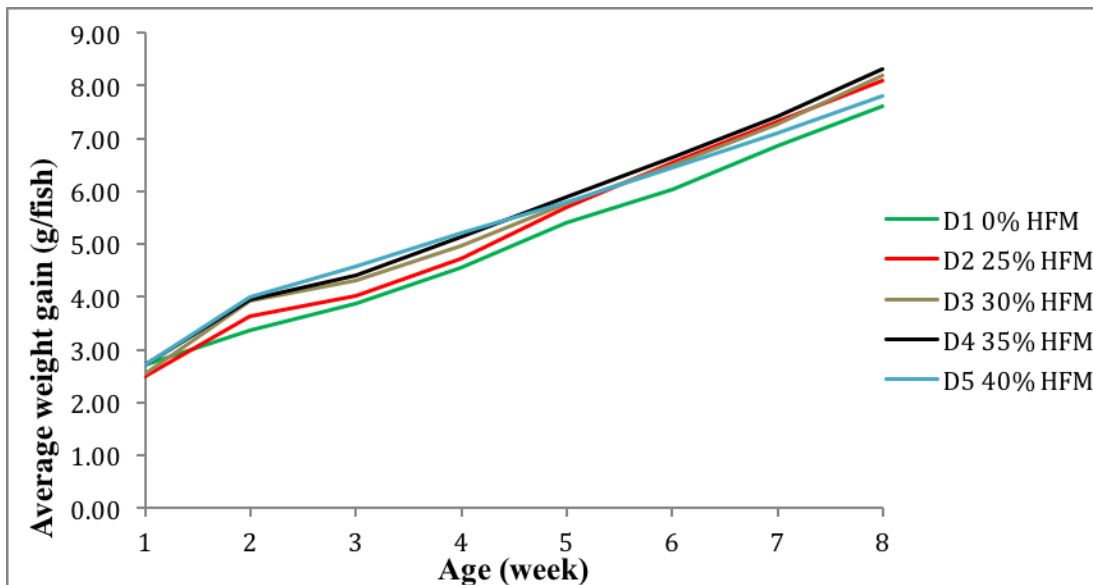
Figure 7. Change in weight of *O. niloticus* fed HFM-based diets.
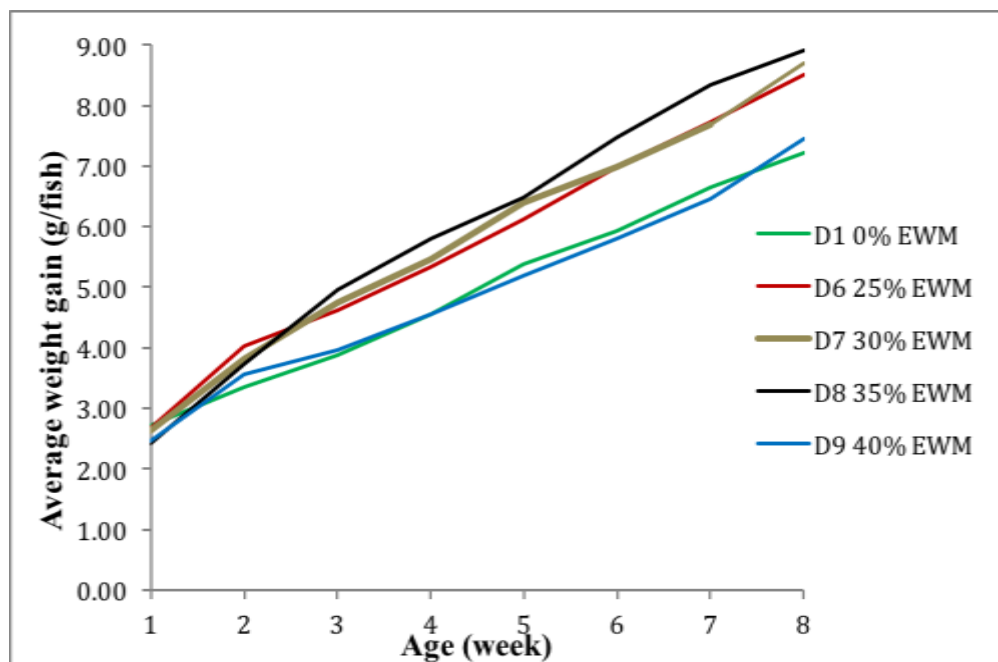
Figure 8. Change in weight of *O. niloticus* fed EWM diets.

Growth, nutrient utilization and survival of fish fed diets containing graded levels of HFM are shown in Table 8. Different dietary treatments had no significant effect on final weight and weight gain. However, average daily gain and feed intake differed significantly with fish fed diet HFM35, which performed significantly higher. Daily feed intake differed significantly with the highest values observed in fish fed HFM35. Similarly, fish fed diet HFM35 had significantly higher FCR and PER.

Table 8. Growth, nutrient utilization and survival of *O. niloticus* fed diets containing graded levels of HFM (Mean ± SD, n=3)

Parameter	Diets				
	HFM0	**HFM25**	**HFM30**	**HFM35**	**HFM40**
IW (g)	2.31±0.18[a]	2.48±0.11[a]	2.54±0.12[a]	2.72±0.09[a]	2.68±0.03[a]
FW (g)	7.71±0.11[a]	8.09±0.45[a]	8.19±0.30[a]	8.33±0.20[a]	7.49±0.32[a]
BWG (g)	5.40±1.21[a]	5.61±1.32[a]	5.65±1.20[a]	5.81±1.31[a]	5.61±1.20[a]
ADG (g)	0.115±0.0[e]	0.117±0.0[d]	0.119±0.0[c]	0.125±0.1[a]	0.122±0.02[b]
DFI (g)	0.59±0.03[c]	0.64±0.05[b]	0.65±0.01[b]	0. 69±0.04[a]	0.50±0.01[d]
FCR	2.65±0.14[b]	2.18±0.16[b]	2.17±0.16[b]	1.87±0.05[a]	1.89±0.02[a]
MGR	1.32±0.03[c]	1.38±0.01[ab]	1.42±0.04[b]	1.48±0.03[a]	1.42±0.05[b]
PER	0.029±0.002[a]	0.029±0.002[a]	0.029±0.001[a]	0.030±0.002[a]	0.030±0.003[a]
Surv (%)	88.09±1.2[b]	97.62±0.3[a]	97.62±0.3[a]	95.24±0.6[a]	97.62±0.3[a]
Cost Effectiveness (Tshs/Kg)	3071	2995	2949	2485	2511

Means with different superscript letters within a row are significantly (p <0.05) different.
IW = Initial body weight, FW = Final body weight, BWG = Body weight gain, ADG = Average daily gain, D FI = Feed intake, FCR = Feed conversion ratio, MGR = Metabolic growth rate, PER = Protein efficiency ratio, Surv = Percentage survival

Growth, nutrient utilization and survival of fish fed diets containing graded levels of EWM are shown in Table 9. Fish fed diets EWM25, 30 and 35 had significantly higher final body weights. Significantly higher body weight gain and average daily gain were observed in fish fed diet EWM35 while the least performance was observed in fish fed diets EWM0 and EWM40. Feed intake was significantly higher in fish fed diets EWM30 and EWM35 while the least intake was observed in fish fed diet EWM40. A significantly low feed conversion ratio was observed in diets EWM30 and EWM35 while that of diet EWM40 was significantly higher.

Table 9. Growth performance and nutrient utilization of *O. niloticus* fed diets containing various inclusion levels of EWM (Mean ± SD, n=3)

Parameter	Diets				
	EWM0	EWM25	EWM30	EWM35	EWM40
IW	2.31±0.18[a]	2.68±0.05[a]	2.63±0.05[a]	2.42±0.08[a]	2.46±0.28[a]
FW	7.71±0.11[b]	8.50±0.28[a]	8.69±0.06[a]	8.92±0.21[a]	7.85±0.58[b]
BWG	5.40±0.01[c]	5.82±0.01[b]	6.06±0.01[b]	6.50±0.01[a]	5.39±0.01[c]
ADG	0.12±0.01[d]	0.13±0.01[c]	0.14±0.01[b]	0.15±0.01[a]	0.12±0.01[d]
DFI	0.59±0.03[b]	0.61±0.02[ab]	0.69±0.04[a]	0.71±0.08[a]	0.42±0.10[c]
FCR	2.65±0.14[d]	2.22±0.07[b]	2.10±0.09[a]	1.85±0.31[a]	2.43±0.04[c]
MGR	1.21±0.03[c]	1.47±0.01[b]	1.52±0.04[a]	1.57±0.01[a]	1.13±0.08[c]
PER	0.02±0.02[c]	0.02±0.01[c]	0.03±0.01[b]	0.04±0.01[a]	0.03±0.01[b]
Surv (%)	88.09±1.2[b]	97.62±0.3[a]	97.62±0.3[a]	97.62±0.3[a]	88.09±0.6[b]
Cost Effectiveness (Tshs/Kg)	3071	3150	2980	2653	3521

Means with different superscript letters within a row are significantly ($p < 0.05$) different.
IW= Initial body weight, FW = Final body weight, BWG = Body weight gain, ADG = Average daily gain,D FI = Feed intake,
FCR = Feed conversion ratio, MGR = Metabolic growth rate, PER = Protein efficiency ratio, Surv = Percentage survival

CONCLUSION

- Chicken manure was found to be the best substrate for production of housefly maggots in terms of yield and protein content. On the other hand, cow manure was found to be the best substrate for producing earthworm in the same context;
- Fish fed diets HFM35 and EWM35 had a superior overall performance. Thus, inclusion of the invertebrate meals at 35% in practical diets containing 5% fish meal and cotton seedcake or any similar plant protein is recommended. Of the two, the HFM is best; and
- Diets HFM35 and EWM35 are more cost effective in producing a unit of fish.

Therefore, it is recommended to include either HFM or EWM at 35% in practical diets containing 5% fish meal and cotton seedcake or any similar plant protein.

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Enhancing the Nutritional Value of Tilapia for Human Health

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT02PU

This research is still ongoing and will be included in a future Technical Report.
See Implementation Plan 2013-2015, page 65.

Production of Periphyton to Enhance Yield in Polyculture Ponds with Carps and Small Indigenous Species

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT08UM

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ABSTRACT

The two best treatments obtained from our on-station trial were tested at farms in Chitwan and Nawalparasi districts. Carp polyculture with 100% feeding and carp+SIS+substrate with 50% feeding were introduced to 19 and 18 women farmers, respectively. Farmers stocked carp and small indigenous species (SIS), fed them with supplementary feed, and fixed bamboo substrate to ponds, as per the protocol of the on-station trial. Farmers were provided with a book to record fish that were consumed, sold, or died. Final harvest was conducted after eight months of culture by netting fish following partial water withdrawal from ponds. Total fish production and gross margin were 19.3% and 51.7% higher in carp+SIS+substrate with 50% feeding than in carp polyculture with 100% feeding. Apparent food conversion efficiency was higher in these ponds than during the on-station trial because ponds were randomly selected for monthly growth measurements and feed adjustment accordingly for all ponds at that farm. Training on carp+SIS+substrate technology was also provided to another 35 farmers through a workshop in Chitwan.

INTRODUCTION

Government of Nepal (GoN) has recognized that chronic malnutrition is a major problem in the country (UNICEF 2012). With the nutrition problem, there is a need to develop an environmentally sustainable and cost effective means of year-round food production that provides adequate nutrients and improves household income to rural poor farmers. Since 2008, the Institute of Agriculture and Animal Science (IAAS) has been promoting an innovative and environmentally sustainable household fish production system "Carp-SIS polyculture" to improve nutrition of poor women and children in Terai (Rai et al. 2012). The approach includes increased intake of nutrient-rich Small Indigenous Fish Species (SIS) to improve health and nutrition of women and children. Vitamin A, calcium, zinc and iron are found to be much higher in the eyes, head, organs and viscera of SIS (Roos et al. 2006). Since SIS are eaten whole, there is no loss of nutrients from cleaning or as plate waste. Moreover, SIS are self-recruiting and therefore, can be harvested weekly and biweekly, favoring household consumption. Carp-SIS polyculture also provides additional income through the sale of surplus fish. Studies revealed that the farming system raised the fish production above that of the national average, doubled consumption rate of owners, and farmers earned Rs 3,025 per household in 270 days which helped them to be empowered economically (Rai et al. 2012).

In commercial fish farming, feed alone accounts for about 60% of total input cost (Bhujel 2009), which is expensive to small-scale farmers, and so it is essential to provide an alternate to reduce feed cost. Adding substrates such as bamboo to carp ponds can increase carp production by facilitating growth of periphyton which serves as food. Since rohu *(Labeo rohita)*, catla (*Catla catla*), and common carp *(Cyprinus carpio)* are periphyton feeders (Rai and Yi 2012), their growth and production are enhanced in ponds with added substrate for periphyton colonization compared to ponds without substrates (Azim et al. 2002, Rai et al. 2008). Azim et al. (2004) showed a 70% increase in Rohu production in ponds for periphyton, compared

to control ponds, a 59% increase in net yield for polyculture carp ponds with feed and periphyton enhancement, and a 28% increase in yield for periphyton enhancement only, compared to ponds with fertilizer only. Since, the combination of species and type of feed influence the yield and income in such a system, it is necessary to test the full combination of feed inputs, periphyton enhancement, and production to truly understand the best system to use for commercial production (Diana 2012).

We conducted trials on-station at AFU to determine the best combination of carps, SIS, and periphyton enhancement choices to maximize net fish yield and profit in ponds. The purpose of this experiment was to use the best treatments found on station and to extend the trial to farmers who would follow some of our protocols but produce fish in their usual manner. Therefore, we assessed the effect of periphyton enhancement on Carp-SIS polyculture with reduced and without feeding systems in Chitwan and Nawalparasi, Nepal.

OBJECTIVES
- To verify findings of the on-station trial through an on-farm trial; and
- To provide training on carp+SIS+substrate technology to nonadopters through workshops.

MATERIALS AND METHODS

On-farm trial. The two best treatments — carp polyculture with 100% feeding and carp+SIS+substrate with 50% feeding — were selected based on fish production and profit from our on-station trial and tested in the field trial. A total of 37 women farmers participated in the on-farm trial: 15 in Majhui, Chitwan (one farmer dropped out in the middle of the project period), and 22 (14 in Seri village and eight in Nandapur village) in Nawalparasi district. Among them, 18 farmers (eight in Chitwan and 10 in Nawalparasi) participated in carp+SIS+substrate polyculture with 50% feeding. Similarly, 19 farmers (7 in Chitwan and 12 in Nawalparasi) participated in carp polyculture with full feeding. Average pond size was 259 m^2 (145–500 m^2) in Chitwan and 413 m^2 (163–552 m^2) in Nawalparasi. All farmers stocked fingerlings of rohu (25%), silver carp (*Hypophthalmichthys molitrix*) (20%), bighead carp (*Aristichthys nobilis*) (5%), mrigal (*Cirrhinus cirrhosis*) (15%), common carp (15%) and grass carp (*Ctenopharyngodon idella)* (20%) at a rate of 1500 fish per hectare and stocking was completed on 8 April 2015. Dedhuwa *(Esomus danricus)* and pothi *(Puntius sophore)* (SIS) were introduced by allowing them to enter the pond from a feeder canal. Those farmers who did not have canal supply collected SIS from nearby canals and stocked some to the pond. Farmers fixed the split bamboo substrates in ponds late in the last week of June (later than originally planned due to the earthquake disruption in Nepal). Substrate preparation was conducted as per protocol of the on-station trial. Sabita, a research student involved in the on-station trial, demonstrated how to construct substrate on both sites. Farmers fed fish with dough of rice bran and mustard oil cake (1:1) in the morning. The feeding rate was 3% fish biomass in ponds without substrates, and feed was reduced to half that level in substrate ponds. Feed quantity was adjusted monthly based on fish sampling conducted in randomly selected ponds. Grass carp were fed on grass, banana leaves, and vegetables. A book was given to each farmer to record the number and weight of fish that were consumed, sold, or died.

Final harvest began on 9 December and ended 19 December 2015. Harvest was conducted without draining ponds, by carefully netting a minimum of three times in each pond; all fish were then weighed. Ponds were not drained because fuel to operate pumps was scarce due to a blockade at the borders of Nepal. Since farmers wished to keep fish for their biggest festival "Maghi" that fell on January 15, netted fish were counted, weighed, and returned to the pond. During Maghi, major sales of fish occur because fish is an important food item in this celebration. Some farmers also may save fish in ponds for year-round consumption and to fetch higher prices later when there is less fish in the village. Gross income from fish sales was calculated from total production, assuming all carp were sold. Selling price of carp was Rs. 250/kg in Chitwan and Rs.270/kg in Nawalparasi, and that of SIS was Rs. 200. Gross margin was

calculated using gross income values for profits and determining the cost of inputs for each farming treatment. This was calibrated to a 100 m^2 pond size and assumed that all SIS were not sold but eaten in the household.

Workshop. A one-day training on periphyton-enhanced carp-SIS polyculture system was provided to 35 (seven men and 28 women) farmers from Chitwan district on 30 November 2015. Experts were from the Agriculture and Forestry University (Dr. Dilip K. Jha, Professor), Directorate of Fisheries Development (Bhagwat Prasad, Senior Fisheries Development Officer), the National Training Centre (Rajan Pd. Poudel, Master Trainer), and an NGO (Sovan Mahato, President and Ms. Usha Chaudhary, staff of Rural Integrated Development Society). The training venue was Kathar, Chitwan. The training was coordinated by Dr. Rai.

RESULTS

Production was higher in farm ponds with SIS treatments and half feeding than in carp ponds that were fully fed (Table 1). Overall production was 25.4% higher in the SIS treatments, and gross income was 51.7% higher (Table 2). Increases in income were the result of both higher overall production and lower costs due to reduced feed costs for the SIS ponds, with gross margin increasing 103% in ponds of the SIS treatment (Table 3).

Table 1. Fish (carp and SIS) production in two treatments in Chitwan and Nawalparasi districts.

Variable	Chitwan		Nawalparasi	
	Carp	Carp+SIS+substrate	Carp	Carp+SIS+substrate
carp production (kg/100 m^2)	26.8	35.3	33.1	37.6
SIS production (kg/100 m^2)	0.10	1.06	0.13	0.48
Total production (kg/100 m^2)	26.9	36.4	33.2	38.1
Extrapolated NFY (t/ha/yr)	4.1	5.5	5.0	5.8
AFCR	4.6	2.3	4.0	1.9

Table 2. Fish production, consumption, sale, and gross income per pond by farmers in Chitwan and Nawalparasi districts.

District	Treatment		Carp sold (kg/pond)	Carp consumed (kg/pond)	SIS consumed (kg/pond)	Total production * (kg/pond)	Gross income from fish sale (Rs./pond)
Chitwan (n=15)	carp polyculture (n=7)	Avg.	10.0	15.0	0.3	75.0	18,761
		Max	60.0	35.0	2.0	150.0	37,530
		Min	0.0	6.0	0.0	24.0	5,974
	carp+SIS+substrate (n=8)	Avg	31.0	23.5	2.6	109.6	27,411
		Max	170.0	45.0	5.0	302.4	75,598
		Min.	0.0	8.0	2.0	30.0	7,510
Nawalparasi (n=22)	carp polyculture (n=12)	Avg.	44.4	15.7	0.5	128.8	34,766
		Max.	260.0	55.0	5.0	261.4	70,575
		Min.	0.0	0.0	0.0	41.0	11062
	carp+SIS+substrate (n=10)	Avg.	25.8	8.7	2.2	164.0	44290
		Max.	155.0	20.0	10.0	275.8	74454
		Min.	0.0	0.0	0.0	107.5	23055

*Includes carp left in the pond and not consumed or sold at harvest.

Table 3. Gross margin (Rs/100 m^2 pond) for the two treatments in Chitwan and Nawalparasi districts.

Variables	Chitwan		Nawalparasi	
	Carp	Carp+SIS+substrate	Carp	Carp+SIS+substrate
Variable cost				
Carp seed	600	600	600	600
Feed	3,744	24,88	3,980	2,184
Total variable cost	4,344	3,088	4,580	2,784
Return				
Carp	6,703	8,853	8,817	10,190
SIS	19	211	27	96
Gross return	6,722	9,064	8,844	10,286
Gross margin	**2,378**	**5,976**	**4,264**	**7,502**
Gross margin (Rs/ha/yr)	361,654	908,850	648,483	1,140,929

<div align="center">DISCUSSION</div>

Fish production was determined based on harvest weight listed by farmers in their record book. Harvest was conducted in shallow ponds without complete draining, so we may not have been successful in removing 100% of the fish. In Chitwan, four ponds had deep water, and some fish may have escaped during netting. Although farmers were clearly instructed not to add water for a month prior to harvesting, four farmers in Chitwan (one SIS, three carp-only treatments) did not drawdown ponds due to water insecurity in the region. In Nawalparasi, the water level was fairly low for effective netting because 90% farmers used well water as the source, and they followed instructions since the water supply was under their control. In Chitwan, almost all farmers used canal water to fill the pond because they did not have wells. The harvesting process may therefore have affected overall fish (carp and SIS) production.

Carp, SIS, and total fish production was also higher in carp+SIS+substrate ponds than carp polyculture in both project sites, indicating increased carp production due to periphyton enhancement. Carp, SIS, and total fish production was 18.0%, 85.4%, and 19.3% higher in carp+SIS+substrate ponds than in carp ponds. Contribution of SIS to total production was small and ranged 1.3-2.6% by weight in carp+SIS+substrate ponds and 0.3-0.4% in carp ponds. SIS production was very low in Nawalparasi because farmers used well water and had no source for SIS colonization. SIS was also harvested in carp-only ponds, as they could enter over time from canal water. AFCR was higher in farm ponds than in the on-station trial, which can be attributed to random selection of ponds for monthly sampling of fish and using it as a basis for calculation of feed ration to all ponds. Sampling in every pond would likely have resulted in more precise measures of fish size and correction of feeding rate. Since farmers did not fertilize the pond, excess feed probably served as fertilizer.

Fish sale and consumption was calculated based on the record books. Despite instruction and format provided for recording sale and consumption by both number and weight, most farmers recorded only by weight, so survival could not be estimated. Carp was the major fish consumed by farmers. Farmers did not sell SIS; it was all consumed at home. Around 84% of farmers (16 carp farmers and 15 carp+SIS+substrate farmers) consumed fish at home, and 41% of farmers (seven carp farmers and eight carp+SIS+substrate farmers) sold carp. Farmers generally sold carp directly to neighbors in the village. Selling price was Rs. 20 higher in Nawalparasi (Rs 270/kg) than in Chitwan (Rs 250/kg), which is reflected in both income and profit. Both return and profit was better in the carp+SIS+substrate treatment due to higher carp yield and reduced feed cost. Gross income was 25.4% higher, and gross margin was 51.7% higher from carp+SIS+substrate ponds than from carp ponds. All farmers returned a profit except one in Chitwan, who lost Rs 1,674 due to poaching of her fish. The gross margin analysis did not take cost of electricity to run pumps into account because it was difficult to separate electricity consumed by

pumps from that of regular household consumption. Similarly, the cost of SIS and bamboo was also not taken into account because SIS were collected free from canal water (labor cost only), and bamboo was freely available in the village. These variable costs would reduce profit but probably only to a small degree.

CONCLUSION

The on-farm trial confirmed that carp+SIS+substrate with 50% feeding gave better yield and profit compared to carp polyculture with 100% feeding.

ANTICIPATED BENEFITS

Using carp, SIS, and periphyton enhancement with reduced feeding produced a 19.3% increase in fish production in farm ponds and a 51.7% increase in gross margin. This technology of polyculture of carp and SIS in a periphyton-enhanced system was shared with 37 women farmers through on-farm training, and 35 (seven men and 28 women) farmers through a workshop.

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Production of Periphyton to Enhance Yield in Polyculture Ponds with Carps and Small Indigenous Species

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT08UM

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ABSTRACT

An experiment was conducted at Agriculture and Forestry University, Chitwan, Nepal to compare fish production between carp-SIS polyculture and periphyton-enhanced carp-SIS polyculture in order to develop a cost-effective means to increase fish production. The experimental period was 210 days from 24 August 2014 to 28 March 2015. The experiment included four treatments: T_1 (carp+100% supplemental feed), T_2 (carp+SIS+100% supplemental feed), T_3 (carp+SIS+50% supplemental feed + bamboo substrate at 1 % of pond surface area) and T_4 (carp+SIS+bamboo substrate with no feed), each with three replications. Silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), common carp *(Cyprinus carpio),* rohu *(Labeo rohita)* and mrigal *(Cirrhina mrigala)* were stocked at a ratio of 4:1:4:3:5 at a rate of 15,000 fish/ha. Additionally, two small indigenous species (SIS) dedhuwa *(Esomus danricus)* and pothi *(Puntius sophore)* were stocked at a ratio of 1:1 at density of 50,000 fish/ha. Carps were fed with freshly made dough of mustard oil cake and rice bran (1:1) daily at 5% of body weight; whereas, grass carp was fed daily with grass at 50% body weight. Growth and yield of common carp was higher in T_3 than the other treatments, indicating it benefits from both periphyton and supplementary feed for better growth. Growth and production of grass carp were significantly higher in ponds without substrate. Total carp yield and combined NFY were higher in T_3, due to higher survival and growth rate of carps caused by periphyton and supplementary feed. Production of SIS was lower in substrate ponds, indicating that they did not use periphyton as a significant food source. Gross margin was highest in T_3, intermediate in T_4, and lowest in T_2. T_3 was found to be the best among treatments, based on fish production and profit.

INTRODUCTION

The government of Nepal has recognized that chronic malnutrition is a major problem in the country (UNICEF, 2012). With the nutrition problem, there is a need to develop an environmentally sustainable and cost-effective means of year-round food production that provides adequate nutrients and improves household income to rural poor farmers. Since 2008, the Institute of Agriculture and Animal Science has been promoting an innovative and environmentally sustainable household fish-production system, "carp-SIS polyculture," to improve the nutrition of poor women and children in Terai region (Rai et al. 2012). The approach includes increased intake of nutrient-rich SIS to improve health and nutrition of women and children. Vitamin A, calcium, zinc, and iron are found to be much higher in the eyes, head, organs, and viscera of SIS (Roos et al. 2006). Since SIS are eaten whole, there is no loss of nutrients from cleaning or as plate waste. Moreover, SIS are self-recruiting and can be harvested weekly and biweekly, favoring household consumption. Carp-SIS polyculture also provides additional income through the sale of surplus fish. The farming system including SIS raised fish production above that of the national average, doubled consumption rate of owners, and farmers earned Rs 3,025 per household in 270 days, which helped them to be empowered economically (Rai et al. 2012).

In commercial fish farming, feed alone accounts for approximately 60% of total input cost (Bhujel 2009), which is expensive to small-scale farmers, so it is essential to provide opportunities to reduce feed cost. Adding substrates such as bamboo to carp ponds can facilitate growth of periphyton, which serves as food for carp and increases their production. Since *rohu (Labeo rohita)*, catla (*Catla catla*), and *common carp (Cyprinus carpio)* are periphyton feeders (Rai and Yi, 2012), their growth and production are enhanced in ponds with added substrate for periphyton colonization compared to ponds without substrates (Azim et al. 2002, Rai et al. 2008). Azim et al. (2002) showed a 70% increase in rohu production in ponds with substrates for periphyton growth, compared to control ponds. Azim et al. (2004) showed a 59% increase in net yield for polyculture carp ponds with feed and periphyton enhancement, and a 28% increase in yield for periphyton enhancement only, compared to ponds with fertilizer only. Since the combination of species and type of feed influence the yield and income in such a system, it is necessary to test the full combination of feed inputs, periphyton enhancement, and production to truly understand the best system for commercial production (Diana, 2012). Therefore, we assessed the effect of periphyton enhancement on carp-SIS polyculture with reduced and without feeding systems in Chitwan, Nepal.

OBJECTIVES

Our overall objective was to compare fish production between carp-SIS polyculture and periphyton-based carp-SIS polyculture in order to develop a cost-effective means to increase fish production. Specific objectives included:

- To compare growth and yield of carps between carp polyculture and carp-SIS polyculture systems;
- To compare the growth and yield of carps and SIS with and without periphyton enhancement;
- To compare water quality among different polyculture systems; and
- To compare profitability among different polyculture systems.

MATERIALS AND METHODS

The experiment was conducted for 210 days (24 August 2014 to 28 March 2015) in 12 earthen ponds at the Teaching and Research Farm of Aquaculture and Fisheries Department, Agriculture and Forestry University, Rampur, Chitwan. The average area of an experimental pond was 150.9±4.1 m^2, ranging from 117.7–168.5 m^2.

The experiment was conducted using a completely randomized design. There were four treatments each, with three replicates. Treatments included: T_1 (carp+100% supplemental feed), T_2 (carp+SIS+100% supplemental feed), T_3 (carp+SIS+50% supplemental feed + bamboo substrate), and T_4 (carp+SIS+bamboo substrate with no feed).

Predatory fish were eradicated by applying bleaching powder at 250 kg/ha to ponds. After 15 days of bleaching, ponds were fertilized with inorganic fertilizer, urea, and DAP at 470 g/100 m^2 and 350 g/100 m^2. Bamboo substrate was installed for growth of periphyton in substrate treatment ponds (T_3 and T_4). Whole bamboo was procured from the AFU farm. These were split into three to five cm broad slats each, with an average length of one m. These slats were then tied onto a rectangular bamboo mat, using string with space between slats to allow fish to browse on attached periphyton. Bamboo mats were constructed and installed so that two mats covered an area equivalent to 1% of total pond surface area. Bamboo mats were suspended vertically in the water column with the top two edges tied to Styrofoam blocks serving as floats, and the bottom two edges tied to bricks serving as weights.

Stocking of fish was initiated seven days after fertilization. Ponds were stocked with silver carp (*Hypophthalmichthys molitrix*) (11.7±0.5 g), bighead carp (*Aristichthys nobilis*) (9.6±0.4 g), grass carp (*Ctenopharyngodon idella*) (4.8±0.2 g), common carp (1.1±0.1 g), rohu (0.8±0.0 g) and mrigal (*Cirrhinus cirrhosis*) (1.8±0.1 g) in all ponds at rates of 3000, 750, 2250, 3000, 3750 and 2250 fingerling/ha,

respectively. Similarly, *pothi (Puntius sophore)* (2.2±0.1 g) and *dedhuwa (Esomus danricus)* (1.2±0.0 g) were also stocked at 25,000 fish/ha for each species in ponds of T_2, T_3, and T_4. Carp and SIS were fed with freshly made dough of mustard oil cake and rice bran (1:1). Feed was provided in traditional bamboo trays, placed in all feeding ponds every morning at 9:00–10:00 h. Feeding rate was 5% BW/day for the initial two months and was then reduced to 2% until the end of the experiment. Since the experiment was carried out during winter, feeding was done only when the feed of the previous day was consumed. In substrate ponds, half of the previous feeding rates was given in T_3 and no supplementary feed in T_4. Also, fertilization was done every two weeks to enhance production of periphyton and maintain the plankton population. Periodic fertilization with inorganic fertilizers was done at similar rate as before. Fertilizers were soaked and dissolved in water a few hours prior to application for better efficiency. Grass carp was fed daily with locally available grass at 50% body weight.

Temperature, dissolved oxygen (DO) and pH were measured every two weeks at 7:00–8:00 h, while transparency, total alkalinity, total ammonia nitrogen, soluble reactive phosphorus, and chlorophyll *a* were analyzed monthly at the AFU laboratory. Periphyton sampling from bamboo substrate was taken randomly from 1 cm^2 and analyzed using methods in Azim et al. (2001). Dry matter, ash content, and ash-free dry matter were estimated using methods from APHA (1980). Fish were sampled monthly for size and feed calculation for the next month. At least 20% of each species were netted and weighed. Final harvest was conducted on 26–28 March 2015 by completely draining ponds using diesel pumps. At final harvest all fish were counted and weighed to assess survival rate and production.

Economic return was calculated using gross margin analysis. Variable costs were estimated for carp seed, SIS seed, bleach, urea, DAP, bamboo, bamboo trays, and feed. Gross return was calculated based on product sold at farm gate prices.

Experimental data were evaluated with one-way analysis of variance (ANOVA) using SPSS (V 16.0) to find significant differences among treatments. Duncan's Multiple Range Test was used when significant differences were found. Differences were considered significant at an alpha level of 0.05. All means are given with ± 1 standard error (SE). Comparison of carp growth and production and of periphyton biomass (i.e., dry matter, ash, and ash-free dry matter) among treatments were done using student t-tests. Data on percent survival, as well as contribution of carp and SIS to total production, were analyzed after square root transformation of original data.

RESULTS

There was no significant difference ($p>0.05$) in mean values of all water quality parameters among different treatments (Table 1).

Table 1: Summary of water quality parameters in different treatments (mean±SE). In most tables, figures in parenthesis show the range, and superscripts of the same value in a row indicate no significant difference between the values.

Parameters	Unit	Treatment			
		T_1	T_2	T_3	T_4
Temperature	°C	23.0 ± 0.0^a	23.0 ± 0.1^a	23.0 ± 0.1^a	23.0 ± 0.1^a
		(14.1-31.6)	(14.1-31.6)	(14.2-32.2)	(14.1-32.1)
pH		6.6	6.5	6.6	6.6
		(5.5-8.3)	(5.7-8.3)	(5.8-8.8)	(5.5-9.3)
Dissolved Oxygen	mg/L	3.6 ± 0.2^a	3.4 ± 0.2^a	3.3 ± 0.2^a	3.0 ± 0.1^a
		(0.6-6.9)	(0.6-6.8)	(0.4-8.2)	(0.1-7.6)
Transparency	cm	25 ± 5^a	23 ± 2^a	21 ± 1^a	21 ± 1^a
		(20-40)	(20-30)	(20-30)	(20-30)
Alkalinity	mg/L as CaCO3	102.7 ± 6.9^a	98.3 ± 1.6^a	95.4 ± 5.0^a	94.5 ± 6.7^a
		(66.0-131.9)	(72.6-128.1)	(68.6-127.4)	(69.0-134.4)
Total Ammonium Nitrogen	mg/L	0.051 ± 0.020^a	0.031 ± 0.008^a	0.053 ± 0.018^a	0.052 ± 0.004^a
		(0.004-0.094)	(0.007-0.099)	(0.009-0.097)	(0.010-0.093)
Soluble Reactive Phosphorus	mg/L	0.030 ± 0.011^a	0.026 ± 0.007^a	0.035 ± 0.004^a	0.020 ± 0.004^a
		(0.004-0.048)	(0.004-0.053)	(0.016-0.049)	(0.003-0.051)
Chlorophyll-a	mg/m^3	20.5 ± 5.8^a	20.9 ± 6.1^a	21.5 ± 1.1^a	23.4 ± 1.0^a
		(8.0-32.6)	(6.4-43.8)	(14.4-27.3)	(17.6-33.7)

Dry matter (mg/cm^2), ash content (%), and ash-free dry matter (mg/cm^2) of periphyton produced on bamboo substrate in treatments T_3 and T_4 were also not significantly different between treatments (Table 2).

Table 2: Periphyton content in different treatments.

Parameters	Treatment	
	T_3	T_4
Dry Matter (mg/cm^2)	3.0 ± 0.2^a	2.5 ± 0.2^a
Ash Content (%)	21.7 ± 1.1^a	22.0 ± 0.2^a
Ash-free Dry Matter (mg/cm^2)	2.3 ± 0.1^a	2.0 ± 0.1^a

Total carp production and combined fish yield was significantly higher in T_3 with periphyton substrate, SIS, and carps than in T_4 ($p<0.05$), and was insignificantly higher than in the other two treatments (Table 3). SIS production was higher in T_2 with SIS and carps at 100% feed than in T_3 or T_4 ($p<0.05$). FCR was best (1.02) in T_3 compared to the other two feeding treatments ($p<0.05$). Among individual species, grass carp had highest production in T_1 and common carp in T_3, while all other species showed no differences among treatments.

Table 3: Growth performance of carps and SIS in different treatments.

Silver carp

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Initial mean weight (g/fish)	12.0±0.2[a]	10.3±2.2[a]	12.2±0.1[a]	12.2±0.2[a]
Initial total weight (g/100m²)	355.9±6.7[a]	308.6±66.1[a]	365.9±4.8[a]	362.3±2.9[a]
Final mean weight (g/fish)	334.5±97.7[a]	278.8±81.1[a]	282.6±13.0[a]	327.4±78.9[a]
Final total weight (kg/100m²)	6.0±1.7[a]	6.4±1.6[a]	7.1±0.4[a]	7.0±0.8[a]
DWG (g/fish/day)	1.5±0.5[a]	1.3±0.4[a]	1.3±0.1[a]	1.5±0.4[a]
TWG (kg/pond)	8.4±2.9[a]	9.5±3.0[a]	9.9±0.6[a]	10.7±1.0[a]
Survival (%)	65.0±13.0[a]	78.2±3.4[a]	84.7±6.4[a]	77.0±9.9[a]
Extrapolated GFY (t/ha/yr)	1.03±0.29[a]	1.11±0.28[a]	1.24±0.07[a]	1.22±0.13[a]
Extrapolated NFY (t/ha/yr)	0.97±0.29[a]	1.05±0.29[a]	1.18±0.07[a]	1.16±0.13[a]

Bighead carp

Initial mean weight (g/fish)	10.7±1.1[a]	9.4±0.5[a]	9.6±0.5[a]	8.9±0.4[a]
Initial total weight (g/100m²)	79.9±8.0[a]	70.0±2.3[a]	72.0±2.3[a]	67.9±3.2[a]
Final mean weight (g/fish)	459.3±28.9[a]	399.4±62.0[a]	466.5±42.7[a]	316.4±55.8[a]
Final total weight (kg/100m²)	1.6±0.1[a]	1.8±0.6[a]	2.2±0.2[a]	1.8±0.3[a]
DWG (g/fish/day)	2.1±0.1[a]	1.9±0.3[a]	2.2±0.2[a]	1.5±0.3[a]
TWG (kg/pond)	2.1±0.1[a]	2.7±0.9[a]	3.1±0.2[a]	2.8±0.5[a]
Survival (%)	45.4±2.5[b]	58.9 ±9.4[ab]	63.6±5.3[ab]	75.6±0.6[a]
Extrapolated GFY (t/ha/yr)	0.27±0.02[a]	0.32±0.10[a]	0.38±0.03[a]	0.32±0.05[a]
Extrapolated NFY (t/ha/yr)	0.26±0.02[a]	0.31±0.10[a]	0.38±0.03[a]	0.30±0.05[a]

Grass carp

Initial mean weight (g/fish)	4.9±0.5[a]	4.5±0.5[a]	5.1±0.1[a]	4.8±0.3[a]
Initial total weight (g/100m²)	145.3±16.4[a]	133.6±13.8[a]	154.3±4.6[a]	141.4±7.8[a]
Final mean weight (g/fish)	465.4±32.1[a]	262.4±24.8[bc]	341.8±81.9[ab]	136.0±39.0[c]
Final total weight (kg/pond)	5.0±0.5[a]	2.9±0.4[b]	4.4±0.1[ab]	2.6±1.0[b]
DWG (g/fish/day)	2.2±0.2[a]	1.2±0.1[bc]	1.6±0.4[ab]	0.6±0.2[c]
TWG (kg/pond)	7.0±0.0[a]	4.2±0.6[ab]	6.2±0.1[ab]	3.9±1.6[b]
Survival (%)	45.4±2.6[b]	36.7±1.5[b]	47.2±9.5[ab]	59.7±6.6[a]
Extrapolated GFY (t/ha/yr)	0.87±0.09[a]	0.50±0.07[b]	0.76±0.01[ab]	0.44±0.17[b]
Extrapolated NFY (t/ha/yr)	0.85±0.09[a]	0.48±0.07[b]	0.73±0.01[ab]	0.42±0.17[b]

Common carp

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Initial mean weight (g/fish)	1.2±0.2[a]	1.1±0.1[a]	1.0±0.1[a]	1.2±0.2[a]
Initial total weight (g/100m²)	26.0±4.3[a]	25.2±2.2[a]	23.2±2.7[a]	26.7±4.0[a]

Final mean weight (g/fish)	1062.8±357.2[a]	666.0±191.0[a]	1167.5±120.2[a]	584.9±183.0[a]
Final total weight (kg/100m^2)	5.9±2.3[b]	3.0±1.1[b]	10.8±1.2[a]	4.8±0.8[b]
DWG (g/fish/day)	5.1±1.7[a]	3.2±0.9[a]	5.6±0.6[a]	2.8±0.9[a]
TWG (kg/pond)	9.1±3.9[ab]	4.5±1.8[b]	15.8±1.6[a]	7.7±1.2[b]
Survival (%)	22.7±3.8[b]	18.4±2.6[b]	41.6±4.4[a]	41.4±7.0[a]
Extrapolated GFY (t/ha/yr)	1.02±0.41[b]	0.53±0.10[b]	1.88±0.21[a]	0.84±0.14[b]
Extrapolated NFY (t/ha/yr)	1.01±0.41[b]	0.52±0.20[b]	1.88±0.21[a]	0.83±0.14[b]
Rohu				
Initial mean weight (g/fish)	0.8±0.0[a]	0.8±0.0[a]	0.8±0.0[a]	0.7±0.1[a]
Initial total weight (g/100m^2)	28.0±0.3[a]	28.5±0.9[a]	31.3±0.7[a]	25.4±4.8[a]
Final mean weight (g/fish)	209.5±15.4[a]	198.9±18.1[a]	225.2±13.7[a]	207.1±13.9[a]
Final total weight (kg/100m^2)	5.2±0.5[a]	4.6±0.5[a]	5.2±1.0[a]	6.0±0.3[a]
DWG (g/fish/day)	1.0±0.1[a]	0.9±0.1[a]	0.7±0.1[a]	1.0±0.1[a]
TWG (kg/pond)	7.7±.4[a]	6.8±0.5[a]	7.5±1.4[a]	9.6±0.6[a]
Survival (%)	66.9±4.0[a]	63.9±11.0[a]	60.1±9.1[a]	77.8±2.1[a]
Extrapolated GFY (t/ha/yr)	0.91±0.09[a]	0.80±0.08[a]	0.90±0.17[a]	1.04±0.05[a]
Extrapolated NFY (t/ha/yr)	0.90±0.09[a]	0.79±0.08[a]	0.90±0.17[a]	1.03±0.05[a]
Mrigal				
Initial mean weight (g/fish)	2.1±0.1[a]	1.7±0.1[b]	1.6±0.1[b]	1.5±0.2[b]
Initial total weight (g/100m^2)	46.1±3.6[a]	39.1±3.2[a]	34.9±1.9[a]	32.8±4.0[a]
Final mean weight (g/fish)	145.3±20.6[a]	150.1±17.0[a]	154.8±21.4[a]	133.4±9.1[a]
Final total weight (kg/100m^2)	2.1±0.4[a]	2.1±0.7[a]	2.3±0.4[a]	2.4±0.3[a]
DWG (g/fish/day)	0.7±0.1[a]	0.7±0.1[a]	0.7±0.1[a]	0.6±0.0[a]
TWG (kg/pond)	3.0±0.6[a]	3.0±0.9[a]	3.3±0.6[a]	3.8±0.4[a]
Survival (%)	66.0±5.3[a]	58.1±16.1[a]	65.7±6.8[a]	78.9±5.1[a]
Extrapolated GFY (t/ha/yr)	0.38±0.08[a]	0.36±0.12[a]	0.40±0.07[a]	0.41±0.06[a]
Extrapolated NFY (t/ha/yr)	0.37±0.08[a]	0.36±0.12[a]	0.39±0.07[a]	0.41±0.06[a]
NFY carp only (t/ha/yr)	4.36±0.47[ab]	3.51±0.42[b]	5.45±0.45[a]	4.15±0.40[ab]
NFY of SIS only (t/ha/yr)	-	0.21±0.07[a]	0.05±0.02[b]	0.05±0.02[b]
Combined NFY (t/ha/yr)	4.36±0.47[ab]	3.72±0.36[b]	5.52±0.43[a]	4.20±0.41[ab]
Feed conversion ratio (FCR)	2.44±0.30[a]	2.44±0.21[a]	1.02±0.06[b]	-

Gross margin was highest for T_3, which was significantly higher than T_1 and T_2 (Table 4). Margins varied from 460 to 966 NRs per pond, and reduced or no feeding provided the highest margins.

Table 4. Gross margin analysis of different treatments (in 1,000 NRs/100 m^2 pond).

Variables	Treatment			
	T_1	T_2	T_3	T_4
Carp seed	498.2±4.3[a]	501.6±2.6[a]	502.7±3.1[a]	498.7±3.0[a]
SIS seed		49.9±0.4[a]	50.2±0.2[a]	49.5±0.2[a]
Bleaching	350	350	350	350
Urea	338.4	338.4	338.4	338.4
DAP	364	364	364	364
Bamboo			250	250
Tray	70	70	70	
Feed	1627.1±166.1[a]	1382.2±7.0[a]	871.5±47.5[b]	
Total variable cost	3247.7±165.8[a]	3056.0±9.9[a]	2796.8±46.7[b]	1850.6±3.2[c]
Return				
Carp	6440.3±680.3[ab]	5200.6±612.6[b]	8031.1±631.2[a]	6135.9±574.3[ab]
SIS		509.5±100.9[a]	323.7±45.4[a]	382.7±29.2[ab]
Gross return	6440.3±680.3[ab]	5710.0±525.2[b]	8354.7±612.9[a]	6518.6±563.1[ab]
Gross margin	3192.6±622.4[bc]	2654.0±519.9[c]	5557.9±588.4[a]	4618.5±566.0[ab]
Gross margin (1,000 NRs/ha/yr)	554.9±108.2[bc]	461.3±90.4[c]	966.0±102.3[a]	802.7±98.4[ab]

DISCUSSION

Adding bamboo substrate to ponds affected the growth and production of grass carp and common carp. Mean harvest weight and DWG of grass carp in T_4 was significantly lower, corresponding to higher survival that caused increased competition for food. Since much of the experimental duration (four months) was in winter, grass was fed at 50% body weight of grass carp, which might be low and cause them to shift to supplemental feed. Better survival of grass carp in substrate ponds was perhaps due to shelter and cover provided by bamboo substrate. Similar to grass carp, survival of common carp was significantly higher ($P<0.05$) in ponds with bamboo substrate (T_3 and T_4) than in ponds without substrate (T_1 and T_2). Higher survival along with availability of periphyton and supplemental feed resulted in higher GFY and NFY for common carp in T_3, indicating that common carp required both supplemental feed and periphyton (Rai et al. 2012) for better growth and production.

Production of SIS was better in fed ponds than in periphyton substrate ponds. Contribution of SIS to total fish production in T_2 was 9.3±2.3%, significantly higher than in T_3 (3.47±0.6%) and similar to results from Gupta (2011) (3.8%–12.6% of total production). SIS was harvested seven times during monthly sampling. NFY of carp in T_3 was significantly higher than in T_2, with no significant differences among other treatments. This demonstrated that yield of carp was enhanced by adding substrate and reducing feed input by 50%. Combined NFY was significantly higher in T_3 than that of T_2, again indicating that periphyton-based culture system produced better yield than conventional carp-SIS polyculture. The overall results showed that periphyton had a positive effect on survival, growth, and production of carp (Azim et al. 2002, Rai et al. 2008).

Feed constituted 45%–50% of total variable cost for carp polyculture, while it was only 31% for periphyton-based polyculture. It is possible this cost could be lowered further with increased substrate area (Azim et al. 2004) and fertilization. Due to lack of feed cost, total variable cost of T_4 (NRs 1850.6±3.2 per 100 m^2) was significantly lower ($P<0.05$) than fed treatments. Similarly, feed cost of T_3 (NRs 871.5±47.5 per 100 m^2) was significantly lower ($P<0.05$) than other treatments with supplemental feeding. Periphyton along with 50% supplemental feed also improved FCR significantly ($p<0.05$) in T_3 (1.02±0.06).

CONCLUSION

Adding bamboo substrate for periphyton production with 50% supplementary feed in a carp-SIS polyculture system enhanced fish production and lowered production cost. Farmers can earn income through carp sales and improve family health and nutrition through consumption of SIS from their ponds. This technology is suitable to rural farmers, as it is cost-effective, simple, and supports family nutrition. Since almost half of the experimental period was in winter, which probably reduced growth and survival of carp, it would be useful to run another trial to verify present findings.

ANTICIPATED BENEFITS

Adding periphyton and reducing feeding rate of carps produced a 74% increase in gross margin for carp polyculture systems. This low-cost technology for small-scale farmers fulfils both the household need of income generation and nutrition. In addition, the substrate provided shelter and cover to protect fish from predators like birds and also hindered poaching. Using substrate and SIS alone, without feed, was much more profitable than traditional farming with 100% feed application. These manipulations can be used by farmers to increase profit and food available without added inputs and with minimal training.

ACKNOWLEDGMENTS

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TOPIC AREA:
CLIMATE CHANGE ADAPTATION: INDIGENOUS SPECIES DEVELOPMENT

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Two Small Indigenous Species to Improve Sustainability in Typical Polyculture Systems in Nepal

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ABSTRACT

Small indigenous species (SIS) grown in polyculture ponds in Nepal have been shown to increase economic and nutritional sustainability for farmers. However, there has been little research determining optimal stocking density of SIS, the resulting production of carp and SIS, effects on water quality, and economic feasibility of purposely stocking SIS. Thus, the overall goal of this research was to identify an optimal stocking density of the SIS punti (*Puntius sophore*) and dedhuwa (*Esomus danricus*) within a typical six-species production system including common carp (*Cyprinus carpio)*, bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys moltrix*), grass carp (*Ctenopharyngodon idella)*, rohu (*Labeo rohita)*, and mrigal (*Cirrhinus cirrhosis)*. We hypothesize this could be done without significant negative impacts on the system itself and with the rationale that these additions will allow farmers to more efficiently use their pond space and will increase economic, nutritional, and environmental sustainability of carp culture. Objectives were evaluated using a controlled production experiment with four stocking densities of SIS: 0/ha, 25,000/ ha, 50,000/ha, and 75,000/ha. SIS stocking density had no significant effect on carp production, water quality, or SIS production, indicating that carp production was not influenced by SIS stocking, but also that there was no advantage to stocking SIS. SIS naturally recruited to all experimental ponds from canal water. Instead of stocking density, isolation and average size of SIS at stocking were strongly correlated to overall fish production. Dedhuwa proved particularly difficult to harvest and contain within ponds and should not be considered for SIS production in ponds. Overall, the high variability of SIS production in this experiment and high harvest of SIS when none were stocked indicate purposeful stocking of the SIS punti and dedhuwa appear to be an additional system cost without increasing profits or SIS production from the system.

INTRODUCTION

Pond culture is the dominant aquaculture system in Nepal and accounts for 90% of aquaculture production (FAO 2013). Similarly, carp are the dominant species used in aquaculture and comprise 90% of the yield. Aquaculture production is fairly new to the country: it began in the 1940s, but did not develop significantly until the 1980s with the creation of the Aquaculture Production Program in 1981 (FAO 2013). Since then, production has increased dramatically from 2,041 tons in 1982 to 36,020 tons in 2013 (FAO 2015). Aquaculture systems, particularly polyculture including SIS, are well received and accepted among rural families and can improve the nutritional and economic well-being of farmers and

their families, with special emphasis on improved welfare for women and children (Kawarazuka 2010, Rai et al. 2014).

SIS may be added to carp polyculture systems to increase pond production, as well as improve household consumption and nutrition. Since SIS are most commonly earmarked for household consumption rather than market sales (Kadir et al. 2006, Roos et al. 2007), SIS production in ponds can directly affect household nutrition. Also, SIS have a higher reproductive rate than carp species and have been known to breed in culture ponds (Kadir et al. 2006). SIS-carp production systems raised production above the national average, doubled consumption rate of household members, and provided $34 USD income per household in 270 days of culture (Rai et al. 2014). Moreover, when compared to carp species, the eyes, head, organs, and viscera of SIS are found to contain higher levels of vitamin A, calcium, zinc, and iron (Roos et al. 2007). This is significant, as SIS are typically consumed whole, whereas carp are gutted and the internal organs discarded. SIS are also a plentiful schooling fish, commonly found in rivers of Nepal, and they are plentiful in the Terai region. Their inclusion in culture should not require additional pond inputs, since they can utilize naturally occurring food sources within the pond, such as plant material, algae, and small insects.

This research focuses on best practices for carp polyculture systems in southern rural Nepal (Terai), with the goal of improving systems to better serve rural farmers and families, without negatively impacting the environment. Given previous evidence for SIS to improve the livelihoods and health of farmers and their families without negative environmental impacts, identifying an optimal stocking density is an important research need. Punti (*Puntius sophore)* and dedhuwa (*Esomus danricus*) were chosen as the focus of this research because they are two SIS commonly found in southern Nepal, they are preferred for consumption in the region, and their inclusion in a carp-SIS culture has shown favorable results in previous studies on improvement of livelihood, income, and nutrition (Rahman 2005, Morales and Little 2007, Rai et al. 2014). The six carp species used in polyculture include common carp (*Cyprinus carpio)*, bighead carp (*Hypophthalmichthys nobilis)*, silver carp (*Hypophthalmichthys moltrix)*, grass carp (*Ctenopharyngodon idella)*, rohu (*Labeo rohita)*, and mrigal (*Cirrhinus cirrhosis)*.

Inclusion of periphyton substrate in ponds has been shown to increase primary production and reduce the need for input of feed, which can lower production costs and increase income for farmers. Rohu is an established periphyton feeder (Wahab et al. 1999, Azim et al. 2002, Rai and Yi 2012), common carp consume periphyton (Rai and Yi 2012) and production of common carp has been shown to increase in ponds with periphyton substrates installed (Wahab et al. 1999, Azim et al. 2002, Rai et al. 2008). Bamboo substrate has been shown to promote the growth of periphyton and can increase carp production (Azim et al. 2002). Because of these results, we used bamboo in ponds to enhance periphyton production in the SIS-carp polyculture system.

The main objective of this study was to compare different SIS stocking densities and their effects on carp production and survival, SIS production, and water quality, in order to identify an optimal stocking density to promote overall pond production, as well as nutritional and economic returns of a typical carp polyculture system in Nepal. We hypothesized that stocking SIS at any density would not negatively impact carp production or survival, or water quality and that inclusion of SIS would provide means to improve economic returns. Moreover, we hypothesized that a density could be identified where pond production and economic return was increased the most by natural reproduction of SIS within the ponds. These objectives were evaluated by investigating carp production and survival, SIS production, and water quality at four different SIS stocking densities.

The overall goal of this research was to identify an optimal stocking density of the SIS punti and dedhuwa within a typical polyculture system, including common carp, bighead carp, silver carp, grass carp, rohu, and mrigal. Specific objectives were:

- To evaluate the impact of adding different densities of two small indigenous fish species (punti and deduwa) to the yield and economic performance of the carp polyculture system in Nepal; and
- To determine the impacts of adding new species on water quality and primary production in these polyculture ponds.

MATERIALS AND METHODS

We used a replicated design with four stocking treatments of SIS to evaluate optimal stocking density of SIS in the carp polyculture system. For all treatments, carp densities, feed composition, and fertilization rate were chosen based on the typical practices in the area, which incorporated six species of carp. Carp density was 15,000/ha and resulted in 300 total carp in each 200 m^2 pond. Surface feeders (silver and bighead carp) were stocked at 50% of total carp density, bottom feeders (common carp and mrigal) at 30%, and column feeders (rohu and grass carp) at 20%. Four treatments with different stocking densities of SIS were evaluated in triplicate ponds. The treatments were: 1) Control, 0 SIS/ha; 2) 25,000 SIS/ha; 3) 50,000 SIS/ha; and 4) 75,000 SIS/ha. Both punti and dedhuwa were stocked at 250 of each species per pond in Treatment 1, 500 in Treatment 2, and 750 in Treatment 3. These are referred to as T250, T500, and T750, respectively.

Ponds were drained, dried, and limed several months prior to stocking. The 12 ponds were stocked in late July and August 2013, with 3 ponds randomly assigned to each of the treatments. Pond depths were maintained at ~1.5m. The 12 ponds were completely harvested in mid-January 2014, giving a 5.5 month grow-out period.

Carp were fed with rice bran and mustard oil cake six days per week at 3% carp body weight per day (excluding grass, bighead, and silver carp). Diammonia phosphate (DAP) was added as fertilizer once a week at 700g per pond, along with urea at 950 g per pond. Bamboo poles were installed as a substrate for periphyton production in all ponds at ~8.64% of the pond surface area. Fertilization was not done on weeks when algae cover became high and morning DO levels were less than three mg/L.

Monthly carp sampling was conducted by seining one to two times to collect fish. All fish caught were identified, counted, and weighed (in g). This sampling was used to estimate carp growth and to recalculate feeding rate based on carp body weight. A few SIS were caught during partial harvests in October; they were counted, measured for length (in cm), and removed from ponds to simulate consumption by the owner. Carp were all returned to ponds after being counted and weighed. During final harvest in January 2014, ponds were drained and all fish identified, counted, weighed, and measured for total length. Survival (%) was estimated using total number of each species at final harvest compared to number stocked. We also determined total number of SIS stocked and harvested in each pond at draining.

Water temperature, DO, pH, and Secchi disk depth were measured weekly in each pond. Diurnal oxygen measurements were made bi-monthly to estimate primary productivity. Weekly water quality measurements were all taken between 6:00–8:00 h (as close to dawn as possible). DO was measured at 25 cm and 75 cm depths, pH measurements were taken near the surface at ~5-10 cm depth, and temperature was taken at ~50 cm depth or at 25 cm and 75 cm and then averaged. Diurnal oxygen measurements were taken at 6:00 h (dawn1 DO) and 18:00 h (dusk DO) on the first day, and then again at 6:00 h (dawn2 DO) the following day at 10, 25, 50, and 75 cm. These DO measurements were then used to estimate primary productivity with the 3-point diel method (Boyd and Tucker 1992). Respiration (RSP: dusk DO – dawn2 DO), net primary production (NPP: dusk DO – dawn1 DO), and gross primary productivity (GPP= RSP + NPP) were calculated (gC * m^{-2} * d^{-1}) at all four depths and then averaged.

Substrate was installed in all ponds to add a natural food source of periphyton. Bamboo was sourced from stands growing around the experimental site, and split in half lengthwise. One set of four split poles with a length of eight m and an average diameter of 30 cm were installed in each pond for surface area coverage of 6.4 m^2. A second set of four split poles eight m long and averaging 24 cm in diameter was installed in each pond for surface area coverage of 7.68 m^2. Bamboo was installed by attaching pairs of half poles, concave side facing up, to two small split bamboo poles, which were pushed into the sediment and anchored at 25 cm depth. The total bamboo surface area coverage in each pond was ~17.28 m^2 or ~8.64% of the total pond surface area.

To estimate periphyton growth in ponds, ceramic tiles were installed in ponds during August 2013 and January 2014. One pond per treatment was randomly chosen (Ponds 3, 5, 8, and 10) and three tiles were installed in each of these ponds at 25, 50, and 75 cm depths. These were enclosed in a mesh net to prevent carp feeding on the periphyton. Tiles were left in ponds for four to five days and then collected. Periphyton was scraped from tiles, water was strained out of the samples, samples were dried in an oven at 100 °C for two hours, and dry weight of periphyton (g) was then determined.

Analysis of variance (ANOVA) was used to determine significant differences in carp production, carp survival, SIS production, and water quality variables between treatments. Carp production was evaluated in grams (g harvested – g stocked) and survival (%). SIS production was examined using counts, or number harvested – number stocked. Alpha was set at 0.05 for all analyses. Any significant ANOVA results were further analyzed using Tukey's HSD post hoc test. In addition to initial ANOVA analysis using pond production data, correlation matrixes, backward stepping multiple regression, and ANOVA on yield residuals created from regression model results were analyzed to further assess and determine variables affecting variation in pond production.

Correlation matrixes were created to explore significant relationships between the independent variables and carp production or SIS production by pond. These variables included number of days DO fell below five mg/L (DO<5mg/L), average Secchi disk depth, average primary productivity, weight of carp stocked, and isolation from disturbance. For weight of carp stocked, both average individual weights of each species and total weight of carp stocked were examined. Isolation — distance from disturbance from a bordering house, road, and footpath — was determined by assigning ponds a score (1-6) based on how far the ponds were from each of the three sources of disturbance. This was done because disturbances caused birds to flee ponds, and bird predation appeared to affect fish survival and production. The scores for each source of disturbance (house, road, and footpath) were weighted at 60%, 25%, and 15%, respectively. These weighted scores were then added together and used as an index of isolation for each pond (Figure 1) with higher numbers indicating more isolation.

Figure 1. Numbers, treatments, and pond isolation scores based on distance from disturbance. Higher Pond scores indicate higher isolation.

In order to identify which physical variables were associated with the variation between ponds in total carp and punti production, backward stepwise multiple regressions were performed. Results from correlation matrixes, Pearson's correlation tests, standard deviations, and inter-quartile ranges (IQRs) were used to help select variables to include in the regression model and to find any correlation between independent variables to avoid issues with co-linearity. Independent variables used for each pond included isolation, number of days DO fell below five mg/L, average Secchi disk depth, average carp stocking size, and SIS stocking density. Four models were produced in each analysis. After analyzing residuals for normality and variance, and using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests for estimating the relative quality of models compared to each other, final models were chosen. Residuals of carp and punti yield (expected – actual yield) were analyzed using ANOVA to explore possible treatment effects on production after accounting for predictive variables identified in regression analysis.

Additionally, average periphyton growth ($g*m^{-2}*d^{-1}$) in test ponds was compared to average primary productivity ($g*m^{-2}*d^{-1}$) using the Pearson's Correlation Test to assess whether periphyton growth promoted higher primary productivity.

The economic feasibility of purposely stocking punti and dedhuwa, and the variance in economic returns resulting from differences in polyculture system design and management in the region were also evaluated using data gathered from this experiment and surveys with local farmers, hatcheries, and market vendors. A partial enterprise budget was calculated using experimental and survey data to assess fiscal viability of adding SIS to carp production systems for local farmers, as well as to evaluate pond management strategies. Information was gathered by interviewing three individual farmers, the Kathar Women's Aquaculture Cooperative, a private hatchery, a government hatchery, and a vendor at a local fish market in Bharatpur, Chitwan. Questions included SIS prevalence and interest, carp, and SIS market price and production, sources of aquaculture information and training, fish production, and pond

preparation costs, including purchase of fingerlings, feed, and fertilizer (Appendix Table 1). The partial enterprise budget evaluated potential changes in income and expenses resulting from stocking of SIS. Reported SIS harvest and costs from all three farmers interviewed were averaged and then corrected to kg/ha. Two results were created: one based on production data gathered in interviews, and the other based on production data collected in this experiment. Additionally, overall production strategies and the resulting variations in costs, production, and profits of various farmers and the experiment were also compared. These comparisons were created assuming 100% sale of carp produced, and all results were adjusted to one hectare of pond area.

RESULTS

As hypothesized, changes in SIS stocking density did not result in significant variations in carp production or survival between treatments (ANOVA, $p>0.05$). Furthermore, total carp production in ponds showed more within treatment than between treatment variation (p-value = 0.823, F-value = 0.302; Figure 2).
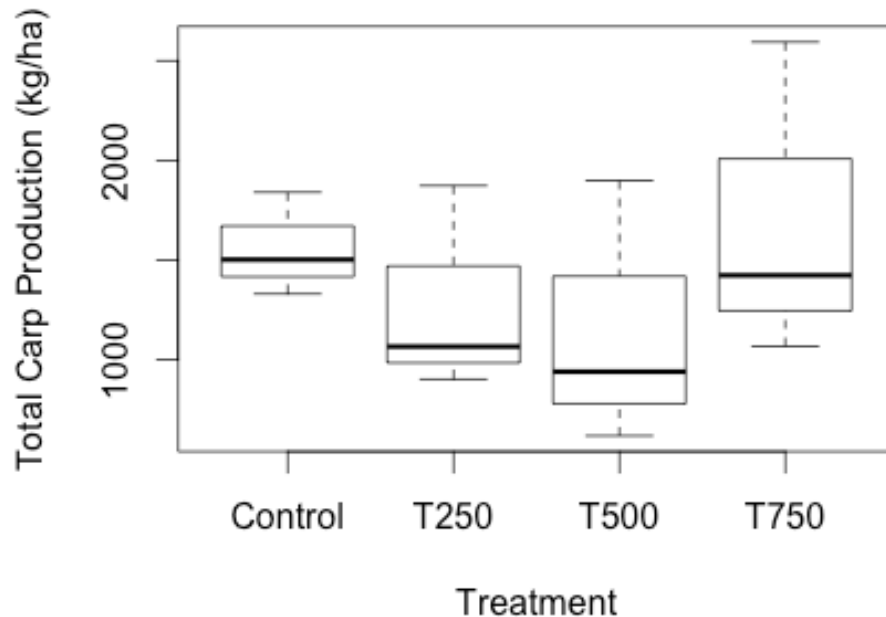
Figure 2. Boxplot of carp production in each treatment. Upper horizontal line: maximum of range, lowest horizontal line: minimum of range, bold horizontal line: median, box: interquartile range, upper and lower limits of box: third and first quartile.

Correlation matrices showed significant correlations between total carp production and isolation, primary productivity, Secchi disk depth, DO, and size at stocking. There was also a significant correlation between isolation index and production of bighead, common, mrigal, rohu, silver carp, and punti ($p < 0.05$), but not grass carp production. Average primary productivity was positively correlated to production of all carp species except grass carp. Secchi disk depth had a negative correlation to production of these same species and with average primary productivity. Average DO showed no significant correlation to production of most species, but a positive correlation to production of grass carp (p = 0.0225). Size at stocking was significantly correlated with common carp production (p = 0.0024).

Regression analysis for total carp production showed isolation was the major factor correlated to pond production, with average carp size at stocking explaining less variance but still significantly correlated (F $(2, 9) = 27.88$, $p < 0.0005$, $R^2 = 0.83$).

Stocking density did not significantly affect punti production. Higher production of punti came from control ponds through natural colonization, and these ponds had higher punti production than T250 or T500 ponds. One-way ANOVA results for production showed more within treatment variation in production than between treatments ($p = 0.177$, Figure 3). Punti were harvested at fairly high numbers in control ponds where none were stocked. Regression analysis for punti production showed only isolation was significantly correlated to production (F $(1, 10) = 14.86$, $p < 0.005$, $R^2 = 0.5575$).
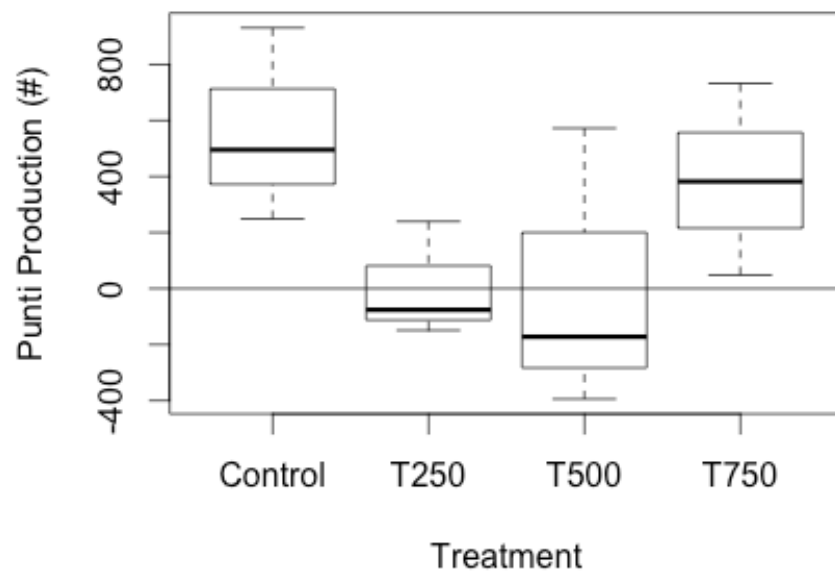
Figure 3. Boxplot of punti production by treatment; notation as in Figure 2.

Stocking density had a significant effect on production of dedhuwa, but this effect was often negative. These negative production values indicated that dedhuwa numbers declined from stocking to harvest. Once again, there was significantly higher production in control ponds than in stocking treatments, while the T750 treatment had the lowest production (Figure 4, p = .00173).
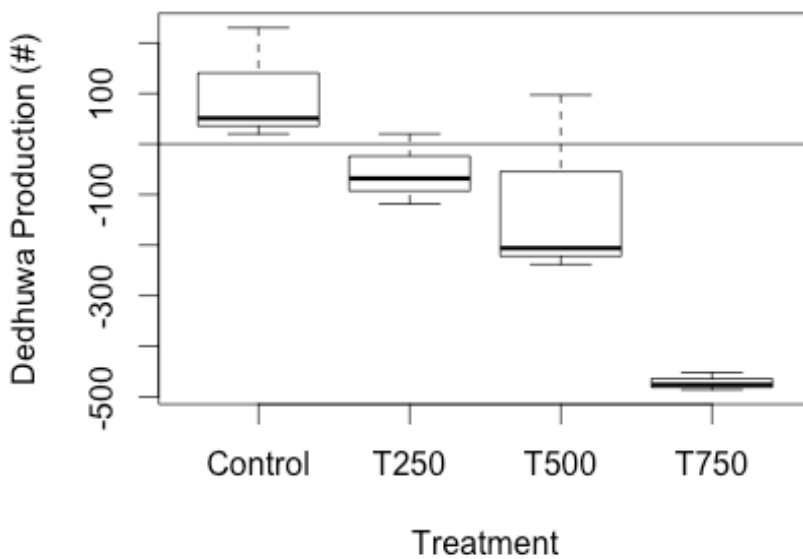
Figure 4. Boxplot of dedhuwa production by treatment; notation as in Figure 2.

There were no adverse effects of SIS stocking density on water quality. For all water quality parameters tested, including primary productivity, average pH, average Secchi disk depth, and DO (days<5 mg/L) there was more within treatment variation than between treatments ($p > 0.05$), and water quality parameters remained within acceptable ranges for carp and SIS survival. Temperature declined over the course of the experiment and reached levels that likely reduced production (T < 20 C) over the last six weeks of grow-out.

Periphyton growth was variable between ponds, at depths, and over time. Neither periphyton growth in August or January were significantly correlated to primary productivity (p = 0.704 and 0.9964, respectively), total carp production, or common carp production. However, a significant correlation was found between periphyton growth in August and rohu production (p = 0.012). Periphyton growth was higher in control, T250, and T750 ponds sampled in January than in August. For primary productivity, ponds showed fair uniformity with productivity oscillating over time and no overall increasing or decreasing trend.

All farmers interviewed reported harvesting SIS, although they did not purposely stock them, and they were all aware of their higher nutritional content and market price compared to carp. Farmers reported harvesting between 180-800 kg/ha. In comparison, the experiment had an average SIS harvest of only 86 kg/ha, but with a wide range from 21 to 202 kg/ha. SIS are not often sold in markets but consumed at home. When they are sold, however, prices are higher/kg than that for carp; (USD $4.00/kg for SIS, $2.00- $3.60 for carp). All farmers interviewed reported selling their carp for Rs 200 ($2.00). Interestingly, farmers with larger ponds did not necessarily report larger harvests of SIS, and when corrected to kg/ha, smaller ponds yielded more SIS per unit area.

Maximum profit was achieved for SIS when no stocking occurred ($1,840 per ha annually), assuming all SIS were sold at market (Table 1). They are usually consumed in the family and not sold, but the value of the produced SIS would be the same. Using average experimental values or maximum values resulted in far less profits ($116–$582). This was partially due to the cost of stocking SIS, but also to higher yields

achieved in farmer ponds. Stocked SIS can either be collected from the wild or purchased from fishermen. Daily collection labor cost was estimated at ~ $3.00/person to collect 1kg SIS, and purchase cost was also ~ $3.00/kg. Purchase and labor costs assumed an average SIS size of 1.5g for this analysis. Thus, when acquiring SIS from either method, $3.00 would equal ~667 SIS. No other costs were increased to include SIS in ponds for experiments or farmers. Our estimates of the costs and benefits of carp production by these same farmers indicated about $3,400 to $4,800 profit per ha annually, indicating that SIS production in their ponds was valued at about 25%–35% of the total value of production, even though SIS were not intentionally stocked or provided any inputs for their added production.

Table 1. Comparison of costs and benefits concerning addition of SIS, dedhuwa and punti, to carp polyculture systems. Values in US dollars.

	Farmers Average	Experiment (Avg. SIS Harvest)	Experiment (Max SIS Harvest)
Purchase/labor Cost	$0	$224	$224
SIS Production (kg/ha)	460	85	201.5
Market price ($/kg)	$4	$4	$4
Total SIS Sales ($)	$1,840	$340	$806
SIS Profit ($/ha)	$1,840	$116	$582

DISCUSSION

The two main objectives in this study were to determine optimal stocking density of SIS and to evaluate the impact of added SIS production on water quality and production. In contrast to our initial hypotheses, stocking SIS into ponds did not increase their production, but rather natural recruitment of SIS into ponds yielded the best production results. Economically, this was also shown by much higher yields and lower costs for farmers who were surveyed from SIS production compared to our experiment. Also, stocking SIS had no impact on water quality or primary production of ponds, and SIS production in the ponds was not correlated with any decline in water quality.

Punti production was not driven by stocking density, and extra effort spent to stock them did not correlate with increased production over ponds with natural recruitment. Movement of SIS between ponds and the connecting canal was evidenced by presence and harvest of punti in control ponds where no punti were originally stocked. Punti production was also highly variable between ponds. Initial ANOVA results on punti production showed that production varied more between ponds of the same treatment than between different treatments. Similar results were seen with dedhuwa. Although at times there were significant differences between treatments, most of the production values for both dedhuwa and many for punti were negative, indicating a loss of stocked fish. Moreover, due to their small size and narrow body structure, dedhuwa were very difficult to harvest and contain within ponds. Based on this, we believe our numbers of dedhuwa harvested are quite biased, so dedhuwa production numbers were not evaluated by treatment or by pond in relation to variation in carp production, punti production, or water quality variables.

SIS stocking density did not significantly affect carp production between treatments. Rather, other variables seemed to be driving variation in carp production, especially isolation and, to a lesser degree, average size of carp at stocking. Similar to punti results, the multiple regression model indicated a strong relationship between carp production and isolation, as well as size of carp at stocking.

Periphyton production was correlated with rohu production, which matched with findings in previous studies showing rohu consumed periphyton (Azim et al. 2004). Thus, addition of periphyton substrate could be beneficial if farmers are especially interested in promoting growth of this species. There was considerable variability in periphyton growth between ponds, at different depths, and between months (August vs. January).

It would be useful to gain a better understanding of what drives natural recruitment of SIS into ponds. Since most ponds in Nepal are filled with canal water, characteristics of the canals and their fish communities are important in setting potential SIS recruitment into ponds. It would benefit farmers to provide means for more SIS to colonize ponds, as long as other damaging fish species do not enter at the same time through natural pathways.

QUANTIFIED ANTICIPATED BENEFITS

The target end users of this system are small-scale rural farmers and their families in the Terai region of Nepal. We anticipated that the addition of SIS to this culture system would increase yield by at least 20%, without reducing carp production, but this was not the case. Natural recruitment of SIS into these ponds was sufficient to seed a population of SIS for household consumption. In fact, average SIS production in farmers' ponds resulted in an added value of about $1,600/ha annually to total fish production. The large carp species are commonly considered cash crops and are sold in local markets, as well as consumed in the home. SIS serve principally as a regular food source for farmers. We believe SIS produced in the ponds will increase household fish consumption by women and children by at least two-fold.

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APPENDIX

Appendix 1. Survey questions and possible responses used for economic evaluation.

Economic Evaluation Survey Questions					
Question	**Answer Categories**				
Area Farmed? (m^2)					
Do you own or lease the pond(s)?	Own			Lease	
Do your ponds contain SIS species? If so, which ones?	Punti		Dedhuwa	Other	
Do you purposefully sock and riase SIS species? If so, which ones and how much(#/pond)?					
If no, are you interested in raising these species purposefully?	Yes			No	
Are you aware of any nutritional content differences between large carp and SIS?	Yes			No	
Which species do you stock?	Carp Species		Tilapia	Other	
Do you purchase hatchlings, fry, or fingerlings?					
Where do you purchase your hatchlings/fry/fingerlings?	Government Hatchery	Private Hatchery	Wild/River		Farmer's Own Production
How much does each species cost to purchase (rs)?					
How much do you sell each species for? (rs/kg)					
How much of each species do you produce per year (kg)?					
What do you use to fertilize your pond(s)?	Organic Matter	Urea	DAP	Manure	Other
What is the cost of the fertilizer(s) you use?					
How much fertilizer do you use per year? (kg)					
What do you feed your fish?	Mustard Oil Cake	Rice Bran	Soybean Cake	Wheat Flour / Fish Meal	Other
How much does your feed cost? (rs)					
How much feed do you use in a year? (kg)					
Where does this feed come from?					
Are there any other costs besides fry/fingerlings, feed, and fertilizer? If so, what are they?	Co-op Fees	Labor	Equipment Rental	0ther	
If so, how much do they each cost? (rs)					
What is your grow-out period to market size? (mo)					
Who do you sell your fish to?	Not Sold	Neighbors	Local Market	Wholesaler	Transporter / Other
Why do you sell to this/these buyer(s)?					
What percentage (or how much(kg)) of the fish you raise are sold to these buyers?	Consumed at home.	Neighbors	Local Market buyers/sellers	Wholesaler	Transporter / Other
What percentage (or how much(kg)) of SIS from your ponds are:	Consumed at home.	Neighbors	Local Market buyers/sellers	Wholesaler	Transporter / Other
How do you learn about new technologies?					

Identifying Local Strains of *Oreochromis niloticus* That Are Adapted to Future Climate Conditions

Climate Change Adaptation: Indigenous Species Development/Experiment/13IND01PU

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ABSTRACT

Climate change can severely impact the food security of tropical developing countries that rely heavily on fisheries and aquaculture for sustenance. Nile tilapia (*Oreochromis niloticus*, or tilapia) is the most widely cultured species of fish in Africa and counted on for future food security. Thus, it is important that *O. niloticus* does not succumb to climate change.

This study was conducted to synthesize information on the ambient water quality (temperature, dissolved oxygen and salinity) for *O. niloticus*, to compare wild populations in the Volta basin and the eighth generation of the selectively bred Akosombo strain from the basin used in fish farming in Ghana under current and future climate conditions, and to develop predictive models to delineate the boundaries of the species' native range to aid in identifying populations with extreme adaptations for future selective breeding in Ghana of the species for aquaculture under climate change. A combination of literature survey, field, and laboratory studies provided data for meta-analysis, growth and genetic analysis, as well as distribution models.

We found variations in water temperature along the latitudinal gradient in Ghana, and temperature was the most informative variable in terms of characterizing the adaptive range and ambient water quality for the species. However, the distribution model for *O. niloticus* did not identify maximum temperature as independently important in delineating the northern limit of the species' range in West Africa. The results of the laboratory growth studies showed no evidence of superior performance of the Akosombo strain over the wild strains under current or predicted future climatic conditions of temperature, dissolved oxygen (DO), or salinity. The results of the genetic analysis also showed that the Akosombo strain was well differentiated from all the wild populations (Aframso, Sabare and Binaba) studied. The combined results of the field, growth and genetic studies show that at least one wild population from the Oti River (Sabare) may possess the traits for superior performance under high temperature and low DO conditions. Further studies should concentrate on comparing the Sabare strain with the Akosombo strain under both lab and pond conditions and increase experimental replications to confirm the suggested differences and the heritability of those performance traits for selective breeding.

The world's climate is changing directionally, and these changes will or are already having severe consequences for fisheries and aquaculture and food security, especially in tropical developing countries (Handisyde et al. 2005, Ficke et al. 2007, Leung and Bates 2013). Among impacts in aquatic systems expected to worsen over time include increased temperature and decreased DO, increased salinization of underground water and intrusion of salt water from sea level rise, and increased incidence of disease outbreaks in culture systems (Handisyde et al. 2005, Ficke et al. 2007, Williams and Rota 2010, Leung and Bates 2013). Recommended solutions emphasize adaptations and interventions that are based as much as possible on local practices and traditions, e.g., developing tolerant strains of existing aquaculture species and enhancing the resilience of communities, ecosystems, and traditional culture techniques (Williams and Rota 2010).

Nile tilapia (*O. niloticus*) is the most widely cultured species of fish in Africa and counted on for future food security. If this species succumbs to climate change, it will be a devastating blow to aquaculture development on the continent. Traditionally, strain selection and breeding has targeted a few traits, primarily fast growth (e.g., GIFT and its derivatives) and other desirable traits are secondary. However, planning for climate change presents a different challenge; cultured strains have to survive the climate and then grow. Temperature and DO tolerance have not been primary traits for selection because the species is considered tolerant. There has been research on hybridization of *O. niloticus* with its salinity-tolerant confamiliar species, e.g., *O. mossambicus* and *Sarotherodon galilaeus* (Kamal and Mair 2005, Yan and Wang 2010) and introduction of marine species' DNA into gonads of *O. niloticus* (El-Zaeem et al. 2011), the goal being to develop more strains that can survive and grow better in high salinity (El-Sayed 2006). The selection and development of better strains locally is encouraged over introduction of strains developed in other parts of the world. On this principle, the Volta strain of *O. niloticus* has been developed in Ghana and there are wide reports of promising performance (Dewedar 2013).

Optimal temperature for survival and growth of *O. niloticus* has been studied under a variety of conditions. Most studies found an optimum of 26-30ºC for growth, FCR and/or survival (Likongwe et al. 1996, Al-Asgah and Ali 1997, Baras et al. 2001, Azaza et al. 2008, El-Sayed and Kawanna 2008, Drummond et al. 2009, Xie et al. 2011). Perhaps, more intriguing is the variation observed in the optimum and the reduced growth and increased mortality past the optimum, well before the upper lethal temperature (preceding references). Strain and acclimation conditions account for some observed variation, but what has not been studied well is whether optimum and lethal temperatures vary within the species. These studies often assume implicitly that the physiological adaptations of the species are the same for all populations and individuals, and that phenotypic plasticity explains observed variation in tolerances. But it is well-known in fishes that geographic cline in traits occur. For example, the mummichog *Fundulus heteroclitus* distributed along the east coast of the United States shows a latitudinal cline in temperature and oxygen use adaptation, with underlying genotypic variation in the allelic isozymes of lactate dehydrogenase (LDH-B) that affect ATP levels (Place 1983). Individual variation in salt tolerance in *O. niloticus* has also been studied at the molecular level, although not in the context of latitudinal clines (Rengmark et al. 2007).

Sub-Saharan Africa pond aquaculture is heavily dependent on natural ambient DO as aeration is rare to nonexistent and mostly unnecessary at moderate stocking densities. Tolerance of *O. niloticus* to low DO should be understood in the context of minimum DO required for acceptable survival, growth, and reproduction. Under recirculating conditions, *O. aureus* had better FCR with intermediate (3.75±0.12 ppm), compared to low and high DOs (Papoutsoglou and Tziha 1996). DO levels do not only influence feed intake in *O. niloticus*, but it also affects growth, size at maturity, gonadosomatic index (GSI), egg size, and absolute fecundity (Kolding et al. 2008; Tran-Duy et al. 2008). There are well known dependencies among temperature and solubility of salt and oxygen, and plastic response of fish to one physicochemical variable also depends on the level of other variables, in addition to interaction of

genetics with environmental factors (Charo-Karisa et al. 2006; Schofield et al. 2011). A quick search of Web of Science and Aquatic Sciences and Fisheries Abstracts databases for the period 1970-2012 revealed more than 1,100 peer-reviewed publications on *O. niloticus* and and confamiliar species that focus on some aspect of growth. However, on close examination, West Africa native strains of *O. niloticus* are under-represented in these studies. Most studies are from Egypt or otherwise from outside of the continent. Identification of better-adapted populations of *O. niloticus*, and degree of adaptation to temperature, DO, and salinity will require synthesis of existing knowledge on the species, a combination of field and laboratory studies, including basic genetic descriptions, and linkage of distribution with biophysical data.

Species exhibit their most extreme adaptations at the tail ends of their range and in response to environmental gradients (e.g., Place 1983). In West Africa, natural climate varies from humid forest to dry savanna and desert as you move from the coast (low latitude) to interior (high latitude). The Volta basin, which spans the entire length of Ghana into Burkina Faso, aligns with this gradient. Reported occurrences (Paugy et al. 2003) indicate that *O. niloticus* range crosses much of the climate gradient. Populations in hot, drought-prone areas are predictably better adapted to high temperature, low DO, and high salinity. The salinity prediction is less intuitive but not when you consider that aquatic systems subject to high evaporation tend to have higher salinities, as drying concentrates dissolved solids. Thus, the predicted climate change scenarios in the southern parts of Ghana where most aquaculture is concentrated is very similar to current conditions in the north, near the upper limit of known *O. niloticus* West African range. A more accurate zoogeographic model for *O. niloticus* would be useful as a tool to identify the best adapted populations and also delineate the species' range from the numerous cichlid species (more than 40 in West Africa, Paugy et al. 2003) that are undoubtedly confused with *O. niloticus*, especially by small-scale farmers who still rely significantly on wild brood and seed to stock their ponds. Identifying these populations and their adaptations will guide future breeding programs that will have to consider climate, and take advantage of individual variation within populations to select for desired traits in addition to fast growth.

OBJECTIVES

- Conduct a comprehensive review, a meta-analysis, and synthesis of the peer-reviewed literature on *Oreochromis niloticus*, with respect to various strains and their adaptive range for temperature, dissolved oxygen (DO), and salinity;
- Conduct laboratory experiments to test the tolerance of the Volta strain and three wild populations of *O. niloticus* to increased temperature, decreased DO, and increased salinity;
- Determine size distribution, sex ratios, and length-fecundity relationships and characterize ambient water-quality (temperature, DO, salinity) of *O. niloticus* in its native habitat along the latitudinal gradient from southern to northern Ghana in the Volta basin;
- Genetically characterize wild populations of *O. niloticus* along the latitudinal gradient of the Volta Lake; and
- Develop a predictive distribution model (i.e., zoogeography) for *O. niloticus* in West Africa and accurately delineate the extremes and boundaries of the species' native range.

Hypotheses for experimental studies

Hypothesis 1: The Volta/Akosombo strain of *O. niloticus* grows faster compared to the average wild strain in Ghana under current climate conditions, but the Volta strain is less tolerant of high temperature, low DO, and high salinity.

Hypothesis 2: Northern populations of *O. niloticus* in West Africa are more tolerant of high temperature, low dissolved oxygen, and high salinity than southern populations; and the northern populations have a higher optimum temperature for growth.

Study area. The study was conducted in the Volta basin of Ghana with field sites at Aframso in the Afram sub-basin, Sabare in the Oti sub-basin, and Binaba in the White sub-basin of the Volta (Figure 1). The distribution model covered the entire West Africa range of *O. niloticus*.

Figure 1: Field sites (Source of base map: www.mapsofworld.com).

Meta-analysis. We surveyed the primary literature for studies that investigated the effect of temperature, DO and salinity on the growth of Nile tilapia, *O. niloticus,* for a meta-analysis. An initial search for papers with the keywords including "growth" and "tilapia" yielded >1,100 hits in Web of Science, of which full text were accessible for 762. Of these, 57 papers were identified to be relevant to the objectives of this study and only 19 provided data that could be reanalyzed (Appendix A). Eight studies investigated temperature effects, nine studies investigated salinity effects and three studies investigated DO effects on growth. We observed almost no consideration of interaction of the three factors among studies, with only one study investigating the combined effect of temperature and salinity on growth on Nile tilapia. We excluded studies that focused only on the egg to fry stages of growth in Nile tilapia, since sample sizes were too small to include analysis of ontogenetic change in tolerance to temperature, DO, and salinity. When data were extracted and catalogued in Excel, the 19 studies together provide 140 rows of data, including direct measurements (or data to calculate) specific growth rate (SGR), length of study, initial and final sizes of fish used in the study, and the specific experimental levels of temperature, DO, and salinity. Although we recoded the tear of study, strain/origin, culture system and other experimental factors, these pieces of information were not available for most studies and samples size did not permit incorporation to a large number of factors in the meta-analysis.

To use an approach that accounted for between study variability without prohibitive cost in degrees of freedom, we first ran a regression with "study" as the predictor of SGR and saved the residual SGR for subsequent analysis. Thus, the models comparing temperature, DO, and salinity effects on SGR each used the residuals. Based on visual inspection of the plot of SGR against each of the three factors, we fit a quadratic regression between temperature and SGR and linear relationships for DO and salinity.

Laboratory challenge and growth experiment. For the laboratory growth experiments, we collected wild broodstock in January 2015 from sampling the Afram River, White Volta and River Oti in Aframso, Binaba and Sabare townships respectively. Broodstock were kept in hapas mounted in a 200 m² pond at Kwame Nkrumah University of Science and Technology (KNUST) fish farm and fed high-protein diets for six months. This period served as acclimation for the fish and provided sufficient time for spawning and growth of juveniles of for the laboratory experiment. All fish were uniquely tagged with elastomer tags at the beginning of the experiment to aid in individual monitoring of fish. We used a factorial design with three factors (temperature, DO, and salinity) with two levels of each factor (low and high) and three replicates, for a total of 24 experimental units (aquarium tanks), each with at least one individual from each river (site, population, or "strain" hereafter) and the and the Akosombo strain. Fingerlings of the Akosombo strain (eighth generation) was obtained from the Pilot Aquaculture Center (PAC) of the Fisheries Commission of Ghana and kept in the same pond with the other strains for acclimation.

High water temperature was set at 33°C with individual aquarium heaters whereas the room temperature (about 24°C) was default for low temperature. High DO was obtained by constant aeration using multiple air stones, in addition to aquarium filters deployed for all experimental units. High salinity was achieved through gradual addition of crude-salt concentrate to the tanks over three days to achieve desired salinity of 15 ppt. A pretest was run for two months prior to the start of the experiment to refine the design and ensure that the desired factor settings were achievable in the experiment. For the main experiment, five uniquely tagged fish (majority between 5g and 8g) were randomly assigned to the treatment tanks, one from each of the three study sites including an extra site in Binaba. The Akosombo strain of *O. niloticus* served as control. We replaced fish that died in the first three days after stocking, after which no further replacements occurred. Fish were fed to satiation 3–4 times daily with a 48% protein commercial feed for the duration of the experiment and the daily feed given was recorded per tank. Temperature, DO, salinity, pH and conductivity were measured daily between 10:00 and 12:00 h with the Hanna multiparameter meter (HI 9828). Ammonia, nitrite and nitrate were measured three times during the experiment with the API Freshwater Master Test Kit. Final fish weights were recorded after 17 days. Growth was expressed in terms of percentage daily weight gain or specific growth rate [(Ln final weight - Ln initial weight) / time × 100]. A general linear model with two-factor interactions was used to analyze the data statistically, in addition to graphical analyses.

Field studies. Fish sampling for the life history studies was conducted with the help of local fishermen between November 2014 and July 2015. Total fish catches varied by month across all three sites, with some sites recording no catches due to high water levels precluding sampling either by seining or cast nets. Fish were measured for total (TL) and standard lengths (SL) to the nearest 1 mm and weighed (total weight) to the nearest 0.1g. Fish for fecundity studies were fixed in 10% formalin solution. Preserved fish were washed thoroughly and stored permanently in 70% ethanol after three days for further analysis. Sex determination was done by physical examination of gonads and fish were described as females, males and juveniles whose sexes were indeterminate. Lengths and weights after preservation were also measured. Fish were dissected to examine gonads. Where present in females, a subsample of eggs were carefully detached from gonads, counted and weighed. Where eggs were not matured, the entire gonad was weighed. Total egg count per female (fecundity) was estimated as weight of gonads multiplied by the number of eggs in the subsample divided by the weight of the subsample. The sexual proportion of males to females was obtained. Females were considered matured if they had eggs or visibly matured gonads. The mean length at sexual maturity for the female across sites was calculated as the length at which 50 percent of all individual become sexually mature, using a logistic regression of length on maturity status.

Water quality was measured monthly at the three sites between August 2014 to July 2015. However, due to logistical constraints, water quality was not measured in December 2014 and May 2015 for Binaba and Sabare, as well as March 2015 for Aframso. The water-quality variables measured each sampling month included temperature, DO, salinity, pH, and conductivity. All water-quality variables were measured with

the Hanna multiparameter probe (HI 9828). When necessary, water samples were sent to the laboratory and the API Freshwater Master Test Kit was used to confirm some of the results obtained with the probe. Differences in average monthly water quality variables were tested with a one-way analysis of variance (ANOVA), with statistical significance set at $p \leq 0.05$.

Genetic characterization. *O. niloticus* fin clips were obtained from fish sampled at five sites in the Volta River system (Sabare on the Oti River, two sites near Binaba on the White Volta River, Aframso on the Afram River, and the widely cultured Akosombo strain from the Fisheries Commission's Pilot Aquaculture Center (PAC) in Kumasi). DNA was isolated from a sample of 30 individuals from each of the five populations. DNA was purified using a commercial kit (PureGene, Gentra Systems), and DNA concentration was quantified using a Nanodrop ☐Lite spectrophotometer. Using polymerase chain reaction (PCR), we screened allelic variation at five microsatellite DNA marker loci for Nile tilapia (*UNH 130, UNH180, UNH858, UNH925*, and *UNH934*) linked to growth rate in other Nile tilapia stocks (Streelman and Kocher 2002, Cnaani et al. 2003). Sequence information for primers for amplifying these microsatellite loci was obtained from GenBank (www.ncbi.nlm.nih.gov/genebank/). Microsatellite amplicon lengths were resolved on an Applied Biosystems (ABI) 3100 automated DNA sequencer at the Virginia Bioinformatics Institute and scored using GeneMarker version 2.6.4. Allele frequencies, expected and observed heterozygosities, pairwise F_{ST} and analysis of molecular variance (AMOVA) were calculated from the data using the Microsat Toolkit version 3.1.1 and Arlequin version 3.5.2.2.

Modeling distribution of *O. niloticus* in West Africa. Thirty-six known presence records were obtained from Paugy et al. (2003) and compared with distribution data available through Fishbase www.fishbase.org. We concluded that the data sources were mostly duplicated. In addition, the reported distributions had large gaps in areas of West Africa where Nile tilapia is known to occur (e.g. the sites sampled in this study). Because of the unreliability of absence records, we adopted the maximum entropy (MaxEnt) presence-only machine-learning modelling approach using MaxEnt version 3.3.3k (http://www.cs.princeton.edu/~schapire/maxent/). Environmental data, principally, a 900-m resolution digital elevation model (DEM) for West Africa was obtained from US Geological Survey (USGS; http://www.webgis.com/terraindata.html), altitude and bioclimatic grids from WorldCLIM (http://www.worldclim.org/; Hijmans et al. 2005), and year 2000 population sizes for cities in West Africa from the Harvard University Center for Geographic Analysis (http://gis.harvard.edu/services/products/harvard-africa). We used the DEM to perform a watershed delineation and delineated about 17,000 subwatersheds for West Africa with areas ranging from 250km^2– 500km^2 and a few >1000km^2. The subwatersed were then used as study units for summarizing environmental variables and attributing the areas of know nile tilapia presence. Final 22 variables used in the MaxEnt modeling included altitude, stream order, population density (interpolated by ordinary Krigging), and 19 bioclimatic variables (BIO1-BIO19) including temperature and precipitation measures (see Appendix E for definition of all variables). Modeling was conducted with 70% of the 36 presence points and tested with the remaining 30%, using the Area under the Receiver-Operating-Characteristic-Curve (AUC) as a measure of model performance. Probability of tilapia presence was calculated for all nearly 17,000 subwatersheds.

<div align="center">

RESULTS

</div>

Meta-analysis. We observed reported SGR across the 19 studies ranging between 0.002%–195%, however, when we recalculated SGR based on beginning and ending sizes reported in individual studies, SGR ranged from 0.002%–15.5%. Temperature ranged between 10ºC–37ºC, DO ranged between 1.33mg/L–6.08mg/L and salinity ranged between 0 ppt–2 ppt for the combined experimental range. For our analysis, we truncated the temperature range to start at 20ºC to approximate tropical temperatures reflective of conditions in Ghana and other West African countries. The regression models showed significant relationships between SGR (%) across studies for temperature and DO, and no relationship

with salinity. Residual plots of SGR and temperature showed a quadratic relationship (Figure 2) and described by the equation:

Residual SGR $= 30.350 + 2.140*Temp − 0.037*Temp^2$; $R^2 = 0.342$, with the highest SGR at 29°C. The relationship of SGR with DO was slightly linear positive and describes by the equation: Residual SGR $= -0.5581 + 0.1638*DO$; $R^2 = 0.104$ (Figure 2).
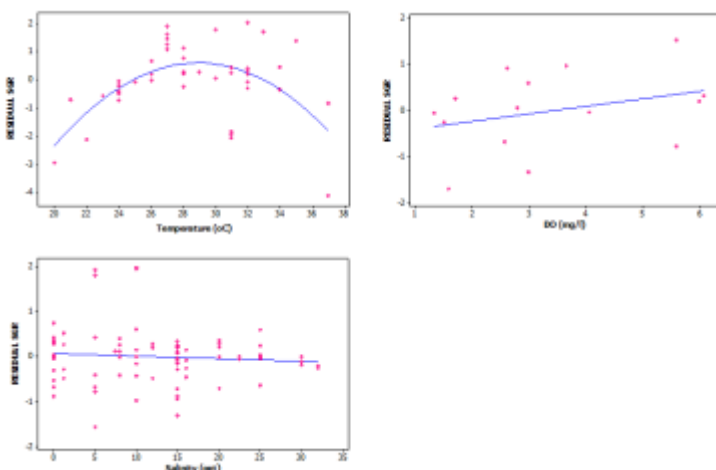
Figure 2. Plots of specific growth rate (residualized) against temperature, DO and salinity

Laboratory experiment. The mean low temperature recorded in the experimental units was 25.73°C and the mean high was 32.75°C. DO measurements recorded were 6.13mg/l and 7.47mg/l for the low and high levels, respectively. Salinity was 0.09 ppt for the low end and 0.15 ppt for the high end. The specific growth rate of *O. niloticus* ranged from -1.7 to 17 and showed varying sensitivity to temperature, dissolved oxygen and salinity across strains. The general linear model indicated an overall significant model, with salinity being the only significant factor at $p = 0.008$. In partial agreement with study hypotheses, SGR under "ideal" conditions (low temperature, high dissolved oxygen and low salinity) were relatively higher regardless of strain (Figure 3). The response to temperature by strain was mixed. The interaction plots from the general linear models showed that overall, the Sabare (SAB) strain tended to grow faster under the "ideal" conditions. Furthermore, compared to the other strains, the growth of the Sabare strain appeared to be insensitive to temperature within the range studied in this experiment, evidenced by comparable specific growth rates under both low and high temperature conditions (Figure 3). The Binaba (BIN) and Akosombo (PAC) strains showed relatively higher growth rates in relation to high temperatures while Aframso tended to be the least tolerant of high temperature (Figure 3). In terms of strain performance, the Akosombo strain was the next best after Sabare, followed closely by the Binaba strain, with the Aframso strain showing high variability in specific growth rates. Most of these trends were obvious, but not statistically significant due to small sample sizes and high variability in the response of the Aframso strain. See Appendix B for additional results.

Interaction Plot for LogSGR
Fitted Means
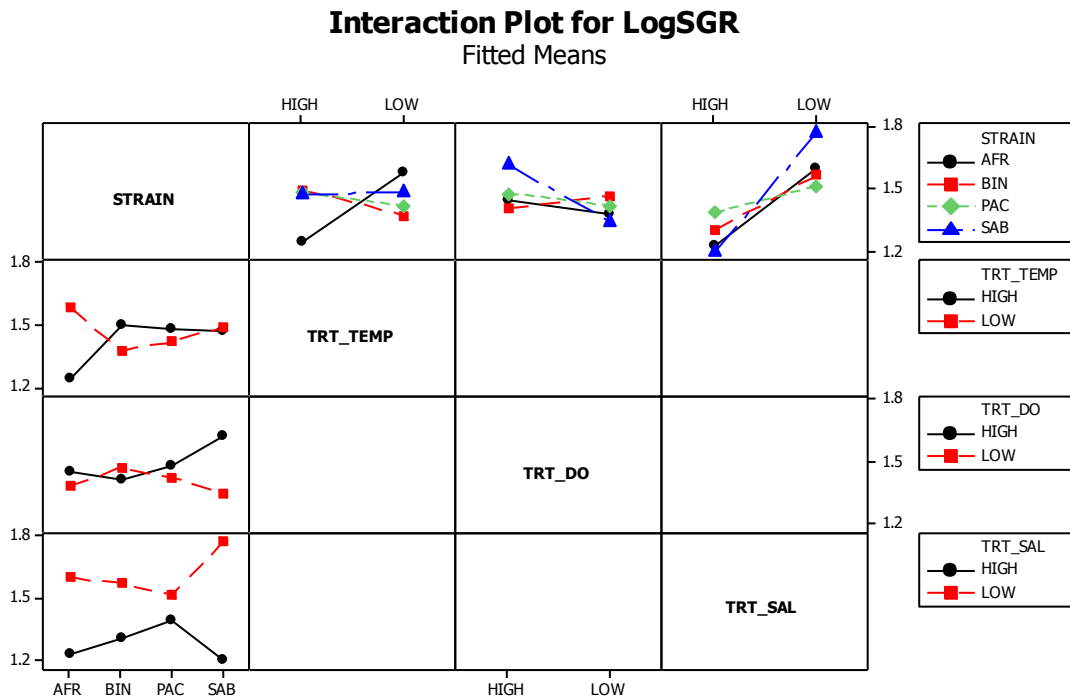
Figure 3. Interaction plots from a general linear model for specific growth rate of the different *O. niloticus* strains across the treatment factors: temperature, dissolved oxygen and salinity.

Field studies. *Life history* — Total length of fish captured in the field ranged from 4 cm to 25 cm with corresponding weights of 1.3g to more than 300g. The length-weight relationships observed from fish sampled at the three sites showed very little variation (Figure 4). The length-weight relation for Aframso is expressed by the regression equation: Log_{10} (Weight) = -1.786 + 3.056 Log_{10} (Length); R^2 = 0.96; N = 23. The length-weight relation for Binaba is expressed by the regression equation: Log_{10} (Weight) = -1.727 + 2.992 Log_{10} (Length); R^2 = 0.95; N = 91. The length-weight relation for Sabare is expressed by the regression equation: Log_{10} (Weight) = -1.788 + 3.054 Log_{10} (Length); R^2 = 0.99; N = 104. Overall, females dominated the samples across the three study populations (Aframso, Binaba, and Sabare). Sex ratio for Aframso was 56% female to 44% male (n = 23), Binaba recorded 66% female to 35% male (n= 97), and Sabare recorded 58% female to 42% male (n = 40). *O. niloticus* from Aframso matured early at about 13 cm, followed by Sabare and Binaba at 14.5 cm and 15 cm, respectively (Figure 5). Mature females were found in all months of the year when observations were made (December to July), with most in April and July at Binaba. Fecundity ranged between 70 to about 5200 eggs per female and had a positive linear correlation with fish size in the Sabare population. However, no clear relationship was exhibited by the Binaba population (Figure 6), and Aframso did not have sufficient mature females to estimate a length-fecundity relationship. See Appendix C for additional results.

Water quality. The water quality variables measured showed high variation but relatively minimal seasonal variation for all three sites except for a marked decrease in temperature and increase in DO in January (the cold, dry season) for the northern sites, Sabare and Binaba. However, the salinity recorded for Aframso was statistically higher compared to Sabare and Binaba across the annual cycle. Temperature was also significantly higher at Sabare on the average compared to Aframso and Binaba (Figure 7).
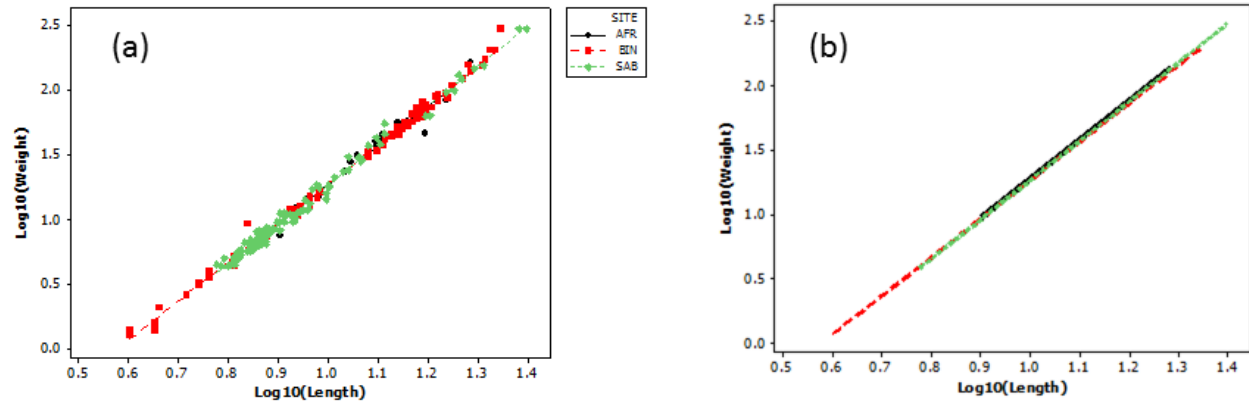
Figure 4. Length-weight relationship *O. niloticus* individuals sampled at Aframso, Binaba and Sabare (a) displayed with observed data (b) displayed without observed data.
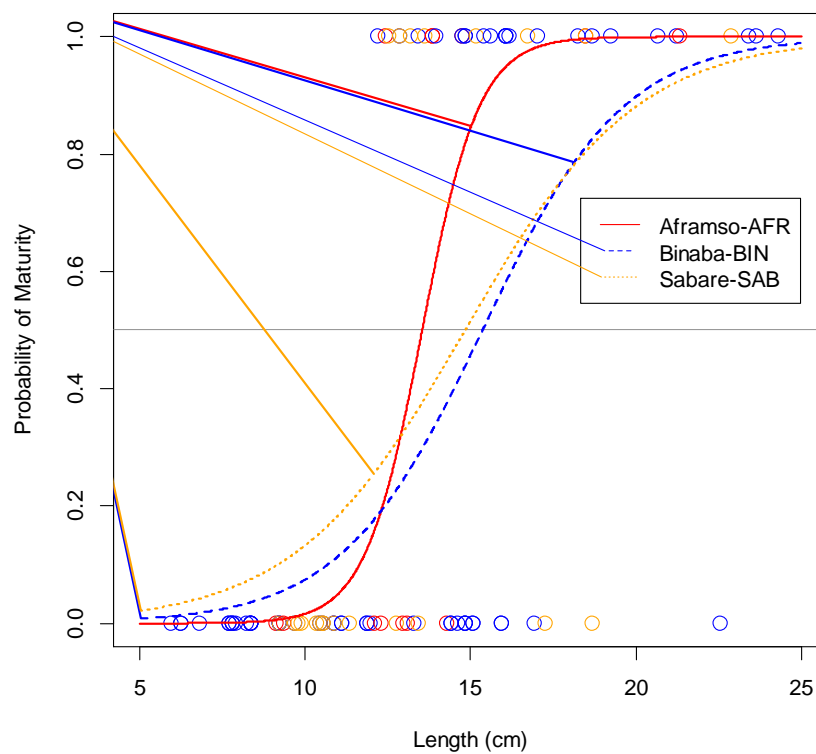
Figure 5. Logistic regression curves showing size at maturity for female *O. niloticus* sampled in Aframso, Binaba and Sabare. The black horizontal line indicates 50% maturity.
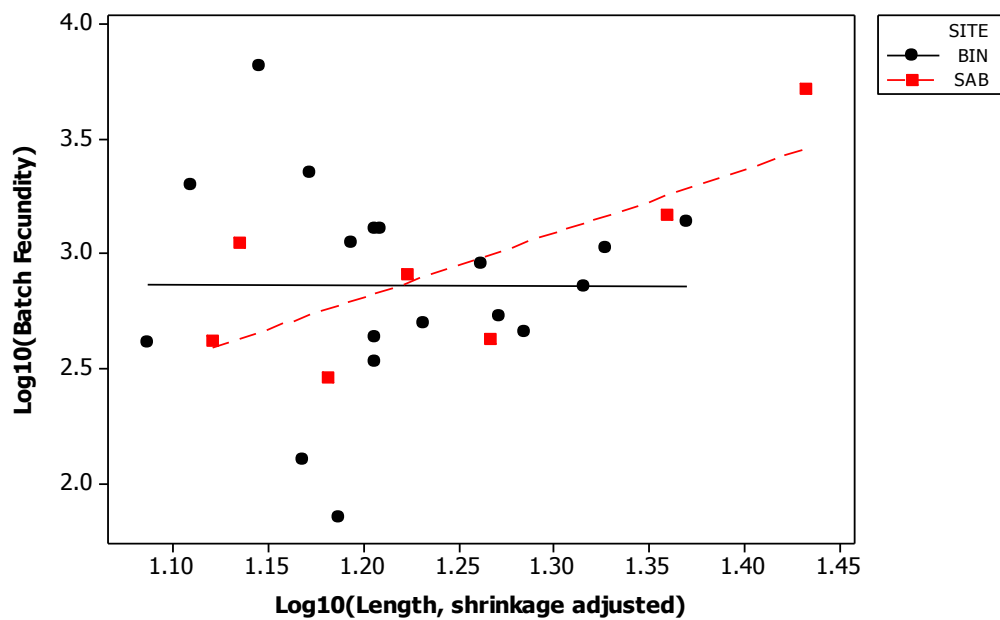
Figure 6. Fecundity versus length for females in the Binaba (BIN) and Sabare (SAB) populations
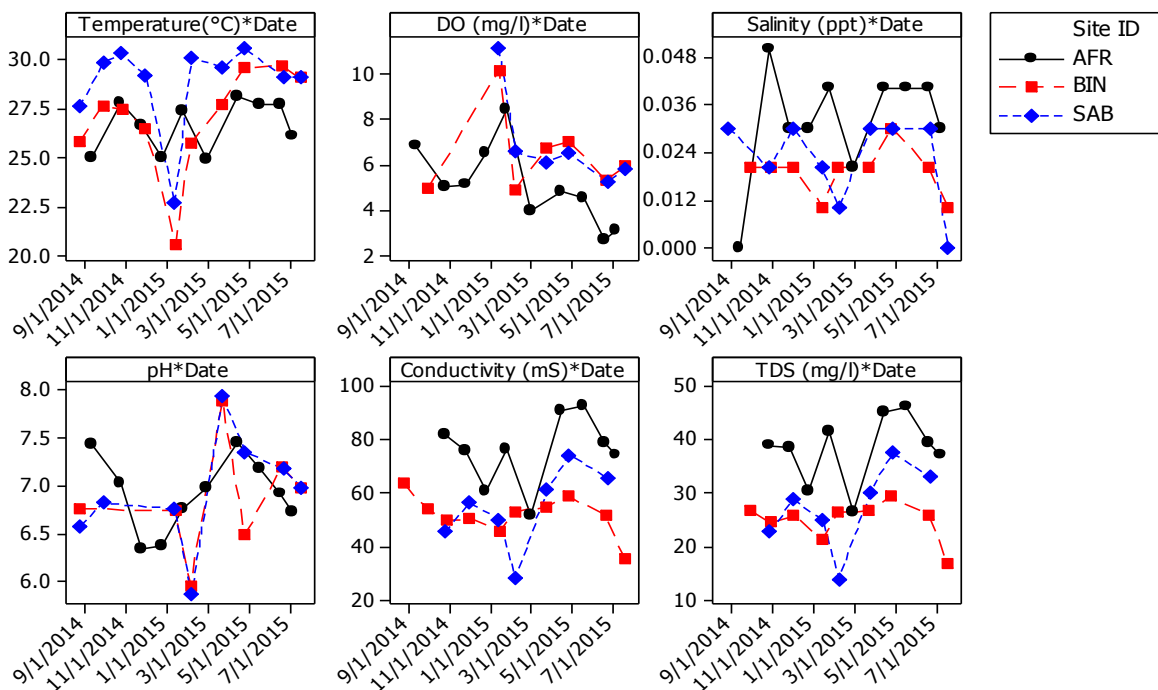
Figure 7. Water-quality variables measured in the Volta system on a monthly basis between September 2014 and July 2015

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Genetic characterization of populations. The results showed that all five microsatellite DNA marker loci screened for Nile tilapia screened to be polymorphic. We found a high allelic diversity (mean $\pm$SD of 9.4$\pm$4.9 alleles per locus per population; Figure 8) and high-expected heterozygosity (0.73 $\pm$0.17). Results of AMOVA showed 93.3% of genetic variance within populations, 2.25% among populations within regions, and 4.47% among regional groupings of populations, a result typical for natural populations. Regional patterns of regional differentiation visualized using classical F_{ST} analysis (Wright 1965) showed considerable differentiation among the Afram and White Volta ($F_{ST} = 0.049$), Afram and Oti ($F_{ST} = 0.027$) and White Volta and Oti ($F_{ST} = 0.054$) populations. At the population level, the widely cultured Akosombo strain was well differentiated from the Afram ($F_{ST} = 0.082$), White Volta ($F_{ST} = 0.080$), and Oti ($F_{ST} = 0.118$) populations. See Appendix D for additional results.
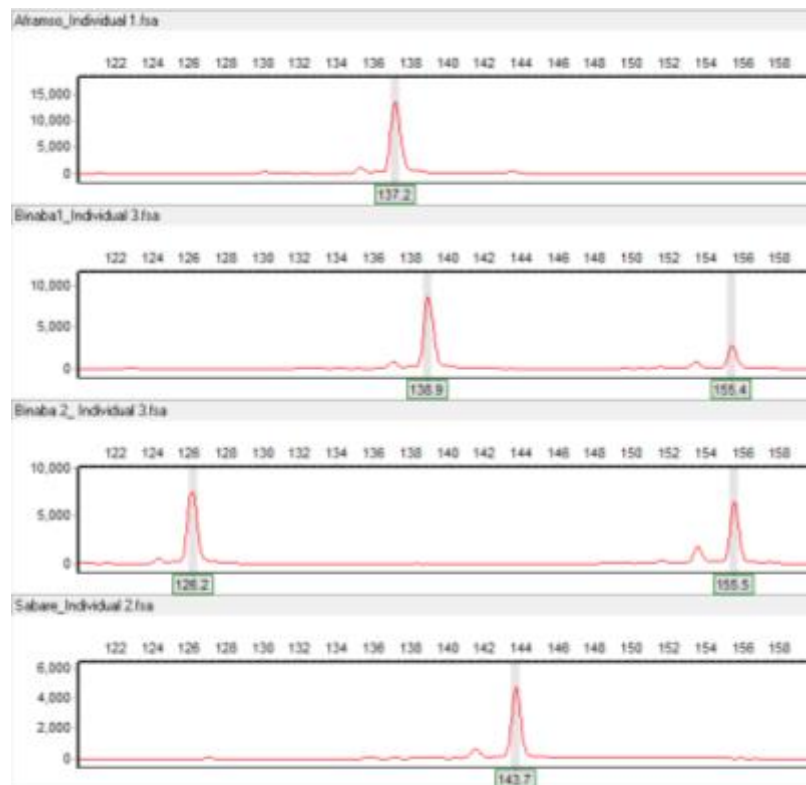
Figure 8. Output screen from Genemarker, used to interpret DNA fragment analysis results. The output above depicts allelic variation in four individuals from four populations at the UNH180 locus. Individuals with a single peak exhibit homozygosity at the locus, whereas two peaks suggest heterozygosity. The number of unique peaks are the different alleles at this locus.

Modeled distribution of *O. niloticus*. Of the 22 variables used in the MaxEent model, the important predictors of *O. niloticus* occurrence in West Africa were stream order, temperature seasonality, annual precipitation, and precipitation of the warmest quarter of the year (Figures 9 to 12); however, the raw temperature measurements by themselves were not important in predicting the species' occurrence. Altitude and population density had a negative relationship with *O. niloticus* occurrence. The resulting predictive model had a good performance, exhibited by a high area under the curve (AUC = 0.87). The model suggests that *O. niloticus* has a northerly distribution in West Africa (Figure 13), with highest probability of presence (0.4–0.91) occurring in the transition zones of the Sahel and savanna areas and furthest from the coastal and tropical forest areas. See Appendix E for additional results.
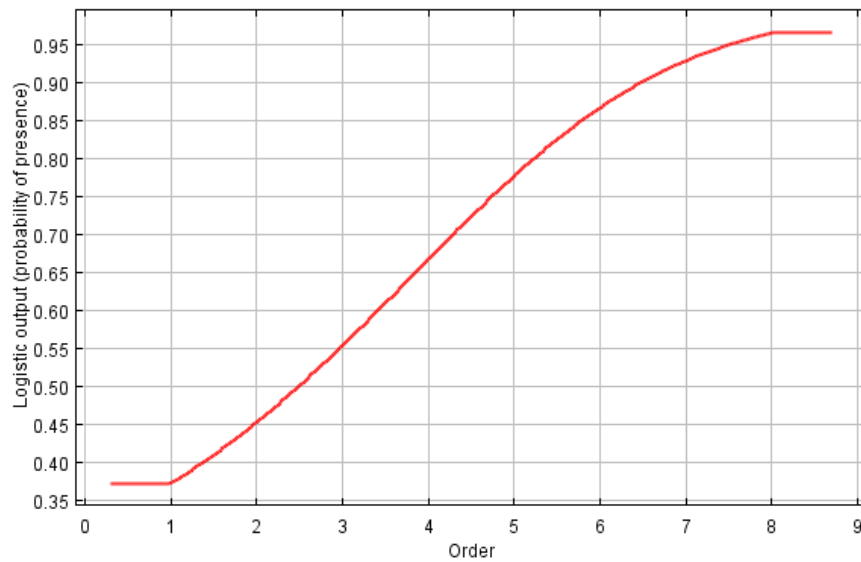
Figure 9. Response of *O. niloticus* to stream order in West Africa using raw variables uncorrected for correlation with other variables. Stream order was calculated by the Strahler method and used 250 km^2 watersheds as first order, which is an underestimation. Partial dependence plots for all variables can be found in Appendix E.
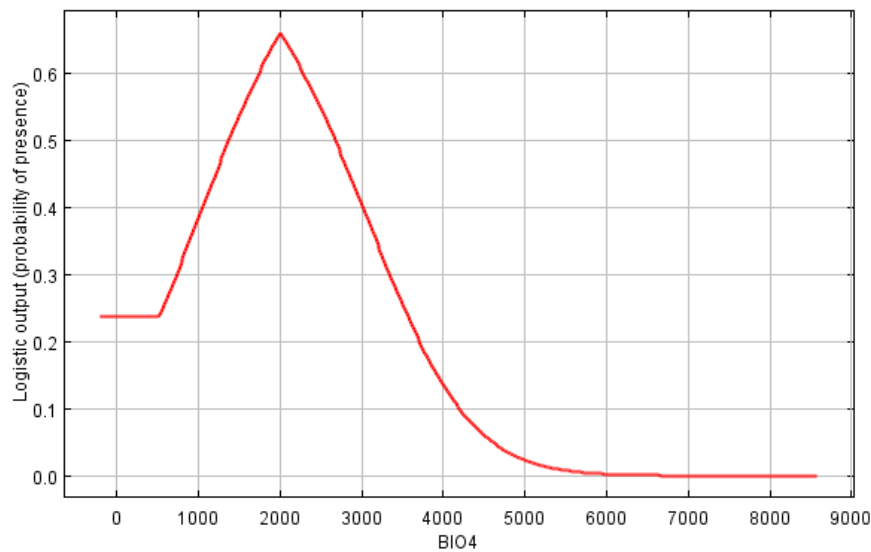
Figure 10. Response of *O. niloticus* to temperature seasonality in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.
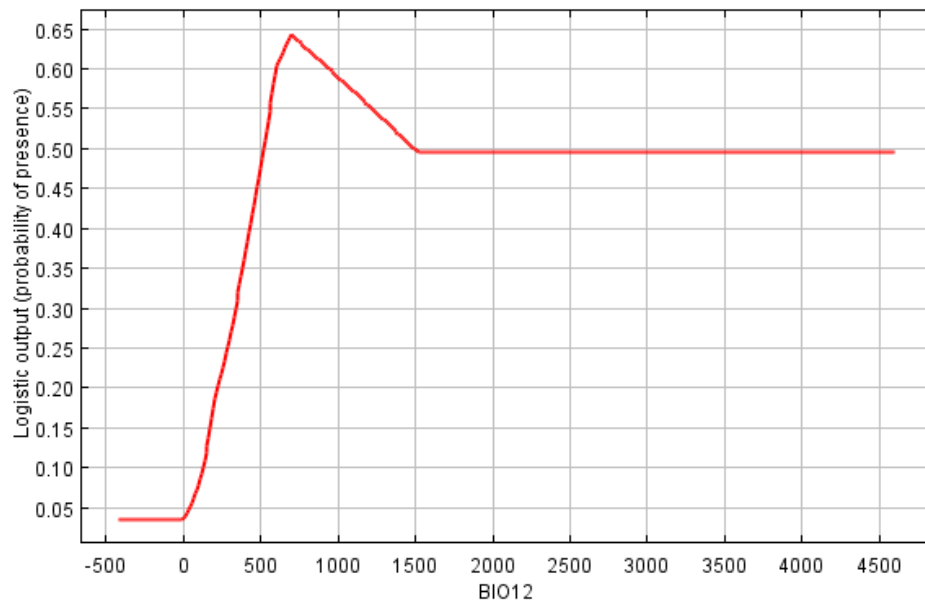
Figure 11. Response of *O. niloticus* to annual precipitation in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.
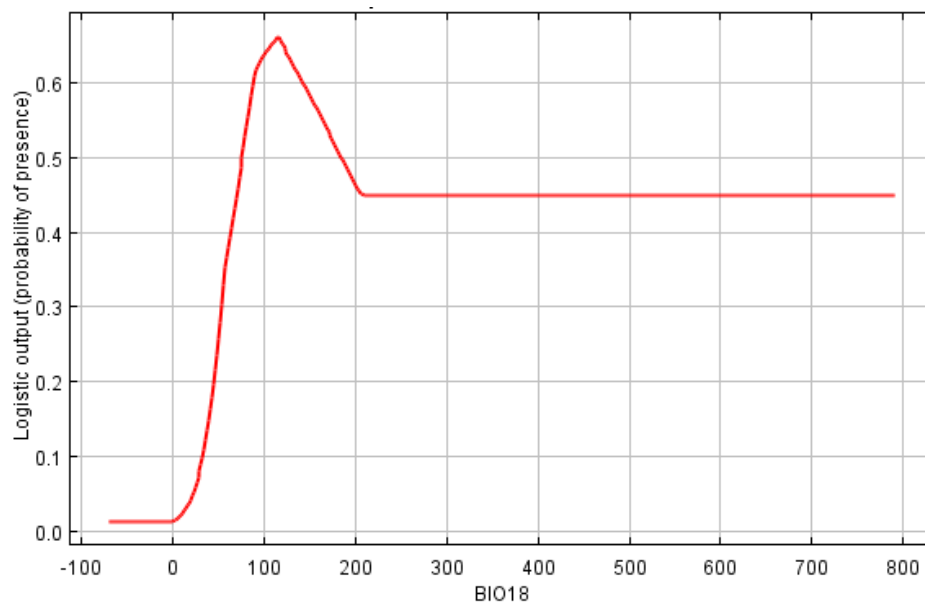
Figure 12. Response of *O. niloticus* to precipitation of warmest quarter in West Africa using raw variables uncorrected for correlation with other variables. Partial dependence plots for all variables can be found in Appendix E.
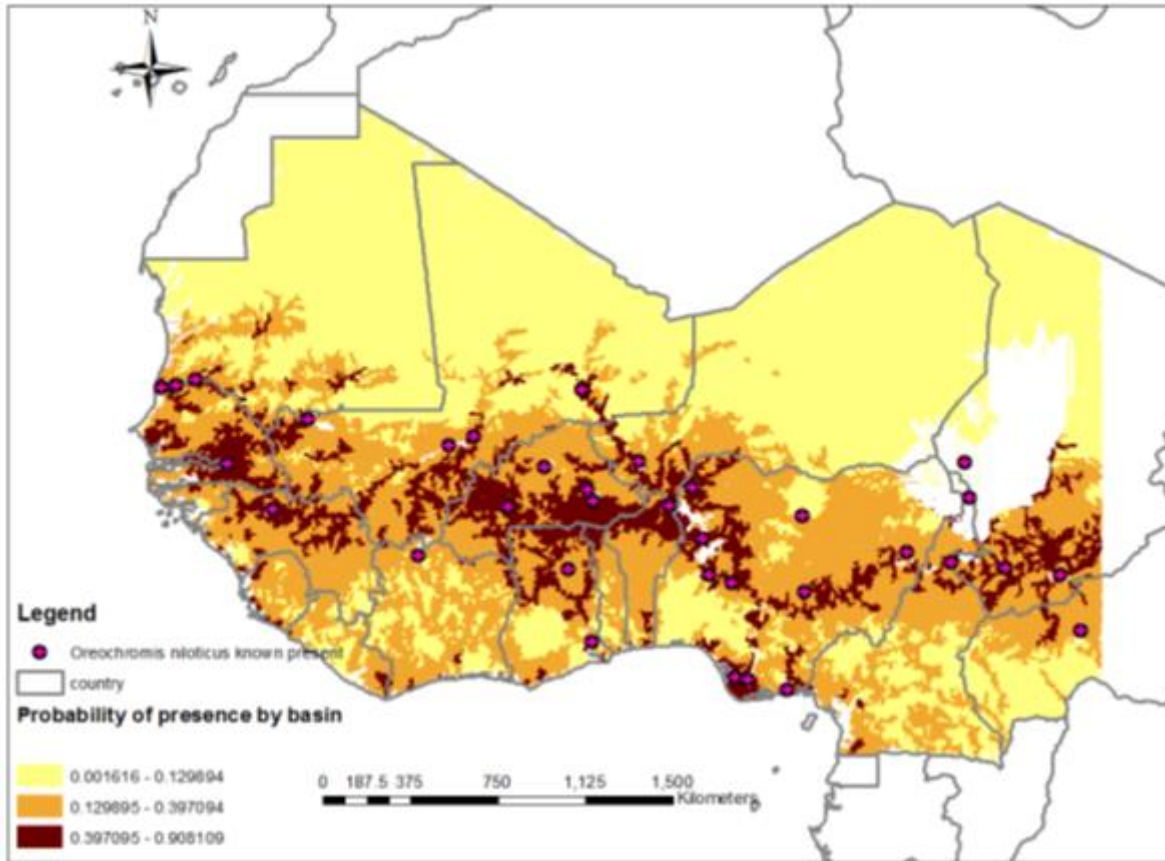
Figure 13. Predicted distribution map for *O. niloticus* in the West African subregion. Map is based on presence-only maximum entropy (MaxEnt) model based on data from Paugy et al. 2003. Brown areas have the highest probability of occurrence.

DISCUSSION

The most significant results of the meta-analysis are that the optimal temperature for growth of *O. niloticus* is about 29ºC, that growth increases linearly with DO, and salinity per se below the upper lethal limit of 32 ppt has no effect on Nile tilapia growth performance. The number of peer-reviewed studies that were available and suitable for this analysis was small and skewed toward North Africa and Asia, making the results potentially less applicable to West Africa. The DO range in the studies was limited as was the number of studies. In the context of the experimental studies section of the current report, the lack of relationship of salinity with SGR points to dependencies of growth on many additional factors, some of which were clearly not included in the meta-analysis because of limited sample sizes. In addition, the lack of studies considering interactions among temperature, DO, and salinity limit the utility of most of the studies for drawing conclusions about the optima of any variable because factor interactions appear to important. In other words, the optimum level of any factor can best be determined at the settings specified for other important factors.

The significant positive linear relationship between fecundity and size of fish observed in the Sabare population was expected. Large fish usually produce relatively more eggs and egg size is often a function of body size. A number of factors could contribute to the pattern observed between fecundity and fish size for the Binaba population. Due to variability in the monthly samples, the number of matured females across seasons was small. Even though we collected significant number of individuals for laboratory

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analysis (n > 250), the size frequencies and the number of eggs per matured females suggested that we sampled more fish during the off peak reproduction season. It is also possible that we collected older females who had fewer eggs due to previous spawning episodes, as well as younger females who had just began spawning and had more eggs. Batch or serial spawning in *O. niloticus* is evidenced by the collection of matured females most months of the year. Thus, our estimate for fecundity is at best, batch fecundity, in most cases, which will tend to have a weak relationship with size. The similar length-weight relationships suggest that there is no significant divergence of morphology among the populations of *O. niloticus* in the Volta basin. The biased sex ratio toward females and the potential differences in size at maturity is worth further investigating with larger sample sizes and more targeted seasonal sampling. For *O. niloticus* in ponds, delayed maturation is a desirable trait.

Our first hypothesis for the laboratory experiment was that the Akosombo strain of *O. niloticus* grows faster under current climate conditions and will be less tolerant of expected future climate conditions: high temperature, low DO, and high salinity. We expected to see the Akosomso strain exhibit significantly high SGR under low temperature, high dissolved oxygen and low salinity conditions. However, no significant differences were observed between the SGR of the Akosombo strain under the low and high conditions of any of the three factors tested. These results suggest that the Akosombo strain may not necessarily be a faster growing strain even under the current climate condition, compared to the wild strains. This is an argument the fish farming community in Ghana continues to make— that the selective breeding of Nile tilapia has not yielded any improvement over the wild strain in terms of growth. On the other hand, the laboratory conditions under which growth was observed could be less than ideal for the selectively bred strain compared to the pond environment to which it has been optimized. Further investigation of the real growth performance differences between the Akosombo strain and its wild counterparts is warranted.

The growth experiment also showed temperature as an important factor differentiating the strains, partly supporting our second hypothesis that northern populations of *O. niloticus* in West Africa are more tolerant of high temperature, low DO, and high salinity than southern populations. The northern populations (Binaba and Sabare) of *O. niloticus* population in Binaba were either more tolerant or insensitive to high temperature conditions, while the southern population (Aframso) was less tolerant to high temperatures. However, the high variability in growth observed in the Aframso strain, which we attribute to small sample size caused by high mortalities during the growth experiment, precludes definitive conclusions about the Aframso population. The fact that the Akosombo strain appeared to be slightly tolerant of high temperature conditions may be attributed to the strain's adaptation to culture conditions over the many years in use in aquaculture in Ghana. More importantly, the result of the growth experiment also suggests that the Sabare strain, though wild, has a slightly better performance than the Akosombo strain even under the experimental conditions. The significantly high water temperatures recorded in Sabare in the field studies, coupled with the Sabare population's temperature insensitivity in the growth studies, suggest that the strain may perform better than the Akosombo strain under stressful conditions such as high temperatures and low dissolved oxygen. The fact that any group does better than or as well as the Akosombo strain under any of the culture conditions is a reason to continue to investigate and Akosombo breed for more tolerant and faster growing strains for Ghana fish farmers.

The results from the genetic characterization showing all five gene loci screened as polymorphic indicates that multiple alleles are responsible for growth both within and among different tilapia populations in Ghana. The high allelic diversity within and among populations is indicative that these populations have a good evolutionary potential and are currently not bottlenecked or at risk of immediate extinction. At the regional level, genetic differentiation observed among the Afram (Aframso) and White Volta (Binaba; $F_{ST} = 0.049$), Afram and Oti (Sabare; $F_{ST} = 0.027$) and White Volta and Oti ($F_{ST} = 0.054$) populations suggest that whereas both the Oti and Afram populations are quite different from the White Volta

population; the Oti and Afram populations are more similar in their genetic make-up. The level of differentiation observed between the Afram and Oti population is interesting and requires further investigation, considering that only the Afram River originates in Ghana, while both the White Volta and Oti have their sources in Burkina Faso. Perhaps, screening additional loci would help clarify the genetic differentiation between the Afram and Oti. The Akosombo strain was well differentiated from Afram (F_{ST} = 0.082), White Volta (F_{ST} = 0.080), and Oti (F_{ST} = 0.118) populations, which confirms that the Akosombo strain was probably developed from crosses involving several different strains from the Volta system. Further studies are required to ascertain this and to investigate the genetic integrity of the broodstock generally referred to as the "Akosombo" strain marketed by hatchery operators in Ghana. The apparent differentiation of the Akosombo strain from the other study strains, coupled with the results from the growth studies, warrant future heritability studies involving the Akosombo strain and the Sabare strain with a goal of developing a high performing strain adapted to stressful future climatic conditions.

The distribution model for *O. niloticus* did not identify maximum temperature as independently important in delineating the northern limit of the species' range in West Africa. The lack of true absence data limits the applicability of a distribution model as, by definition, presence-only models overestimate distribution range. Future extensions of distribution modeling of *O. niloticus* would benefit from inclusion of reliable absences and more samples from smaller water bodies which are probably undersampled by the Paugy et al. (2003) collections derived mostly from fishing (large river, reservoir, lake) data.

CONCLUSION

This study used a multifaceted approach to study the Nile tilapia populations of the Volta basin and the eighth generation of the selectively bred Akosombo strain from the basin used in fish farming in Ghana. We found no evidence of superior performance of the selected strain over the wild strains under current or predicted future climatic conditions of temperature, DO, and salinity. At least one wild population from the Oti River may possess the traits for superior performance under high temperature and low DO conditions. Further studies should concentrate on comparing the Sabare strain with the Akosombo strain under both lab and pond conditions and increase experimental replications to confirm the suggested differences and the heritability of those performance traits for selective breeding.

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APPENDICES

Appendix A. List of papers used in the meta-analysis, in EndNote library.

Appendix B. Additional results for lab experiments.

Main Effects Plot for LogSGR
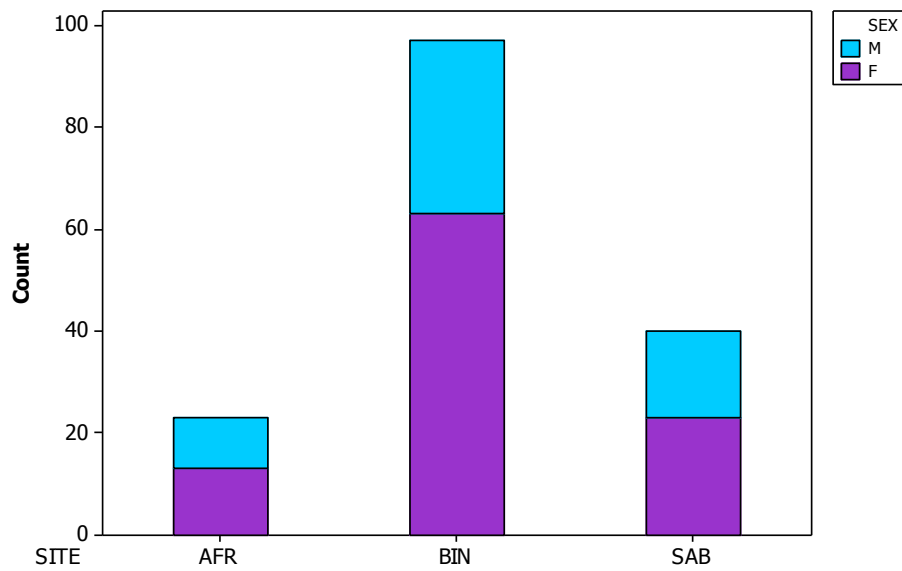Fitted Means
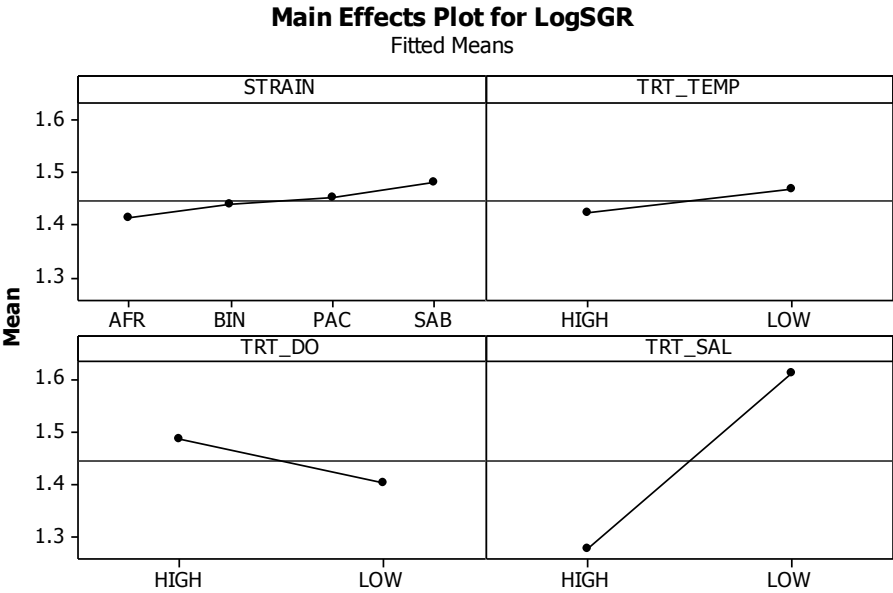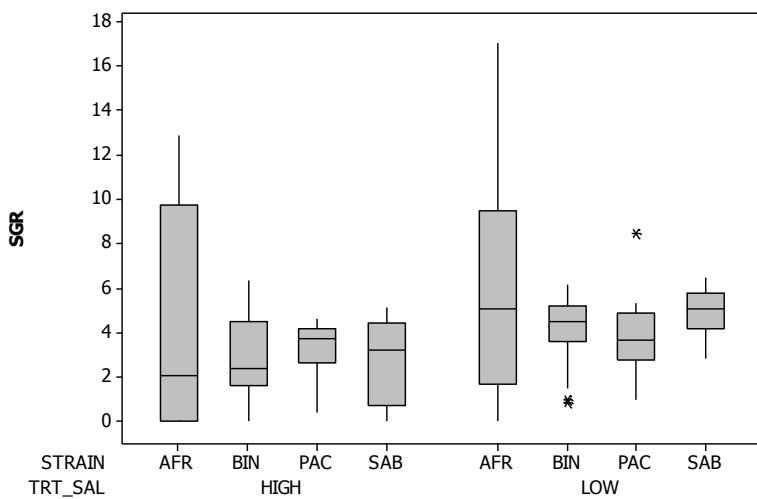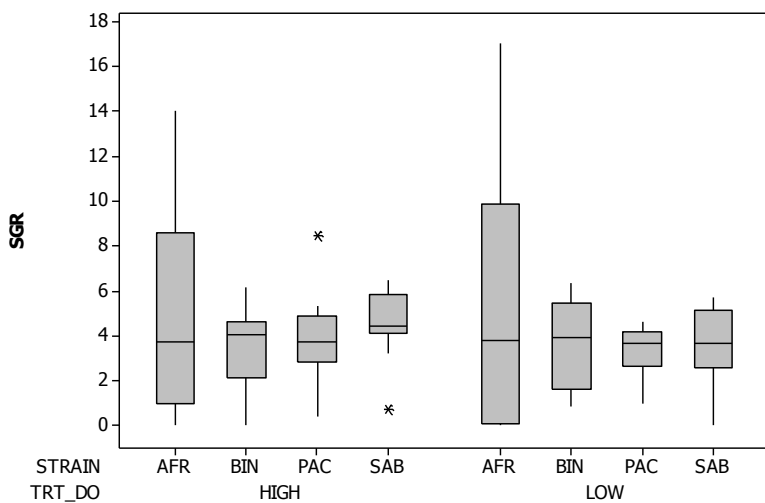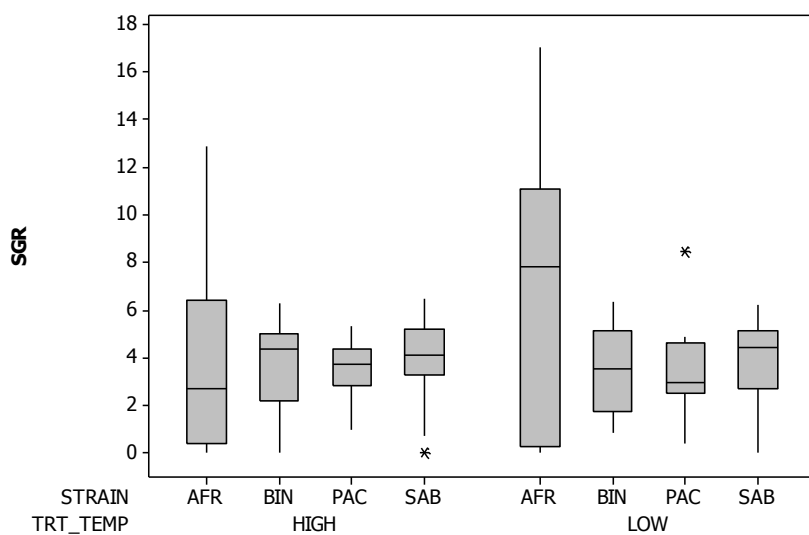
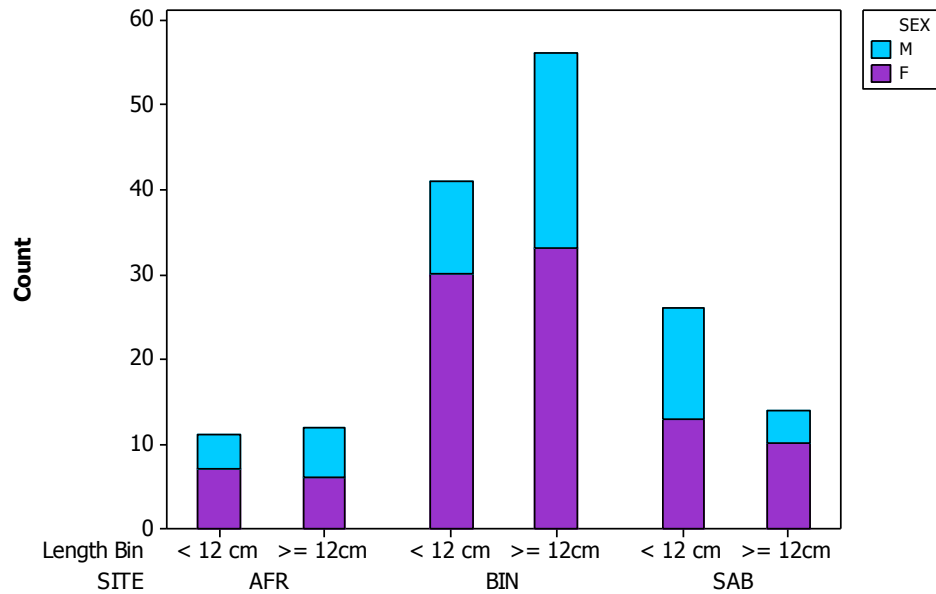**Appendix C.** Additional life-history results.

(i). Length distribution of preserved specimen sexually identified, N = 160
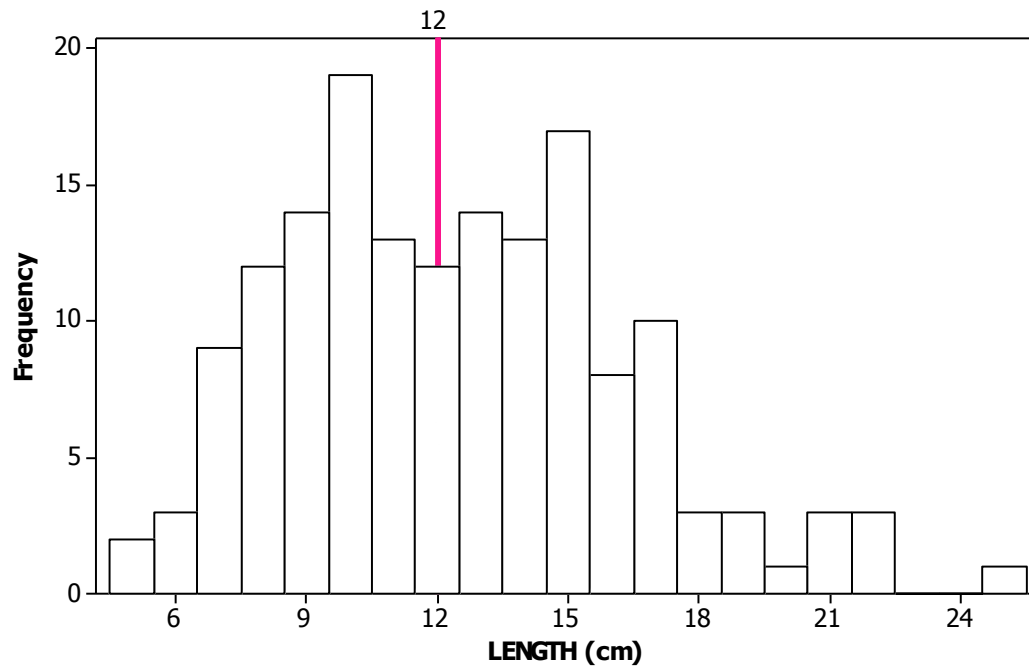
(ii.) Sex Ratios

(iii.) Sex ratio by site & size

Appendix D. Additional genetics results

(i). Numbers of alleles per locus per population.

```
----------------------------------------------------------------------
---------
 Locus     Afram    W. Volta   Akosombo      Oti        Mean
s.d.
----------------------------------------------------------------------
---------
UNH130        5         4          5          4        4.500
0.577
UNH180        7         6          7          9        7.250
1.258
UNH858       15        20         11         15       15.250
3.686
UNH925       14        16         11         14       13.750
2.062
UNH934        8         8          4          6        6.500
1.915
----------------------------------------------------------------------
---------
  Mean     9.800    10.800      7.600      9.600      9.450
1.340
   s.d.    4.438     6.870      3.286      4.827      4.856
1.494
```

(ii). Expected heterozygosity per locus per population.

```
--------------------------------------------------------------------------
 Locus      Afram    W. Volta   Akosombo        Oti         Mean
s.d.
--------------------------------------------------------------------------
UNH130     0.65706    0.67541    0.58701     0.60254      0.63051
0.04241
UNH180     0.70960    0.61263    0.74802     0.72429      0.69864
0.05948
UNH858     0.92316    0.93598    0.90226     0.86497      0.90659
0.03103
UNH925     0.89353    0.86072    0.87175     0.86025      0.87156
0.01558
UNH934     0.73277    0.60046    0.21751     0.57401      0.53119
0.22035
--------------------------------------------------------------------------
 Mean      0.78323    0.73704    0.66531     0.72521      0.72770
0.04854
  s.d.     0.11793    0.15232    0.27940     0.13755      0.17180
0.07310
--------------------------------------------------------------------------
```

(iii). Analysis of Molecular Variance (AMOVA) design and results.

Source of variation	d.f.	Sum of squares	Variance components[1]	Percentage of variation
Among groups	3	32.589	0.08574 V[a]	4.47
Among populations within groups	1	4.682	0.04305 V[b]	2.25
Among individuals within populations	144	308.555	0.35493 V[c]	18.52
Within individuals	149	213.500	1.43289 V[d]	74.76
Total	297	559.326	1.91660	

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(iv). Population[1] pairwise F_{ST} values.

	1	2	3	4
1	0.00000			
2	0.04893	0.00000		
3	0.08288	0.07963	0.00000	
4	0.02682	0.05364	0.11770	0.00000

1 Populations: 1-Afram River, 2-White
 Volta River, 3-Akosombo strain, 4-Oti
  River.
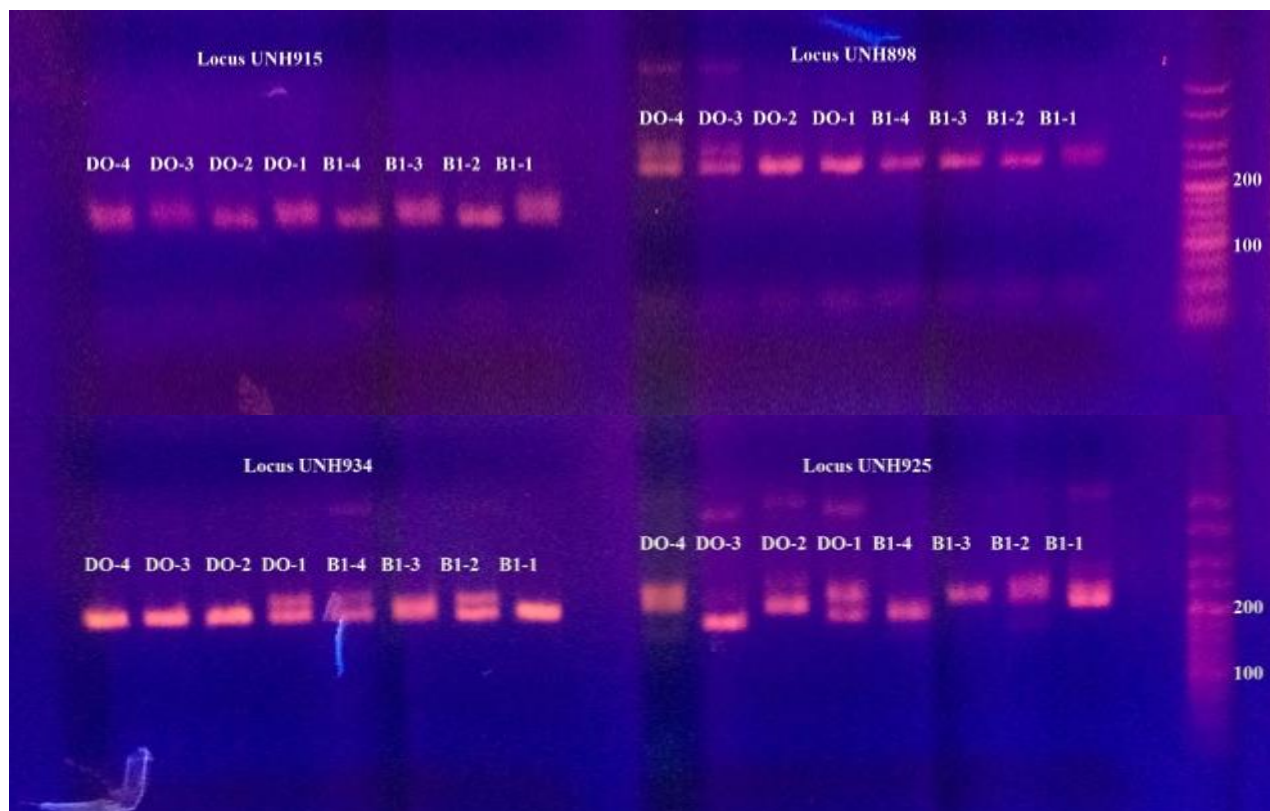
PCR products of individuals from Akosombo (DO) and Binaba (B1) populations at four microsatellite loci. Product size (base pairs) averaged around 200 bp; labels were added to ladder (right) for reference. After initial testing, loci UNH898 and UNH915 showed little to no variation and were excluded from future analyses. Both UNH925 and UNH934 exhibited moderate variation and were selected for DNA fragment analysis.

Appendix E. Additional methods and results of the Nile tilapia distribution models.

Definition of bioclimatic variables used in the models

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (BIO2/BIO7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

Additional plots and results from the MaxEnt tilapia distribution model
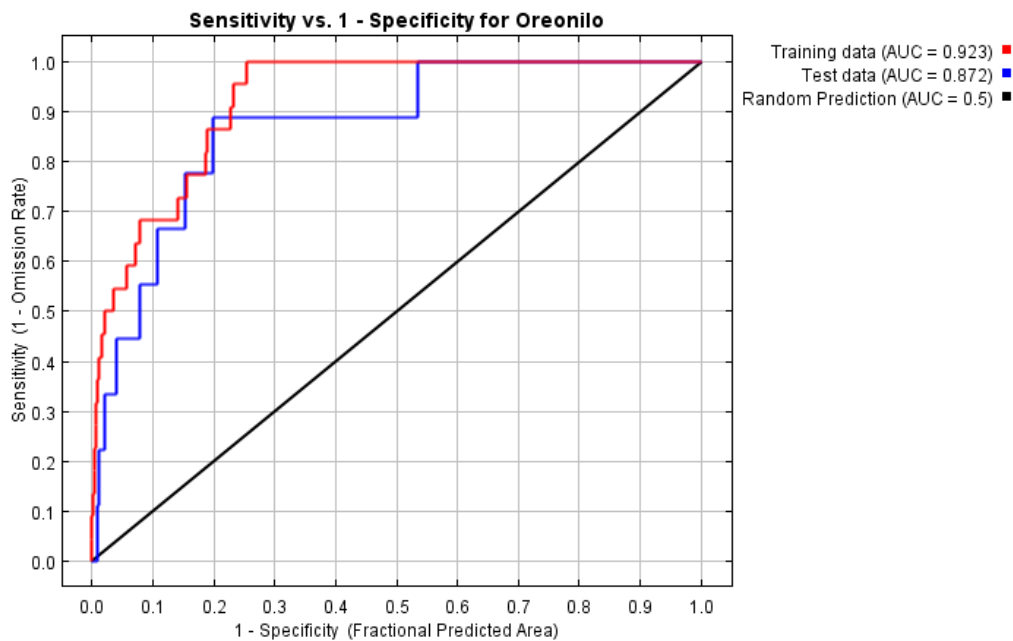


Analysis of variable contributions
The following table gives estimates of relative contributions of the environmental variables to the Maxent model. To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable, or subtracted from it if the change to the absolute value of lambda is negative. For the second estimate, for each environmental variable in turn, the values of that variable on training

presence and background data are randomly permuted. The model is re-evaluated on the permuted data, and the resulting drop in training AUC is shown in the table, normalized to percentages. As with the variable jackknife, variable contributions should be interpreted with caution when the predictor variables are correlated.

Variable	Percent contribution	Permutation importance
Order	48.9	18
BIO12	19.3	20.5
BIO18	14.9	37.3
BIO14	3.7	0.1
BIO11	3.3	0
Altitude	3	5.1
BIO4	2.1	14.1
Population	2	0
BIO19	1.1	0
BIO17	0.6	0
BIO1	0.6	2.3
BIO7	0.5	2.3
BIO2	0.1	0.2
BIO16	0	0
BIO9	0	0.2
BIO3	0	0
BIO5	0	0
BIO15	0	0
BIO6	0	0
BIO13	0	0
BIO8	0	0
BIO10	0	0

Partial response curves

These curves show how each environmental variable affects the MaxEnt prediction. The curves show how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. Note that these represent 'partial' or 'marginal' plots correcting for the effect of all other variables in the model. In other words, the curves show the marginal effect of changing exactly one variable, whereas the model may take advantage of sets of variables changing together.
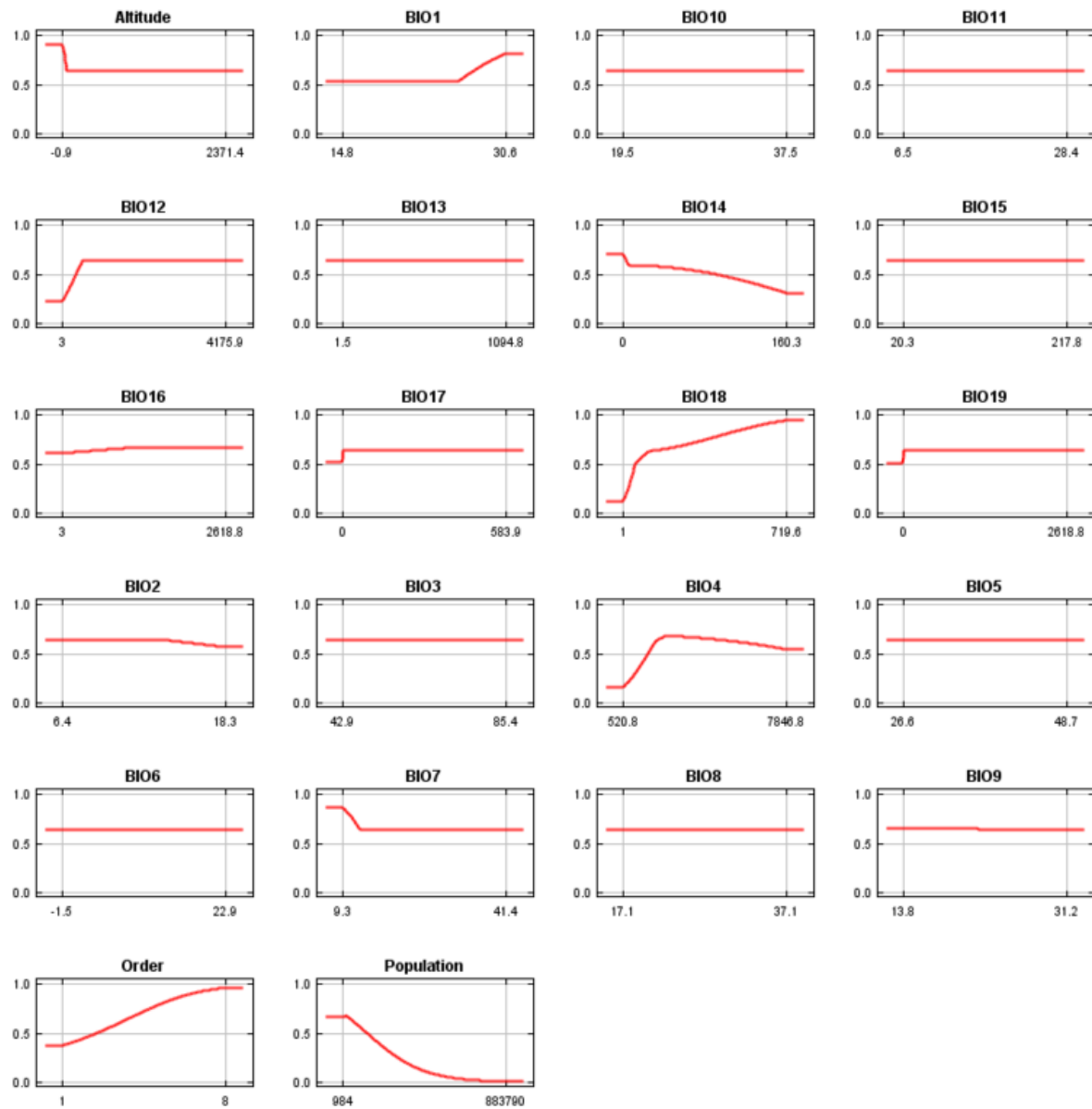
Sustainable Snakehead Aquaculture Development in the Lower Mekong River Basin of Cambodia

Indigenous Species Development/Experiment/ 13IND02UC

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ABSTRACT

Farming snakehead is prohibited in Cambodia due to its dependence on freshwater small-sized fish (FSF) for sourcing key dietary nutrient inputs and seed collected from the wild, while lack of technologies on developing of snakehead hatcheries through breeding, weaning, and grow-out on formulated or pelleted diets. This study was conducted to investigate weaning and grow-out performance of the wild indigenous *Channa striata* (non-domesticated) in Cambodia compared to those of domesticated snakehead imported from Vietnamese hatcheries on formulated or pelleted feed (FF or PF) and to assess economic efficiency and product quality of the two types of snakehead fed on different diets at the end of experimental grow-out. In the experiment 1 (weaning): three day-old larvae of both types of both *C. striata* were stocked in 50 L-tank at a density of five fish L-1 and fed on Moina, FSF, and FF (45% CP) to satiation four times daily for 45 days. In experiment two (grow-out): the experiment was conducted in 18 hapa-nets (1.8m x 2.5 m x 1.8 m) placed in three earthen ponds (300 m^2 each) at a density of 100 fingerlings hapa-1 (three replicated hapas for domesticated fingerling and three replicated for non-domesticated). Snakehead fingerlings (12-13 g fish-1) were fed on three diets: 1) FSF (Pond 1); 2) PF (40% CP, Pond 2); and 3) 50:50 mixtures of FSF and PF (Mix, Pond 3). The fish was fed to satiation twice daily for six months. The results of the study showed that weaning of non-domesticated and domesticated *C. striata* larvae on FF can start at 17 days after hatch with replacement ratio 10% FF day-1 for substituting FSF. Feed intake (107 mg fish-1 day-1) and final weight (170 mg) of domesticated snakehead was higher than the ones (85 mg fish-1 day-1 and 146 mg, respectively) of non-domesticated snakehead, while survival rate (29%) and cannibalistic rate (47%) of the domesticated was lower than the ones (36% and 51%, respectively) of the non-domesticated. In grow-out experiment, both snakeheads can accept formulated or pelleted feed. However, the domesticated snakehead showed higher survival rate (75%), better growth performance (final body weight 367 g fish-1), higher feed intake (3 g fish-1 day-1), and food conversion ratio (FCR; 1.5) than the non-domesticated snakehead (69% and 233 g fish-1, 2 g fish-1 day-1 and 1.7, respectively) since the domesticated hatchery snakehead has gone through more than two-decade domestication. Considering economic efficiency, replacing freshwater small-sized fish by pelleted feed up to 100% is possible and profitable for both snakeheads. However, the domesticated snakehead (about US$ 0.35/kg fish produced) showed higher profit than the non-domesticated snakehead (US$ 0.25/kg fish produced). In regards to product quality, pelleted feed does not significantly affect the fillet quality of both cultured snakeheads compared to a diet of FSF and a mixture.

INTRODUCTION

In Cambodia wild snakeheads are generally cultured in smaller cages and ponds. Feed represents more than 70% of the total operational cost and the main type of feed for wild snakehead culture is small-sized or low -value fish, representing 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers (So et al. 2005). During the dry season (October to May), the most important source of feed is freshwater small-sized or low-value fish, while more marine small-sized or low-value marine fish species are used during the rainy season (June to September) (So et al. 2005). Importantly, the snakehead production contributes more than 70% of total aquaculture production in Cambodia due to its popularity as food and high market and trade demand in Cambodia as well as in Vietnam, being found in most Cambodian and Vietnamese dishes at all wealth class levels (i.e. from poor, medium, to rich people). During the first phase of AquaFish CRSP (2007-2009), the Investigation # 2 revealed that nearly 200 freshwater small-sized fish species were detected in the Mekong River Basin of Cambodia and Vietnam, and these freshwater small-sized fish species, including juveniles of commercially important fish species, contribute more than 70% to total freshwater capture fisheries production.

The government of Cambodia put a ban on snakehead farming in September 2004 by the Announcement No. 4004 kor.sor.ko.sor.chor.nor. The reason for this was the potential negative impacts on wild fish populations from wasteful snakehead seed collection and on other fish species diversity, particularly the small-sized fish used as feed for snakehead aquaculture, and also potential negative effects on poor consumer groups from decreased availability of small-sized or low-value fish due to dependency of snakehead aquaculture on small-sized or low-value fish (So et al. 2007).

After the ban on snakehead culture in Cambodia, snakeheads have illegally been imported from the neighboring countries, particularly from Vietnam, to supply high local market demands in Cambodia. Furthermore, the study showed that freshwater small-sized fish have illegally been exported to Vietnam for feeding the significantly and commercially developed snakehead aquaculture in Vietnam. The first phase study also indicated that the incentives for choosing snakehead before other fish species by tens of thousands of fish farmers are strong as it generates more than 10 times higher profits than other fish species. Therefore, the ban does not only result in positive impacts on poor consumer groups from increased availability of freshwater small-sized fish in Cambodia, but also providing negative effects on food and nutrition security and livelihood of tens of thousands of snakehead farmers who depend on this livelihood for improving household food and nutrition security and generating household income. In other words, these snakehead fish farmers have lost their important livelihoods and household income. Moreover, the ban also does not provide positive impacts on snakehead wild stocks as fishing pressure on wild snakehead using illegal and destructive fishing gears particularly electro-shockers has increased in recent years in order to supply local and external markets.

In order to remove this ban, the same announcement mentioned that successful technologies of domesticated breeding, weaning and rearing or growing-out of snakeheads using formulated diets should be developed and applicable in on-station and on-farm levels in Cambodia.

During the second phase of AquaFish CRSP (2009-2011), the wild striped snakehead *Channa striata* broodstocks were successfully developed, matured, and semi-artificially induced spawning using the hormone HCG on-station in Cambodia was accomplished (So et al. 2011). The striped snakehead *Channa striata* aged 30 days old after hatch could gradually and successful accept AquaFish CRSP Snakehead Formulated Feed developed by AquaFish CRSP project (Hien & Bengtson, 2009; 2011) in replacement of small-sized fish in the rate of 10% every three days for a period of 30 days of feeding (So et al. 2011).

The objective of the study was focused on weaning performance and grow-out of the wild *C. striata* (non-domesticated) from Cambodia compared to those of domesticated snakehead from Vietnam with assessment of survival rate and growth performance of the two types of *Channa striata* larvae or fingerling on formulated feed. Furthermore, the assessment of economic efficiency and product quality

during grow-out of the both snakehead on different diets will also be conducted in a purpose to provide policy recommendations for snakehead farming in Cambodia.

To meet the objective, the experiments of weaning and grow-out of both snakeheads were conducted at Freshwater Aquaculture Research and Development Center (FARDeC). The experiments conducted were:

- Weaning on formulated feed as comparing the growth performance and survival of domesticated snakehead versus non-domesticated snakehead.
- Grow-out on three diets (pelleted feed, freshwater small-sized fish, and 50:50 mixture), as comparing the growth performance and survival of domesticated snakehead versus non-domesticated snakehead.
- Sensory analysis of snakehead products fed different diets

RESULTS
Experiment 1: Weaning performance of domesticated versus non-domesticated snakehead C. striata using formulated feed.

Introduction. Snakehead fish have been domesticated for almost two decades in Vietnam (So, 2009). In the past two decades, aquaculture of this domesticated snakehead fish has commonly been practiced by using freshwater and marine small-sized fish as directed feed. Farming snakehead is prohibited in Cambodia due to its dependence on small-sized fish in the diet and seed from the wild stock, while domestication of snakehead in the hatchery is just recently new. To address the key issues on using freshwater small-sized fish, Tran Thi Thu Hien and her colleagues at Can Tho University in Vietnam and University of Rhode Island, USA (Tran Thi Thu Hien and Bengtson, 2009) had successfully developed weaning methods and cost-effective and high-performing compounded feeds under laboratory and on-farm trial conditions that would allow less reliance on FSF and would have lower environmental impacts, the so-called AquaFish CRSP Snakehead Formulated Feed. Recently, aquaculture of snakehead has been shifted and wisely practiced by using the formulated or pelleted feeds in different cultured systems.

This study, therefore, was designed to wean non-domesticated snakehead *C. striata* (from wild stock through induced spawning) and domesticated snakehead using this formulated feed and weaning method to compare the growth performance and survival of the two snakehead strains.

Methods. A batch of *C. striata* larvae which was simultaneously produced from domesticated and non-domesticated snakehead brooders was used for weaning experiment at the same time using the same protocol (Hien and Bengtson 2009; 2011). After absorbing the yolk on day 3 after hatch, larvae were fed with *Moina* for 7 days till 10 day-old after hatch, and then larvae were fed with a mixture of dead *Moina* and ground freshwater small-sized fish, FSF (replacing Moina 20% per day by freshwater small-sized fish) for 7 days more till larvae were 17-day old after hatch. At 17-day old, weaning on formulated feed were started with replacement of freshwater small-sized fish 10% per day by formulated feed until FSF was completely substituted by formulated feed. Formulated feed contained 45% crude protein (Table 2.1; Figure 2.1).

There were two treatments with five replicated tanks each (Figure 2.2). Larvae were stocked in 50-L tanks with stocking density of five fish L^{-1}. The fish were fed to satiation by hand at 07:00, 10:00, 14:00 and 17:00 h. The uneaten feed and faeces were siphoned out before every feeding. Fish mortality, food consumption including water qualities, such as temperature, pH, and dissolved oxygen were recorded daily. Temperature ranged from 27.7-29 ℃, pH ranged from 7-8, DO ranged from 3-5 mg L^{-1}, NH_3 ranged from 0-0.1 mg L^{-1} and NO_2^- ranged 0 mg L^{-1}.

Larvae were weighted and measured (on wet basis) at biweekly intervals. At the end of the experiment, final body weight (FBW, mg), wet weight gain (WWG, mg), daily weight gain (DWG), specific growth rate (SGR) and feed intake (FI) including survival rate (SR) were determined. Weaning lasted 45 days. Difference between the treatments on growth and survival were determined by two sample t-test at p= 0.05 using SPSS 16.0.

Data calculation
SR (%) = (Numbers of fish at the end of experiment/ numbers of initial fish) x 100
WG (g) = Final body weight – Initial body weight
DWG (g fish^{-1} day^{-1}) = (Final body weight- Initial body weight)/ Experiment time
SGR (% of body weight day^{-1}) = ((ln final weight — ln initial weight)/ number of culture day) x100
FI = (Feed intake/no. fish)/No. days

Results and discussions. There was a significant difference observed between the treatments in growth performance, daily weight gain, feed intake and survival rate. Growth performance, daily weight gain, and feed intake in domesticated larvae were significantly higher ($p < 0.05$) than those in non-domesticated larvae (Table 2.2; Figure 2.3), while the survival rate was significantly lower ($p < 0.05$) in domesticated treatment (28.9%) than in the non-domesticated (35.7%) (Table 2.2). The cannibalism rate was not significant different between the treatments ($p > 0.05$; Table 2.2), while the mortality rate was significantly higher in the domesticated (24.5%) than in the non-domesticated (13%) (Table 2.2). Thus, domesticated snakehead are more adapted to formulated feed in term of growth and feed intake than non-domesticated snakehead. The survival of domesticated larvae was lower due to its higher mortality. The mortality might be caused due to satiation rate.

The survival rates were comparable to the start of weaning at 20-day old larvae (36.2%), but lower than fish weaned at 30-day old (68.9%) with replacement of small-sized fish 10% per day by formulated feed reported by (So Nam et al. 2011) and lower than on farm trial weaning (72.5-81.7%) at 6-7 g/fish initial weight reported by (Hien and Bengtson, 2011), however, it was higher than the weaning at 10-day old (15%) with 10% /day replacement reported by (Hien and Bengtson, 2009).

In this study, *C. striata* showed a high cannibalism (46% and 51% for domesticated and non-domesticated, respectively) in weaning to formulated feed. Hien et al. (2015) observed that *C. striata* is much more aggressive toward conspecifics in a tank. In fishes, cannibalism is usually associated with heterogeneous size variation, lack of food, high density, lack of refuge area, and light condition. Among these variables, size variation and unsuitable food are considered the primary causes of cannibalism (Hien and Bengtson, 2009).

Experiment 2: Grow-out performance of domesticated versus non-domesticated snakehead C. striata using pelleted feed, freshwater small-sized fish and 50:50 mixtures.

Introduction. Aquaculture of snakeheads in Cambodia is mainly dependent on freshwater small-sized fish (FSF) for sourcing key dietary nutrient inputs (So Nam et al. 2009; So Nam et al. 2005), and feeding cost is the highest cost for the fish farmers. The recent study by So Nam et al. (2009) revealed that more than 200 FSF species, with nearly 50,000 ton (accounting more than 10% of total freshwater fisheries production in Cambodia; So Nam et al. 2005) are used for aquaculture in Cambodia. Many problems are raised among many snakehead farms. The main problems are poor quality of FSF and variable nutritional composition because of inappropriate storage. Risk of disease introduction and out breaks, environmental pollution, and high feed conversion in snakehead rearing contributed more concerns. Moreover, the growing competition between human and aquaculture usage of FSF led to increasing its price to the fish famers (Le Xuan Sinh et al, 2009; So Nam et al. 2009; Rachmansyah et al. 2009; So Nam et al. 2007).

One key constraint and challenge to the culture of this species is the ban on snakehead culture by the government of Cambodia due to the lack of formulated diets (So Nam et al. 2009).

This experiment, therefore, was designed to grow non-domesticated snakehead (*C. striata*) and domesticated snakehead using pelleted feed, FSF, and 50:50 mixtures in order to compare the growth performance and survival of the both snakehead strains.

METHODS

A batch of the two types of snakehead fingerlings accepting formulated feed from the above weaning were used in grow-out experiments. The snakehead fingerlings were fed with three diets: 1) FSF; 2) pelleted feed (PF); 3) 50:50 mixture of FSF and PF (Mix). There were six treatments with 3 replicated-hapas each as follows:

- Domesticated fed freshwater small-sized fish (d-FSF)
- Nondomesticated fed freshwater small-sized fish (n-FSF)
- Domesticated fed pelleted feed (d-PF)
- Nondomesticated fed pelleted feed (n-PF)
- Domesticated fed 50:50 mixture (d-Mix)
- Nondomesticated fed 50:50 mixture (n-Mix)

The experiments were conducted in 18 hapa-nets (1.8m x 2.5 m x 1.8 m) with a stocking density of 100 fingerlings hapa^{-1}. The hapas were placed in three earthen ponds (300 m^2) with six hapas each assigned three replicated hapas for domesticated fingerling and three replicated hapas for non-domesticated (Figure 2.5). In pond one, snakehead fingerlings (12-13g fish^{-1}) were fed ground freshwater small-sized fish only (control); in pond two, snakehead (12-13g fish^{-1}) were fed commercial-snakehead pelleted feed (Super floating AquaFeed containing 40% crude protein) only ; and in pond three, snakehead (12-13g fish^{-1}) were fed 50:50 mixture of freshwater small-sized fish and the pelleted feed. The fish were fed to satiation twice daily at 09:00 h and 16:00 h. The amount of feed consumption and fish mortality were recorded daily. Water qualities include transparency, temperature, pH, dissolved oxygen, ammonia, nitrite were monitored weekly. Fish were weighted and measured monthly (wet basis) by sampling 30 fish per hapa. The grow-out lasted for six months. At the end of experiment, survival, final body weight (FBW, g), and wet weight gain (WWG, g), daily weight gain (DWG, g fish^{-1} day^{-1}), specific growth rate (SGR, % BW day^{-1}), feed intake (FI, g fish^{-1} day^{-1}), food conversion ratio (FCR) and survival rate (SR, %) were determined. Economic efficiency and fish product quality of different diet treatments were also evaluated.

Treatment means of the above parameters were subjected to one-way analysis of variance (ANOVA) at a 5% significance level and Duncan's multiple-range test. The effects of snakehead strains, diets and their possible interactions on the growth parameters and survival were determined using two-way ANOVA at a 5% significance level. Statistical analyses were performed using the statistical package (SPSS 16.0).

Data calculation
SR (%) = (Numbers of fish at the end of experiment/ numbers of initial fish) x 100

WG (g) = Final body weight — Initial body weight

DWG (g fish^{-1} day^{-1}) = (Final body weight — Initial body weight)/ number of experimental day

SGR (% of body weight day^{-1}) = ((ln final weight — ln initial weight)/ number of culture day) x100

FI (g fish^{-1} day^{-1}) = (Feed intake/no. fish)/ number of experimental day

FCR= Feed intake/ Weight gain

Profit (KHR; Khmer Riel) = Total income — total cost

Sensory test of fillets (see below)

RESULTS AND DISCUSSION

Water quality parameters. Water quality parameters are presented in Table 2.3. The average concentrations of the different water quality parameters remained within the safe limits for snakehead growth.

Effect of snakehead strain and diet on growth performance. The data on growth performance, feed intake, food conversion, and survival are presented in Table 2.4. At the end of the experiment (six months), the growth parameters were significantly affected by strain and diet. The FBW, WG, DWG, and SGR were significantly greater in the domesticated strain than the non-domesticated in all diet treatments (Table 2.4 and 2.5). In both strains, freshwater small-sized fish treatments were significantly lower than pelleted feed and mixture treatments, while both treatments were not significant difference (Table 2.4; 2.6). The d-PF showed the highest value for FBW (366.7 ± 8.8 g), WG (353.2 ± 8.8 g) and DWG (2.0 ± 0.0 g fish^{-1} day^{-1}), followed by the d-Mix treatment (346.7 ± 8.8 g; 333.2 ± 8.8 g; and 1.9 ± 0.0 g fish^{-1} day^{-1}) respectively). The lowest value was showed in the n-FSF treatment (210.0 ± 5.8 g**;** 196.5 ± 5.8 g; and 1.1 ± 0.0 g fish^{-1} day^{-1} respectively). In non-domesticated, the n-Mix showed the highest value (255.0 ± 2.9 g; 241.5 ± 2.9 g; and 1.3 ± 0.0 g fish^{-1} day^{-1} respectively), followed by the n-PF treatment (233.3 ± 3.3 g; 219.8 ± 3.3 g; and 1.2 ± 0.0 g fish^{-1} day^{-1} respectively) (Table 2.4). The highest SGR value was obtained in the d-PF and d-Mix (1.8 ± 0.0 % day^{-1}; 1.8 ± 0.0 % day^{-1} respectively); the lowest value in the n-FSF (1.5 ± 0.0 % day^{-1}); and intermediate in the n-PF, n-Mix, and d-FSF (1.6 ± 0.0% day^{-1}; 1.6 ± 0.0 % day^{-1}; and 1.7 ± 0.0 % day^{-1} respectively) (Table 2.4). Two-way ANOVA showed a significant interaction ($P < 0.05$) between strain and diet in FBW and WG.
In this study, the final body weight of the snakehead stains in the three diets showed a trend of linear increase with time (Figure 2.4).

Growth performance was significantly affected by strain and diet and their interaction. In general domesticated strain had significantly higher individual FBW, WG, DWG, and SGR than the existing non-domesticated strain. Pelleted feed and mixture treatments had significantly higher FBW, WG, DWG, and SGR than freshwater small-sized fish treatment. The results obtained in this study show FBWs and DWGs were lower than a range 391-403 g; 2.67-2.75 g fish^{-1} day^{-1} respectively for 12-13 g snakehead stocked in hapas using freshwater small-sized fish versus formulated feed respectively reported by Hien and Bengtson (2011), and lower than 562 g; 3.05 g fish^{-1} day^{-1} respectively for 2.5 g snakehead cultured in pond using commercial pellet feed at density 100 fingerlings m^{-2} for six months in demonstration farms in Vietnam reported by Hien and Bengtson (2011). However, FBWs of this study were higher than a range 136-199 g for snakehead stocked in hapas for four months in Dong Thap reported by Hien and Bengtson (2011). So Nam et al. (2011) reported FBW and DWG ranged from 313.5-467.9 g; 1.0-1.5 g fish^{-1} day^{-1} respectively for non-domesticated snakehead cultured on station in hapas fed formulated feed and freshwater small-sized fish respectively for 10 months.

Effect of snakehead strain and diet on feed intake, feed conversion, and survival. Feed intake (FI) and feed conversion ratio and production differed significantly among the six treatments. The highest FCR value was observed in the n- FSF (4.2 ± 0.1), followed by the d-FSF (3.7 ± 0.0) treatment. The best (lowest) FCR was observed in the d-PF (1.5 ± 0.0), followed by the n-PF (1.7 ± 0.1). The n-Mix and the d-Mix showed intermediate values (Table 2.4). The highest FI value was observed in the d-FSF (6.0 ± 0.1 g fish^{-1} day^{-1}), followed by the n-FSF (4.6 ± 0.2 g fish^{-1} day^{-1}) and the d-Mix (4.5 ± 0.1 g fish^{-1} day^{-1})

treatments; both were comparable. The lowest FI value was observed in the n-PF (2.1 ± 0.1 g fish^{-1} day^{-1}). The n-Mix (3.8 ± 0.1 g fish^{-1} day^{-1}) and the d-PF (3.1 ± 0.1 g fish^{-1} day^{-1}) showed intermediate values (Table 2.4). Feed intake and feed conversion ratio pooled across diets were significantly higher in freshwater small-sized fish feeding (Table 2.6). However, data pooled across strains showed the FI and FCR to have no significant difference (Table 2.5). Survival was significantly different among treatments (Table 2.4). However, data pooled across stains and diets (Table 2.5 and 2.6) showed no significant differences. Survival ranged from 61.3 % to 77.5 %. Two-way ANOVA showed a significant interaction between strain and diet in FI, FCR, survival rate, and production.

In aquaculture, the cost of feed is a major component of the operating cost of fish farms. In Cambodia, snakehead feed cost accounts for more than 70% of the total expenses (So et al. 2005). Therefore, any improvement in FCR would have a positive impact in reducing the production cost. In this experiment, the d-PF and n-PF showed significantly lower FCR values than other treatments. The FCR range of 1.5-4.2 obtained in this study was comparable with the range of 1.4 - 4.4 reported by Hien and Bengtson (2011), and the range of 1.7 – 4.2 reported by So Nam et al. (2011) for formulated feed and freshwater small-sized fish feeding, respectively. In the present study, FCR was significantly affected by diet (Table 2.6), but not significantly affected by fish strain. Freshwater small-sized fish feeding showed higher FCR than other treatments, while lower FCR was in pelleted feed treatment. In this study, the survival rate was intermediate in all treatments (61.3% - 77.5%) and was not influenced by strain and diet, but their interaction showed significant difference among the treatments. The survival rate of the study was comparable with the range of 54.6% - 79.7% reported by Hien and Bengtson (2011), and the range of 56% - 60% reported by So Nam et al. (2011).

Economics. The economics of experimental snakehead was presented in Table 2.7 and 2.8. In this experiment, since feed cost was accounted for more than 70% of the total expenses, we focused only feed cost and income from selling snakehead on farm get price in the current market to make economic analysis. The n-FSF and n-Mix showed lost profit (-0.5 $\pm$0.1 x1000 KHR/kg fish produced and -0.8 $\pm$0.3 x1000 KHR/kg fish respectively). The highest profit value showed in the d-PF treatment (1.3 $\pm$ 0.2 x1000 KHR/kg fish respectively), followed by the d-Mix and n-PF (0.9 $\pm$ 0.4 x1000 KHR/kg fish respectively; 0.9 $\pm$ 0.3 respectively x1000 KHR/kg fish respectively).

Fish product quality

Method. At the end of the experiment, all fish were killed, filleted, and washed, and then they were steamed for three minutes. First, these fish were used to determine the difference in the quality of fish fillet between the control and experimental groups by triangle test (two controls and one sample) with three replacements per test (Hien and Bengtson, 2011). And the control sample was the snakehead which was bought at the local market. There were six samples named n-FSF, n-PF, n-Mix, d-FSF, d-PF, and d-Mix.

If less than six out of nine detected the odd sample correctly, we determined that there was no significant difference and therefore no need to conduct a sensory test. A multiple range-test was run if there were any difference in any sensory attributes for texture or taste even if they were minor—called a "descriptive test". On the other hand, if seven out of nine people detected the odd sample correctly, there was a significant difference at $p<0.01$ or six out of nine $p<0.05$. In this case, it was necessary to do a comprehensive multiple range-tests on appearance, texture, and taste. Tests are hedonic and score on an intensity scale (one to-9 points) on appearance such as liking (1, least like—5, o.k.—9, like very much, whiteness (1, dark—5, medium—9, very white), and structural integrity (uniformity: 1, very irregular—5, medium—9, very uniform); taste, for example liking (1, least like—5, o.k.—9, like very much); snakehead-like taste (1, very little—5, o.k.—9, very much); presence of objectionable taste (yes or no) and presence of objectionable odor (yes or no); texture, for instance, liking (1, least like—5, o.k.—9, like

very much); firmness (1, very soft—5, medium—9, very firm); moistness (1, very dry—5, medium—9, very moist); chewiness (1, mushy—5, medium—9, very chewy); and flakiness (1, least or rubbery—5, medium—9, very flaky). Mean values of results in different treatments were compared by Duncan's multiple-range test using SPSS 16.0 software. Treatment effects were considered with the significance level at $p < 0.05$.

RESULTS

Sensory analysis. The sensory analysis was presented in Table 2.9 and 2.10. In appearance, both fish fed freshwater small-sized fish, pelleted feed and mixture feed received scores of approximately six to seven, meaning that the fillets were relatively likeable for liking; rather white for whiteness and relatively uniform for structural integrity. In taste, the fish fillets had snakehead-like taste without the presence of objectionable taste or odor. In texture examination, for liking score was from six to seven meaning fairly or relatively like. For firmness, the scores were around six, medium or rather firm fish fillet. The fillet moistness was medium (no dry and not moist). The fillet chewiness and flakiness was judged to medium (not mushy and not chewy; not rubbery and not flaky).

The result showed that there were no significant differences between samples in triangle tests (less than six out of nine people detected the odd sample correctly). These samples were then subjected to descriptive multiple tests, with the result that the quality of fish fillet samples among the treatments did not significantly differ.

In summary, snakehead fillet quality was relatively like and did not significantly differ among samples in triangle tests. In descriptive multiple tests, there were also no significant differences among the samples. So, the diets and snakehead strains did not effect on the quality of fish fillet for fish in these experiment.

CONCLUSION

The study concludes that both Vietnam hatchery snakehead (domesticated) and Cambodia indigenous wild snakehead (non-domesticated) can accept formulated feed, with similar product quality. However, Vietnam hatchery snakehead show higher growth rate and profit than Cambodia wild capture snakehead because Vietnam hatchery snakehead has been undergone domestication and selection breeding for more than 20 years. Considering economic efficiency, replacing freshwater small-sized or low-value fish by formulated feed up to 100% is possible for both Vietnamese domesticated snakehead and Cambodian non-domesticated snakehead, however, the domesticated snakehead (about US$0.35/kg fish) shows higher profit than Cambodian non-domesticated snakehead (US$ 0.25/kg fish).

Using formulated or pelleted feed for snakehead culture provides significantly better growth performance, FCR and higher profit than using freshwater small-sized fish and mixture diets.

In regards product quality, formulated or pelleted feed does not significantly affect the fillet quality of both Vietnamese and Cambodian cultured snakehead compared to a diet of freshwater small-sized low value fish or 50:50 mixtures of small-sized fish and pelleted feed. In a quality test, diets and fish strains do not affected the fillet quality among the samples.

RECOMMENDATIONS

The following recommendations should be carefully considered for policy and action plan development in order to lift the ban on snakehead and achieve sustainable development snakehead aquaculture in Cambodia:

- The availability of hatchery broodstock has" very important implications" for protecting the small sized fishes that are usually fed to snakeheads

- Collecting striped snakeheads from different natural water bodies across the country to develop sufficient numbers of broodstock at hatcheries for research into breeding and weaning techniques to produce high-quality seed
- Characterizing biologically striped snakeheads from different populations in the Tonle Sap Lake, the upper and lower stretches of the Mekong and Bassac rivers and their associated floodplains to determine favorable traits for aquaculture development.
- Assessing genetic diversity and populations in different locations to maintain the diversity of wild stocks, conserve the species and enhance broodstock diversity when conducting domestication and breeding programs
- Substituting small-sized fish with formulated feed would help lift Cambodia's ban on snakehead farming which has now been in force for 10 years
- Optimizing survival and growth rate of Cambodia indigenous snakehead through development of F_2 and F_3 generation broodstock and genetic selection of wild capture snakehead collected from different natural water bodies in Cambodia
- Developing practical formulated diets for snakehead broodstock, fry and fingerlings to replace small-sized fish from capture fisheries
- Providing extension services to farmers on techniques for snakehead breeding, weaning and grow-out using formulated diets
- Encouraging the government, business and development partners to invest in the value chain of snakehead aquaculture development, especially the private sector to formulate and improve commercially manufactured feed that is better integrated into local economy with fewer imported ingredients and lower prices

QUANTIFIED ANTICIPATED BENEFITS

This research provides information on domesticated breeding, weaning and growing out of snakehead fish, especially development of Cambodia's snakehead aquaculture technologies, in order to lift the ban on snakehead culture in Cambodia. The following are quantifiable anticipated benefits:

- Scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staffs, private sector and university lecturers and students working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries were better informed about research methods and findings, and have better recommended policies and strategies for sustainable snakehead aquaculture
- Three undergraduate students were supported and trained by this investigation through their B.Sc. thesis researches
- Benefits to the US include improved knowledge and technologies on domestication of freshwater fish species for aquaculture and this aquaculture is considered as a climate change adaptation measure

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TABLES AND FIGURES

Table 2.1 Formulated feed formulation (about 45% CP) for weaning.

Main ingredients	(g)
Fish meal	570
Soy bean meal	140
Rice bran	100
Cassava meal	130
Vitamin C	2
Premix mineral-Vitamin	15
Fish oil	25
Phytase	0.2
Binder	17.8
Total	1000

Table 2.2 Survival rate (%), Cannibalism rate, feed intake (%), body weight (mg.fish[-1]), and daily weight gain (mg.day^{-1}) of non-domesticated vs. domesticated *Channa striata* weaned to formulated feed (FF) for 45 days.

Parameters[1,2]	Treatments	
	Nondomesticated	Domesticated
Stocking		
Density (larvae l[-1])	5	5
Total larvae (larvae tank[-1])	250	250
Initial weight (mg fish[-1])	2.1	2.1
Initial length (mm fish[-1])	12	12
Harvest		
Total fish (fish tank[-1])	89	72
Final body weight (mg fish[-1])	145.5[a] ± 5.2	170.2[b] ± 2.6
Weight gain (mg fish[-1])	143.4[a] ± 5.2	168.1[b] ± 2.6
Daily weight gain (mg fish[-1] day^{-1})	3.2[a] ± 0.1	3.7[b] ± 0.1
Length (mm fish[-1])	23.0[a] ± 0.6	24.5[a] ± 0.7
SGR (% day[-1])	9.4[a] ±0.1	9.8[b] ± 0.03
Feed intake (mg fish[-1]day[-1])	84.9[a] ± 3.7	106.6[b] ± 8.3
Survival (%)	35.7[a] ± 1.6	28.9[b] ± 1.9
Mortality (%)	13[a] ± 2.5	24.5[b] ± 2.3
Cannibalism (%)	51.3[a] ± 3.4	46.6[a] ± 2.8

[1]In each row, data having different superscripts are significantly different ($p < 0.05$).
[2]Data are means ± SE. (n=5).

Table 2.3 Water quality parameters in ponds fed freshwater small-sized fish, pelleted feed and mixture.

Ponds	Temperature (ºC)	pH	DO (ppm)	Transparency (cm)	NH$_3$ (mg L^{-1})	NO$^-_2$ (mg L^{-1})
Freshwater small-sized fish	29-34	7-8	3-5	25-30	0.003-0.002	0.01-0.05
Pelleted feed	29-35	8-9	4-6	18-25	0.02-0.09	0.01-0.05
Mixture	29-35	8-9	4-6	20-30	0.02-0.08	0.01-0.08

Table 2.4 Final body weight (FBW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed intake (FI), food conversion ratio (FCR) and survival (SR) of two strains of snakehead fed three diets for 6 months.

Parameters[1,2]	Treatments					
	n-FSF	n-PF	n-Mix	d-FSF	d-PF	d-Mix
Stocking						
Density (fish hapa^{-1})	100	100	100	100	100	100
Initial weight (g fish^{-1})	13.5 ± 0.7	13.5 ± 0.7	13.5 ± 0.7	13.5 ± 0.7	13.5 ± 0.7	13.5 ± 0.7
Harvest						
FBW(g fish^{-1})	210.0 ± 5.8[c]	233.3±3.3[d]	255.0 ± 2.9[d]	305.0 ± 4.1[a]	366.7 ± 8.8[b]	346.7 ± 8.8[b]
WG (g fish^{-1})	196.5 ± 5.8[c]	219.8±3.3[d]	241.5 ± 2.9[d]	291.5 ± 4.1[a]	353.2 ± 8.8[b]	333.2 ± 8.8[b]
DWG (g fish^{-1}day^{-1})	1.1 ± 0.0[c]	1.2 ± 0.0[d]	1.3 ± 0.0[d]	1.6 ± 0.0[a]	2.0 ± 0.0[b]	1.9 ± 0.0[b]
SGR (% day^{-1})	1.5 ± 0.0[c]	1.6 ± 0.0[d]	1.6 ± 0.0[d]	1.7 ± 0.0[a]	1.8 ± 0.0[b]	1.8 ± 0.0[b]
FI (g fish^{-1} day^{-1})	4.6 ± 0.2[cd]	2.1 ± 0.1[e]	3.8 ± 0.1[f]	6.0 ± 0.1[a]	3.1 ± 0.1[b]	4.5 ± 0.1[c]
FCR	4.2 ± 0.1[d]	1.7 ± 0.1[e]	2.8 ± 0.1[f]	3.7 ± 0.0[a]	1.5 ± 0.0[b]	2.4 ± 0.1[c]
SR (%)	77.5 ± 3.9[b]	68.5 ± 5.5[c]	61.3 ± 0.9[d]	64.9 ± 2.2[ac]	75.7 ± 4.1[b]	64.9 ± 1.6[ac]
Production (Kg hapa^{-1})	15.2 ± 0.6[c]	15.0 ± 1.2[c]	14.8 ± 0.3[c]	18.9 ± 0.4[a]	26.7 ± 1.0[b]	21.6 ± 1.1[a]

[1]In each row, data having different superscripts are significantly different ($p < 0.05$).
[2]Data are means ± SE. (n=3)
n, nondomesticated; d, domesticated; FSF, freshwater small-sized fish; PF, Pelleted feed; Mix, 50% mixture of FSF and PF.

Table 2.5 Effect of snakehead strain on growth, Feed Intake, FCR and survival rate.

Parameter[1,2]	Nondomesticated	Domesticated
FBW(g fish^{-1})	232.8 ± 6.8[a]	342.2 ± 8.9[b]
WG (g fish^{-1})	219.3 ± 6.8[a]	328.7 ± 8.9[b]
DWG (g fish^{-1}day^{-1})	1.2 ± 0.04[a]	1.8 ± 0.05[b]
SGR (% day^{-1})	1.6 ± 0.01[a]	1.8 ± 0.02[b]
FI (g fish^{-1} day^{-1})	3.5 ± 0.4[a]	4.6 ± 0.4[b]
FCR	2.9 ± 0.4[a]	2.6 ± 0.3[a]
SR (%)	69.1 ± 3.1[a]	68.5 ± 2.3[a]
Production(kg hapa^{-1})	15 ± 0.4[a]	22 ± 1.2[b]

[1]In each row, data having different superscripts are significantly different ($p < 0.05$).
[2]Data are means ± SE. (n=9).
FBW, final body weight; WG, weight gain; DWG, daily weight gain; SGR, specific growth rate; FI, feed intake; FCR, food conversion ratio; SR, survival rate.

Table 2.6 Effect of diet on growth, Feed Intake, FCR and survival rate.

Parameter[1,2]	FSF	PF	Mix
FBW(g fish^{-1})	261.7 ± 23.6[b]	300.0 ± 30.1[a]	300.8 ± 20.9[a]
WG (g fish^{-1})	248.2 ± 23.6[b]	286.5 ± 30.1[a]	287.3 ± 20.9[a]
DWG (g fish^{-1}day^{-1})	1.4 ± 0.1[b]	1.6 ± 0.2[a]	1.6 ± 0.1[a]
SGR (% day^{-1})	1.6 ± 0.05[b]	1.7 ± 0.05[a]	1.7 ± 0.05[a]
FI (g fish^{-1} day^{-1})	5.4 ± 0.3[a]	2.6 ± 0.3[b]	4.2 ± 0.2[c]
FCR	4.0 ± 0.2[a]	1.7 ± 0.04[b]	2.7 ± 0.1[c]
SR (%)	71.2 ± 3.4[a]	72.1 ± 3.5[a]	63.1 ± 1.1[a]
Production (kg hapa^{-1})	17.3±0.98[a]	20.8±2.7[a]	18.2±1.6[a]

[1]In each row, data having different superscripts are significantly different ($p < 0.05$).
[2]Data are means ± SE. (n=6).
FBW, final body weight; WG, weight gain; DWG, daily weight gain; SGR, specific growth rate; FI, feed intake; FCR, food conversion ratio; SR, survival rate; FSF, freshwater small-sized fish; PF, pelleted feed; Mix, Mixture of freshwater small-sized fish and pelleted feed.

Table 2.7 Economics of experimental snakehead culture per hapa.

Treatments	Total cost (feed) (thousand KHR/hapa)	Total income (thousand KHR/hapa)	Profit (thousand KHR/hapa)
n-FSF	129.0 ± 4.7[a]	121.6 ± 5.1[a]	-7.4 ± 1.8[a]
n-PF	106.1 ± 4.2[b]	120.4 ± 9.5[a]	14.3 ± 5.4[b]
n-Mix	130.1 ± 1.7[a]	118.4 ± 2.3[a]	-11.7 ± 3.9[c]
d-FSF	140.4 ± 2.2[c]	151.2 ± 3.0[c]	10.8 ± 0.9[d]
d-PF	178.6 ± 9.4[d]	213.3 ± 7.7[d]	34.7 ± 3.9[e]
d-Mix	162.3 ± 1.4[e]	181.1 ± 3.7[e]	19.1 ± 2.5[f]

Table 2.8 Economics of experimental snakehead culture per kg fish produced

Treatments	Total cost (feed) (thousand KHR/Kg fish)	Total income (thousand KHR/Kg fish)	Profit (thousand KHR/Kg fish)
n-FSF	8.5±0.1[a]	8.0±0.00	-0.5 ±0.1[a]
n-PF	7.1±0.3[b]	8.0± 0.00	0.9± 0.3[b]
n-Mix	8.8 ±0.3[a]	8.0 ±0.00	-0.8 ±0.3[c]
d-FSF	7.4±0.03[c]	8.0± 0.00	0.6 ±0.03[d]
d-PF	6.7 ±0.2[d]	8.0± 0.00	1.3 ± 0.2[e]
d-Mix	7.5±0.4[e]	8.0± 0.00	0.9 ± 0.4[f]

Table 2.9 Triangle test for difference (number from a 9-person sample who detected the odd sample correctly, data are mean ± SD.

n-FSF	n-PF	n-Mix	d-FSF	d-PF	d-Mix
3.0 ± 1.0	2.7 ± 0.6	3.3 ± 1.2	2.3 ± 0.6	3.7 ± 1.5	4.0 ± 1.0

Table 2.10 Channa striata sensory analyses, data are mean ± SD.

Content	Scores					
	n-FSF	n-PF	n-Mix	d-FSF	d-PF	d-Mix
Appearance						
Liking	6.1 ± 0.8	6.4 ± 0.9	7.0 ± 0.9	6.6 ± 1.3	6.7 ± 1.3	6.9 ± 0.9
Whiteness	6.5 ± 1.2	6.9 ± 0.8	6.5 ± 1.0	6.6 ± 0.9	6.5 ± 1.2	6.3 ± 1.0
Structural integrity	6.4 ± 2.1	7.0 ± 1.3	6.9 ± 1.1	6.5 ± 1.7	6.3 ± 1.9	6.6 ± 2.1
Taste						
Liking	6.6 ± 1.6	7.4 ± 1.0	6.9 ± 0.8	7.0 ± 0.8	7.0 ± 1.3	7.1 ± 0.8
Snakehead-like taste	8.0 ± 0.9	7.6 ± 0.9	7.5 ± 0.9	7.5 ± 0.8	7.7 ± 0.9	7.6 ± 1.0
Presence of objectionable taste	No	No	No	No	No	No
Presence of objectionable odor	No	No	No	No	No	No
Texture						
Liking	5.9 ± 1.5	7.0 ± 1.1	6.8 ± 1.0	7.0 ± 1.1	6.9 ± 1.1	6.9 ± 1.3
Firmness	6.3 ± 1.0	6.9 ± 1.1	6.0 ± 1.1	6.1 ± 1.2	6.1 ± 1.1	6.5 ± 0.8
Moistness	4.9 ± 1.0	5.1 ± 1.2	5.3 ± 1.3	4.8 ± 1.0	5.7 ±1.4	4.8 ± 1.3
Chewiness	5.3 ± 1.4	5.1 ± 0.8	6.0 ± 1.3	4.8 ± 0.7	5.4 ± 1.1	5.0 ± 1.2
Flakiness	4.8 ± 1.0	4.9 ± 2.0	5.0 ± 2.3	4.6 ± 1.1	5.9 ± 1.1	4.9 ± 1.2

Figure 2.1. Formulated feed preparation for weaning experiment.
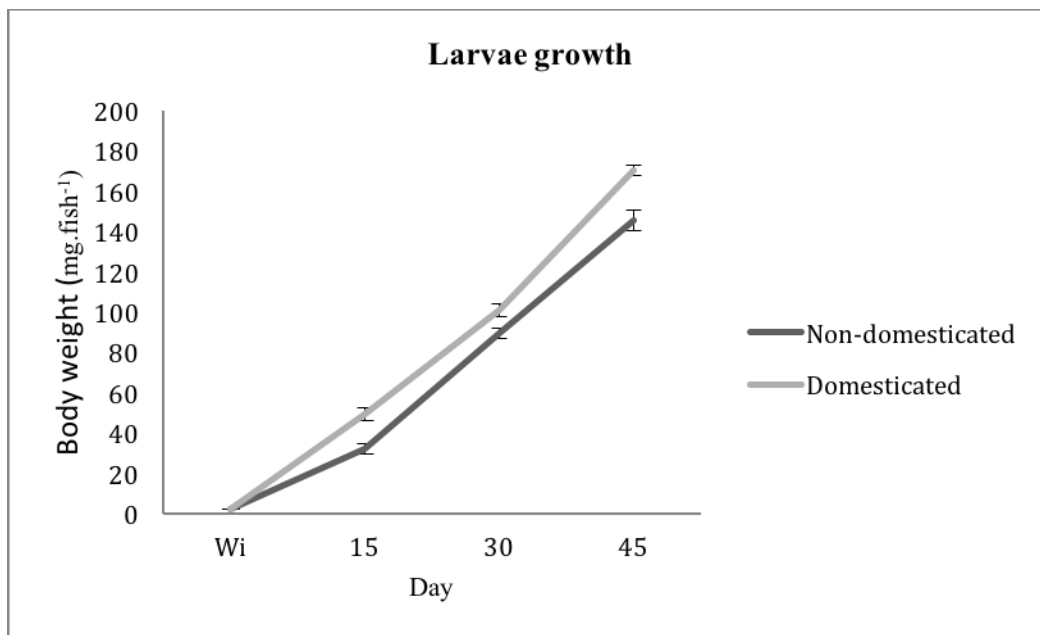
Figure 2.2. Weaning experiement replicated tanks.

Figure 2.3 The growth performance of nondomesticated vs. domesticated *C. striata* weaned to formulated feed (Wi = Initial weight).
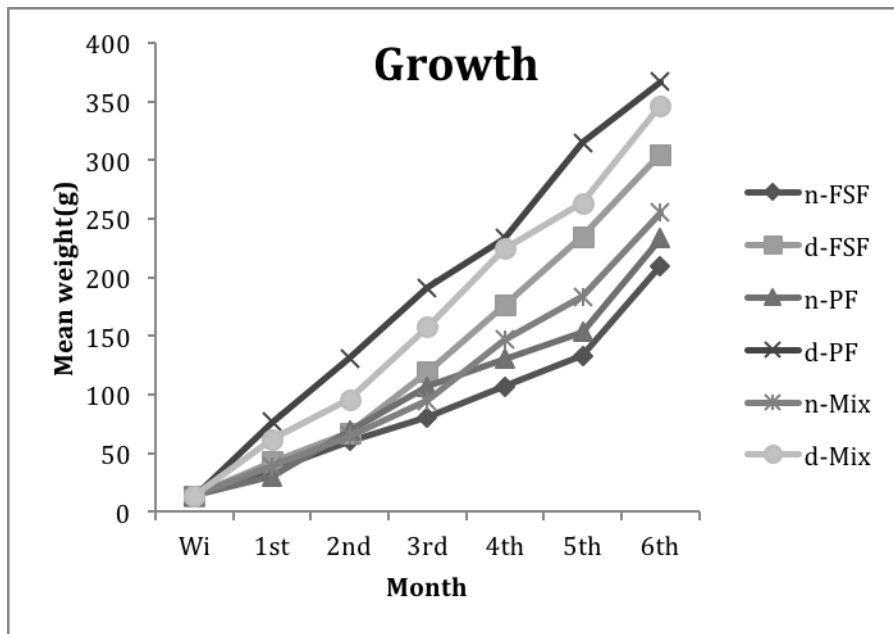
Figure 2.4 Growth graph for domesticated and non-domesticated strains of snakehead fed three diets: nondomesticated fed freshwater small-sized fish (n-FSF), Pelleted feed (n-PF), 50:50 mixture of freshwater small-sized fish and formulated feed (n-Mix); and domesticated fed freshwater small-sized fish (d-FSF), formulated feed (d-PF), 50:50 mixture of freshwater small-sized fish and formulated feed (d-Mix).

Figure 2.5. Grow-out experiment (left: freshwater small-sized fish fed pond; middle: pelleted feed-fed pond; right: mixture-fed pond).

Figure 2.6 Snakehead fish sampling at harvest of six month grow-out (Fish: upper: domesticated snakehead; lower: non-domesticated snakehead; from left to right: Pelleted feed fed-fish, 50:50 mixture fed fish and freshwater small-sized fish fed fish).

Figure 2.7 Snakehead fillet quality test (sensory test).

APPENDIX
Questionnaires
Snakehead Fish *Channa striata* Sensory Analysis

Fish_____; Diet_____   Name_____

Content	Scores									
I. Appearance	• Liking	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, least like--- 5, o.k.--- 9, like very much)									
	• Whiteness	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, dark--- 5, medium--- 9, very white)									
	• Structural integrity	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(Uniformity: 1, very irregular--- 5, medium--- 9, very uniform)									
II. Taste	• Liking	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, least like--- 5, o.k.--- 9, like very much)									
	• Snakehead-like taste	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, very little--- 5, o.k.--- 9, very much)									
	• Presence of objectionable taste	Yes ☐ No☐									
	• Presence of objectionable odor	Yes ☐ No ☐									
III. Texture	• Liking	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, least like--- 5, o.k.--- 9, like very much)									
	• Firmness	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, very soft--- 5, medium--- 9, very firm)									
	• Moistness	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, very dry--- 5, medium--- 9, very moist)									
	• Chewiness	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, mushy---5, medium--- 9, very chewy)									
	• Flakiness	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐
	(1, least or rubbery--- 5, medium--- 9, very flaky)									

Development of Low-cost Captive Breeding and Hatching Technologies for the African Lungfish (*Protopterus aethiopicus* and *P. amphibius*) to Improve Livelihoods, Nutrition, and Income for Vulnerable Communities in Uganda

Climate Change Adaptation: Indigenous Species Development/Experiment/13IND03AU

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ABSTRACT

Culturing resilient species in the prevalent variable climate conditions will be beneficial to African aquaculture. Air breathing fish like the African lungfish (Protopterus sp.) will be desirable, but fish farmers lack aquaculture technologies to propagate and manage this fish. This report summarizes experimental results on diversity, breeding, and management of lungfish reared in aquaculture systems. Relatively higher survival and maturity rates were achieved when lungfish is kept in captivity. A novel SNP panel that will guide a comprehensive lungfish-breeding program is partially generated. Hermaphroditism in lungfish is first reported in this investigation study. These results will guide the generation low-cost technologies for propagating and producing cultured African lungfish to improve household nutrition, food security, and income. Consequently, natural stocks will be protected through this intervention.

INTRODUCTION

Marbled lungfish (Protopterus aethiopicus) is native to Ugandan waters, but its natural stocks are declining mainly due to overexploitation, environmental degradation, and the large-scale conversion of wetlands to agricultural land. Furthermore, climate change continues to influence regional rainfall patterns and temperature regimes, which directly affects aquaculture production. For example, seasonal water deficits caused by prolonged droughts usually constrain management of aquaculture systems. Rearing fish species that are tolerant to drought and poor water quality conditions would be a significant future for African aquaculture development (Allison et al. 2007, Daw et al. 2009, Wagle et al. 2011, Williams and Rota 2011). Air breathing fishes would be suitable candidates in poor water quality conditions because of their ability to obtain and utilize atmospheric oxygen to meet all or part of their metabolic demands (Myers 1986, Pethiyagoda 1991, Graham 1997, Thomson 2013).

The African lungfish, however, is an air breather that may offer some distinct advantages when water quality for fish growth is poor, like low-dissolved oxygen. Lungfish is valued in Uganda and consumer acceptance seems high and widespread, but appropriate aquaculture technologies are not available for fish farmers engaged its culture. Lungfish farmers currently collect seed from the wild environments and either raise them in earthen ponds or tanks. This is not sustainable ecologically, since its captive breeding technology is not known or documented. The absence of breeding and production technologies limits possibilities to explore its potential to generate income and improve nutrition for small-scale holders.

This study seeks to develop low-cost sustainable breeding and culture techniques for African lungfish in the region. Therefore, the underlying molecular information that supports its diversity, reproduction, and/or propagation is important. However, there is insufficient lungfish genomic data since it has the

largest genome (> 130 Gb) among vertebrates (Metcalfe et al. 2012). Hence, novel approaches like Single Nucleotide Polymorphisms (SNPs) panel will facilitate, i) genetic diversity of lungfish, ii) whole genome sequencing, iii) breeding program, and iv) conservation programs.

OBJECTIVES

- Determine the genetic diversity of the endemic African lungfish (Protopterus aethiopicus) fingerlings sourced from four agro-ecological zones (East, North, South western and Central) of Uganda;
- Domesticate the African lungfish using simple, adoptable, and productive captive breeding techniques that integrates indigenous knowledge;
- Assess the reproductive performance of the African lungfish in captivity; and
- Evaluate the culture performance of African lungfish raised to market size in small-scale fish ponds.

METHOD

Study 1: Determining genetic diversity of African lungfish (Protopterus aethiopicus) sourced from six lakes of Uganda using mtDNA and morphometry.

Morphometry. A total of 254 fish samples were collected from Lakes Bisina, Nawampasa, Edward, George-Kazinga, Wamala, and Kyoga in 2014. Sampling stations included open lake ecosystem, in-river, and lake-river interface.

Whole fish samples (Figure 1) of adult and juvenile fish were obtained from fishing grounds using monofilament gill nets (size = 2 to 8"), and long lines. Using truss dimensions (Strauss and Bookstein 1982) each fish was constructed, using calipers, as described by Flemming et al. (1994, 1995).

From each fish, the total weight, total length, truss, and non-truss parameters (Figure 2 and Table 1) were measured to compute morphological disparity among and between the populations. These were done using a digital weighing scale, graduated meter rule, and Vernier calipers. From each fish, a tissue sample of about 1x1 cm was extracted, mainly from the left posterior claspers and preserved in an absolute ethanol and kept at -32OC at ARDC Kajjansi before transferring to BecA-ILRI Hub laboratory facility in Nairobi, Kenya for genomic analysis.

Principle Component Analysis (PCA) was used to determine morphometric differences from each source using XLSTAT software, version 2012. A total of six truss parameters, mainly were fin (claspers), lengths of the head, snout, gape size and orbital distance, and four nontruss morphometric variables were measured on each specimen. All morphometric measurements were transformed in PCA to adjust for variations in fish size (Reist 1985).
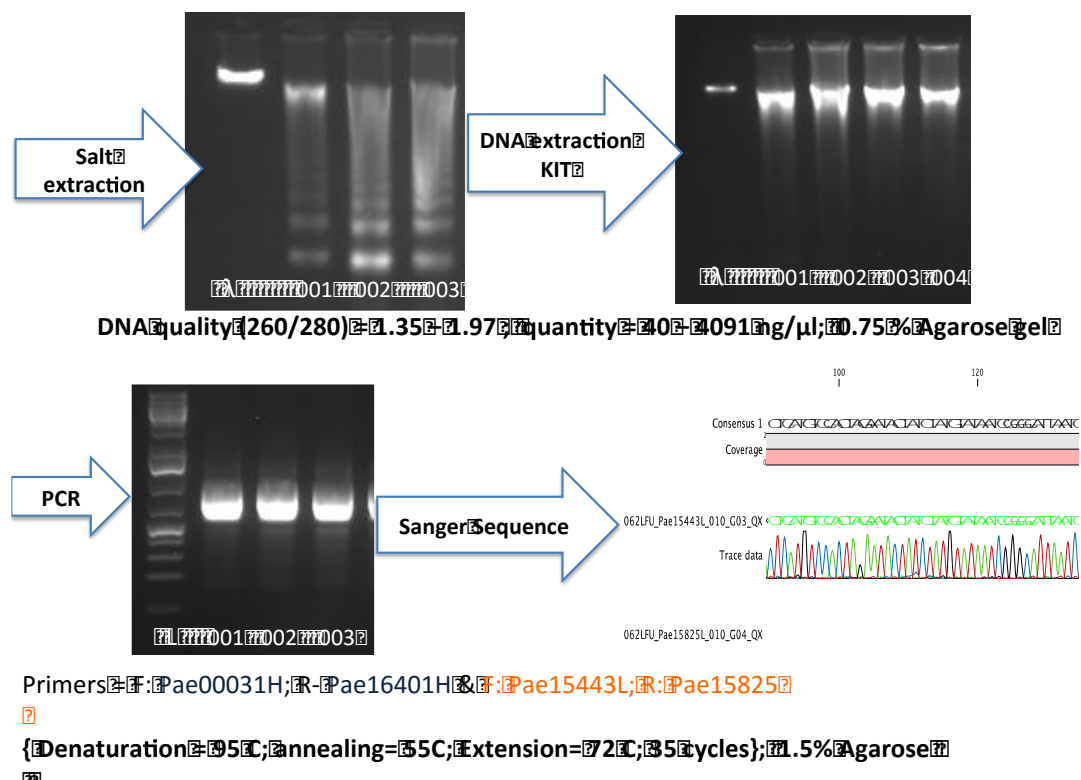
Figure 1. Sources of lungfish samples in Uganda.

Figure 2. Landmarks used for the morphometric analysis of P. aethiopicus.

Table 1. Morphometric characters used for analysis of African lungfish (P. aethiopicus).

Character	Description
Anal fin length (AFL)	Distance between anterior of anal fin to tip of caudal fin.
Dorsal fin length (DFL)	Distance between anterior of dorsal fin to tip of caudal fin.
Gap size (EL)	Maximum height of mouth cavity
Head length (HL)	Distance from tip of snout to posterior margin of opercula
Orbital distance (OBD)	Distance between the eyes
Right and Left pectoral fins (RAC and LAC)	Length of pectoral fin measured from proximal to distal end
Right and Left pelvic fin (RPC and LPC)	Length of pelvic fin measured from proximal to distal end
Snout length (SL)	Mouth size
Total length (TL)	Distance from the tip of the snout to the longest caudal fin ray

D-loop determination. Genomic DNA was extracted from fin clips of 254 fish samples using protocols described by Life technologies (Invitrogen) kit®, but also compared to salt extraction method. The mtDNA control region was amplified using Bioneer kit® conditions and primers developed Protopterus dolloi Boulenger (Zardoya and Meyer 1996).

Amplified products were visualized on electrophoresis through 1.5% agarose gels, amplified fragments were purified using Qiaquick Gel Extraction columns (Qiagen) then mtDNA Sanger sequenced at BecA-ILRI, Nairobi, Kenya. Phylogenetic analysis was applied to further understand the genetic variability of this fish in Ugandan waters. Information generated will enhance strategies to conserve its biodiversity and support the aquaculture program in the region.

DNA quality (260/280) = 1.35 – 1.97 ; quantity = 40 – 4091 ng/µl; 0.75 % Agarose gel

Primers = F: Pae00031H; R- Pae16401H & F: Pae15443L; R: Pae15825

{ Denaturation = 95 C; annealing= 55C; Extension= 72 C; 35 cycles}; 1.5% Agarose

Figure 3. Workflow of DNA extraction and quality assessment of genomic material.

D-loop data generated was analyzed using CLC Main Workbench Version 7.5 (QIAGEN Aarhus, A/S www.clcbio.com) 2014. The phylogenetic tree was constructed using MEGA 6.06 software.

Experiment 1.1: Genetic diversity of African lungfish in Uganda— Relatedness based on single nucleotide polymorphisms (SNPs) and microsatellite markers. This panel comprises a small set of markers that will guide in the selection of broodstock to be recruited into the breeding program. Secondly, changes in the genetic profile of this fish can be determined as the species is developed. In this study, fin clips from 200 lungfish samples (50 per site) were collected from four AEZs (i.e,. Lake- Edward, Kyoga, Bisina, Nawampasa, Wamala, and George) were used and flash frozen in liquid nitrogen and stored at - 80°C. Total RNA was isolated from each sample using TRIzolTM (Invitrogen, Carlsbad, CA) protocol. Equal masses of total RNA from the samples of each group were pooled and used for RNA-Seq sequencing.

cDNA libraries were prepared and sequenced through Illumina Genome Analyzer (single-end, 300 bp read length) at the International Livestock Research Institute-molecular laboratory (Nairobi, Kenya) as described by Severin et al. (2010). Variant detection pipeline (Miller et al. 2008) was used to map sequence reads to a reference transcriptome of a double-haploid Latimeria chalumnae fish (Amemiya et al. 2013). SNP detection was done using the Next Generation Sequencing (NGS) pipeline described in Figure 4.
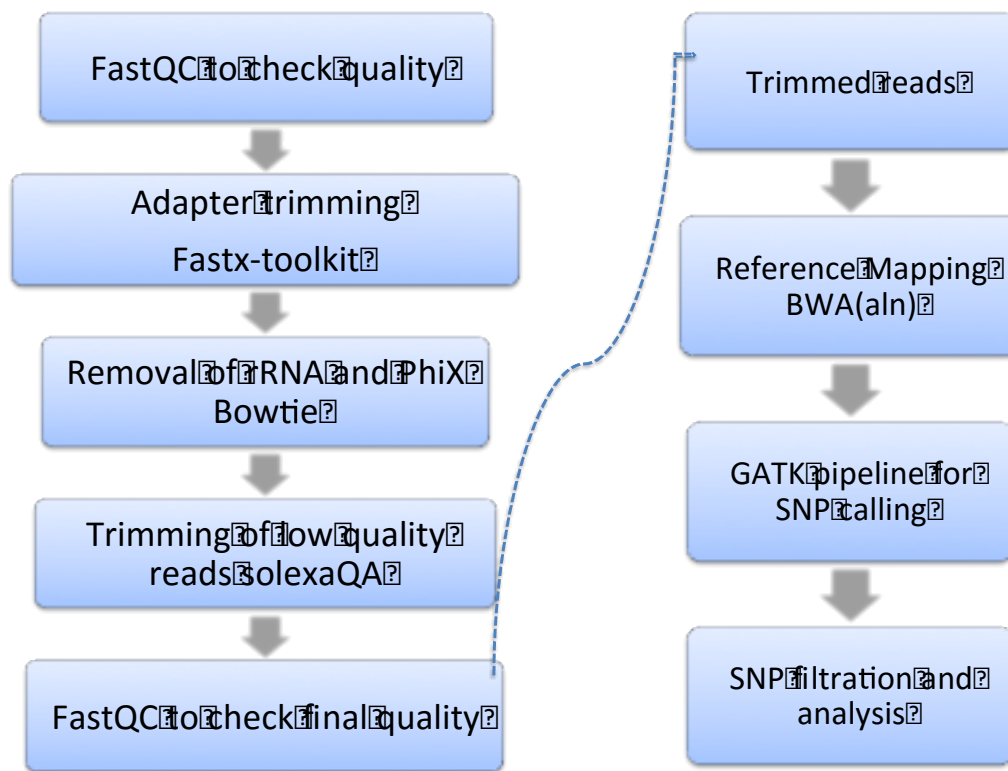
Figure 4. Schematic of transcript assembly and SNP detection pipeline.

Experiment 1.2: Reproductive biology of African lungfish in captivity. To ensure an environmentally sustainable supply of African lungfish seed to fish farmers, artificial breeding technologies have to be well developed. Low-cost breeding technologies will be ideal for rural communities that are dependent on this fish. Lungfish collected from wild populations were visually checked for maturity, and subsequently subjected to artificial reproduction techniques; natural and artificial breeding.

Study 2: Domesticating the African lungfish using simple captive breeding techniques that integrate indigenous knowledge.

Experiment 2.1: Artificial breeding of African lungfish in captivity. Following Vijay Kumar et al. (1998) protocol, mature broods were caught from wild lake environments (Lakes Edward, Bisina, and Kyoga) during rain seasons and conditioned in concrete tanks at NaFIRRI for two weeks. Fish were injected with two selected hormones; human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone analogue (LHRHa) to induce spawning. Response to hormone treatment was observed, measured, and recorded. Water quality parameters were monitored to understand environmental factors affecting artificial breeding.

Results on reproduction of lungfish held in captivity at NaFIRRI-Kajjansi show males reach maturity at 300 g (37–57cm) compared to wild conditions where first maturity occurs between 65–85 cm, according to Mosille and Mainoya (1988) and Greenwood (1958).

Lungfish that weighed more than 300 g responded positively to doses of HCG and LRHa synthetic hormones, but failed to breed under captive conditions. Anatomy of mature fish samples revealed that hermaphroditism exists in lungfish populations. Hermaphroditism in lungfish presents a challenge in propagating this fish, but comprehensive research is tracked to understand this phenomenon.

Experimental fish gradually accepted the sinking fish feed pellets. However, lungfish slightly increased in average body weight having highest growth attained using feeds with high protein content. With this diet, lungfish grew with a specific growth rate (SGR) of ($0.50 \pm 0.06\%$/d), and feed conversions ratios ranging from 1.61 ± 0.26 to 2.07 ± 0.11 were obtained. The highest survival rate under this experiment condition was $57.50 \pm 2.85\%$, which is higher than previously documented by Mlewa et al. (2009). Cannibalism predisposed most experimental fish to aquatic pathogens; primarily water molds and bacteria causing mortalities.

Common diseases encountered included: bacteria (Aeromonas sp. and Flavabacterium columnaris), fungus (Fusarium spp., Aspergilus sp. and Saprolegnia sp.), and parasite (Dactylogurus sp., Trichodina sp., Tetrahymena sp., Heterorchis sp., and cestodes). Nevertheless, regeneration of injured appendages (fins) was observed, as previously described by Tamura et al. 2010. However, lungfish grows better in aquaculture outdoor tanks when poly-cultured with tilapia.

Cultured lungfish juveniles reached market size of average weight of 138 ± 42.46g in six months when polycultured with mixed-sex tilapia. Size at harvest ranged 50.2–512.9g, indicating a genetic variation within this species, and therefore, a comprehensive selecting program for this fish species is important. Survival rates improved to 86%, compared to indoor experiment, and generally fish appeared healthy.

Experimental 2.2: Natural breeding of African lungfish in captivity. Selected mature brood-fish collected were kept in two concrete tanks and water surface covered with water hyacinth (Eichornia crassipes) to simulate a natural breeding habitat. Water levels were manipulated (0.2 to 0.4m) every week to stimulate natural ovulation, spawning, and fertilization. Water quality parameters [Temperature and Dissolved oxygen (DO)] were monitored weekly to understand environmental factors affecting breeding in these tanks.

Study 3: Evaluating the performance of African lungfish reared in ponds under different management practices.

Indoor experiment was conducted in twelve Crest fiberglass tanks (60 L): testing three formulated diets replicated four times. Each replicate was stocked with 30 lungfish fingerlings of mean weight 9.74 ± 0.12 g and mean length of 13.74 ± 0.33 cm. Systems were aerated with 50% of water exchanged three times per week. Sampling was done weekly.

Outdoor trial was conducted in concrete tank (2 x 7 x 0.5) m3, stocking 100 juveniles (40.84 ± 19.24g) together with 50 mixed-sex tilapia (Nile tilapia and Tilapia zilli) fingerlings. Fish were fed ad libitum with formulated fish feeds (30% Crude protein).

Morphometry. Findings indicate lungfish from six lakes generally exhibit intrinsic and extrinsic homogeneity. However, samples from Lake Nawampasa showed variations in gape size, snout length, orbital distance, dorsal fin length, and anal fin length.

D-loop (mtDNA) diversity. The phylogenetic tree showing 70 mtDNA haplotypes, 13 reference groups, and one outgroup is shown in Figure 5. The lungfish haplotypes cluster into three distinct groups (henceforth referred to as A, B, and C clades) that differ at about 9.8% sequence divergence. The distinction between lineages was supported at a bootstrap value of 100%. Tajima's D values were

negative for all populations, indicating a low frequency polymorphism compared to expected value, hence, an expanding population.
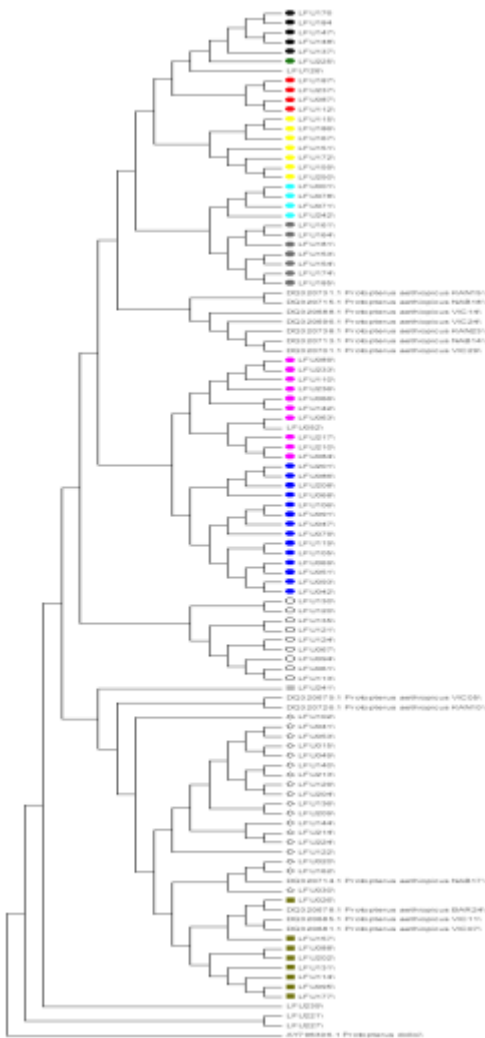

Figure 5. Phylogenetic tree generated showing clades of lungfish collected from six lakes of Uganda. (Different colors indicate haplotypes of P. aethiopicus).

Conversely, Fu's FS tests showed negative values for all populations, indicating an excess of rare haplotypes/alleles expected from a recent population expansion, largely contributed from Lakes Bisina and Edward. However, the nucleotide (Pi) diversity values averaged 0.010 ± 0.001, indicating less diversification between the six populations.

Geographical structure of African lungfish results from the AMOVA (Table 2) show that overall genetic variation within populations (79.01%) is much larger than the variation between populations (20.91%).

Table 2. Comparison of geographical structure of populations by assessments with Analysis of Molecular Variance (AMOVA).

Sources of Variation	d.f.	Sum of Squares	Variance Components	Percent of Variation
Among populations	5	182.9	1.0 V_a	20.9
Within populations	197	751.6	3.8 V_b	79.9
Total	202	934.5	4.824	
Fixation Index	$F_{ST} = 0.209$			

Single nucleotide polymorphism (SNP) panel for genetic diversity of lungfish from six lakes in Uganda. A total of 80,358,264 Single-end short reads (301-bp) through RNA-Seq using Mi-Seq pipeline were generated (Table 3). After filter using NGS QC Toolkit, a total of 75,647,110 high quality reads with 285 bp were generated from transcriptome. A total of 64,666,158 high quality reads with 285 bp were obtained after mapping to the reference Latimeria cholumnae.

Table 3. Statistics of raw reads with high quality and mapped reads ratio of transcriptome.

	Reads
Total number of reads: raw	80,358,264
Total number of reads: clean	75,647,110
Mapped to reference	64,666,158
Mapped read ratio	85.5%
Average GC content after trimming	43.4%

A total of 12,085 putative SNPs with high quality were identified in the transcriptome. Using the four sets of data together (Wamala, Kyoga Nawampasa, and Bisina), a total of 121 SNPs with high quality were predicted (Figure 6).

The estimated SNP frequency was 0.21% (one per 400 bp). Within the identified SNPs, more transition substitution (41.7%) was found than transversion substitution (68.3%) (Table 4 and 5). In terms of transition substitution, the amount of A/G transitions was similar to that of C/T transition. In terms of transversion substitution, the frequency of four types (G/C, G/T, A/C, and A/T) was equal. The estimated ratio for transition to transversion was 0.7.

Table 4. Statistics of transition and transversion type in the total SNPs.

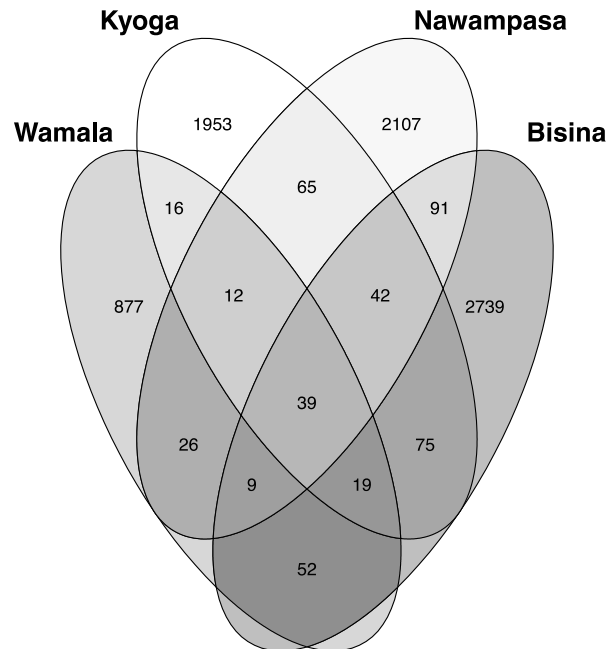| Type	Transition		Transvertion		
	GA	CT	GC	GT	AC
Number	2433	2614	1374	1613	1912
Percent	20.1	21.6	11.4	13.3	15.8
Total	5047		7038		

Figure 6. Venn diagram of SNPs using reads of the four datasets. Lakes Wamala + Kyoga + Nawampasa + Bisina represented SNPs discovered using the reads of the four transcriptomes together.

Table 5. Summary of SNPs per lake.

Lake	Number of SNPs
Wamala	831
Edward	1,430
Kyoga	1,811
Nawampasa	2,009
George	2,070
Bisina	2,602

As the read depth in SNPs position was closely related to the prediction accuracy of SNPs, the statistics of read depth for each SNP was calculated. It showed the estimated average read depth was 33. SNPs with read depth between 10 and 50 account for the majority (98%), while SNPs with read depth range from >200 account for nearly 1%. The average read depth was 33.

Table 6. Statistics of read depth represented the number of SNPs with the corresponding read depth.

SNP Read Depth	Number of SNPs
>10-50	11,984
60-100	117
110-150	43
160-200	5
>200	33

CONCLUSION

This study assessed indigenous practices and understanding about lungfish as a potential culture species in Uganda. Fish farmers inadvertently farm lungfish that enter their ponds during flood periods and have realized they can survive and grow alongside other fish (i.e., tilapia). However, optimal feed composition and lungfish grow-out strategies remain to be established. At present, growers rely on wild-caught lungfish fingerlings for the limited culture currently taking place.

Research must clarify the reproductive cycle of lungfish to facilitate farm-based spawning and produce uniform seed stock batches of genetically advantaged fish. A clear foundation to establish a lungfish industry, as well as the biology and manipulation of reproduction processes, is not fully understood.

An experimental program is needed to establish production parameters, since little is known about the growth cycle and nutritional needs of farm-reared lungfish. For example, optimal water temperatures, salinity tolerance, and other basic parameters of the species are not known.

Farmers have developed indigenous means of handling and managing lungfish in natural water bodies and farm ponds. These techniques are beginning to be discovered and codified. Promoting wider levels of lungfish production will require articulation of model production strategies and management systems that account for the burrowing and mobility of lungfish. Clearly, cage culture would overcome some known difficulties, but this work has not yet been accomplished.

Lungfish is a delicacy among groups in northern and eastern Uganda, and in some parts of the western region. Thus, present and potential consumer demand for the species is fairly well established. Field work has assessed potential paths of production and training for lungfish as a cultured species and managed water resource.

Lungfish may be raised on artificial diets since all fish farms feed commercial pellets to catfish or tilapia stock. Efforts to domesticate African lungfish are fundamental to advance a commercial industry capable of providing a valuable food item to people in need of affordable protein.

QUANTIFIED ECONOMIC BENEFITS

- Basic guidance on management of lungfish expressed in a farmer-oriented leaflet is being developed;
- Basic nutrition profile of lungfish grow out was expressed in a technical report for extension;
- Basic fingerling supply and grow out information expressed in a journal article; and
- Inform the merits of continuing research into developing low-cost, artificial breeding technologies for these species.

ACKNOWLEDGMENTS

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TOPIC AREA:
QUALITY SEEDSTOCK DEVELOPMENT

❖

Spat Collection and Nursery Methods for Shellfish Culture by Women

Quality Seedstock Development/Experiment/13QSD01PU

This research is still ongoing and will be included in a future Technical Report.
See Implementation Plan 2013-2015, page 40.

Reproduction and Seed Production of Sahar
(*Tor putitora*) in Chitwan Nepal

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ABSTRACT

Sahar (*Tor putitora)* is a high-value indigenous riverine species of Nepal that is declining in its natural habitat and has been declared an endangered species. Limited seed production of this species in the temperate region has restricted its expansion in culture and rehabilitation in natural waters. An experiment was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, from August 2014 to April 2015 to explore and assess breeding performance of sahar in the Terai region of Nepal, which has a subtropical climate. Twenty-eight male (0.5–1.5 kg) and 35 female (0.8–2.5 kg) brood fish were reared in ponds at 1000 kg/ha and provided 35% protein feed at 3% body weight per day. Maturity was observed by sampling fish and applying pressure to the abdomen to express gonads every two weeks during the off-season; this frequency was increased to every third day as breeding season approached. One female sahar (3–5 years old) was ready for breeding in March when water temperature was 23.3–25.2 °C. In the same month, another female responded to an injection of inducing hormone (ovaprim) when the temperature was 25.3–28.7 °C. Males about 1–2 years old expressed milt in almost all months. Ova from mature females were obtained by simple hand stripping and fertilized with milt manually collected from males. The fertilized eggs were incubated in Atkin hatching trays. Survival and growth of fry were high and maturation details were similar to fish spawned under temperate conditions. This study demonstrated that natural and induced breeding and fry rearing is possible in the Terai region of Nepal. However, further studies on synchronization of breeding time and mass seed production are needed.

INTRODUCTION

Sahar (*Tor putitora*), also known as "mahseer," is an important fish species of the torrential water of the Himalaya. It is a popular, economically important, and high-value indigenous fish species. Sahar is important as a game and food fish and is widely distributed in rivers, streams, and lakes (Rai et al. 1997). The price of sahar in the Nepalese market is almost double that of commonly cultivated carps and tilapia. Sahar is still taken in capture fisheries in lakes and rivers, and no commercial cultivation has begun in Nepal. This species is declining from its natural habitat mainly due to urbanization, poaching, over-fishing, and ecological alterations of physical, chemical, and biological conditions in the natural environment (Bista et al. 2007). Hence, there is a need for conservation of this species. In recent years, success in artificial breeding at some research stations has provided the additional enthusiasm on developing sahar for commercial cultivation, as well as the rehabilitation in natural waters (Rai et al. 2006).

Attempts to culture and conserve sahar have been initiated in Nepal with major efforts to develop culture technology and propagate the species (Gurung et al. 2002, Joshi et al. 2002). This has led to better knowledge of spawning biology, ecology, and behavior, as well as preliminary growth performance in

captive conditions. Enhanced growth in tropical and subtropical ponds, as well as the recent breeding success in hatcheries, has raised new hopes on the prospects of sahar aquaculture in Nepal (Shrestha et al. 2005, Bista et al. 2001, 2007, Rai 2008). In addition to the culture of fish to adult size for consumption, these new developments can contribute to rearing individuals that can be stocked into natural waters to replenish populations there. Due to its omnivorous and predatory feeding, sahar has also proven to be a good candidate to co-culture with mixed-sex tilapia to control tilapia recruits and provide better size at harvest and yield of tilapia (Shrestha et al. 2011). Inclusion of sahar in polyculture of mixed-sex tilapia with carps has enhanced production in these ponds.

Sahar is known to be intermittent in spawning behavior. In Nepalese context, it can spawn year-round, except January, under cultured conditions. However, in natural waters, it spawns during the monsoon when rivers and streams are at peak flows. Sahar typically migrate a long distance from large rivers to streams for spawning. The Fisheries Research Center in Pokhara is the key center that produces sahar fry in limited quantity. Demand for sahar fry has increased for restocking in rivers and lakes, as well as for aquaculture production. Lack of availability of fish seed is a major bottleneck for commercial production and conservation.

OBJECTIVES

- To extend sahar breeding technology to Chitwan from work conducted in Pokhara;
- To develop protocols for sahar reproduction and mass-scale seed production in Chitwan;
- To establish nursing and rearing management practices of sahar fry in Chitwan; and
- To make sahar fry available for culture and restocking.

MATERIALS AND METHODS

The experiment for sahar breeding was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, for nine months (1 August 2014 to 30 April 2015). For response studies, female sahar broods were transferred from the Fisheries Research Center, Pokhara, and reared at AFU. Male fish of more than one year age were collected from the aquaculture farm at AFU. Females of approximately 1.0–2.5 kg body weight were stocked in brood pond at the rate of 1,000 kg/ha. Brood fish were fed with 30%–40% protein diets. The feeding rate was 2%–5% body weight per day. Pond water quality parameters, such as temperature, pH, and dissolve oxygen, were measured every morning using Lutron Oxygen Meter DO-5510 and Lutron YK-21 PH model. Maturity of female fish was monitored at regular intervals. They were checked biweekly before the breeding season (May–August). The male broods were always found ripe with oozing milt after pressing their belly, but females were not ripe during May–August. As the breeding season approached during August– November and February–May, maturation testing was increased to every third day. Maturity observation was performed once a month for the remaining two months (December and January).

For maturity tests, fish collected from ponds were held in a hammock and readiness for spawning was examined by applying gentle hand pressure near the genital opening. Ripe males released milt, and females brood ova on 9 March. This female fish and two males were transported to the hatchery. The clean and dried female was stripped gently to empress eggs into a clean and dried bowl. Milt from both males was also collected in another bowl, then mixed with eggs for dry fertilization. The fertilized eggs were washed several times and spread in Atkin incubators by allowing one layer of eggs to settle on a single mesh screen in the flow- through system, and water flow was maintained at 7–9 L/min. The incubation trays were covered with a towel to reduce light levels in the tray. The eggs were observed after 24 hours during incubation, and unfertilized eggs were counted. Dead eggs were counted and removed each day to protect the healthy eggs from fungal infection. After four days (96 hours) hatching occurred and was completed within 24 hours when distinct eyes were seen in hatchling fish. Early hatched larvae had large amounts of yolk sac and settled around stones or near corners of the incubation tray. After

attaining free-swimming stage, the larvae were transferred into a tank of 2.5 m x 0.4 m x 0.3 m dimension.

On 26 March, another two female fish were injected with ovaprim hormone at the rate of 0.5 mg/kg body weight. Along with those females, four mature male fish were also kept in the spawning tank. After 26 hours, when the females were checked by pressing gently on the stomach, one female brood released eggs. Milt from two males was used to fertilize the eggs. Fertilized eggs were incubated as before, and a similar process was repeated. Hatching occurred after 60–72 h.

Reproductive parameters, such as relative fecundity, fertilization rate, hatching rate, and survival rate, were measured to analyze breeding performance. After fertilization, the total number of eggs and egg number per kg body weight were calculated. Egg size, mean weight of eggs and mean weight of swim-up fry were measured using an electronic balance. Fertilization rate, hatching rates, incubation period, hatchling survival rate, yolk absorption time, and time of hatchling to fry were also recorded.

The fry-rearing experiment was conducted for 45 days using two types of feed — commercial feed (T_1), and locally prepared feed (T_2) — for two batches of fry (from a naturally spawned brood and from a hormonally induced spawning). Each treatment was replicated three times. Sahar fry of 0.073–0.077 g body weight were stocked at 200 fry/m^2 in a hapa and water depth was maintained about 60 cm in each hapa. Fish fry were fed three times a day at 5% body weight throughout the experimental period.

RESULTS

Out of 35 female fish reared, one was observed overly matured on 21 November and another on 24 November 2014. On 8 December, two females were found overmatured, and, again on 9 December, two females showed overmaturation. Another female showed over maturation on 4 January 2015. In February, four females were overmature. In March, four females were overmature. In total, 15 female fish were found overmature during regular observations (Table 1).

Table 1. Result of maturity tests in different months from November 2014 to March 2015.

Date	Number of over mature females	Number of over spawned females	Water temperature of brood pond (°C)
21 November	1		21.2–23.0
24 November	1		19.7–22.8
8 December	2		18.2–21.2
9 December	2		17.5–20.5
4 January	1		15.5–17.2
21 February	1		19.1–21.3
24 February	2		19.4–22.0
27 February	1		20.6–22.8
4 March	2		22.5–26.0
7 March	1		22.2–25.4
9 March	1	1	23.3–25.2
27 March		1	25.3–28.7

One female was ready to spawn without injecting hormone (natural breeding) on 9 March 2015 when minimum temperature of water was 23.3°C and maximum was 25.2°C. Similarly, breeding of hormone-induced females occurred on 26 March when minimum temperature was 25.3°C and maximum was 28.7°C (Table 2). The total number of eggs obtained from the natural breeding female was 2585, while it was 4738 for hormone-induced breeding. Relative fecundity was 2,119 and 3,746 for natural and hormone-induced breeding, respectively. For natural breeding, 1g of ovulated eggs contained 94 eggs, while in hormone-induced breeding, 103 ovulated eggs were in 1 g of egg.

Fertilization rate of natural and hormone-induced breeding was 98% and 99%, respectively. The incubation period for natural and hormone-induced breeding was 96–104 h and 80–88 h, respectively. In the case of natural breeding, hatching rate was 95%, while that was 97% in hormone-induced breeding. Hatchling survival of natural breeding was 81%; whereas survival was 90% for hormone-induced breed hatchling (Table 2).

Newly hatched larvae were 9.4±1.2 mm long and 13.01±0.53 mg in weight. Similarly, newly hatched larvae of hormone-induced breeding were 8.9±0.7 mm long and 13.19±0.49, mg in weight (Table 3). It took 6 days to complete yolk sac absorption in natural breeding at 19.4–26.2 °C, but only 5 days to absorb yolk sac for induced larvae at 24.8–27.2 °C. Average yolk sac absorbed larvae from natural spawning were 11.5±0.5 mm long and 10.09±1.12 mg in weight. Similarly, average yolk sac absorbed larvae from hormone-induced breeding were 11.5±0.5 mm long and 9.87±1.41 mg in weight (Table 3).

Table 2. Breeding performance of sahar with and without induced maturation.

Description	Natural breeding	Induced breeding
Date	9 March	27 March
Temperature (°C)	23.3 –25.2	25.3–28.7
Female body weight (kg)	1.2	1.3
Male body weight (kg)	0.65, 0.80	0.72, 0.87
Total egg spawned	2585	4738
Egg number per kg body weight	2119	3746
Ovulated eggs per g	94	103
Fertilization rate (%)	98	99
Incubation period (hour)	96–104	80–88
Hatching rate (%)	95	97
Hatchling survival (%)	81	90
Yolk sac absorption period (days)	6	5
Time to swim-up fry (days)	17	17

Table 3. Mean and range of egg diameter, and length and weight (mean±SE) of larvae and fry from two breeding parents.

Parameter	Brood	
	Natural breeding	Induced breeding
Mean fertilized eggs diameter (mm)	2.9±0.2	3.1±0.3
	(2.8–3.5)	(2.8–3.3)
Mean fertilized eggs weight (mg)	12.37±0.80	12.69±0.78
	(11.57–13.17)	(11.97–13.47)
Mean newly hatched larvae length (mm)	9.4±1.2	8.9±0.7
	(8.2–10.6)	(8.2–9.6)
Mean newly hatched larvae weight (mg)	13.01±0.53	13.19±0.49
	(12.48–13.54)	(12.70–13.68)
Mean yolk sac absorbed larvae length (mm)	11.5±0.5	11.5±0.5
	(11–12)	(11–12)
Mean yolk sac absorbed larvae weight (mg)	10.09±1.12	9.87±1.41
	(8.97–11.21)	(8.46–11.28)
Mean swim-up fry (17 days) length (mm)	14.6±0.5	13.3±0.5
	(14.08–15.12)	(12.8–13.8)
Mean swim-up fry (17 days) weight (mg)	20.96±1.08	14.04±0.40
	(19.1–22.5)	(13.5–14.6)

During brood rearing, temperature ranged from 14.3°C to 38.2°C (Figure 1), DO from 1.4–13.5 mg/L, and average pH from 5.9 to 10.4. Natural spawning occurred when temperature was between 23.3°C and 25.2°C, while induced spawning occurred when temperature of pond was between 25.3°C and 28.7°C.
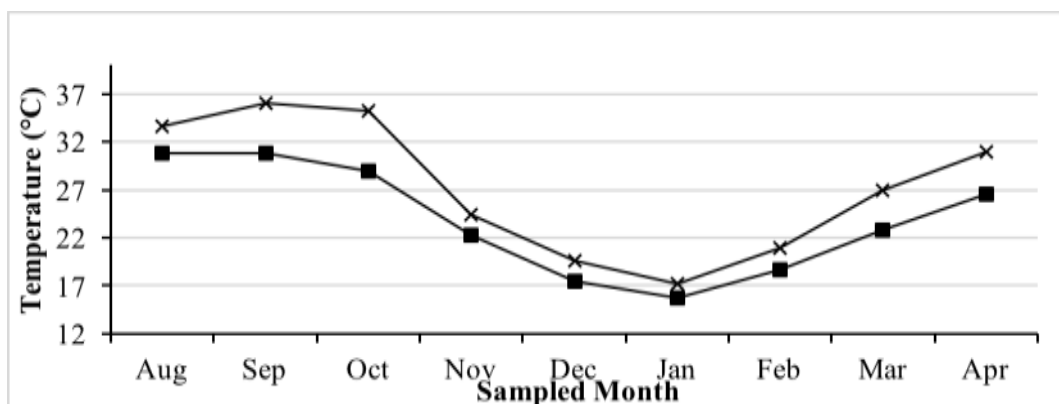
Figure 1. Average water temperature of the brood pond at morning and evening each month during brood rearing.

Growth parameters of swim-up fry after 17 days were obtained from two broods fed with two different types of feed (Table 4). In the case of fry obtained from natural breeding, there were no significant differences in mean initial weight, mean final weight, specific growth rate (SGR), daily weight gain (DWG), or survival rate for fry fed with two different types of feed. Similarly, in the case of fry from hormone-induced breeding, there was no significant difference in any growth parameters for fry fed with two different types of feed. Growth for both types of fry was linear over the rearing period (Figures 2 and 3).

Table 4. Growth performance of fry from two breeding parents fed with different feeds.

Brood	Natural breeding (B_1)		Induced breeding (B_2)	
Feed type	F_1	F_2	F_1	F_2
Mean initial wt. (mg)	76.47±7.65[a]	71.00±3.38[a]	80.83±4.14[a]	73.87±11.99[a]
Mean Final wt. (mg)	252.11±40.82[a]	330.88±39.49[a]	282.17±62.00[a]	272.79±12.73[a]
Culture days	40	40	40	40
SGR (%/day)	2.97±0.61[a]	3.84±0.25[a]	3.09±0.44[a]	3.29±0.30[a]
DWG (mg/fish/day)	4.39±1.17[a]	6.50±0.94[a]	5.03±1.46[a]	4.97±0.13[a]
Survival (%)	98.67±2.31[a]	98.00±1.80[a]	94.67±5.77[a]	95.17±3.33[a]
AFCR	1.7±0.2[a]	1.2±0.1[b]	1.8±0.4[a]	1.6±0.1[a]

Mean values with same superscript in the same row are not significantly different ($p<0.05$).
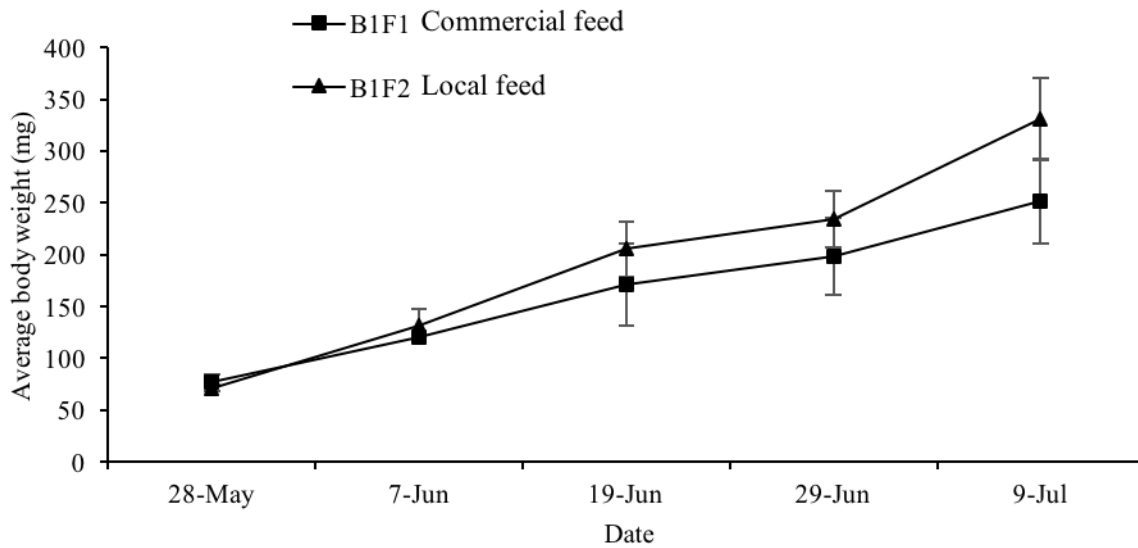
Figure 2. Growth of fry from natural breeding fed with two types of feed.
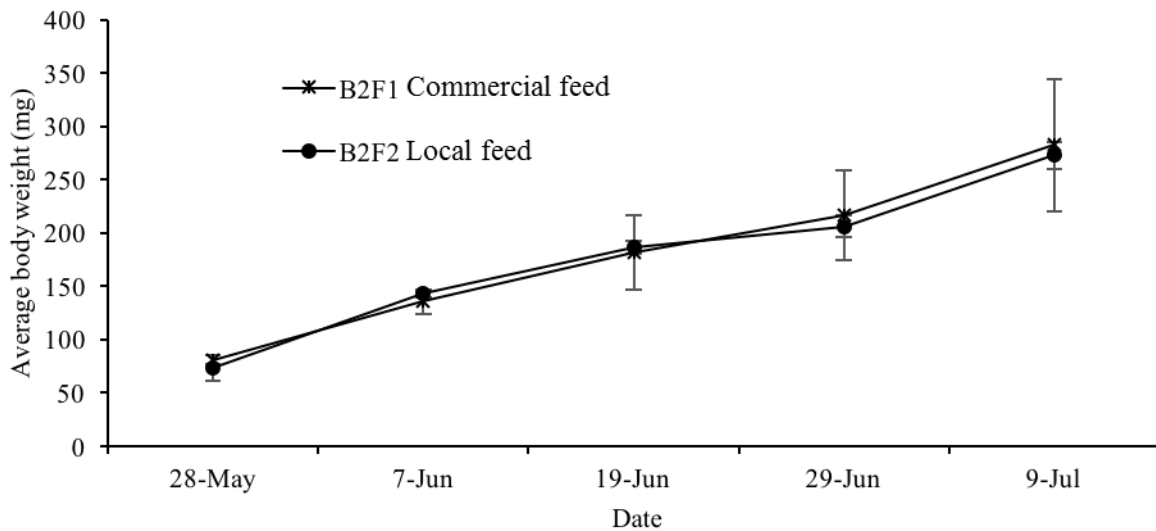

Figure 3. Growth of fry from induced breeding fed with two types of feed.

DISCUSSION

Regular maturity observation of sahar showed that getting the correct spawning time is critical for sahar breeding, and thus, examination of female fish for maturity must be done frequently. As reported by Bista et al. (2010), pond reared sahar show intermittent spawning characteristics, and determining optimum timing for egg stripping by frequent checking of brood fish may result in a spawning success rate of more than 50%. Although frequent examination of brood for maturity was done in this study, 15 females were found over mature, while successful breeding was attained only in 2 females. Overripe females were recorded even when temperature ranged between 15.5–28.7°C, from the last week of November to the last week of February. Bista et al. (2010) reported that spawning occurred when temperature ranged

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between 26–27.4°C on one occasion, and 20–21°C on a second occasion in Pokhara, and there were more spawners in February and March compared to September and October. Pandey et al. (1998) reported successful spawning induced by hormonal injection when water temperature in the pond was 18–24 °C. However, the dose administered was lower (0.2 ml/kg body weight) than in this study. Bista et al. (2010) also reported that diameter and weight of fertilized eggs were 2.87±0.13 to 2.98±0.08 mm and 13.90±0.91 to 15.38±1.26 mg, respectively. They also reported an incubation period of 45–125 hours at water temperatures from 19–28 °C. The shorter incubation period for the second lot of eggs can be attributed to a higher temperature. The length and weight of newly hatched larvae were similar to data from Bista et al. (2010).

Female rearing was done in temperatures ranging between 14.3–38.2°C, DO ranging between 1.4–13.5 mg/L and average pH ranging between 5.9–10.4. There did not appear to be any stress of the female fish in response to these varying conditions. Our results for temperature at spawning were similar to Bista et al. (2010), who documented natural breeding in autumn when the temperature was 22–27°C, and in spring when the temperature was 19–25°C. Similarly, Pandey et al. (1998) documented induced breeding of sahar when temperature was 18–24°C. Bista et al. (2010) also reported that pH ranged from 7–9 and DO from 3–9 mg/L when natural breeding occurred.

There were no significant differences in mean final weight, SGR, DWG, and survival rate of fry from natural breeding when fed with commercial feed (F_1) and farm-made feed (F_2). Similarly, there were no significant differences in these values for fry from induced breeding when fed with two different types of feed. The DWG of fry were considerably lower than reported by other authors, such as FRC (1995, 0.02–0.03 g/day), Nepal et al. (1996, 0.52 g/day), Paudel (2003, 0.10–0.13 g/day), Acharya (2004, 0.32 g/day) and Bista et al. (2007, 0.18–0.28 g/day). Survival rate of fry during present study was considerably higher than values found by Paudel (2003, 33–51.3%) and Islam (2002, 83.8–89.4%), but similar values documented in FRC, Pokhara (1995, 90.4–92.1%), and Acharya (2004, 92%).

CONCLUSIONS

Breeding of sahar is possible in the subtropical climate of Nepal. The best temperature lies between 17.5°C and 28°C. During testing, we observed that maturity time is very short (12 to 24 h), and many fish were not found until they were overly mature. Testing should be conducted more frequently to find ripe female fish during the spawning interval. The success of hormone-induced spawning shows new hope for possible mass production of sahar seed.

QUANTIFIED ANTICIPATED BENEFITS

Sahar were successfully bred at the new site in Rampur, and, as a result, about 5,000 fry were produced. Five farms used sahar in carp polyculture trials during our on-farm trial with mixed-sex tilapia. We conducted a workshop on production of sahar seed with hatchery operators as well as development and research personnel to help extend this information to more users.

ACKNOWLEDGMENTS

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