EFFECT OF STOCKING DENSITY AND FERTILIZATION ON THE GROWTH PERFORMANCE OF TILAPIA (*Oreochromis spp.*) FED RICE BRAN, WATER SPINACH AND DUCKWEED IN POND AND PADDY FIELD

By

Sen Sorphea

SLU
Institutionen for husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

MSc. Thesis

Uppsala 2008-2010
ISBN 978-91-86197-98-8

Effect of stocking density and fertilization on the growth performance of tilapia (*Oreochromis spp.*) fed rice bran, water spinach and duckweed in pond and paddy field

Sen Sorphea

CelAgrid (Centre for Livestock and Agriculture Development),
P.O Box 2423, Phnom Penh 3, Cambodia.
E-mail: sorphea_sen@yahoo.com

SLU	**MSc. Thesis**
Institutionen for husdjurens utfodring och vård	
Swedish University of Agricultural Sciences	**Uppsala 2008-2010**
Department of Animal Nutrition and Management	**ISBN 978-91-86197-98-8**

Dedication

To my mother Chheng Khhim,
brother Sen Sovann,
sister Sen Chhan Mouny,
and father Chhim Sen

Effect of stocking density and fertilization on the growth performance of tilapia (*Oreochromis spp.*) fed rice bran, water spinach and duckweed in pond and paddy field

Sen Sorphea

CelAgrid (Centre for Livestock and Agriculture Development),
P.O Box 2423, Phnom Penh 3, Cambodia.
E-mail: sorphea_sen@yahoo.com

Abstract

Two experiments were conducted at CelAgrid, Kandal Province, each for a period of 80days.

In Experiment 1, 12 ponds, each with area of 10m^2,were used to compare four treatments arranged in a 2 x 2 factorial with a completely randomized design with 3 replicates. The first factor was fertilizer with effluent and no effluent; the second factor was stocking density of 3 or 5 fish per m^2. The fish in each pond were provided with feed at 5% of the fish biomass (DM basis). The feed contained 25% water spinach, 25% duck weed and 50% rice bran (DM basis). The effluent from a bio-digester was applied every 7 days, at rates equivalent to 150 kg N/ha.

Survival rate was higher in ponds fertilized with effluent and in ponds with lower fish density. There were no differences for gain in weight and length due to treatment, and no interaction between fertilizer and stocking density. However, these data were derived from random samples of fish taken at 20 day intervals and the results were partially confounded by differences among treatments in survival. Net fish yield was higher for the higher stocking density but there was no effect of fertilization with effluent. Feed conversion (DM offered/net fish yield) did not differ among treatments, but this measurement was also confounded by differences in survival, as amounts of feed offered were based on initial numbers of fish and the average weight estimated from the sampling at 20 day intervals. Final fish weight and net fish yield were negatively correlated with survival rate.

In Experiment 2, 12 plots in a paddy field, each with an area of 209m^2, were used to compare 4 treatments in a 2 x 2 factorial in a completely randomized design with 3 replicates. The first factor was with or without feed supplement; the second factor was different stocking densities of 3 or 5 fish per m^2. In each plot of paddy there was a trench 11m wide x 1m in length x 1m deep along one side of the plot. The feed supplement was the same as in Experiment 1. All paddy plots were fertilized with effluent from a bio-digester every 7 days at the rate of 150 kg N per ha.

Survival rate was not affected by supplementation but there was a tendency (P = 0.10) for it to be lower on the higher stocking rate. Both final weight of fish and the net fish yield were increased by supplementation and by stocking rate with no interaction between the treatments. The FCR (for those paddies that received feed supplementation) was not affected by stocking rate.

In conclusion it would seem that in rice-fish systems, supplementation is not an appropriate intervention, in view of the lower efficiency of use of the supplement. Thus, for the additional 43 kg of net fish yield (123-80 kg) in Experiment 2, the amount of feed provided was on average 358 kg (7.5/209*10000), that is about 7.5 kg feed per 1 kg of net fish yield. Measures that lead to enhancement of the natural feed supply (e g: fertilization with bio-digester effluent) would seem to be more appropriate technology.

Key words: Effluent, feed conversion, rice-fish culture, supplementation,

Table of Contents

1. Introduction ……………………………………………………………………………….8
2. Objectives ……………………………………………………………………………….8
3. General discussion ……………………………..……………………………………….8
 3. 1 Tilapia……….……………………………………………………………………...8
 3. 2 Biodigester effluent as fertilizer for fish ponds..……………………………….....9
 3. 3 Duckweed and water spinach………….…………………………………………9
 3. 4 Fish stocking density……………………………………………………….....10
 3. 5. Integrated Rice-Fish system...…..10
4. Conclusions……………………………………………………………………….10
5. Acknowledgements……………………………………………………………….10
6. References…………………………………………………………………………..11

Paper I……………………………………………………………………………………13

Abbreviations

ADG	Average daily weight gain
CP	Crude protein
DM	Dry matter
N	Nitrogen
FE	Feed fertilized with bio-digester effluent
FNE	Feed not fertilized with bio-digester effluent
EF	Effluent from bio-digester with feed supplement
ENF	Effluent from bio-digester without feed supplement

1. Introduction

The natural fish harvest has declined during the last 10 years in Cambodia due to illegal fishing and environmental changes. This has a significant effect on the livelihoods of Cambodian people whose main protein source is deriving from fish. At present, much effort from government, development institutions and the private sector has been made to the development of aquaculture in order to match the shortfall of fish protein for human consumption. It has been observed that around Phnom Penh City, and elsewhere with access to water and ponds, fish culture has increased. Farming of common carp, silver carp, silver barb, tilapia and catfish is encouraged by government and development organizations so that farmers in the rural areas can have fish for their own consumption and income generation.

Fish culture in the rice field is especially interesting as a method for maximizing land use, combining the production of both rice and fish. Fish culture in the rice fields is mainly concentrated in Southeast Asia, where it has been practised for centuries. A number of advantages are obtained by applying integrated rice-fish cultivation. The presence of fish in a rice field generally increases the rice yield by 10 to 15% (Hilbrands et al 2004). Fish is a source of protein and by integrating production with rice food security is improved. In addition, raising fish contributes to the improvement of public health because they can eat insects like mosquitoes. Raising fish in a rice field is also a biological way of reducing weeds, insects, snails and some rice diseases. This is a safe and cheap alternative to using chemical pesticides to control insects and algae. In areas where rice production is not profitable in all seasons, fish production forms an alternative source of income from the field (Hilbrands et al 2004).

The major factors that influence the economics of fresh water fish production in pond culture are:

- fertilization of the ponds in order to increase the feed available by stimulating the natural food web chain;
- supplementation with commercial fish pellets or with local feed resource; and
- stocking density of the fish in the pond

When fish are raised in the rice field, the same factors apply, but in addition there are the direct and indirect effects of the fish on the yield of rice.

2. Objectives

The aims of the experiments reported in this thesis were:

- To study the effect of stocking density and fertilization on the growth performance of tilapia raised in ponds supplemented with water spinach and duckweed
- To study the effect of stocking density and supplementation on the growth performance of tilapia raised in rice paddies.

3. General discussion

3. 1 Tilapia

The advantages of using Tilapia are that this species can digest natural food organisms, such as plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, larva fish, detritus, and decomposing organic matter. They are thus well suited to systems which depend to a major extent on stimulating the natural feed chain and use of vegetative supplements such as duckweed and water spinach.

3. 2 Bio-digester effluent as fertilizer for fish ponds

The potential for using animal wastes in fish culture has been demonstrated for many years in China, where animal manure has been used as the main fertilizer in fish culture. Processing of manure by anaerobic bio-digestion results in the production of biogas, which is used as fuel mainly for cooking, and effluent, which contains all the plant nutrients present in the original manure while the greater part of the nitrogenous compounds is converted to ammonium salts (San Thy and Preston 2003). This ionization of the nitrogenous fraction has been shown to improve the fertilizer value of the effluent compared with the original manure. The biomass yields and crude protein content of duckweed and cassava foliage were increased when bio-digester effluent rather than the manure put into the bio-digester was used as fertilizer (Le Ha Chau 1998a, b). Similar positive results have been reported for the use of bio-digester effluent in fish culture. Pich Sophin and Preston (2001) reported that tilapia, silver carp, bighead carp, silver barb and Mrigal grew faster in ponds fertilized with effluent than with urea-DAP or manure. Increased productivity in poly-culture fish ponds when bio-digester effluent was the fertilizer rather than raw manure was also reported by Ding Jieyi and Han Yujin (1983). Yields of fish were increased by 26% when the effluent was applied compared with the original manure.

Surprisingly, there was no effect of bio-digester effluent in the experiment reported in Paper I. The reason may have been the over-riding effect of the supplementation with duckweed, water spinach and rice bran, which besides serving as direct feed to the fish would also have acted partially as fertilizer for the ponds.

3. 3 Duckweed and water spinach

Duckweed is a small floating aquatic plant that grows very well on stagnant ponds and is commonly found throughout tropical countries (Leng et al 1995). Crude protein yields of between 6 and10 tons/ha/yr have been recorded when the N content in the water was in the range of 10 to 30 mg/liter (Nguyen Duc Anh 1997). Not only the yield but also the crude protein of duckweed responds to the nutrient content of the water, increasing from 15% in DM with 10 mg N/litre to 40% crude protein in DM with 60 mg N/litre (Rodríguez and Preston 1996). Many trials have been carried out using duckweed as the major feed to raise fish, with good results (Journey et al. 1991). Fasakin et al (1999) found that duckweed meal (from *Spirodela polyrrhiza*) could replace up to 30% of the total diet of the blue tilapia (*Oreochromis niloticus*), and Hasan and Edwards (1992) grew tilapia in static water concrete tanks and found that the fish slowly consumed *Spirodela polyrrhiza* while *Lemna perpusilla* was rapidly consumed.

Water spinach and duckweed were compared as supplements to a poly-culture (Tilapia, Silver carp and Mrigal) in ponds fertilized with bio-digester effluent at 120 kg N/ha (San Thy et al 2008). Net fish yields were 1888 and 2493 kg/ha for water spinach and duckweed compared with only 848 kg/ha when only effluent was used.

In Experiment 2 in Paper I, supplementation with a combination of duckweed, water spinach and rice bran (25, 25, 50% DM basis) increased the net fish yield from 81.4 to 122 kg/ha. However, the system was different from that described by San Thy et al (2008) in that the fish (Tilapia) were cultivated in a rice paddy.

3.4 Fish stocking density

There appear to be no studies on the effect of fish stocking density in culture systems of the kind described in Paper I. In the present study, when the Tilapia were raised in $10m^2$ ponds, net fish yield was increased from 1466 to 2248 kg/ha. However, at the same time, the fish survival rate declined from 78 to 60%. In the rice paddy system, increased stocking density from 3 to 5 fish/m^2 also increased net fish yield (from 80 to 123 kg/ha) but this was also associated with a reduction in survival rate from 66 to 42%. The indirect effect of a decrease in survival rate was to increase the feed availability to the survivors which grew faster such that at the end of the experiment, the total fish weight was greater, although numbers were smaller in the treatments with lowest survival rate.

3. 5 Integrated Rice-Fish systems

Most studies on integrated rice-fish system have employed stocking densities of between 1500 and 6000 fish/ha of rice paddy. In the study with no supplement (Rothius 1998), the net fish yield increased linearly (from 60 to 180 kg/ha) as the fish density was increased from 1500 to 6600/ha. In the report of Bocek (no date), the increase in fish density from 1500 to 4500/ha resulted in the net yield increasing from 500 to 1000 kg/ha, but in this case the fish were supplemented with soybean, copra meal and rice bran.

In the study reported in Paper 1, the net fish yield increased from 80 to 123 kg/ha when the fish density increased from 3300 to 5500 fish/ha.

4. Conclusion

- As a general conclusion it would seem that in rice-fish systems, supplementation is not an appropriate intervention, in view of the lower efficiency in use of the supplement.
- Measures that lead to enhancement of the natural feed supply (e g: fertilization with bio-digester effluent) would seem to be a more appropriate strategy.

5. Acknowledgements

The authors are grateful to the MEKARN program, financed by Sida (Swedish International Development Agency) for supporting this study.

6. References

Bocek A No date Water harvestinng and aquaculture for rural development. http://www.ag.auburn.edu/fish/international/introrice.htm

Ding Jieyi and Han Yujin 1983: Comparative studies on the effects of fresh pig manure and anaerobically fermented pig manure upon fish farming, p: 288-296.

Fasakin E A, Balogun A M and Fasuru B E 1999 Use of duckweed, Spirodela polyrrhiza, L. Schleiden, as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* L. Aquaculture Research 30:313-318

Hasan M S and Edwards P 1992 Evaluation of duckweed (L. perpusilla and S. polyrrhiza) as feed for Nile tilapia (Oreochromis niloticus). Aquaculture 104:315-326

Hilbrands A and Yzerman C 2004 Agrodok 21: On farm fish culture http://books.google.com.vn/books?id=1N1H9-KG2nUC&printsec=frontcover&dq=Aldin+Hilbrands+and+Carl+Yzerman+2004&source=bl&ots=9a5SJqGlKC&sig=So8Lwg7a1Mh7dhLTcLC7HNkusYo&hl=en&ei=ezfaS8i4EJeXkQWtg7x7&sa=X&oi=book_result&ct=result&resnum=1&ved=0CAYQ6AEwAA#v=onepage&q&f=false

Journey W K, Skillicorn P and Spira W 1991 Duckweed Aquaculture - A New Aquatic Farming System for Developing Countries. The World Bank. 76pp. Washington DC) http://www.p2pays.org/ref/09/08875.htm

Leng R A, Stambolie J H and Bell R 1995: Duckweed - a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development (7) 1 Livestock Research for Rural Development. (7) 1 http://www.lrrd.org/lrrd7/1/3.htm

Le Ha Chau 1998a Biodigester effluent versus manure from pigs or cattle as fertilizer for production of cassava foliage (*Manihot esculenta*). Livestock Research for Rural Development. (10) 3 http://www.lrrd.org/lrrd10/3/chau1.htm

Le Ha Chau 1998b Biodigester effluent versus manure, from pigs or cattle, as fertilizer for duckweed (*Lemna* spp.) Livestock Research for Rural Development. (10) 3 http://www.lrrd.org/lrrd10/3/chau2.htm

Nguyen Duc Anh and Preston 1997 Effect of management practices and fertilization with biodigester effluent on biomass yield and composition of duckweed**.** Livestock Research for Rural Development: 9 (1) http://www.lrrd.org/lrrd9/1/anh91.htm

Pich Sophin and Preston T R 2001: Effect of processing pig manure in a biodigester as fertilizer input for ponds growing fish in polyculture. Livestock Research for Rural Development. (10) 6: http://www.lrrd.org/lrrd13/6/pich136.htm

Pich Sophin 2001 Liturature review : Waste recycling and fish culture. http://www.utafoundation.org/utacambod/msc99thes/Sophinlr.htm

Rodriguez L and Preston T R 1996 Use of effluent from low-cost plastic biodigesters as fertilizer for duck weed ponds Livestock Research for Rural Development: 8 (2) http://www.lrrd.org/lrrd8/2/lylian2.htm

Rothuis A 1998 The effect of rice seeding rate on rice and fish production in direct-seeding rice-fish culture. Page 43. Chapter 7: Polyculture of Silver Barb, *Puntius Gonionotus* (Bleeker), Nile Tilapia, *Oreochronis Niloticus* (L) and Common Carp, *Cyprinus Carpio* (L) in Vietnamese rice fields: 2. Fish production parameters. Page 90. http://www.google.com.vn/search?hl=en&source=hp&q=Arjo+Rothuis+1998+RICE-FISH+CULTURE+IN+THE+MEKONG+DELTA%2C+VIETNAM%3A+CONSTRAINT&meta=&rlz=1R2GPEA_en&aq=f&aqi=&aql=&oq=&gs_rfai=

San Thy and T R Preston 2003 Effluent from biodigesters with different retention times for primary production and feed of Tilapia (*Oreochromis niloticus*) Livestock Research for Rural Development: 15 (9) 2003

San Thy, Khieu Borin, Try Vanvuth, Pheng Buntha and Preston T R 2008 Effect of water spinach and duckweed on fish growth performance in poly-culture ponds. Livestock Research for Rural Development. Volume 20, Article #16. http://www.lrrd.org/lrrd20/1/sant20016.htm

Effect of stocking densities and feed supplements on the growth performance of tilapia (*Oreochromis spp.*) raised in ponds and in the paddy field

Sen Sorphea, Torbjorn Lundh, T R Preston and Khieu Borin
CelAgrid (Center for Livestock and Agriculture Development)
sorphea_sen@yahoo.com

Abstract

Two experiments were conducted at CelAgrid, Kandal Province, each for a period of 80days.

In Experiment 1, 12 ponds, each with area of 10m^2, were used to compare four treatments arranged in a 2 x 2 factorial with a completely randomized design with 3 replicates. The first factor was fertilizer with effluent and no effluent; the second factor was stocking density of 3 or 5 fish per m^2. The fish in each pond were provided with feed at 5% of the fish biomass (DM basis). The feed contained 25% water spinach, 25% duckweed and 50% rice bran (DM basis). The effluent from a bio-digester was applied every 7 days, at rates equivalent to 150 kg N/ha.

Survival rate was higher in ponds fertilized with effluent and in ponds with lower fish density. There were no differences for gain in weight and length due to treatment, and no interaction between fertilizer and stocking density. However, these data were derived from random samples of fish taken at 20 day intervals and the results were partially confounded by differences among treatments in survival. Net fish yield was higher for the higher stocking density but there was no effect of fertilization with effluent. Feed conversion (DM offered/net fish yield) did not differ among treatments but this measurement was also confounded by differences in survival as amounts of feed offered were based on initial numbers of fish and the average weight estimated from the sampling at 20 day intervals. Final fish weight and net fish yield were negatively correlated with survival rate.

In Experiment 2, 12 plots in a paddy field each with an area of 209m^2, were used to compare 4 treatments in a 2 x 2 factorial in a completely randomized design with 3 replicates. The first factor was with or without feed supplement; the second factor was different stocking densities of 3 or 5 fish per m^2. In each plot of paddy there was a trench 11m wide x 1m in length x 1m deep along one side of the plot. The feed supplement was the same as in Experiment 1. All paddy plots were fertilized with effluent from a bio-digester every 7 days at the rate of 150 kg N per ha.

Survival rate was not affected by supplementation but there was a tendency (P = 0.10) for it to be lower on the higher stocking rate. Both final weight of fish and the net fish yield were increased by supplementation and by stocking rate with no interaction between the treatments. The FCR (for those paddies that received feed supplementation) was not affected by stocking rate.

In conclusion it would seem that in rice-fish systems, supplementation is not an appropriate intervention, in view of the lower efficiency of use of the supplement. Thus, for the additional 43 kg of net fish yield (123-80 kg) in Experiment 2, the amount of feed provided was on average 358 kg (7.5/209*10000), that is about 7.5 kg feed per 1 kg of net fish yield. Measures that lead to enhancement of the natural feed supply (e g: fertilization with bio-digester effluent) would seem to be more appropriate technology.

Key words: Effluent, feed conversion, rice-fish culture, supplementation,

Introduction

Cambodian people prefer their protein to come from freshwater fish that is eaten fresh, salted, smoked or made into fish sauce and paste. Tonle Sap, Tonle Mekong and Basak rivers are the main capture fisheries in Cambodia (Agriculture in Cambodia 2010).

Tilapia has become popular for farmers as it is easy to culture and there is a good demand in the market. Moreover, tilapias (*Oreochromis spp.*) adapt well to the local environment and local feed, and have high productivity. The fish are usually kept in a pond near to their houses, as in addition to having the fish as protein source, farmers can grow vegetables and use the water from the pond to water the vegetables. The feeds used for the fish depend on the resources available in the area. Duckweed and water spinach are available almost everywhere in the villages, while rice bran is the by-product from rice milling.

The combination of rice and fish can be a very profitable system, since it was observed that the fish feed on organisms, such as insects and larvae which grow and live in the rice fields. This system provides both rice and fish. Besides economic benefits, the biological benefit is also a factor. Weeding and use of chemical fertilizers and pesticides are reduced when this system is practiced. Moreover, the movement of the fish stirs the water, which increases the oxygen level and improves the development of the roots of the rice. Rice-fish culture improves the income of farmers in the rural areas, as the system requires very little inputs and farm labor. Farmers can harvest rice or fish at the same time or harvest only the rice and keep the fish, or alternatively harvest the fish before the rice (Mackay 1995).

Hypothesis

- In pond culture the net fish yield of tilapia will be increased by fertilizing with bio-digester effluent and by increasing the stocking density.
- When tilapia is raised in the rice paddy there will be advantages from supplementation with duckweed, water spinach and rice bran.

Objectives

Two experiments were carried out to study the effects of stocking density and fertilization/supplementation on the growth performance of Tilapia raised in ponds and in a paddy field.

Location and climate

The experiments were carried out at the Center for Livestock and Agriculture Development (CelAgrid) experimental farm, located in Prah Theat village, Rolous commune, Kandal Steung district, Kandal Province, approximately 19 km from Phnom Penh City.

In Cambodia, the rainy season is from June to October, while the dry season is from November to May. This climate provides good conditions for the animal and rice production system. Average temperature is around 25^{o}C, with a maximum of about 40^{o}C in April, while the coldest month is January, when the temperature is around 21^{o}C, with a maximum of about 31^{o}C. Average annual rainfall in Cambodia varies from 1,500 mm or less in the central plain and 1,500 to 2,500 mm in the surrounding mountains. Over most of the South West coastal region, average annual rainfall is in excess of 3,000 mm. The rainfall to the East of the Mekong River is generally between 1,800 mm and 3,000 mm, while the lower Mekong valley and basin of the Tonle Sap Lake are relatively dry, with rainfall averaging between 1,200 and 1,500 mm.

Experiment 1. Effect of stocking density and fertilization with bio-digester effluent on the growth performance of tilapia (*Oreochromis spp.*) fed rice bran, water spinach and duckweed in pond culture

Materials and methods

Study duration

The experiment was conducted from 12 January to 1 April 2010.

Pond preparation and management

In total 12 ponds were prepared at the CelAgrid centre, each with an area of 10 m^2 (4 m in length x 2.5 m wide) and a depth of 1.5 m. The ponds were lined with plastic to avoid filtration of water, and then water was pumped in from nearby canals and pond. Lime (CaO) at 200 g/m² was applied before stocking the fish, in order to kill parasites and pathogenic organisms and also to increase water pH.

Experimental design

The experiment was conducted for 80days and designed as a 2 x 2 factorial arrangement: the factors were with or without application of bio-digester effluent, and two stocking densities (3 and 5 fish per m^2) (Table 1). A Complete Randomized Design (CRD) was used. Each treatment was replicated 3 times. In total 640 fingerlings were bought from a commercial fish farm and randomly distributed into the ponds. Weight and length of a sample of the fish were recorded as the initial weight and length.

Table 1: Experimental layout

1	2	3	4	5	6	7	8	9	10	11	12
5F-NE	3F-E	5F-E	3F-E	3F-NE	5F- NE	3F-NE	3F-E	5F-NE	5F-E	5F-E	3F-NE

F = Feed, E = Effluent, NE = Not fertilized with effluent; 3 and 5 = density 3 or 5 fish/m^2

Experimental diets

The fish were fed a mixture of rice bran, water spinach and duckweed as the basal feed. All ponds were provided with the same feed (Table 2).

Table2: Ingredient composition of the diet, % DM basis

Water spinach	25
Duckweed	25
Rice bran	50
Total	100
% Crude protein (in DM) #	18.5

 # Calculated on basis of observed composition of the ingredients (Table 3)

Duckweed was cultivated in CelAgrid, while water spinach was bought from a local market. In order to reduce the moisture content, duckweed was collected in the evening and wilted for the morning feeding, while the duckweed collected in the morning was wilted and fed to the fish in the evening.

Water spinach was chopped into small pieces and mixed with duckweed and rice bran before feeding. The feed was provided twice daily at 8:00h and 16:00h. The amount of feed was 5% (DM basis) of the fish body weight. The amount of feed was adjusted by the average of fish sampling multiplied with the initial number of the fish at the beginning. The feed was mixed and put in a floating feeding frame to avoid the feed spreading in the pond (Photo 1).

Fertilization

Ponds were fertilized with effluent from a plastic bio-digester loaded with pig manure at a rate of 150 kg of N/ha/year (San Thy et al 2006). The effluent from the bio-digester was pumped into containers (Photo 2). Before applying it to the fish pond, a sample was taken to determine N. The amount of effluent applied to the ponds was calculated according to the concentration of N.

Photo 1: Individual pond with floating feeding frame

Photo 2: Effluent storage

Measurements

The oxygen and pH of the water in the fish pond were measured every 5 days. Each measurement was taken 2 times at 06:00h and 16:00h using a pH meter (pHep by HANNA) and a DO_2 meter (Model 9150), respectively. The water temperature was measured 3 times every 5 days at 06:00h, at 12:00h and at 16:00h using thermometers. A thermometer was placed permanently in each pond. Water transparency was measured at 12.00h every 2 days using a Secchi disk.

Every 20 days a sample of the fish was caught with a seine net and ten individuals chosen at random. These were weighed using an electronic scale and measured with a ruler from the mouth tip to the caudal fin. Survival rate was measured at the end of the experiment by the following equation:

$$X (\%) = (N_t / N_0) \times 100$$

Where: N_0 : initial number of the fish; N_t : final number of the fish

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using the General Linear Model (GLM) of the Minitab software (version 2000 release 13.1). Sources of variation were: effluent, density, effluent * density interaction and error.

Gain in weight and length were measured as the linear regression of weight (or length) on days in the experiment, using the SLOPE command in the Minitab software.

Results

Chemical composition of the feed

During periods of 20 days, water spinach and duckweed were harvested at the same place to make sure that the nutrient content was not so different. The protein content of the duckweed was higher than of the water spinach (Table 3).

Table 3: Chemical composition of the diet ingredients

	DM, %	CP, % of DM
Water spinach	13.6	26.0
Duckweed	6.86	30.5
Rice bran	90.2	8.65

Nitrogen requirement of the pond

Effluent from the bio-digester was analyzed for N, and the amount calculated based on the rate of N application of 2.88 g N per pond per week (Table 4).

Table 4: Nitrogen requirement in each pond #

Application level of N, kg/ha	150
Area of pond, m^2	10
N requirement, g/pond	150
N requirement per pond per week, g	2.88

 # Source: San Thy et al 2006

Water quality

The treatments had no effect on water quality, measured by pH, temperature or dissolved oxygen levels (Table 5), all of which were within the normal range for culture of Tilapia (Swingle 1969). There was an interaction between treatments for water density (Table 6), which was more transparent (less phytoplankton) with the lower fish stocking rate when effluent was applied. In contrast, in the absence of effluent, the water was more transparent at the higher stocking rate.

Table 5: Mean values for water quality in ponds stocked with Tilapia at different densities and fed supplements of rice bran, water spinach, and duckweed with addition of bio-digester effluent or none

	Effluent (E)		Density (D)		Probability			SEM
	Effluent	No effluent	3	5	E	D	E*D	
pH								
06:00h	7.8	7.9	7.9	7.9	0.79	0.98	0.77	0.058
16:00h	8.8	8.1	8.8	8.1	0.30	0.30	0.30	0.514
DO, mg/liter								
06:00h	3.1	3.0	3.0	3.1	0.74	0.63	0.75	0.104
16:00h	4.2	4.2	4.1	4.3	0.77	0.28	0.73	0.155
Temperature, ^oC								
06:00h	28.9	28.8	28.8	28.8	0.49	0.94	0.31	0.106
12:00h	31.5	31.4	31.4	31.4	0.68	0.85	0.12	0.199
16:00h	33.1	33.0	33.1	33.0	0.72	0.68	0.38	0.201
Water transparency, cm								
12:00h	18.1	18.7	18.7	18.1	0.29	0.26	0.001	0.380

Table 6: Mean values for water density in ponds stocked with Tilapia at different densities and fed supplements of rice bran, water spinach, and duckweed with addition of bio-digester effluent or none

Effluent	**Yes**		**No**		**SEM**	**P**
Fish. m^2	**3**	**5**	**3**	**5**		
Water transparency, cm	19.8^a	16.6^b	17.7^b	19.7^a	0.53	0.001

a, b Mean values without common superscript differ at P<0.05

Changes in weight and length, and in the ratio of weight: length

There were no differences for gain in weight and length due to treatment (Table 7), and no interaction between fertilizer and stocking density. However, these data were derived from random samples of fish taken at 20 day intervals and, as will be discussed later, the results were partially confounded by differences among treatments in mortality/survival.

Table 7: Mean values in live weight of tilapia fertilized with and without effluent

	Effluent (E)		Density (D)		Probability			SEM
	Effluent	No effluent	3	5	E	D	E*D	
DWG, g/day	0.700	0.607	0.698	0.608	0.223	0.238	0.891	0.049
DLG, mm/day	0.102	0.092	0.097	0.097	0.320	1.000	0.733	0.006
W: L ratio	0.044	0.039	0.041	0.042	0.211	0.793	0.544	0.002

The best measure of the overall effect of the treatments is the net yield of fish expressed as the weight of all the fish at the beginning of the experiment subtracted from the final weight of the survivors (Tables 8 and 9). On this basis there was no effect of the effluent but a significantly higher yield for the greater stocking density. However, as feed offered was based on average live weights in each pond multiplied by the numbers of fish at the beginning, those with the highest density received more feed. Also in the ponds with highest mortality, the survivors received more feed per fish than in the ponds with higher survival rate.

Table 8: Mean values (main effects) for total weight gain, feeds offered and feed utilization for Tilapia (*Oreochromis spp.*) stocked at different densities and fed supplements of rice bran, water spinach, and duckweed, with addition of bio-digester effluent or none

	Effluent (E)		**Density (D)**		**Probability**		**SEM**
	Effluent	**No effluent**	**3**	**5**	**E**	**D**	
Water spinach, g/pond/d	15.2	14.8	11.4	18.6	0.914	0.035	2.34
Duckweed, g/pond/d	15.2	14.8	11.4	18.6	0.914	0.035	2.34
Rice bran, g/pond/d	9.47	9.19	7.12	11.54	0.90	0.051	1.55
Total DM, g/pond/d	39.8	38.8	29.9	48.7	0.91	0.038	6.23
Total DM, g/pond in 80d	3183	3103	2390	3895	0.841	0.005	273
Initial wt, g/pond	142	162	123	180	0.203	0.004	10.2
Final wt, g/pond	2010	2006	1589	2427	0.986	0.006	161
Net fish yield, g in 80 days	1869	1845	1466	2248	0.918	0.009	160
FCR	1.71	1.71	1.67	1.74	0.999	0.725	0.131
Survival, %	74	64	78	60	0.045	0.003	3.03

FCR = Feed DM offered/net fish yield

Table 9: Mean values for weight gain, feeds offered and feed utilization for Tilapia (*Oreochromis spp.*) stocked at different densities and fed supplements of rice bran, water spinach, and duckweed with addition of bio-digester effluent or none

	Effluent		**No effluent**		**SEM**	**P**
	3	**5**	**3**	**5**		
Water spinach, g/pond/d	11.6	18.7	11.1	18.5	3.31	0.208
Duckweed, g/pond/d	11.6	18.7	11.1	18.5	3.31	0.208
Rice bran, g/pond/d	7.28	11.67	6.96	11.4	2.20	0.27
Total DM, g/pond/d	30.5	49.0	29.2	48.4	8.81	0.221
Total DM, g/pond in 80d	2444	3922	2336	3869	386	0.94
Initial wt, g/pond	116	167	130	193	14.43	0.656
Final wt, g/pond	1672	2348	1505	2507	228.74	0.496
Net fish yield, g	1556	2182	1375	2314	227.3	0.511
FCR	1.61	1.81	1.74	1.68	0.19	0.888
Survival, %	87.7	60.0	68.0	59.3	4.29	0.058

FCR = Feed DM offered/net fish yield

Net yield of fish and survival rate

Survival rate was higher in ponds fertilized with effluent and in ponds with lower fish density (Tables 8 and 9). This in turn affected the net fish yield which is measured as the weight of surviving fish less the weight of fish at the beginning.

Discussion

Growth rate

Growth rates in weight and in length were calculated from measurements on a random sample of 10 fish taken at 20 day intervals. The growth rates are affected by the density of fish in the pond, which was determined not only by the experimental treatments (3 or 5 fish/m^2), but also by the survival rate of the fish population. This varied among treatments, and thus in some cases the feed available per fish was influenced by the numbers of fish surviving in the pond. When the survival rate was low the feed offer per surviving fish was increased, which would stimulate faster growth rate. The problem is that survival rates could only be measured at the end of the experiment, and it is not known at what stage of the experiment the fish died.

For this reason productivity was measured as the net fish yield, expressed as weight of fish at the end of the experiment less the weight at the beginning. Using this criterion it was clear that the survival rate had a determining effect on fish productivity. As the survival rate increased the final weight per fish and the net fish yield both decreased (Figures 1 and 2). In contrast, the feed DM offered per pond was determined by the stocking density at the start of the experiment. The relation to survival is incidental and not causal (Figure 3). The final result was that the feed conversion, measured as DM offered/net fish yield, was not affected by the survival rate (Figure 4).

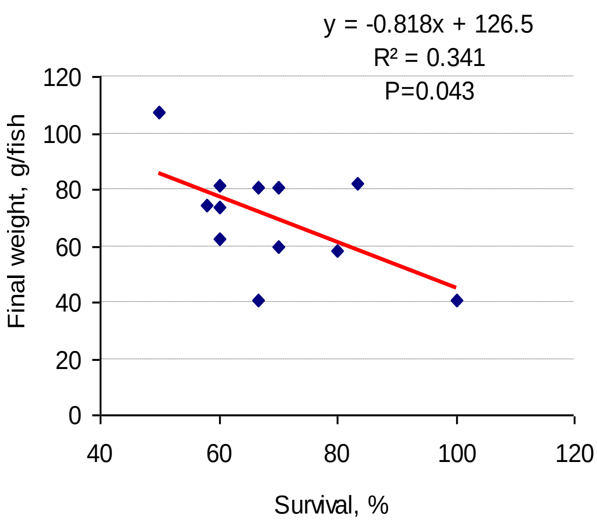

Figure 1. Relationship between survival rate and final weight per fish

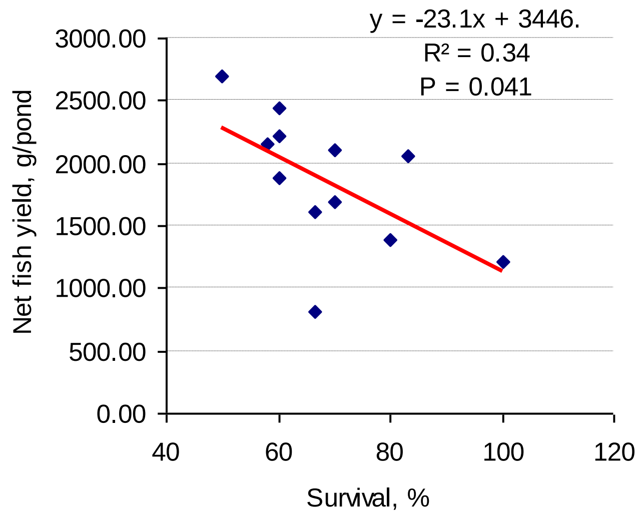

Figure 2. Relationship between survival rate and net fish yield per pond

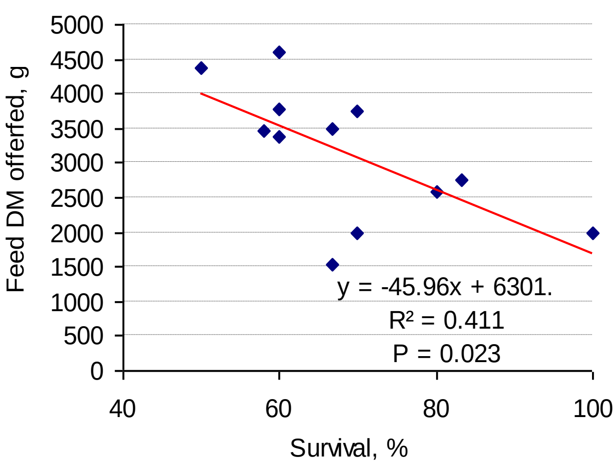

Figure 3. Relationship between survival rate and DM offered per pond

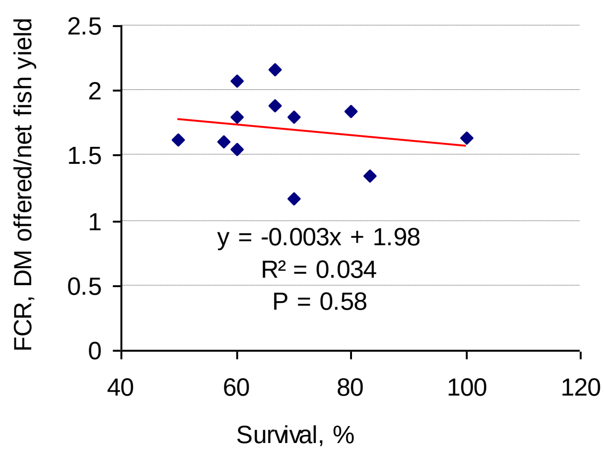

Figure 4. There was no relationship between survival rate and FCR (DM offered /net fish yield per pond)

The net fish yield in this experiment (a range of 1500 to 2200 kg/ha) was twice as high as the yields (760 – 1200 kg/ha) reported by San Thy and Preston (2003). These researchers also used Tilapia but in ponds of $6m^2$ and at a lower stocking rate of 2 fish/m^2. They applied bio-digester effluent to the ponds at a similar rate (160 kg N/ha) but the fish received no supplementary feed. Nguyen Duy Quynh Tram et al (2007) fertilized ponds with raw pig manure or bio-digester effluent (derived from the same manure) at 240 kg N/ha over 120 days. The net fish yield of a mixture of Tilapia, Silver carp and Hybrid Catfish was 1700 kg/ha with the effluent and 2100 kg/ha with the raw manure.

Conclusions

- The net yield of fish was not affected by addition of effluent to the ponds but was higher for the greater stocking density.
- Survival rate was higher in ponds fertilized with effluent and in ponds with lower fish density.
- As the survival rate increased the final weight per fish and the net fish yield both decreased

Experiment 2. Effect of stocking density and feed supplementation on the growth performance of tilapia (*Oreochromis spp.*) raised in the paddy field

Materials and methods

Study duration

The experiment was conducted in the dry season, from 23rd January to 13th April 2010.

Pond preparation and management

Twelve plots were prepared in a paddy field. The total area of each plot was 11m x 19 m with a trench 11m wide x 1m in length x 1m deep at one side of each plot. In total 528 fingerlings were purchased from a fish hatchery farm, Preak Phnov, near Phnom Penh City. They were raised in a nursery pond in CelAgrid for 15 days before being introduced into the plots of paddy rice, which was done 7 days after rice transplanting.


Photo 3: Rice transplant


Photo 4: Rice shooting


Photo 5: Rice maturity

Experimental design

The experiment was carried out as a 2 x 2 factorial arrangement. The factors were: with and without supplementary feed; and stocking densities of 3 and 5 fish per m^2 (Table 10). A Complete Randomized Design (CRD) was used. Each treatment was replicated 3 times.

Table 10: Experimental layout

1	2	3	4	5	6	7	8	9	10	11	12
3E-F	3E-NF	3E-F	5E-NF	5E-F	5E-NF	5E-F	3E-NF	5E-NF	3E-NF	3E-F	5E-F

E = Effluent, F = Feed supplement, NF = No feed supplement, 3 and 5 = density 3 or 5 fish/m^2

Feed processing

Fish were fed on a mixture of rice bran, water spinach and duckweed (Photo 6, 7 and 8; Table 11).

Table 11: Ingredient composition of the diet, % DM basis

Water spinach	25
Duckweed	25
Rice bran	50
Total	100
% Crude protein (in DM)#	18.5

 # Calculated on basis of observed composition of the ingredients (Table 12)

The feed was provided twice daily at 8:00h and 16:00h at an estimated rate of 5% (DM basis) of the fish body weight. The amount of feed was adjusted by the average of fish sampling multiply with the initial number of the fish at the beginning. Duckweed was cultivated in CelAgrid, while water spinach was bought from the market. In order to reduce the moisture content, duckweed was collected in the evening and wilted for the morning feeding, while duckweed collected in the morning was wilted and fed to the fish in the evening. Water spinach was chopped into small pieces and mixed with rice bran and duckweed before feeding to the fish, in the feeding frame (Photo 1). During successive periods of 20 days, water spinach and duckweed were harvested at the same place to make sure that the nutrients contained in water spinach and duckweed were not so much different.

Photo 5: Duckweed

Photo 6: Chopped Water spinach

Photo 7: Rice bran

Measurements

Fish weight and length were measured on random samples (n=10) of fish taken at 08:00h every 20 days before they were given feed and before application of effluent. Dry matter and CP of feeds were analyzed every 20 days before the fish sampling. Survival rate was measured at the end of the experiment. Water quality was measured following the same procedures as in Experiment 1. The fish were harvested after 80 days. Total weight, length and the number of the fish were measured.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using the General Linear Model (GLM) of the Minitab software (version 2000 release 13.1). Sources of variation were: feed, density, feed * density interaction and error.

Results

Chemical composition of the feed and fertilization

The crude protein content in duckweed was higher than in water spinach (Table 12).

Table 12: Chemical composition of the diet ingredients

	DM, %	CP % of DM
Water spinach	13.6	26.0
Duckweed	6.86	30.5
Rice bran	90.2	8.65

All the ponds in the paddy field were fertilized with effluent from a bio-digester at a rate of 150 kg N per hectare/year (San Thy et al 2006). The amount of effluent was calculated on the basis of its content of N (Table 13). It was supplied directly into each paddy at intervals of 7 days.

Table 13: Total Nitrogen requirement in each pond #

Proportion of N, kg/ ha	150
Area of pond, m^2	11
N requirement, g	165
N requirement per week, g	3.17

 # Source: San Thy et al 2006

Water quality

There were no treatment effects on water quality (Table 14).

Table 14: Mean values for indices of water quality (main effects)

	Supplement (S)		Density (D)		Probability			SEM
	Feed supplement	**No feed supplement**	**3**	**5**	**S**	**D**	**S*D**	
			pH					
06:00h	8.0	8.0	8.0	8.0	0.540	0.858	0.664	0.057
16:00h	8.2	8.3	8.3	8.2	0.691	0.794	0.937	0.064
			DO, mg/liter					
06:00h	3.2	3.2	3.2	3.2	0.659	0.792	0.642	0.090
16:00h	4.3	4.2	4.3	4.2	0.668	0.477	0.775	0.104
			Temperature, ^oC					
06:00h	27.9	27.9	28.0	27.9	0.9955	0.691	0.709	0.116
12:00h	30.4	30.5	30.5	30.4	0.642	0.636	0.877	0.241
16:00h	32.0	32.0	32.1	31.9	0.801	0.681	0.534	0.195
			Water transparency, cm					
12:00h	11.6	10.7	10.8	11.5	0.102	0.149	0.541	0.353

Gain in weight and in the ratio weight: gain

Growth rate in weight was increased by supplementation (Table 15), with no effect due to stocking rate. However these results were confounded by differences in survival rate (Table 16).

Survival and net fish yield

Survival rate was not affected by supplementation, but there was a tendency (P = 0.10) for it to be lower on the higher stocking rate (Table 15). Both final weight of fish and the net fish yield were increased by supplementation and by stocking rate with no interaction between the treatments. The FCR (for those paddies that received feed supplementation) was not affected by stocking rate.

Table 15: Mean values for initial and final fish numbers, survival rate, feed offered, total weight gain and feed conversion for Tilapia in the paddy field at two densities and with and without supplements of duckweed, water spinach and rice bran

	Feed supplement		Prob.	Fish/m^2		SEM	Prob.
	Supplement	No supplement		3	5		
Initial number	44	44		33	55		
Final number	22.2	22.7	0.931	21.8	23.0	3.93	0.839
Survival, %	51.2	56.8	0.686	66.2	41.8	9.4	0.104
Growth rate, g/day #	0.677	0.458	0.010	0.573	0.562	0.046	0.863
Initial weight, g	716	705		501	919	85.2	
Final weight, g	3256	2406	0.003	2174	3488	142.7	0.001
Net fish yield, g	2540	1702	0.016	1672	2570	196	0.012
Net fish yield, kg/ha	122	81.4		80	123		
DM offered (80days) per paddy, g ##				5802	9116		
FCR, DM offered/net fish yield #				2.85	3.02		

 # Based on weights of samples of fish taken at 20 day intervals
Data are for the paddies that received the feed supplement

Discussion

The average growth rates in Experiment 1 for the supplemented fish were higher (0.70 g/day) and feed conversion better (1.61) than in the present study. It is possible that some of the feed supplement was washed into the rice field, which would make it more difficult for the fish to consume it. The two systems are not comparable as the net outputs are quite different. In Experiment 1 the only output was the fish, whereas in Experiment 2 the outputs were fish and rice. The comparisons in Table 16 are more relevant, although here also the conditions varied in terms of quantity of supplement supplied and fish densities. In general, the net fish yield in our study can be said to be within the range reported in Table 16.

As a general conclusion it would seem that in rice-fish systems, supplementation is not an appropriate intervention, in view of the lower efficiency in use of the supplement. Thus, for the additional 43 kg of net fish yield (123-80), the amount of feed provided was on average 358 kg (7.5/209*10000), that is about 7.5 kg feed per 1 kg of net fish yield. Measures that lead to enhancement of the natural feed supply (e g: fertilization with bio-digester effluent) would seem to be more appropriate.

Table 16: Reports from other research on rice-fish systems compared with data from the present study

Systems	Treatments	Growth rate, g/day	Net fish production, kg/ha	Authors
Rice – fish (No feed supplement) with different seeding rate/ha	100kg rice/ha	0.80	32.5	Rothuis, 1998[a]
	200kg rice/ha	0.89	23.8	
	300kg rice/ha	0.80	16.7	
Rice - fish Poly-culture with different stocking density (No feed supplement)	6600fish/ha	0.25	177.4	Rothuis 1998[b]
	5400fish/ha	0.28	125.1	
	3400fish/ha	0.33	110.5	
	1400fish/ha	0.59	53.8	
	3800fish/ha	0.25	62.4	
Rice – fish (supplement rice bran)	6000fish/ha	0.48	132	Rasowo et al 2006
Rice – fish with different stocking density (rice bran 72%, copra meal 20% and soybean meal 8%)	1500fish/ha		508	Bocek
	3000fish/ha		913	
	4500fish/ha		1044	
Rice-fish culture with different stocking density #	Supplement	0.67	122	This study
	No supplement	0.45	81.4	

 # Mean values for densities of 1578 and 2631 fish/ha

Conclusions

- Net fish yield was increased by supplementation and by the higher stocking density,
- The survival rate tended to be reduced (P=0.10) by the increased stocking density but was not affected by supplementation
- Tended (P=0.10) to reduce the rate affected to the survival rate, with lower stocking density resulting in higher survival rate

Acknowledgements

The authors are grateful to the MEKARN program, financed by Sida (Swedish International Development Agency), for supporting this study.

References

References

Agriculture in Cambodia 2010: http://en.wikipedia.org/wiki/Agriculture_in_Cambodia#Rice_production

AOAC 1990: Official Methods of Analysis. Association of Official Analytical Chemists. 15th edition (K Helrick, editor). Arlington pp 1230

Bocek A: Water harvestinng and aquaculture for rural development. http://www.ag.auburn.edu/fish/international/introrice.htm

Kenneth T M 1995: Rice-Fish culture in China http://www.idrc.ca/openebooks/313-5/

Nguyen Duy Quynh Tram, Le Duc Ngoan and Ogle B 2007: Effect of processing pig manure through a biodigester as fertilizer for fish ponds on water quality and growth performance of three fish species. http://www.google.com.vn/search?num=100&hl=en&rlz=1R2GPEA_en&q=Nguyen+Duy+Quynh+Tram%2C+Le+Duc+Ngoan+and+Brian+Ogle%3A+Effect+of+processing+pig+manure+through+a+biodigester&btnG=Search&meta=&aq=f&aqi=&aql=&oq=&gs_rfai=

Rasowo J and E O Auma, 2006: On farm trials with rice-fish cultivation in the west Kano rice irrigation Scheme, Kenya http://myais.fsktm.um.edu.my/2514/

Rothuis A 1998: Chapter 5: The effect of rice seeding rate on rice and fish production in direct-seeding rice-fish culture. Page 43.
Chapter 7: Polyculture of Silver Barb, *Puntius Gonionotus* (Bleeker), Nile Tilapia, *Oreochronis Niloticus* (L) and Common Carp, *Cyprinus Carpio* (L) in Vietnamese rice fields: 2. Fish production parameters. Page 90.
http://www.google.com.vn/search?hl=en&source=hp&q=Arjo+Rothuis+1998+RICE-FISH+CULTURE+IN+THE+MEKONG+DELTA%2C+VIETNAM%3A+CONSTRAINT&meta=&rlz=1R2GPEA_en&aq=f&aqi=&aql=&oq=&gs_rfai=

San Thy, Khieu Borin, Try Vanvuth, Pheng Buntha and Preston T R 2006: Effect of water spinach and duckweed on fish growth performance in poly-culture ponds. Cambodian Journal of Agriculture, Volume 7(1). pp. 12-16. http://www.lrrd.org/lrrd20/1/sant20016.htm

Swingle H S 1969: Methods of analysis for waters, organic matter and pond bottom soils used in fisheries research. Auburn University, Auburn, AL. http://www.google.com.vn/search?hl=en&source=hp&q=Methods+of+analysis+for+waters%2C+organic+matter+and+pond+bottom+soils+used+in+fisheries+research.+Auburn+University%2C+Auburn%2C+AL&meta=&rlz=1R2GPEA_en&aq=f&aqi=&aql=&oq=&gs_rfai=