Aquaculture CRSP and AquaFish CRSP Technical Sessions at
World Aquaculture 2008
Busan, Korea
19-23 May 2008

Proceedings

Assembled by Briana Goodwin
Edited by Kat Goetting
2017

AquaFish Innovation Lab Management Office
Oregon State University
Corvallis, OR USA 97330

USAID FROM THE AMERICAN PEOPLE
Oregon State University
AQUAFISH INNOVATION LAB

Program activities are funded in part by the United States Agency for International Development (USAID) under CA/LWA No. EPP-A-00-06-00012-00 and by participating US and Host Country institutions.

The mission of the AquaFish Innovation Lab is to enrich livelihoods and promote health by cultivating international multidisciplinary partnerships that advance science, research, education, and outreach in aquatic resources. Bringing together resources from Host Country institutions and US universities, the AquaFish Innovation Lab emphasizes sustainable solutions in aquaculture and fisheries for improving health, building wealth, conserving natural environments for future generations, and strengthening poorer countries' ability to self-govern.

Acknowledgements
The AquaFish Management Entity acknowledges the contributions of researchers and the support provided by participating US and Host Country institutions, including the associated collaborators involved in this work. The editors acknowledge the contributions of Ford Evans, Jenna Borberg, Stephanie Ichien, and Amanda Hyman.

This publication may be cited as:
AquaFish Innovation Lab. 2017. AquaFish Technical Sessions at World Aquaculture 2008. AquaFish Innovation Lab, Oregon State University. Corvallis, Oregon, USA.

AquaFish Innovation Lab
College of Agricultural Sciences
Oregon State University
Strand Agriculture Hall
Corvallis, Oregon 97330-1643 USA

Table of Contents

PREAMBLE

World Aquaculture 2008
Busan, Korea
19-23 May 2008

The 2008 Annual International Conference and Exposition of the World Aquaculture Society held in Busan, Korea, was themed, "Aquaculture for Human Wellbeing – The Asian Perspective." CRSP researchers presented over 50 talks and posters at the Conference. On May 21, Dr. Egna co-moderated a well-attended CRSP session featuring 12 presentations on CRSP global successes in genetics, reproductive biology, physiology, production, health and safety, marketing, and capacity building. Also on May 21, the African Aquacultural Session, with CRSP Co-Moderators Nancy Gitonga (Kenya) and Khalid Salie (South Africa), featured additional talks on CRSP projects in Ghana, Kenya, and South Africa.

Aquaculture and AquaFish CRSP Session Agenda
Wednesday, May 21, 2008, 09:00 - 14:50

Chairs: Kevin Fitzsimmons and Gyung-Soo Park
Moderator: Hillary Egna

09:00 **Assessment of size distribution, sex conversion rate, growth and survival of Nile tilapia (*Oreochromis niloticus*) fry collected from artificial incubation units, hapas, and ponds**
Remedios Bolivar

09:20 **Evaluation of the use of Insulin-like Growth Factor-I gene expression as an instantaneous growth indicator in the tilapia, *Oreochromis niloticus***
Christopher L. Brown

09:40 **Incorporation of the native cichlid, *Petenia splendida*, into sustainable aquaculture: reproduction systems, nutrient requirements, and feeding strategies**
Wilfrido Contreras-Sanchez

10:00 **Aquaculture research in Guyana: the work of the Mon Repos Aquaculture Station**
Tejnarine S. Geer

10:20 **Elimination of methyltestosterone from intensive masculinization systems: use of solar irradiation and bacterial degradation**
Wilfrido Contreras-Sanchez

11:30 **Management strategies for oyster culture, *Crassostrea gigas* and *C. cortesiensis*, in Sinaloa and Nayarit, Mexico**
Maria C. Haws

11:50 **Bivalve market study in Pacific Mexico**
Francisco J. Martinez-Cordero

12:10 **Contributions of the Aquaculture Collaborative Research Support Program to the development of fish farming in Kenya**
Charles C. Ngugi

13:30 **Influence of indispensable amino acid imbalanced diets on growth and free amino acid levels in body tissues of tropical fish pacu, *Piaractus mesopotamicus***
Maria Ceclia Portella

13:50 **Limnological characteristics and cage culture practice in Indrasarobar Reservoir of Nepal**
Madhav K Shrestha

14:10 **Enhancing local capacities on tilapia seed production through education in Honduras**
Suyapa Triminio Meyer

14:30 Dynamics of increase in Insulin-like Growth Factor-I mRNA expression in Nile tilapia, *Oreochromis niloticus*, in response to elevated temperature
Emmanuel M. Vera Cruz

African Aquaculture Session Agenda
Wednesday, May 21, 2008, 14:50 - 17:40

Chairs: Khalid Salie and Jong-Man Yoon
Moderator: Nancy Gitonga

14:50 Small-scale aquaculture and credit use in Ghana
Kwamena Quagrainie

15:10 The use of credit in fish production in Kenya
Kwamena Quagraine

16:00 Improved principles and practices for small-scale cage culture on irrigation reservoirs in South Africa
Khalid Salie

16:20 Integration of GIS, remote sensing, and USLE to predict soil erosion distribution in Nzoia Basin (Kenya)
Ernest W. Tollnar

16:40 Adding value to weeds *Tithonia diversifolia* and *Chromolaena odorata* through their use as fishpond fertilizer
Rodrigue Yossa Nouaga

Aquaculture and AquaFish CRSP Session
Abstracts and Presentations

Assessment of size distribution, sex conversion rate, growth and survival of Nile tilapia (Oreochromis niloticus) fry collected from artificial incubation units, hapas, and ponds

Remedios Bolivar*, Hernando Bolivar, Antonio Taduan, Lourdes Dadag, Roberto Miguel Sayco, and Russell Borski

Freshwater Aquaculture Center-College of Fisheries, Central Luzon State University, Science City of MuNueva Ecija 3120, Philippines
rbolivar@mozcom.com

The increase in demand for tilapia fingerlings is a constraint to the expansion of tilapia growout production. Tilapia hatcheries in the Philippines use various techniques in fry production that includes ponds, hapas and tanks. Anecdotal observation by tilapia growout farmers indicates that tilapia fingerlings collected from pond facilities perform better than fingerlings nursed from tanks or hapas. However, tilapia hatchery operators allegedly claim that sources of fingerlings do not affect growout performance of the fish. This study evaluated the size distribution, sex conversion ratio, growth and survival of Nile tilapia raised in incubation jars, ponds or hapas. The GIFT strain of Nile tilapia was used in these studies. Breeding of tilapia broodstock was conducted in 2.5 x 10 x 1 m net enclosures (hapas) and in 100 sq. m. ponds. After 15 days, fry were collected. Fifteen day prior to collection of fry, eggs were collected from breeders in hapas and were incubated in artificial incubation jars until yolk sac fry stage. Fry from different sources (artificial incubation, hapas, ponds and mixed sources [hapas and ponds]) were designated as treatments. Preliminary results showed no significant difference on growth and survival among treatments. However, fingerlings from the artificial incubation units were found to be of two sizes only (size 22: mean wt. = 0.2-0.25 g and size 20: mean wt. = 0.3-0.35 g) while tilapia fingerlings from hapas and ponds were more variable in sizes (size 24, 22, 20 and 17).

ASSESSMENT OF SIZE DISTRIBUTION, GROWTH AND SURVIVAL OF NILE TILAPIA, *Oreochromis niloticus* L. FRY COLLECTED FROM DIFFERENT HATCHING SYSTEMS

Investigators

Bolivar, R. B.[1], H. L. Bolivar[2], R.M. V. Sayco[1], E.B. T. Jimenez[1], R.L. B. Argueza[1], L. B. Dadag[2], A. G. Taduan[2] and R. Borski[3]

AFFILIATIONS

[1] Freshwater Aquaculture Center-College of Fisheries, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines

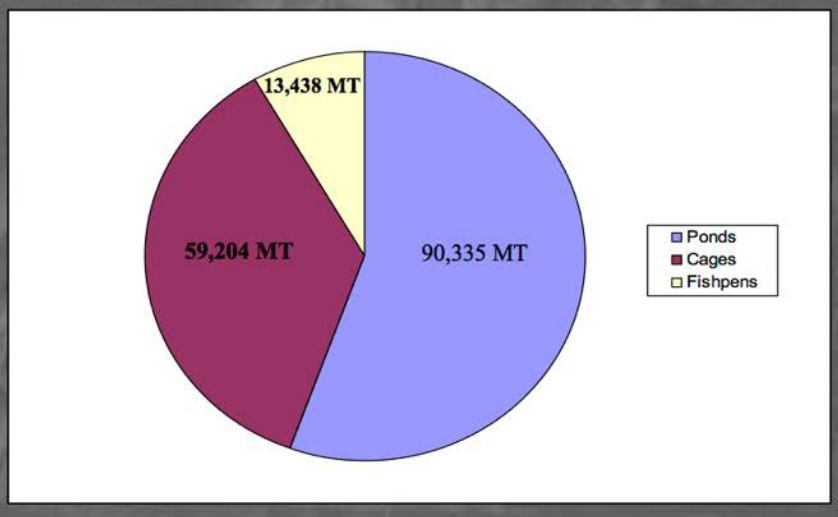
[2] GIFT Foundation International, Incorporated (GFII), CLSU Compound, Science City of Muñoz, Nueva Ecija, Philippines

[3] Department of Zoology, North Carolina State University (NCSU), Raleigh, NC 27695-7617

INTRODUCTION

- Tilapia is the main cultured finfishes in freshwater pond production and the third among the major cultured species for aquaculture in the Philippines
- The main production systems for tilapia aquaculture are ponds, cages and fishpens

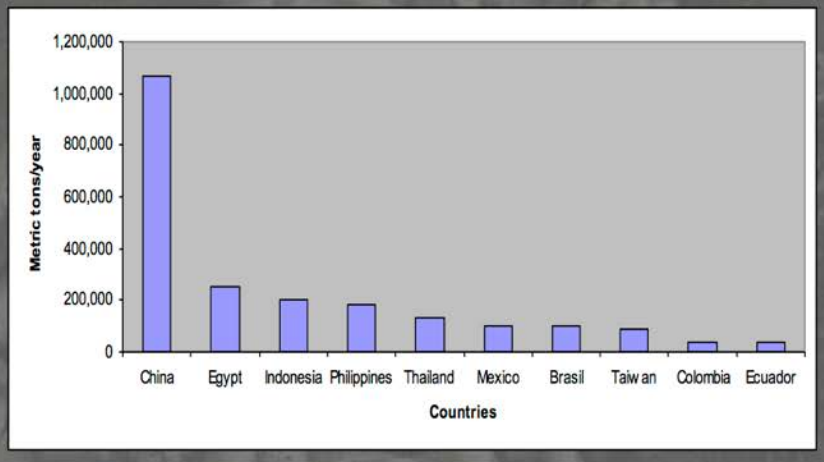
Tilapia Production in the Philippines (2005) by Major Types of Culture System



INTRODUCTION

- The development of genetically improved tilapia strains such as GIFT, FaST, GMT, BFAR-GET ExCEL, GENOMAR, etc. paved the way in the increase of tilapia production in the Philippines
- Seed production is an essential component of successful production of any organism
- The study was conducted from October to December, 2007 at the GFII facility

Top Ten Tilapia Producing Countries (2006)



STATEMENT OF THE PROBLEM

- Shortage of tilapia fry has remained an important constraint to further the development of aquaculture in many parts of the world

OBJECTIVES OF THE STUDY

To assess the size distribution, growth and survival of fry collected from artificial incubation units, hapas and ponds after 23 days of sex reversal treatment

METHODOLOGY

Experimental Units

Breeding

- Fourteen (14) 2.5 x 10 x 1 m fine mesh hapas
- Four (4) 100 m^2 earthen ponds

Sex-Reversal Treatment

- Twelve (12) 2 x 4 x 1 m fine mesh hapas

METHODOLOGY

Treatments

I – Incubation-hatched fry

II – Hapa-hatched fry

III – Pond-hatched fry

IV – Combination of hatched fry from TI, II and III

There were 3 replicates per treatment

Hatching Systems

Artificial Incubation Units

Hapas

Ponds

METHODOLOGY

- Tilapia fry were stocked in hapas at the rate of 850 m^2
- Feeding with androgen-treated fry mash was done six (6) times a day for 23 days
- Fish sampling was done once a week
- Data analysis was done using analysis of variance (ANOVA) in Randomized Complete Block Design (RCBD) with three replications followed by Least Significant Difference for comparison of means

Fry collection

Collection of fry in breeding hapas

Collection of fry from mouthbrooding females

Collection of fry in ponds using a dip net

RESULTS AND DISCUSSION

Initial and final mean length and weight of fry reared in hapas

Treatment	Initial Length (mm)	Final Length (mm)	Initial Weight (g)	Final Weight (g)
I	8.45	17.41	0.014	0.071
II	8.40	17.30	0.012	0.081
III	8.30	17.40	0.014	0.068
IV	8.45	17.57	0.013	0.072

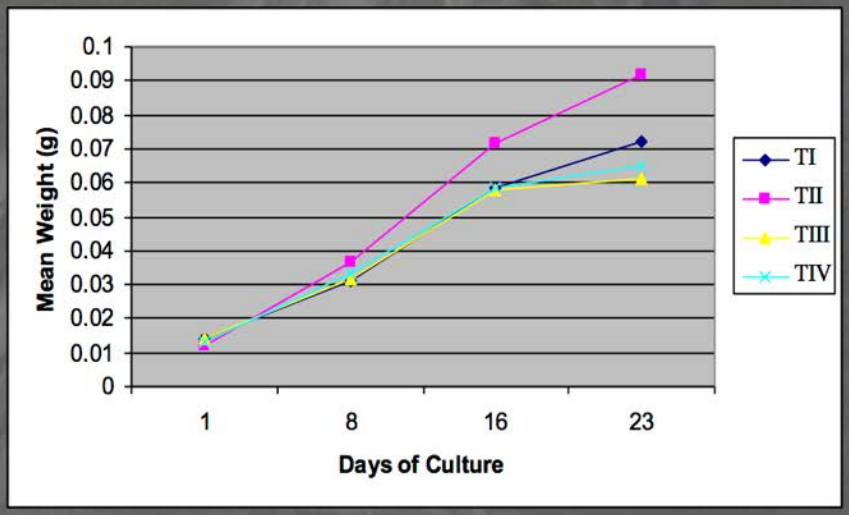
Note: Analysis of variance showed no significant difference among treatment means ($P>0.05$)

Gain in length, weight and specific growth rate of tilapia fry in hapas

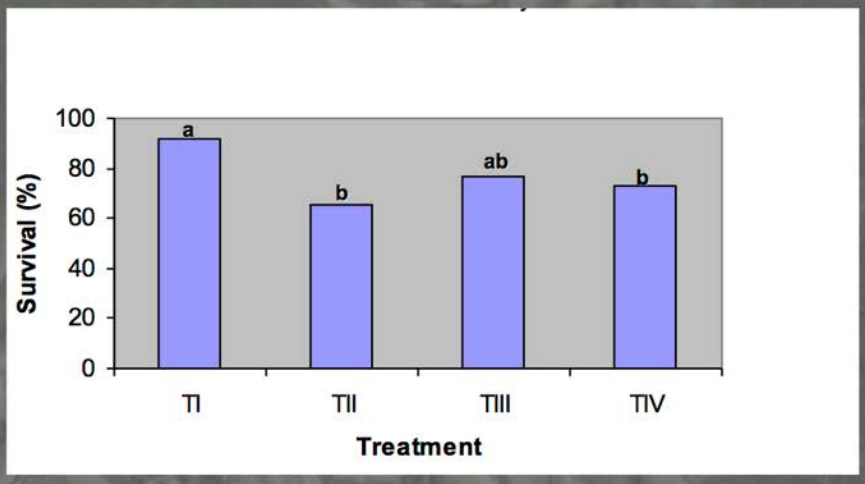
Treatment	Gain in Length (mm) ± S.D.	Gain in Weight (g) ± S.D.	Specific Growth Rate (%) ± S.D.
I	6.82 ± 1.47	0.06 ± 0.02	6.97 ± 1.41
II	8.37 ± 1.80	0.08 ± 0.03	8.70 ± 1.34
III	6.33 ± 1.52	0.05 ± 0.02	6.32 ± 1.14
IV	6.38 ± 1.12	0.05 ± 0.01	6.92 ± 0.98

Note: Analysis of variance indicated no significant differences among treatment means for length, weight and specific growth rate ($P>0.05$)

Growth pattern of fry in hapas

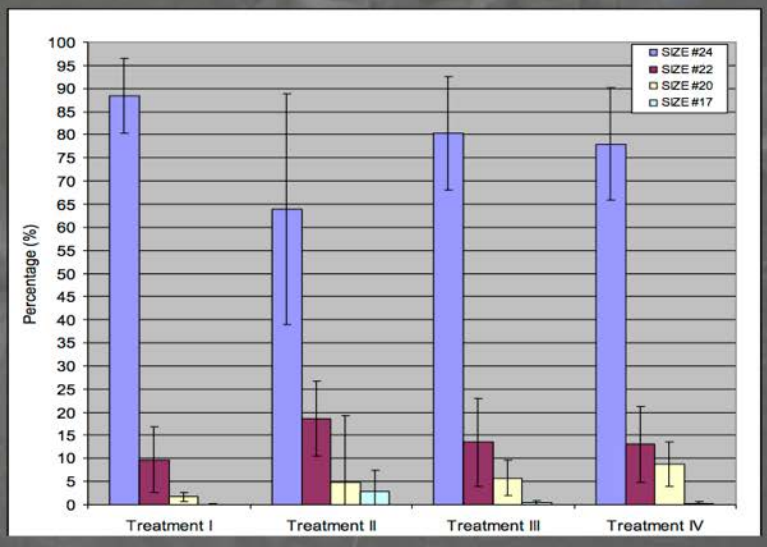


Percent survival of fry in hapas



Note: Treatment means with the same letter/s are not significantly different (P>0.05)

Size distribution of tilapia fry in hapas



SUMMARY

- The experiment was conducted to evaluate size distribution, growth and survival of tilapia fry collected from different hatching systems
- Collected fry were sex-reversed in 2 x 4 x 1 m hapas and were stocked at a stocking density of 850 per m^2
- Data on length, weight, size distribution and survival were gathered along with the water quality parameters

SUMMARY

- Treatment I had the least size variability of fry produced after the sex- reversal treatment
- Treatment I also had the highest percent survival among treatments
- There were no significant difference on the gain in length, gain in weight and specific growth rate among treatments

CONCLUSIONS

- Tilapia fry from artificial incubation units had a significant advantage in terms of survival and uniformity in size as compared to the fry from the other hatching systems

RECOMMENDATIONS

- Further study is recommended to assess other hatching systems like cages and tanks in the production of tilapia fry
- It is also recommended to conduct the study at different season to see the possible effect of weather condition on the hatching systems used in the production of tilapia fry

Funding for this research was provided by the

Aquaculture and Fisheries Collaborative Research Support Program

The AquaFish CRSP is funded in part by United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00 and by participating institutions.

Thank You!

Evaluation of the use of Insulin-like Growth Factor-I gene expression as an instantaneous growth indicator in the tilapia, *Oreochromis niloticus*

Christopher L. Brown*, Emmanuel Vera Cruz, Remedios B. Bolivar, and Russell J. Borski

US Department of Commerce, NOAA Aquaculture and Enhancement Division, The Milford Laboratory, 212 Rogers Avenue, Milford, CT. USA 06460
christopher.l.brown@noaa.gov

Insulin-like growth factor-I (IGF-I) is an important compound in the regulatory cascade that controls growth rate in vertebrates, including teleost fishes. Hepatic IGF-I synthesis is tightly correlated with growth rate in the Nile tilapia, as confirmed by the detection of gene activity through the quantification of mRNA. Alterations of the nutritional status of Nile tilapia result in comparable changes in the rate of growth and in the abundance of IGF-I mRNA in liver tissue.

Experimental variations of environmental conditions also result in alterations of IGF-I mRNA abundance in tilapia hepatic tissues, which are clearly reflective of the variable growth status of fish. Temperature elevation and the lengthening of photoperiod enhance the expression of the IGF-I gene, and result in a correlative increase in growth rate. Temperature elevation to a level considered optimal for cultivation of this species (~28^{o}C) more than tripled the abundance of detectable hepatic IGF-I mRNA, also in a close relationship with the promotion of growth. The sensitivity and rapidity of IGF-I mRNA as a growth indicator was underscored by the time course of changes in detectable levels; relative increases in mRNA abundance was seen after two days of temperature alterations, just as initial changes in specific growth rate first became detectable.

The relative social status of tilapia has also been found to influence growth rate, and that too produces marked alterations in IGF-I gene expression. Social dominance results in accelerated growth, as compared with the growth rates of subordinate animals, which are repressed. The levels of IGF-I mRNA quantified in hepatic tissue correlate closely with growth rate, and in turn also correlate with the social rank of male tilapia.

In sum, these experiments indicate that the quantification of IGF-I gene expression can provide a precise, rapid, and reliable indication of growth rate in tilapia. Conditions altering growth change IGF-I production within days, as opposed to the conventional comparative growout studies, which typically require thousands of animals and a much longer period of time. The measurement of IGF-I gene activity may provide a useful and practical alternative means of evaluating growth for the assessment and elaboration of effective rearing conditions for tilapia production.

IGF-1 GENE EXPRESSION: an instantaneous growth indicator for tilapia

Emmanuel Vera Cruz, Christopher Brown, Remedios Bolivar, Russell Borski, students, and colleagues

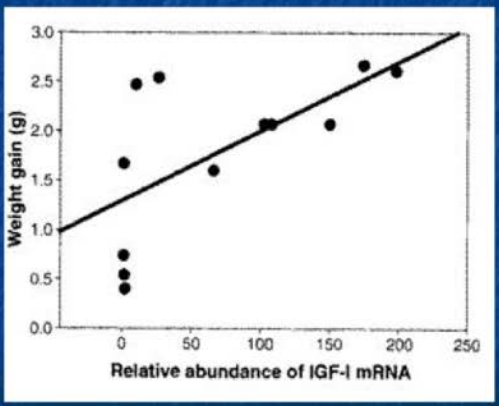
- Cloning, diet (2006) Aquaculture 251:585-595.
- Social Status (2007) Hormones and Behavior. 51:611-619.
- Temperature dependence, in press
- Influence of photoperiod, in press

Isolation of IGF-1 mRNA, *Oreochromis niloticus*

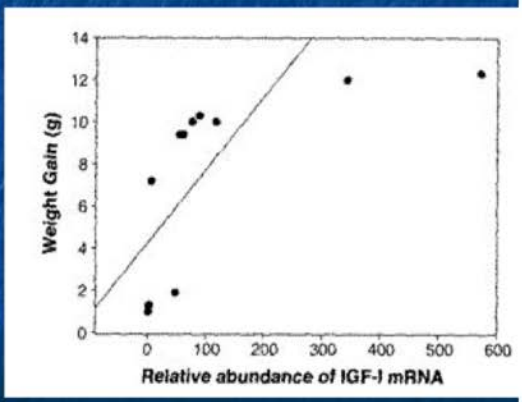
1 TTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCGGAATATTGAGATGTGACATCGCC 59
60 CGCATCTCATCCTCTTTCTCCCTGTTTTAATGACTTTAAACAAGTTCATTTTCGTCGGGC 119
Signal Peptide (144-275)
120 TTTGTCTTGTCGGAGACCCGTGGGGATGTCTAGCGCTTTTTCCCTTCAGTGGCATTTATGT 179
M S S A F S L Q W H L C
180 GATGTCTTCAAGAGTGCGATGTGCTGTATCTCCTGTAGCCACACCCTCTCACTACTGCCG 239
D V F K S A M C C I S C S H T L S L L P
B domain(276-362)
240 TGGGTCCTCACCCTGACTCCGACGGCAACAGGGGCGGGCCCTGAGACCCTGTGCGGGGCG 299
C V L T L T P T A T C A G P E T L C G A
300 GAGCTGGTCGACACCTGCAGTTTGTCTGTGGAGAGCGAGGCTTTTATTTCAATAAACCA 359
E L V D T L Q F V C G E R G F Y F N K P
C domain (363-392) **A domain** (393-455)
360 ACAGGCTATGGCCCCAGTGCAAGGCGGTCACGTGGCATCGTGGACGAGTGCTGCTTCCAA 419
T G Y G P S A R R S R G I V D E C C F Q
D domain (456-479)
420 AGCTGTGAGCTGCAGCGCCTTGAGATGTACTGTGCACCTGTCAAGACTCCCAAGATTTCT 479
S C E L Q R L E M Y C A P V K T P K I S
E domain (480-539)
480 CGCTCTGTGCGTTCACAGCGGCACACAGACATGCCAAGAGCACCCAAGGTTAGTAGCAGA 539

Close (84-90%) sequence identity with other IGF-1s

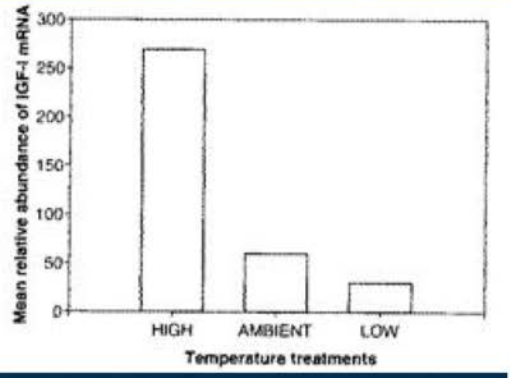
Hepatic gene expression as related to weight gain



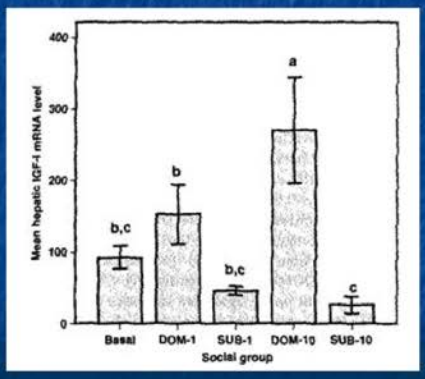
Temperature-dependent differences in weight gain and IGF-1 expression



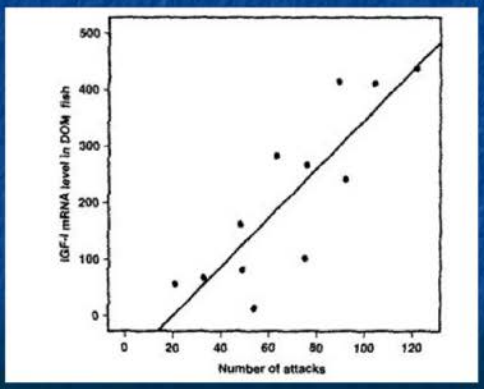
Effect of environmental temperature on IGF-1 expression



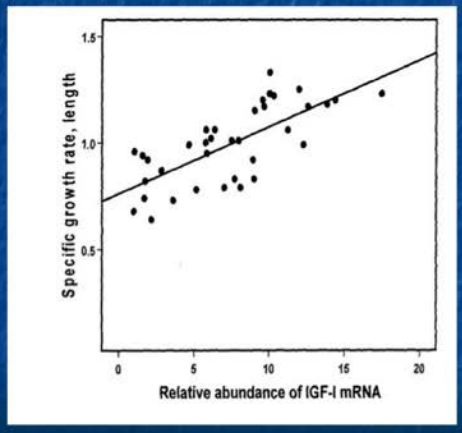
Social dominance and IGF-1 expression



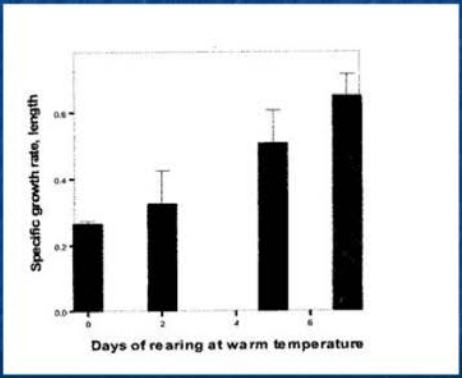
Correlation of aggression w/ IGF-1 expression



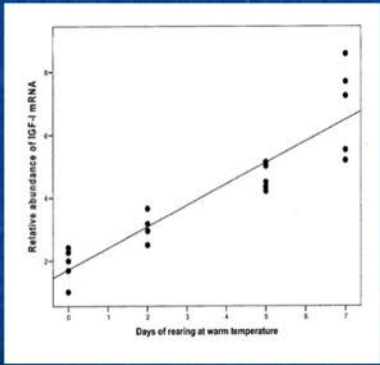
Photoperiod-induced changes, growth & IGF-1



Temperature influence on growth rate



Correlative change in IGF-1 expression…



… resulting from tight association of growth with IGF-1 expression

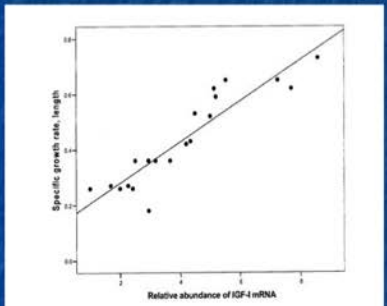


Figure 4. Correlation of relative abundance of IGF-I mRNA and specific growth rate (length) of the fish (n = 20), r = 0.92 (P<0.001)

Acknowledgements

- Funding agencies
  - USAID/PDA CRSP
  - The Fulbright Foundation
- Institutions
  - Central Luzon State University
  - North Carolina State University
  - Florida International University
  - NOAA, The Milford Laboratory

Incorporation of the native cichlid, *Petenia splendida*, into sustainable aquaculture: reproduction systems, nutrient requirements, and feeding strategies

Wilfrido M. Contreras-Sánchez, Carlos Alfonso Alvarez González, Arkady Uskanga-Martínez, Luis D. Jímenez-Martínez, José Angel Almeida-Madrigal, and Lander Lara-Aguilar

Laboratorio de Acuacultura
División Académica de Ciencias Biológicas
Universidad Juárez Autónoma de Tabasco
Carretera Villahermosa-Cárdenas Km. 0.5
Villahermosa, Tabasco, México. CP 86000

Our experiments have significantly contributed to the development of the technological package for the culture of the native cichlid tenhuayaca (*Petenia splendida*). Information on reproduction in captivity, larval rearing conditions and feeding during different stages of development has generated an important starting point for the management and conservation of native cichlids. The aim of this investigation was to address three research areas: 1) reproductive performance with different sex ratios; 2) intensive fry culture using high stocking densities and 3) protein requirements for fry, juvenile and adult growth using practical diets. To determine the best broodstock stocking rates, three male/female sex ratios were evaluated (1:1, 1:2, and 1:3). Each treatment consisted of three 2 m-diameter tanks that were divided into six spawning compartments. Fertilization rates, hatching success and larval survival were evaluated from each spawning. Reproductive behavior was also observed in each tank. The effect of stocking density was evaluated using sex reversed *Petenia* fingerlings. Fish were stocked at densities of 0.5, 1, 5, 10 and 20 fish/L using 70-L cylindrical-conical fiber glass tanks connected to a recirculating system. The use of vegetable meal at different life stages (larvae, juveniles and adults) was also studied by replacing fish meal with wheat gluten at different percentages (0, 25, 50, 75 and 100%). The control groups consisted of Artemia nauplii for larvae, or commercial feeds for carnivorous species (Silver CupThe 1:2 male/female ratio produced the largest number of fry, reaching 81,364 over 70 days of experimentation. This treatment produced more than 5,000 fry/Kg of female than the other ratios and more than one thousand fry per day. The average number of eggs produced per female (2,325), fertilization and hatching rates (above 97%), and survival during the early stages (100%) were high for this species. The results obtained using different stocking densities indicated that the optimal density for *P. splendida* was between five and ten larvae/L. This density resulted in good growth and survival. Stocking densities of 0.5 and one larvae/L provided the best growth, but the number of fish produced per tank was significantly reduced. The diet study produced important results in two areas: a) the development of a practical diet that can be used for larvae, juveniles and adults and b) the utilization of alternative ingredients in the diets (i.e. wheat gluten) which reduces costs by using lower amounts of fish meal. Experiments using larvae, juveniles and adults provided similar results regarding the amount of fish meal that can be replaced with wheat gluten. Even though *P. splendida* is considered to be a carnivorous cichlid, fish meal replacement in diets ranging from 25 to 50% (in relation to protein) can be used.

Aquaculture research in Guyana: The work of the Mon Repos Aquaculture Station

Tejnarine Shawn Geer*

Mon Repos Aquaculture Station, Fisheries Department,
Ministry of Agriculture,
Regent and Vlissengen Road, Georgetown, Guyana.
tejnarinegeer@yahoo.com

The Mon Repos Aquaculture Station, the first freshwater aquaculture station in Guyana, was commissioned in 2001, with a mandate to conduct research and training, and produce seedstock for small farmers.

In the six years of its existence, this facility has trained over 150 persons in the basics of fish farming, and has produced over hundreds of thousands of fingerlings of several species, to support small scale aquaculture development.

Research activities at the Mon Repos Aquaculture Station are centered on basic, adaptive and practically oriented activities, for easy adoption by farmers. Important areas of focus are spawning, fry nursing and growout, and feed and stocking trials.

Ongoing research is conducted on introduced species: Nile Tilapia (*Oreochromis niloticus*), Jamaican Red Tilapia (*Oreochromis spp.*) and the Giant Malaysian Freshwater Prawn (*Macrobrachium rosenbergii*), as well as Native South American species: Sweetwater Pacu (*Colossoma macropomum*), Hassar (*Hoplosternum littorale*) and Arapaima (*Arapaima gigas*).

Of particular interest is the research conducted in polyculture of Tilapia and Hassar, which has demonstrated significant increase in earnings for farmers over monoculture of either species, while simultaneously increasing the protein production from the same pond area.

The station supported two rice-fish projects, from 2004 to 2006, funded by the UN-FAO and IFAD, with the provision of fingerlings, training and technical field support. These projects demonstrated that integrated rice-fish farming, using Tilapia, resulted in increased yield and quality of rice, reduced input costs, and increased income for farmers from both the rice and Tilapia crop.

In 2006 and 2007, activities have focused on the evaluation of the YY or Supermale Tilapia, for supply of all male fingerlings to small farmers.

2008 and 2009 will see the Mon Repos Aquaculture Station implementing activities associated with the Aquaculture-Fisheries Collaborative Research Support Programmes Evaluation of local feed ingredients for Tilapia and Pacu production.

Elimination of methyltestosterone from intensive masculinization systems: use of solar irradiation and bacterial degradation

Wilfrido M. Contreras[1], Grant W. Feist[2], Carlos A. Alvarez Gonz[1], Rosa M. Padr[1], Ulises Hern[1], Gabriel M[1], Carl B. Schreck[3], and Guillermo Giannico[2]

Graduate Research Student: Arkady Uskanga-Mart[1]
Undergraduate Research Students: Beatriz[1]

[1]Laboratorio de Acuacultura
División Académica de Ciencias Biológicas
Universidad Juárez Autónoma de Tabasco
Carretera Villahermosa-Cárdenas Km. 0.5
Villahermosa, Tabasco, México CP 86000

[2]Department of Fisheries and Wildlife
104 Nash Hall
Oregon State University
Corvallis, OR 97331-3803 USA

[3]Biological Resources Division, USGS
Oregon Cooperative Fish and Wildlife Research Unit
Department of Fisheries and Wildlife
Oregon State University
Corvallis, OR 97331-3803 USA

It is well known that one of the major problems in aquaculture is the elimination of culture wastes from water. The amount and type of residues will depend on the species cultured, the stage of development and the feeds used. Methods for the elimination of synthetic steroids from aquaculture facilities are important for keeping safety standards in the industry. We have previously reported that considerable amounts of MT leak into the environment during dietary treatments, remaining in the water for several minutes and potentially accumulating in sediments. The goal of this investigation was to determine the capabilities of biofiltration and/or charcoal sunlight present within our Recirculating Aquaculture Systems (RAS) for degrading MT; evaluated in absence or presence of sunlight. Two experiments were conducted at the Laboratory of Aquaculture at UJAT, in Tabasco, Mexico. MT determination was conducted by Radioimmunoassay at the fish physiology and genetics at Oregon State University. We were able of following the steroid closely, obtaining two very distinctive patterns of elimination. Results from this research indicate that large amounts of MT in the water can be completely removed when activated charcoal is used in a RAS and partially removed by either sunlight exposure and/or biofiltration. Activated charcoal in RAS can efficiently remove MT in less than 24 hours of treatment. Both sunlight and biological filtration follow a very similar pattern of MT degradation, suggesting that these systems can eliminate the synthetic steroid if exposed for a significant amount of time. The results from this investigation encourage us to keep promoting the use of Recirculating Aquaculture Systems in aquacultural facilities that conduct masculinization of fish using synthetic steroids.

Management strategies for oyster culture, *Crassostrea gigas* and *C. cortesiensis*, in Sinaloa and Nayarit, Mexico

Maria C. Haws*, Gaxiola-Camacho, E., G. RodrO.Calvario-Martinez, M.C. VelJ. Supan, F. Martand Q.S.W. Fong 2007

Pacific Aquaculture and Coastal Resources Center (PACRC)
University of Hawaii-Hilo
200 W. Kawili St.
Hilo, HI 96720
haws@aol.com

Oyster culture has been practiced in Nayarit State on the Pacific Coast of Mexico for 35 years, while in Sinaloa State, this activity is beginning. Preliminary microbiological water quality monitoring demonstrated that poor sanitation conditions in commercial shellfish growing waters exist. This is due in part to inadequate waste water treatment of domestic wastes that are discharged directly into growing areas. Since the goal is to produce safe shellfish for local consumption as well as potentially for export, improving water quality in shellfish growing areas is a priority for a collaborative effort involving the Autonomous University of Sinaloa, Pacific Aquaculture and Coastal Resources Center/University of Hawaii Hilo, Louisiana State University, and the Research Center for Food and Development/Mazatlan (CIAD), sponsored by the AquaFish Collaborative Research and Support Program (CRSP) of Oregon State University with funding from the United States Agency for International Development (USAID).

Monitoring revealed that in the case of the principal growing areas for *Crassostrea cortesiensis* in Nayarit State, total and fecal coliform levels for most sampling stations exceeded standards established by Mexico, which are similar to those used in the United States. On the other hand, at Santa Maria Bay (Bahia Santa Maria), located in Sinaloa State, levels fell well below the maximum allowable limits in all areas sampled. These results were presented in public meetings involving a wide range of stakeholders in both states in order to develop strategies to manage water quality in contaminated areas and maintain good water quality in pristine areas. In Nayarit, one result of the public involvement was the formation of the Council for the Conservation and Development of the Boca de CamichEstuary (CCDEBC), a major oyster cultivation area. This Council subsequently established strategies to reduce the bacterial levels in the growing areas including: an environmental education program for the communities within the watershed area; efforts to find areas for oyster relaying and depuration which could be classified as acceptable within the Mexican standards; and development of depuration capacity for large volumes of production.

In the case of Sinaloa State, *C. gigas* is currently cultured in Altata Bay, part of the larger Santa Maria Bay system, and efforts are underway to extend cultivation into other parts of the State, beginning with sites in Santa Maria Bay. Training with women and fishers in small communities is also conducted to promote so oyster culture as an alternative livelihood. *C. gigas*, although a proven culture species, is an introduced species which has a higher production costs due to the need to import seed, so grow-out trials of the native *C. cortesiensis* are being conducted since spat could be collected from the wild.

Collaborators and Sites

University of Hawaii Hilo

Maria Haws (Sea Grant)

William Steiner

Sharon-Ziegler Chong

Louisiana State University

John Supan (Sea Grant)

Autonomous University of Sinaloa (UAS)-Mexico

Eladio Gaxiola Camacho

Guillermo Rodriguez Dominguez

Center for Food and Development Research (CIAD)-Mexico

Omar Calvario

Center for Aquatic Ecosystems Research/Central American University (UCA)-Nicaragua

Agnes Saborio Coze and Nelvia Hernandez

Summary of Goals

- BMPs for improved aquaculture sanitation
- Environmental aspects of bivalve culture
- Bivalve culture as diversification and alternative using native species
- Capacity building for bivalve culture and sanitation
- Pilots for bivalve culture and depuration

Activities and Investigations

Bivalve culture and sanitation research/environmental aspects

- Spat collection, growth rates and survival of the native oyster species, *Crassostrea corteziensis* at Santa Maria Bay, Mexico.
- Oyster-relaying and depuration in an open-water locations, Boca Camichin, Mexico
- Determination of carrying capacity of the Boca Camichin Estuary, Mexico in reference to oyster culture.
- Microbiological quality of bivalve growing waters and tissues (Nicaragua)*

Capacity building and institutional strengthening

- International Workshop for Aquaculture Sanitation
- Regional workshop on shellfish culture and sanitation
- Training in Best Management Practices for the production of molluscs in the States of Nayarit and Sinaloa.
- Intensive Training and Internship in Bivalve Culture and Shellfish Sanitation (LSU)

Progress

Microbiological quality of bivalve growing waters and tissues (Nicaragua)

Water quality and tissue analysis results for three estuaries:

- *E. coli* levels exceed permissible levels in all estuaries during August-December period
- *Salmonella* not detected in any of the three estuaries
- *Vibrio parahaemolyticus* within permissible levels in all estuaries
- Hepatitis A found in tissues in one estuary

Results indicate need to relay/depurate and focus on sources of contamination sources

Work plan change requested: to focus on cleanest estuary to develop relay/depuration methods, conduct further water quality testing

Progress

Carrying capacity in oyster growing areas of Boca Camichin

Collection of water and monitoring of oyster growth/filtration parameters initiated

Progress

Capacity building and institution strengthening activities

June 8-16	***Intensive Training and Internship in Bivalve Culture and Shellfish Sanitation (LSU)***
August 14-18	***International workshop on aquaculture sanitation & Regional workshop on bivalve culture and sanitation***
Beginning in June	***Training in Best Management Practices for the production of molluscs in the States of Nayarit and Sinaloa***

Progress

Community-based management efforts in all three sites are on-going

Development of management strategies to improve quality of life and environmental conditions

Collaborators and Sites

University of Hawaii Hilo

Maria Haws (Sea Grant)

William Steiner

Sharon-Ziegler Chong

Louisiana State University ✓subcontract

John Supan (Sea Grant)

Autonomous University of Sinaloa (UAS)-Mexico ✓MOU, site descriptions

Eladio Gaxiola Camacho

Guillermo Rodriguez Dominguez

Center for Food and Development Research (CIAD)-Mexico

Omar Calvario

Center for Aquatic Ecosystems Research/Central American University (UCA)-Nicaragua ✓MOU, site descriptions

Agnes Saborio Coze and Nelvia Hernandez

Mexico Sites

Bahia Santa Maria, Sinaloa

- 57,909 ha
- 70 km of coast line
- ~200,000 inhabitants
- Near major city of Culiacan
- Sinaloa-26% of Mexican aquaculture production
- Four community based oyster culture projects

Boca Camichin, Nayarit

- **Part of Marismas Nacionales with 200,000 ha**
- **80% of mangroves of Pacific Coast (113,248 ha)**
- **Annual oyster production: 800-1200 mt**
- **Major fishing and agricultural area**

Nicaraguan Estuaries

- **Approximately 150,000 ha mangrove**
- **Two are protected areas**
- **One is adjacent to protected areas**
- **Livelihoods: farming, cockle collection and shrimp farming**
- **Among the most impoverished communities in Nicaragua**

Funding for this research was provided by the

AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM

USAID FROM THE AMERICAN PEOPLE

OSU Oregon State

AQUAFISH COLLABORATIVE RESEARCH SUPPORT PROGRAM

The AquaFish CRSP is funded in part by United States Agency for International Development (USAID) Cooperative Agreement No. EPP-A-00-06-00012-00 and by US and Host Country partners.

Bivalve market study in Pacific Mexico

F.J. Martinez-Cordero, Q.S.W Fong, M.C. Haws, E. Gaxiola-Camacho, and G. Rodriguez-Dominguez

Centro de Investigacien Alimentaciy Desarrollo, A.C.
AP.711, Mazatl
Sinaloa, M82000
cordero@ciad.mx

A market study for oysters grown by social (cooperatives) groups of farmers, in BahSanta MarSinaloa, Mwas carried out as part of a multi-component effort conducted with the beneficiaries in order to help them to successfully produce and commercialize their oyster production. This study focused on information from the demand side (including consumer preferences) specifically the market segment of local buyers, since this is considered the most feasible option for marketing given the small-scale nature of the stakeholders aquaculture operations. The work was conducted by the Research Center for Food and Development/Mazatlan (CIAD), Fishery Industrial Technology Center/University of Alaska Fairbanks-Kodiak, Autonomous University of Sinaloa and the Pacific Aquaculture and Coastal Resources Center/University of Hawaii Hilo, sponsored by the AquaFish Collaborative Research and Support Program (CRSP) of Oregon State University with funding from the United States Agency for International Development (USAID).

The results show that selling directly to local buyers (restaurants and owners of mobile point of sales) is the best marketing strategy to follow for the stakeholders, considering their current low production capacities. Analysis of the characteristics of this local market revealed preferences for the local regional oyster (*Crassostrea cortesiensis*), and a market window for product with consistent year-round supply that is high-quality (larger sizes), and safe. The stakeholders are advised to take advantage of a possible 0.50-1.00 peso increase in price per piece that buyers will pay when these desired characteristics are met. Stakeholders from this project may consider taking a price premium offer by survey respondents from local markets by delivering a high quality, larger sized oyster with safety guarantees. With products that include the said characteristics, a long-term commercial relationship that is based on trust and personal communications can then be established with buyers.

The timing may be right for the stakeholders to develop markets and buyer-seller relationships in the markets surveyed based on the results of the one-on-one interviews, which guarantees the price premium offered by the buyers. In a few years, there will be more products in the market, and the price elasticity of demand may turn negative. Finally, wholesale markets are not recommended to the stakeholders, since the local market is significant enough to absorb current production, but also due to a reduced margin profit in La Viga and Zapopan wholesale markets. The stakeholders would find it very difficult to sustain a high-volume supply of oyster, which is what these markets demand.

Transferring Market Information to Oyster Growing Stakeholders in Bahia Santa Maria: Synopsis of the Oyster Market in Guamuchil, Mexico

Martínez-Cordero*, F.J., Q.S.W Fong, M.C. Haws, E. Gaxiola-Camacho, and G. Rodríguez-Domínguez

USAID

Aquaculture & Fisheries CRSP
Collaborative Research Support Program

Francisco Javier Martínez-Cordero, Ph.D.
Center for Food and Development Research (CIAD)
Mazatlan, Mexico

Quentin S.W Fong, Ph.D.
Fisheries Industry Technology Center
University of Alaska Fairbanks
Kodiak, Alaska, USA

Maria C. Haws, Ph.D.
Pacific Aquaculture and Coastal Resources Center (PACRC)
University of Hawaii Hilo
Hilo, Hawaii, USA

Eladio Gaxiola Camacho and Guillermo Rodríguez Domínguez, Ph.D.
Autonomous University of Sinaloa
Culiacan and Mazatlan, Sinaloa, Mexico

Background

- Bivalves are an important fishery and aquaculture resource in Mexico
- Most oyster culture on the Pacific Coast consists of *Crassostrea gigas* and *C. cortesiensis* (native species)
- Bivalves, along with tilapia, have been prioritized by Mexico Government for diversification of aquaculture
- Since 2003, this CRSP project team has worked to expand and improve bivalve culture as an alternative for poor coastal communities

Oyster Production

Mexico, 1993-2003

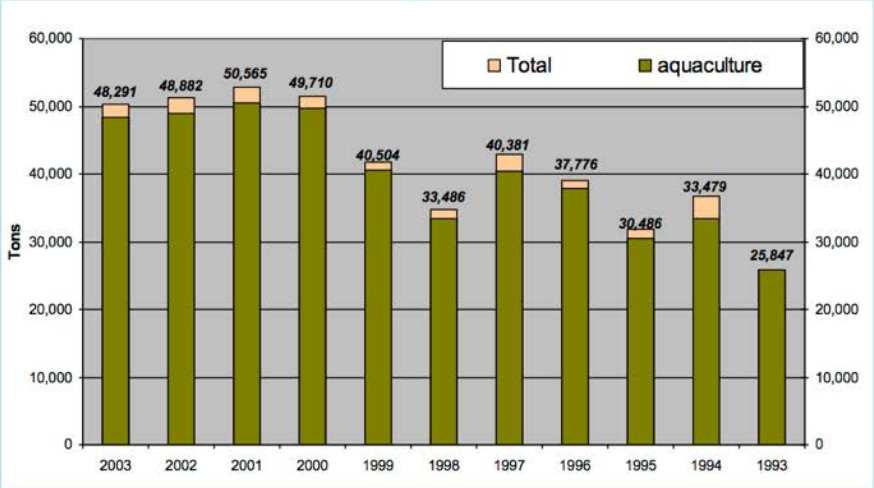


Figure on top of each bar is annual aquaculture oyster production

Oyster species

Crassostrea cortesiensis

(Pleasure Oyster)

Crassostrea gigas

(Japanese Oyster)

Improvement of bivalve culture requires market information

For example:

- What size of oyster should be grown?
- What prices would the buyer would pay?
- What is the preferred minimum shelf-life for the products?
- Is sanitation an important attribute?
- Does harvest location matter?

This information will help farmers in market identification and development of market penetration strategies

Objective

Overall:

- Assist oyster growing cooperatives in BSM to identify opportunities for marketing of oysters within the state of Sinaloa

Specific:

- Elicit the preference structure of managers/owners of seafood restaurants for oysters
- Recommend steps to be taken by growers for market penetration strategies based on the findings
- Use the process to educate Mexican small-scale oyster growing stakeholders in market research techniques and application of results.

Research Process

- Assess stakeholder's production capabilities, market knowledge, and market informational needs.
- Survey prepared that consists of structured and open ended questions.
- Survey administered to 15 restaurant managers and/or owners that serve oysters as part of their menu.
- Open-ended questions to consumers
- Feed back research results to producers

Type of restaurants and vendors

Results:
Importance of Oyster Attributes

Attributes	Average Score
Consistency in Supply	10.00
Uniformity in Size	10.00
Water Quality at Product Origin	10.00
Price	9.73
Mode of Transportation	9.64
Meat Fill	9.45
Oyster Size	9.36
Product Origin	9.00
Shape of Oyster	8.18

Results:
Highest Quality Oyster

Attribute with Highest Response vs Other	Percent
Live Shell-on Oyster vs. Other Products	84% vs. 16%
Native Oyster vs. Japanese Oyster	82% vs. 18%
Year Round vs. Intermittent Supply	70% vs. 30%
3 days vs. 1day and 10 days Shelf-Life	66.6%
Large vs. Medium and Small Size	61.5% vs. 39.5%
Wild Harvest vs. Cultured	57% vs. 43%
Northern Sinaloa State vs. Other Region in Origin	54% vs. 46%

Results:
Relationship with Supplier

Duration	Main Supplier	Secondary Supplier
> 3 Years	46%	18%
1 to 3 Years	27%	27%
6 to 12 Months	18%	37%
< 6 Months	9%	18%

Results:

Consumer's Preference for Oysters

Attributes	Strongly Agree	Agree	Disagree	Strongly Disagree
Prefer local oysters	37%	9%	18%	36%
Price is Important	91%	9%	0%	0%
Prefer Live, Shell-on Oyster	100%	0%	0%	0%
Harvest Date Important	73%	9%	18%	0%

Conclusions

BSM producers should:

- **Focus on producing large, live shell-on oysters as product form**
- **Adopt best sanitation management practices to ensure minimum preferred shelf-life**
- **Develop 3rd party water quality certification**
- **Develop relationship with potential buyer early**
- **Ensure year-round consistent supply**

Note: If the above attributes are met, all respondents are willing to pay higher prices)


Funding for this research was provided by the
AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM
USAID
FROM THE AMERICAN PEOPLE
OSU
Oregon State
AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM
The AquaFish CRSP is funded in part by United States Agency for International Development (USAID)
Cooperative Agreement No. EPP-A-00-06-00012-00 and by US and Host Country partners.
The contents of this presentation do not necessarily represent an official position or policy of the United States Agency for International Development (USAID). Mention of trade names or commercial products in this presentation does not constitute endorsement or recommendation for use on the part of USAID or the AquaFish Collaborative Research Support Program. The accuracy, reliability, and originality of the work presented are the responsibility of the individual authors.

Contributions of the Aquaculture Collaborative Research Support Program to the development of fish farming in Kenya

Charles C. Ngugi* and James R. Bowman

Moi University Department of Fisheries and Aquatic Sciences
P.O. Box 1125, Eldoret, Kenya
cngugi@africaonline.co.ke

Research and outreach efforts conducted under the Aquaculture Collaborative Research Support Program (ACRSP) have resulted in a number of aquacultural advances since the program began work in Kenya in 1997. Workers from Moi University, the Kenya Fisheries Department, Oregon State University, and Auburn University have collaborated closely in this effort, which has focused primarily on increasing production of farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Extensive training for extension workers, fish farmers, and university students, focusing on pond design, pond construction, and pond management, has been a key component of the effort. Other noteworthy elements of the Kenya Project have included pond and hatchery experiments at Sagana Aquaculture Centre and the Moi University Fish Farm, economic studies, and testing and disseminating new technologies through on-farm trials conducted in central and western Kenya.

Experimental work conducted under the ACRSP Kenya Project has included evaluations of alternative organic and inorganic fertilizer and feed application rates and regimes for use in earthen ponds, an examination of the growth and reproduction capacities of three strains of Nile tilapia suitable for fish farming in Kenya, and work on feed formulation and production using locally available raw materials. ACRSP studies were also conducted on risk analysis and the economics of tilapia production in Kenya and commercialization of small-scale fish farming, including aspects of enterprise budgeting. Capacity building efforts have resulted in tremendous improvements at both Sagana Aquaculture Centre and Moi University's Department of Fisheries and Aquatic Sciences. This has occurred mainly through training to equip university students and Fisheries Department personnel with knowledge on proper pond construction and the skills needed for improved fish handling and pond management, but also through physical improvements of the facilities at the two sites.

Research results are now being shared with extension specialists, advanced farmers, and students. Improved technologies developed through ACRSP investigations are leading to better fish pond management by individual fish farmers, resulting in turn in increased fish production. New short courses are being developed to focus specifically on the production of fingerlings. Much additional research is necessary to further improve growth and survival of Nile tilapia and African catfish juveniles in the hatchery and in ponds. Several management strategies will be assessed to determine other means to increase pond fish production.

CONTRIBUTIONS OF THE ACRSP TO THE DEVELOPMENT OF FISH FARMING IN KENYA

Charles C. Ngugi*
Moi University, Eldoret, Kenya
and
Jim Bowman
Oregon State University, Corvallis, Oregon

*Presenting Author cngugi@africaonline.co.ke

Highlights –Aquaculture in Kenya:

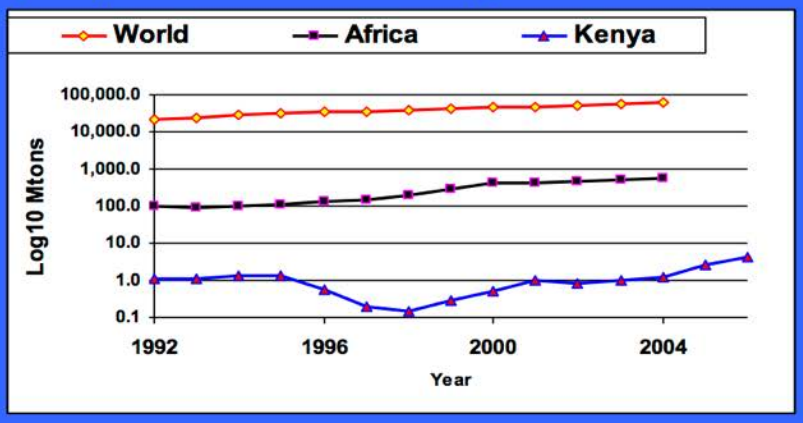
- Current fisheries situation in Kenya
- Development of Fish Farming in Kenya
- ACRSP practices and technologies developed in Kenya
- Future direction in tilapia and catfish research and extension

Fisheries Situation in Kenya

- **Current annual fish production stands at 145849MT (FD 2005 Annual Report)**
- **Fish supplies from both fresh and marine waters appear to be declining in terms of volume and catch-per unit effort. Recent rise resulted from Participatory Management instituted by FD**

1990	1995	2000	2001	2002	2003	2004
201,778	193,789	202,639	164,276	128,227	119,688	134,737

Kenya's Aquaculture Contribution to Global production from 1992 to 2004



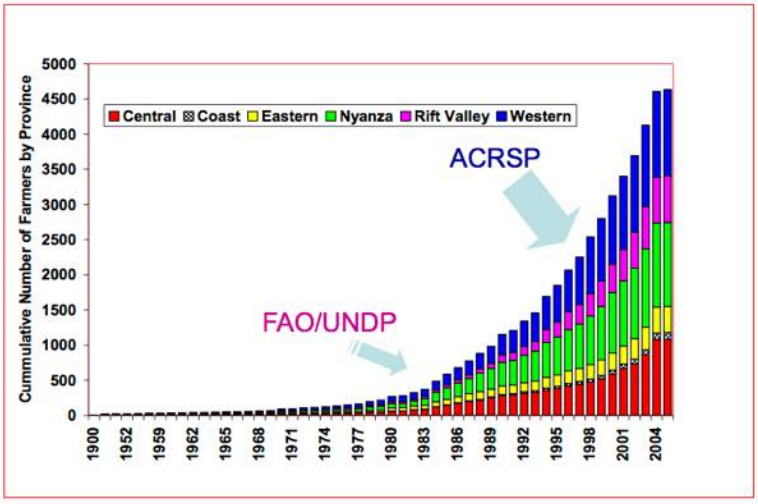
- In Kenya Aquaculture contribute to only 1,000T of the total fish production annually
- Since 2006 production has risen to 4,000T resulting from ACRSP efforts

Growing interest is in polyculture of culture of Nile tilapia and Africa catfish

Small holder farmers ponds are stocked with tilapia and catfish

Efforts to promote Aquaculture in Kenya include but not limited to:

- Government setting up Research and Demonstration fish farms (1940s; Sagana Fish Farm)
- Eat More Fish Campaign (1960s -1970s)
- Government continued efforts in Aquaculture in the 1970s and 1980s but low investment
- Revival efforts in 1990s to present, include: ACRSP, USAID,FAO, UNDP, and Belgian Survival Fund, among others



Annual cumulative number of fish farmers classified by province

Efforts By ACRSP:

- A CRSP started working in Kenya in 1997.
- Since then, several ACRSP practices and technologies have been developed.
- These "technologies" have impacted the aquaculture industry in Kenya in various ways.

"Technologies" Developed Include:

1. **Pond design, construction and mgt**
2. **Feed formulation and production using locally available raw materials**
3. **Inorganic fert/feed application rates in ponds**
4. **On-farm trials disseminating technology to fish farmers**

Cont…

5. **Training farmers, university students and extension workers (Training evaluation)**
6. **Evaluation of growth and reproduction capacity of three strains of Nile tilapia suitable for fish farming in Kenya**
7. **Economics and risk analysis of tilapia production in Kenya**
8. **Commercialization of small-scale fish farming (Enterprise Budgeting)**

1. Pond Design, Construction & Management

- A total of 380 individuals received hands-on training on pond construction methods and pond management techniques.
- 2-3 three-week short courses in pond construction and management were conducted for University students, Fisheries personnel and fish farmers.
- Construction of research and production ponds both at Sagana fish farm and Moi University
- Individual farmers assisted to construct a considerable number of small ponds

Pond design, construction and mgt Cont'd

Benefits:

- Research and production ponds constructed both at Sagana Fish Farm and Moi University
- Increased fish farmers pond surface area
- Capacity built for tilapia and catfish research and production

Water Quality and Lab Equipment from ACRSP funding

Weather Station, Hatchery and Feed equipment funded by ACRSP for Research and Training

2. Feed Formulation and Production using locally available raw materials

Observed that:

Farmers using costly feed (dairy meal) received low returns. Cost of feed and fertilizers was Kshs 19.00 (~ US $ 0.25) and so need to develop their own mixer

- Evaluate the growth performance and economic feasibility of feeding Nile tilapia with diets made up of locally available feedstuffs
- Develop a simple feed pelleting feed mixer which could easily be adopted by rural fish farmers to manufacture their own feeds

Benefits:

- **Simplified feed mixer and fish feed pelleting machine**
- **Increased fish production, farm income, and employment opportunities, resulting from better pond management by farmers**

3. Inorganic fertilizer/feed application rates in ponds

- Experiments were conducted to characterize the productive capacity of ponds and determine least-cost combinations of rice bran and inorganic fertilizer
- Stocking of tilapia has risen from one per m^2 to 2-4 per m^2. Net returns were highest for farms that combined fertilizer and feed applications

Benefits:

- Provided data on the comparative value of inorganic fertilizers and low-cost supplemental feeds as pond inputs for tilapia production
- Provided the basis for the development of more efficient production strategies for pond systems in Kenya and similar areas of Africa

4. On-Farm Trials Disseminating Tilapia technology to fish farmers

- On-farm testing was adopted as a logical step in transferring research based technologies to the farm
- Strengthened linkages between research and extension activities in Kenya.
- Farmers from central and western regions were selected to participate in the on-farm trials in 1999-2001.

Better management increased yield

Management Options	Gross Yield Kg/Ha/Yr	Net Revenue $US/Ha /Yr	Number of Ponds
Intensity			
High	8,455	6,896	11
Medium	7,274	5,017	5
Low	5,153	3,968	5
Stocking			
Clarias Only	10,417	3,637	2
Tilapia + Clarias	9,498	8,625	6
Tilapia	5,948	4,751	13
Harvest Strategy			
Total	7,006	4,746	13
Partial	8,007	7,385	8

On-farm trials in Central and Western Kenya

The average increase in fish harvested during these trials was 330% (3.5 t ha-1, as compared with an estimate of just over 1 t ha –1 prior to trials).

Almost two-thirds of the ponds gave net revenues exceeding Kshs 250,000 (US $ 3333) ha -1 yr –1; the average was Kshs 310,832 (US $ 4144) ha -1 yr –1.

Best Farmers Rewarded for highest revenue

Benefits:

- On-farm testing of various alternatives of pond management allowed farmers to assess their costs and benefits under local conditions.
- 86% of the farmers related production to improved extension effort and over 480 people new what farmers were doing and expressed interest in farming fish
- Provided opportunities for project personnel to work with extensionists, improved technical confidence and boosted morale among fisheries personnel in extension work.
- Farmers learned that improved management lead to increased production and learned new techniques on pond record keeping, fish sampling etc.

5. Training Farmers, University Students and Extension Workers

- Both undergraduate and graduate students have been trained at Sagana and Moi University
- Farmers education days have been held in which 360 farmers and 40 extension workers have participated

Benefits:

- Training enabled FD personnel and other extension workers to train potential farmers on the economic viability of fish farming
- Resulted in increased awareness of fish farming as an avenue for wealth creation in rural areas

Fish farming techniques demonstrated during farmers' field days

6. Evaluation of Growth and Reproduction of three strains of Nile tilapia

- Three strains of Nile tilapia (Sagana, Turkana, and Victoria) found in Kenya were evaluated to determine their culture characteristics
- Preliminary results showed that Victoria strain had highest growth performance, survival and relative fecundity while the Sagana was an inbred strain

Benefit:

- Provided the basis for the development of more efficient production strategies through use of best strain in Kenya

7. Economics and Risk analysis of tilapia Production in Kenya

Observed that :

There was lack of organized economic information needed for preparing enterprise budgets and business plans:

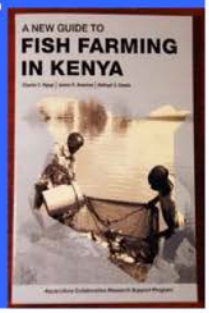
- Which limited expansion of fish production due to lack of financial resources
- Need for better understanding of farm economic & financial performance
- Need for farm data & information to estimate economic and financial indicators

Benefits:

- Provided economic data and models useful to lending agencies
- Developed pro forma financial statements for use as components of business plans
- Developed feasible business plans & enterprise budget needed for financial assistance

Benefits:

- **Increased lending to recipient commercial fish farm industry in Kenya**
- **Published a training and instruction tilapia manual**
- **Increased income and employment opportunities, resulting from better record keeping and good enterprise budgets**



8. Commercialization of Small Scale Fish Farming (Enterprise Budgeting)

- Emphasis was placed on viewing fish ponds and fish farms as commercial enterprises rather than subsistence activities

Benefits:

- Commercialization of small-scale fish farming have since recorded attractive profits.
- Fish farming is seen as a viable enterprise by micro finance institutions and banks
- Some fish Farmers have bought extra pieces of land as a direct benefit.
- Moi University has incorporated enterprise budget and business plan in their curricula

Future Research and Outreach Directions:

- **Selective Breeding Programme to develop fast growing tilapia strains**
- **Further work on quality seed and feeds**
- **Roll out more documentaries, manuals, brochures and other outreach methodologies**
- **Increased efforts in organizing farmers into more clusters for better extension, pond fish production and marketing**

ACRSP Spin offs include:

New facilities built by FD, KBDS bait fish Production in Western Kenya and the Dominion commercial fish farm

Participating team in Tilapia work

Oregon State University, Corvallis, Oregon (Lead US Institution)
- Christopher Langdon Lead US Principal Investigator
- James Bowman US Co-Principal Investigator

Auburn University
- *Karen Veverica* US Principal Investigator
- *Lim Chhorn*
- *Phelps*

University of Arkansas, Pine Bluff, (US PI Institution)
- *Kwamena Quagrainie*
- *Carole Engle*

Moi University, Eldoret, Kenya (Lead Host Country Institution)
- Charles Ngugi Lead Host Country Principal Investigator
- David Liti Lead Host Country Principal Investigator
- Mucai Muchiri Lead Host Country Principal Investigator

Kenya Fisheries Department, Nairobi, Kenya (collaborating Host Country Institution)
- Nancy Gitonga Host Country Co-Principal Investigator
- Bethuel Omolo Host Country Co-Principal Investigator
- Benson Thiga Host Country Co-Principal Investigator

Moi University ---Theses

- Leah Cherop
- Barasa Echessa
- Robison Mugo
- Maina Gichuiri
- James Mugo
- Enos Mac'Were,
- Elizabeth Nyanchiri
- Robert Olendi

Tilapia Publications

- Ngugi, C. C.; Bowman, J. and Omolo, B. (Eds.) 2007. A New Guide to Fish Farming in Kenya. ACRSP 100p.
- Kaliba, A.R., **Ngugi, C.C.**, Kajitanus, O.O., Mnembuka, B. V., Amisah, S. Fosu, A.K., and Makambo, J., 2007. Potential Impact of Aquaculture Promotion on poverty reduction in Sub-Saharan Africa. - Aquaculture International Vol. 15: No 5-6. DOI: 10.1007/s.10499-007-9110-5.
- Kaliba, A. R., **Ngugi, C.C**. Makambo, J. and Quagrainie, K.K. 2007. Economic profitability of Nile Tilapia (*Oreochromis niloticus*) Production in Kenya. In press, Aquaculture Research Vol. 38: DOI: 10.1111/j.1365-2109.2007.01772.x.
- Mac'Were, O.E., Ngugi, C.C., and Veverica, K.L., 2006. Yield and Economic Benefits of Tilapia and Catfish Production from two Locally Available Feeds in Tropical Fertilized Ponds. Journal of East African Resources Management, JEANARM. Vol. (1) (2) 1-13.

Tilapia Publications, cont..

- Liti, D., Cherop, L., Munguti, J., and Chhorn, L., 2005. Growth and Economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on two formulated diets and two locally available feeds in fertilized ponds. Aquaculture research 2005, 36, 746-752.
- Muchiri, M., **Ngugi, C**. and Hickley, P. 2004. A Community Project for Aquaculture in Kenya. A Paper presented at the *Institute of Fisheries Management Annual Conference 7th-9th September 2004, Cardiff University,* **UK Conference Proceedings** on Sustainable Fisheries 2004 pages 15-23.
- Ngugi, C.C., J. Amadiva, K. Veverica, J. Bowman, S. Imende, B. Nyandatt, and G. Matolla. 2003. On farm trials in Kenya change attitudes of fish farmers and extensionists. Samaki News, Vol. 2, July 2003.
- Ngugi, C.C. and J.O. Manyala, 2002. Review of extension service in Kenya. In: Aquaculture Extension in Africa.
- Omondi, J.G., Gichuri, W.M., and Veverica, K. 2001, A partial economic analysis for Nile tilapia *Oreochromis niloticus* L. and sharptoothed catfish *Clarias gariepinus* (Burchell 1822) polyculture in central Kenya. Aquaculture Research, 2001, 32. 693-700.

Acknowledgement:

Our travel and this work supported by the Aquaculture Collaborative Research Support Program (AquaFish CRSP), in part funded by the United States Agency for International Development.

Influence of indispensable amino acid imbalanced diets on growth and free amino acid levels in body tissues of tropical fish pacu, *Piaractus mesopotamicus*

Rodrigo Takata, Maria C. Portella*, Karolina Kwasek and Konrad Dabrowski

*SPaulo State University - Aquaculture Center. Via Prof. Paulo Donato Castellane, 14884-900 Jaboticabal, SP, Brazil. E-mail: portella@caunesp.unesp.br
The School of Environment and Natural Research, The Ohio State University, Columbus, OH, 43210, USA.

The pacu, *Piaractus mesopotamicus*, is a native species extensively cultured in Brazil because of its fast growth and high consumer acceptance. Almost two decades of research on rearing techniques, reproduction, nutrition and, more recently, larviculture have resulted in the development of pacu farming. However, specific information about the role of indispensable amino acids in the physiology of the species is missing. The present study aimed to compare pre-feeding and postprandial body free amino acid (FAA) levels in fish fed protein-based (CG), free amino acid (FAA)-based, indispensable amino acid (IDAA)-balanced and IDAA-imbalanced diets and to understand how disproportional amounts of IDAA in pacu diets can affect free amino acid levels in fish tissues.

Pacu juveniles (800 fish of the mean weight: 97.6 10.0 mg) were stocked randomly in 16 polyethylene tanks (100L capacity). Four isonitrogeneous and isolipidic diets were used in the experiment, each one assigned to four tanks. The diets were: (a) casein-gelatin-based diet (CG, protein control), (b) FAA-based diet (with all indispensable amino acids), (c) (-)Lys diet (deficient in Lys, His, Ile, Phe and Trp and (d) (-)Arg diet (deficient in Arg, Thr, Val, Leu and Met). Amino acids were supplied in the form of L-free amino acids in FAA, (-)Lys and (-)Arg diets. Juveniles were fasted for 48 hours and then fed *ad libitum* with different diets. Postprandial sampling took place 30 min after. Subsequently, the experiment was carried out in two different schedules: fish were fed restricted amounts (5% body weight per day) for 20 days and later for 40 days more (10% body weight per day). Mortality was monitored and moribund fish were removed to avoid cannibalism. Growth performance was assessed by using mean individual weight (Fig. 1). Free amino acids were quantified by the Waters PicoTag method with pre-column derivatization and reverse-phase high performance liquid chromatography (RP-HPLC).

In postprandial samples, levels of free amino acid (FAA) in body tissues showed no difference ($P>0.05$).

INFLUENCE OF INDISPENSABLE AMINO ACID IMBALANCED DIETS ON GROWTH AND FREE AMINO ACID LEVELS IN BODY TISSUES OF TROPICAL FISH PACU *Piaractus mesopotamicus*

Rodrigo Takata[1], Maria Célia Portella[1], Karolina Kwasek[2] and Konrad Dabrowski[2]

[1] Sao Paulo State University - Aquaculture Center. Via Prof. Paulo Donato Castellane, 14884-900 Jaboticabal, SP, Brazil. E-mail: portella@caunesp.unesp.br.

[2] The School of Environment and Natural Resources, The Ohio State University, Columbus, OH, 43210, USA.

May, 2008

Introduction

- Species – pacu *Piaractus mesopotamicus*

  Tropical fish – important native from South America

  Difficult feed management during the early phases

  ↳ Altricial larvae

  Some studies on initial feeding (Jomori et al., 2003, 2007; Tesser et al. 2005)

  ↳ Conclusion – Live food is very important in the initial phase

Figure. Pacu with 95 mg and 19 mm TL.

Figure. Pacu with 241 mg and 26 mm TL.

JOMORI, R.K; CARNEIRO, D.J; MALHEIROS, E.B.; PORTELLA, M.C. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. **Aquaculture**, 221, p.277-287. 2003.

JOMORI, R.K., DUCATTI, C., CARNEIRO, D.J., PORTELLA, M.C. Stable carbon (d13C) and nitrogen (d15N) isotopes as natural indicators of live and dry food in *Piaractus mesopotamicus* (Holmberg, 1887) larval tissue. **Aquaculture Research**, p. 1-12, 2007.

TESSER, M.B.; CARNEIRO, D.J.; PORTELLA, M.C. Co-feeding of pacu (*Piaractus mesopotamicus*, Holmberg, 1887) larvae with *Artemia* and microencapsulated diet. **Journal of Applied Aquaculture**, v.17, p.47-59, 2005.

Introduction

- Nutrition and Development
  20 amino acid of importance for food and tissue proteins
  Amino acids – Dispensable - DAA
  Indispensable - IDAA

- Dabrowski and Guderley (2002) - The majority of indispensable amino acid is identical in all animals, including fish.

- Some problems are known in mammals fed imbalanced diets
  Reduced feed intake and growth
  Disturbance in the assimilation of nutrients, specially of amino acids

DABROWSKI, K. & GUDERLEY, H. Intermediary metabolism. **In: Fish Nutrition**, 3 rd. (Halver, J. E. and Hardy, R. W. eds.), 310-367. Academic Press, San Diego, CA, 2002.

Introduction

- Previous studies – Midas fish - have shown a high tolerance in amino acid imbalanced diets and increased feed intake of these diets compared to the group fed with IDAA-balanced diets. (Dabrowski et al., 2007)

- Nutritional requirement in pacu
  Few studies
  Focus – growth and assimilation of nutrients using practical diets (Abimorad and Carneiro, 2007)

  No studies related to amino acid metabolism in pacu juveniles and the influence of imbalanced diets on the species.

ABIMORAD, E. G.; CARNEIRO, D. J. Digestibility and performance of pacu (*Piaractus mesopotamicus*) juveniles — fed diets containing different protein, lipid and carbohydrate levels. **Aquaculture Nutrition**. v. 13, p. 1-9, 2007.

DABROWSKI, K; ARSLAN, M.; TERJESEN, B. F.; ZHANG, Y. The effect of dietary indispensable amino acid imbalances on feed intake: Is there a sensing of deficiency and neural signaling present in fish?. **Aquaculture**. v. 268, p. 136-142, 2007.

Objective

- **In the present study we investigated how disproportional amounts of IDAA in juvenile pacu diets affects the free amino acid (FAA) levels in body tissues and fish growth. Specifically we:**

- **a) Compared pre-feeding and postprandial body FAA levels in fish fed a protein-based, FAA-based IDAA-balanced diets and IDAA-imbalanced diets;**
- **b) Compared the response of FAA levels in fish body following ingestion of the balanced or imbalanced diets during an extended period of 60 days;**
- **c) Evaluated fish growth and survival.**

Material and Methods

- Culture Conditions at the Aquaculture Center, Brazil

Pacu juvenile
~100 mg

Temperature: 28 ºC

Dissolved Oxygen: 6.18 mg/L

Constant aeration

Initial density: 0.5 fish/L

Composition of the four experimental diets for the experiment (g/100g).

Ingredients	Protein	Free AA	(-)Lys	(-)Arg
Casein	29.6	4.5	4.54	4.54
Gelatin	2	2	2	2
Dextrin	23.8	25.6	24.68	26.59
Cellulose	30.1	30.1	30.1	30.1
AA mix*	0	23.3	24.2	22.3
CMC[1]	2	2	2	2
Phosphitan C[2]	0.05	0.05	0.05	0.05
Vitamin mix[3]	2.4	2.4	2.4	2.4
Mineral mix[4]	4	4	4	4
Cod liver oil	3	3	3	3
Soybean oil	3	3	3	3

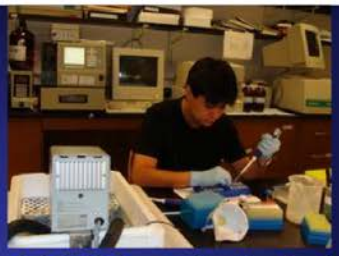
Free amino acid composition for Free AA, (-)Lys and (-)Arg diets (g/100g).

Free amino acid composition	Free AA	(-)Lys	(-)Arg
L-Lysine HCl	1.529	0	3.058
L-Arginine	1.251	2.502	0
L-Histidine	0.613	0	1.226
L-Threonine	1.258	2.516	0
L-Valine	1.623	3.246	0
L-Leucine	2.128	4.256	0
L-Isoleucine	1.364	0	2.728
L-Methionine	0.923	1.846	0
L-Cysteine	0.135	0.135	0.135
L-Phenylalanine	2.372	0	4.744
L-Tyrosine	0.073	0.073	0.073
L-Tryptophan	0.351	0	0.702
L-Glycine	0.51	0.51	0.51
L-Aspartate	2.658	2.658	2.658
L-Glutamate	2.829	2.829	2.829
L-Serine	1.929	1.929	1.929
L-Proline	0.385	0.385	0.385
L-Alanine	1.365	1.365	1.365
Total AA	23.296	24.25	22.342

Experimental protocol: dietary treatments, feeding strategy and sampling.

- Analyses:
  - Weight
  - Biomass gain
  - Specific Growth Rate (SGR)
  - Feed Conversion (FC) – restricted feeding
  - Survival
  - Free amino acid levels in body tissue
- Interpretation:
  - The results were presented as mean ± standard deviation.

Free amino acid concentrations were expressed in μmol/kg wet weight.



- Free amino acid analyses:

Samples:

Homogenized for 60s at the speed of 10,000 rpm in the buffer (0.1mol/L HCl containing 160 mmol/L norleucine internal standard) with appropriate dilution factor.

Filtered in Millipore Ultrafree-MC centrifugation tubes with 10 kDa ultrafiltration membranes and span 90 min at 4°C with the speed of 2000 x g.

Prepared blanks (0.1M HCl + 160 mmol/l nLeu) (Terjesen et al., 2004) and external standards (Sigma acid/neutral and basic amino acids, the same concentration of glutamine were prepared at the same time and add into the basic amino acids).

Terjesen, B.F., Park.K, Tesser, M.B., Portella, M.C., Zhang, Y. & Dabrowski, K. Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish pacu (*Piaractus mesopotamicus*). **J. Nutr.** 134:2930-2934, 2004.

- Free amino acid analyses:

Free amino acids were qualified by:

* One Waters Pico Tag RP-HPLC equipped with an application-specific column (3.9×30 cm).
* One Waters 717 auto sampler.
* Two Waters 501 pumps.
* One Waters 441 absorbance detector at 254 nm.
* One column heater set at 46°C.

Each amino acid were identified using spiking with known amino acids and retention time of external standards.

FAA concentrations were calculated using internal and external standards (Cohen et al., 1989).

Cohen, S., Meys, M. & Tarvin, T. **The Pico-Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis.** Millipore Corporation Milford, MA., **1989.**

- Statistical analyses
  The fish group in one tank were considered as the experimental unit.

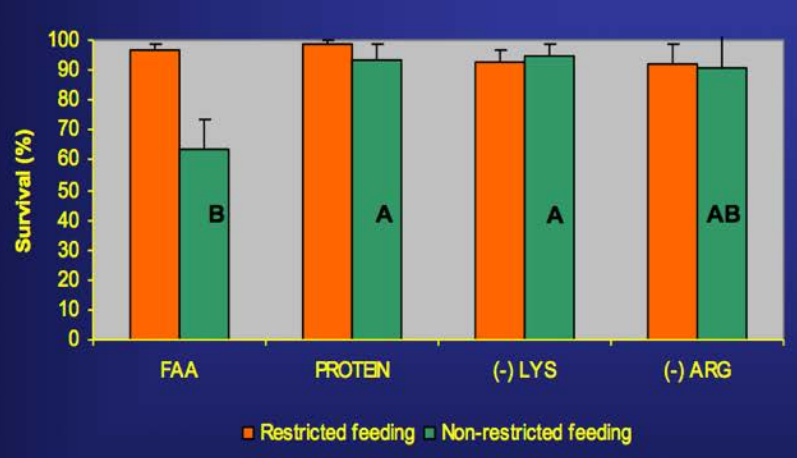
  Experimental design : Entirely randomized, following a subdivision scheme with 4 main treatments (diets) and 7 secondary treatments (periods of evaluation), with 4 replicates.

  The differences in free amino acid concentrations were analyzed in pre-feeding (fasting), and among dietary treatments in postprandial and long-term feeding.

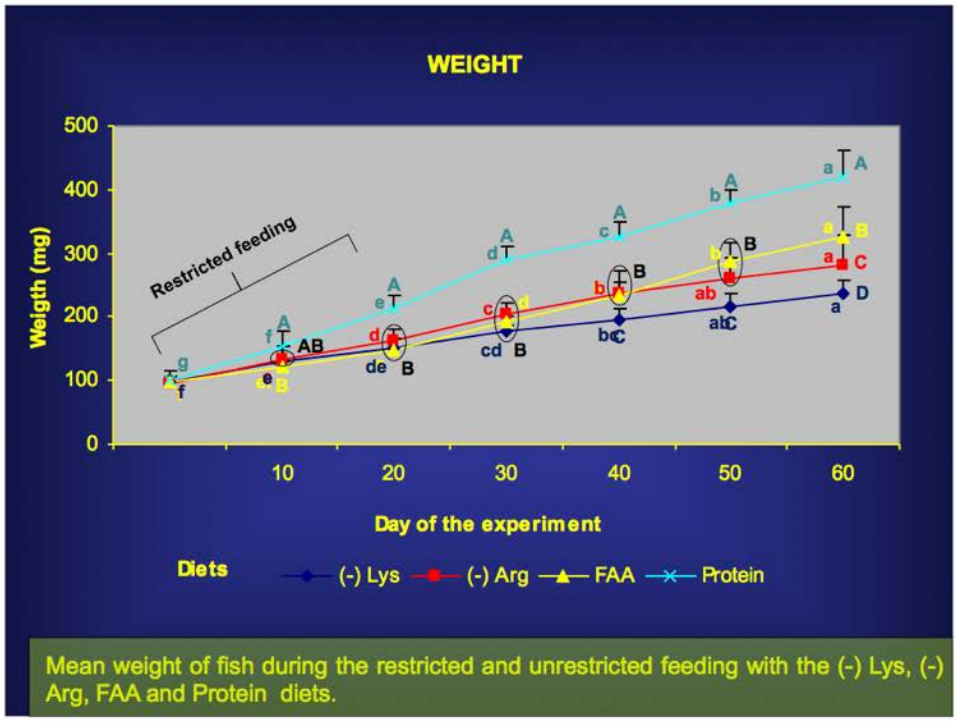
  The growth parameters and survival were analyzed among dietary treatments.

  For the analyses of the results were used one-way or two-way ANOVA at P = 0.05. All procedure were performed using Statistical Analysis System, SAS, version 8.0.

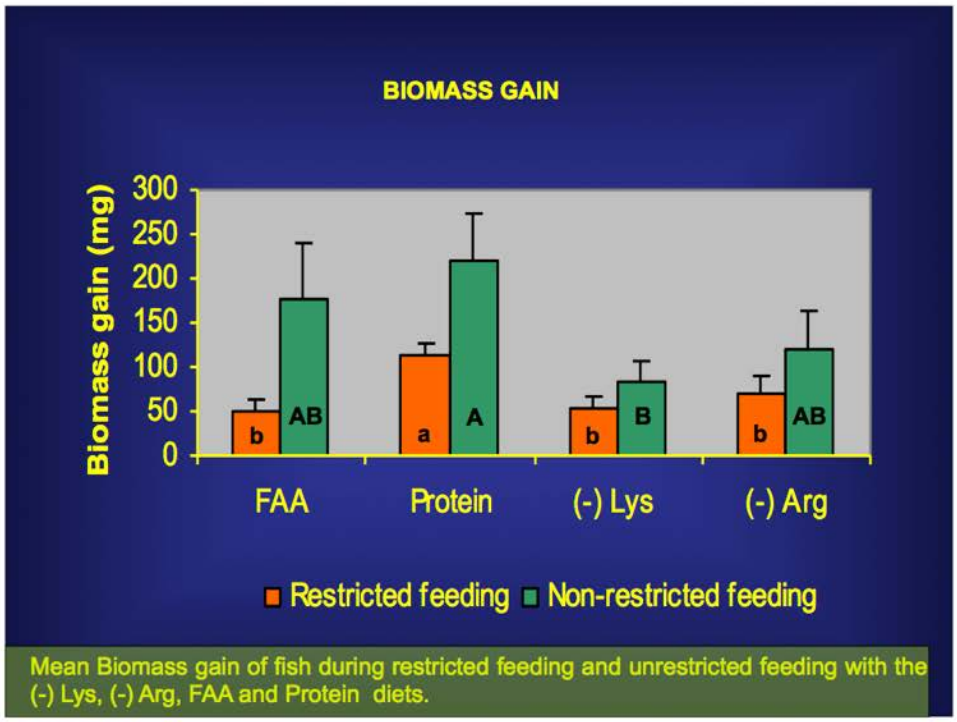
Results



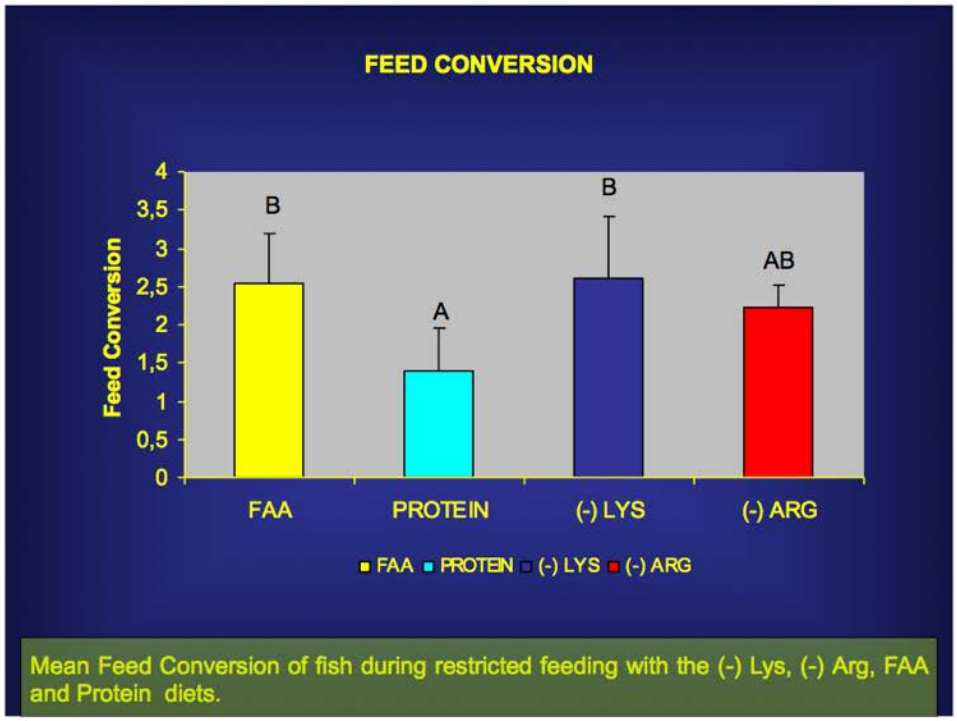
Mean survival of fish during restricted feeding and unrestricted feeding with the (-) Lys, (-) Arg, FAA and Protein diets.



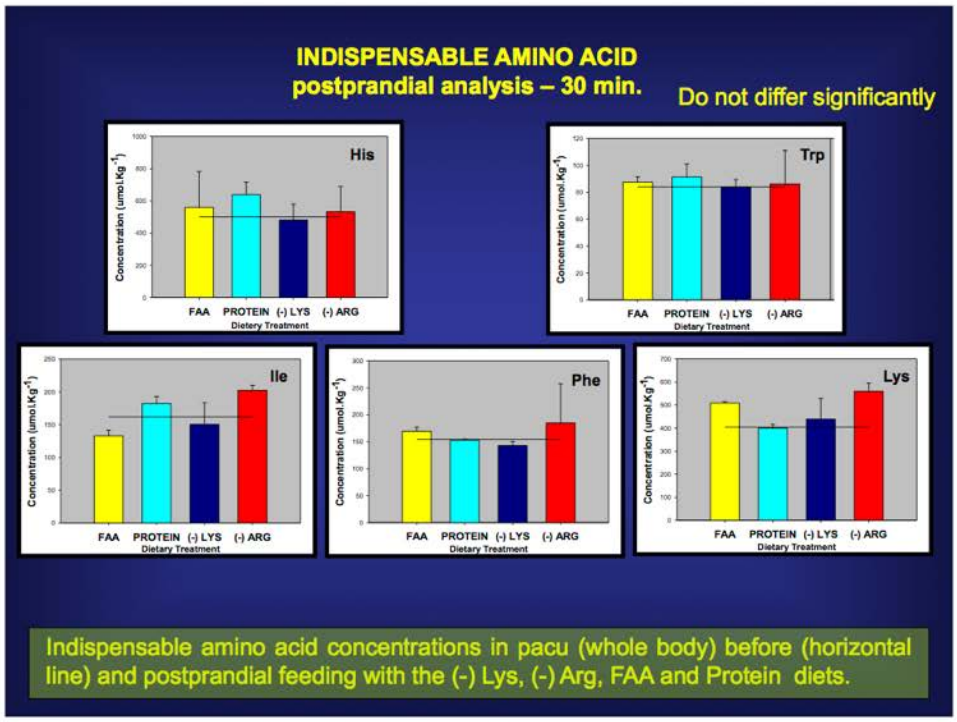
Mean weight of fish during the restricted and unrestricted feeding with the (-) Lys, (-) Arg, FAA and Protein diets.



Mean Biomass gain of fish during restricted feeding and unrestricted feeding with the (-) Lys, (-) Arg, FAA and Protein diets.



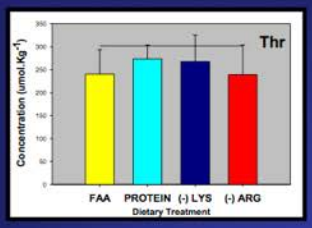
Mean Feed Conversion of fish during restricted feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

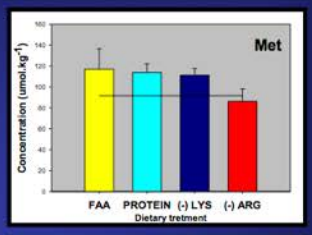


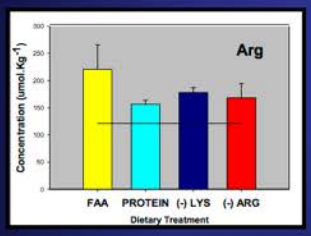
Indispensable amino acid concentrations in pacu (whole body) before (horizontal line) and postprandial feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

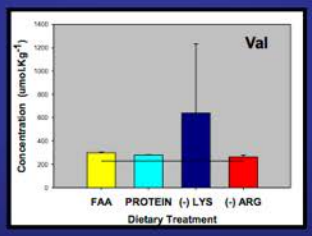
INDISPENSABLE AMINO ACID postprandial analysis -30 min.

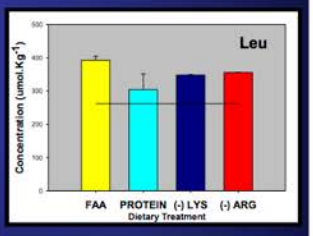
Do not differ significantly







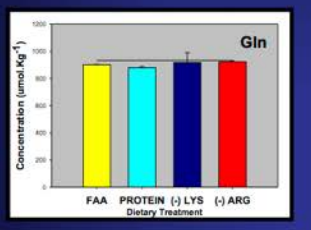


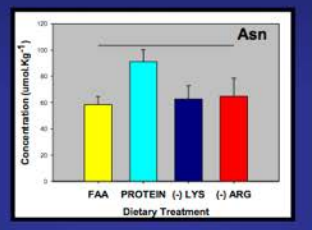


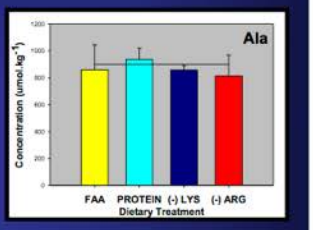
Indispensable amino acid concentrations in pacu (whole body) before (horizontal line) and postprandial feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

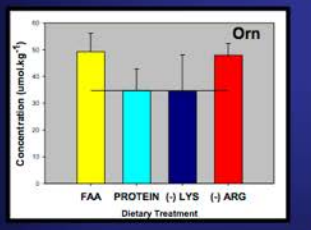
DISPENSABLE AMINO ACID postprandial analysis – 30 min.

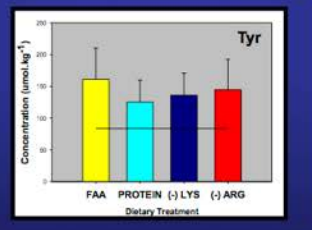
Do not differ significantly

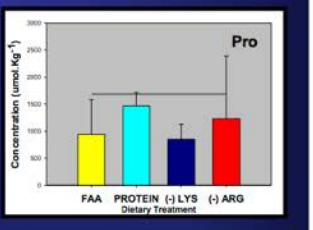








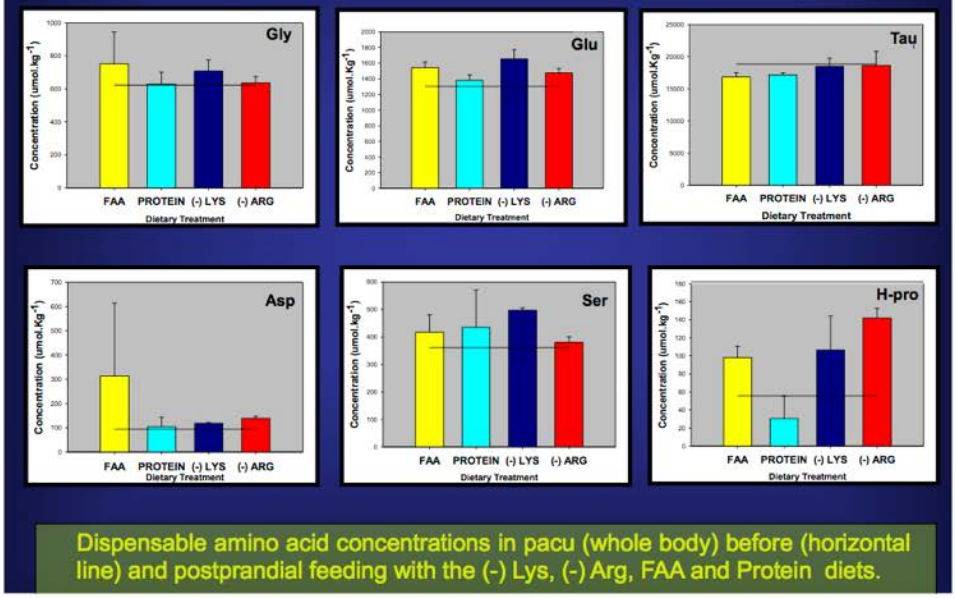




Dispensable amino acid concentrations in pacu (whole body) before (horizontal line) and postprandial feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

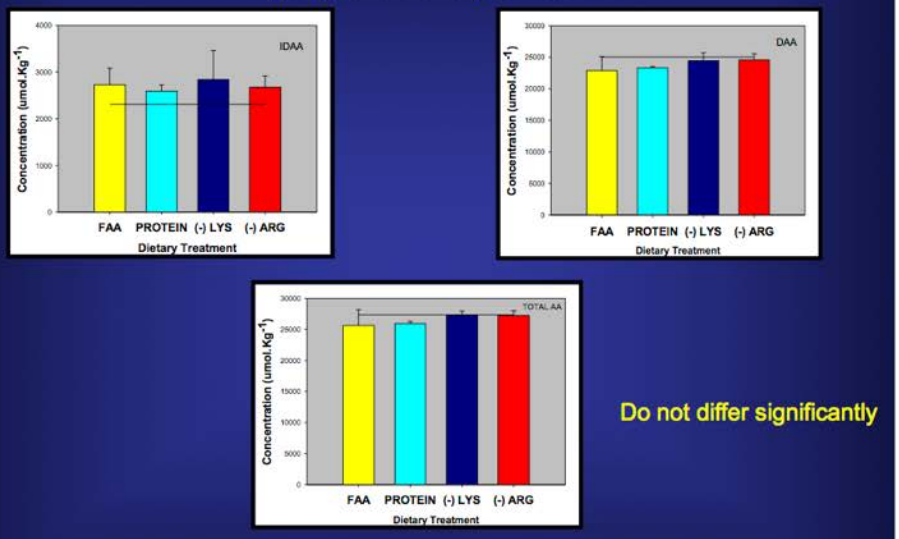
DISPENSABLE AMINO ACID
postprandial analysis – 30 min.

Do not differ significantly



Dispensable amino acid concentrations in pacu (whole body) before (horizontal line) and postprandial feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

AMOUNT OF AMINO ACID
postprandial analysis – 30 min.



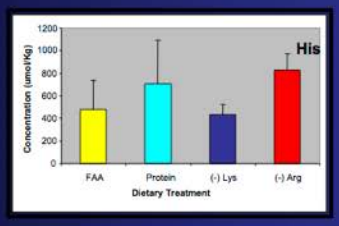
Do not differ significantly

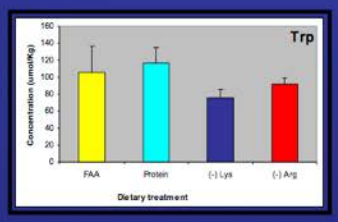
Free amino acid concentrations in pacu (whole body) before (horizontal line) and postprandial feeding with the (-) Lys, (-) Arg, FAA and Protein diets.

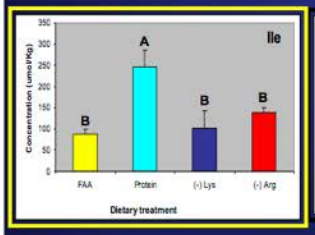
Results

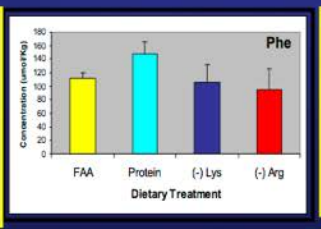
INDISPENSABLE AMINO ACID

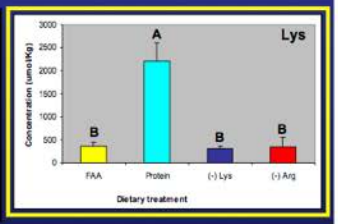
60th experimental day – 3h after meal









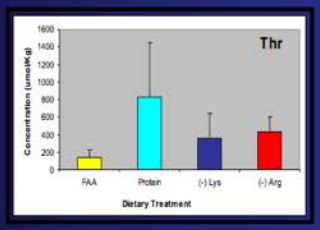


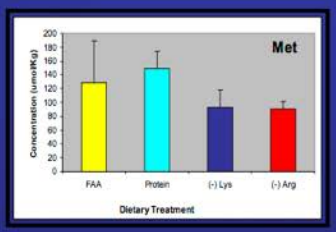
Indispensable amino acid concentrations in pacu (muscle) after 60 days of experiment with the (-) Lys, (-) Arg, FAA and Protein diets.

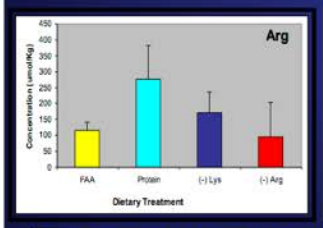
Results

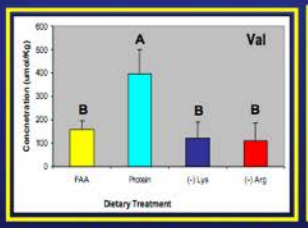
INDISPENSABLE AMINO ACID

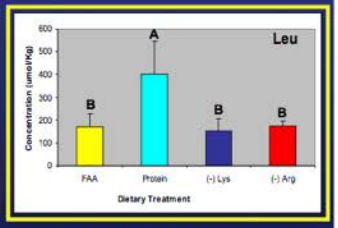
60th experimental day – 3h after meal









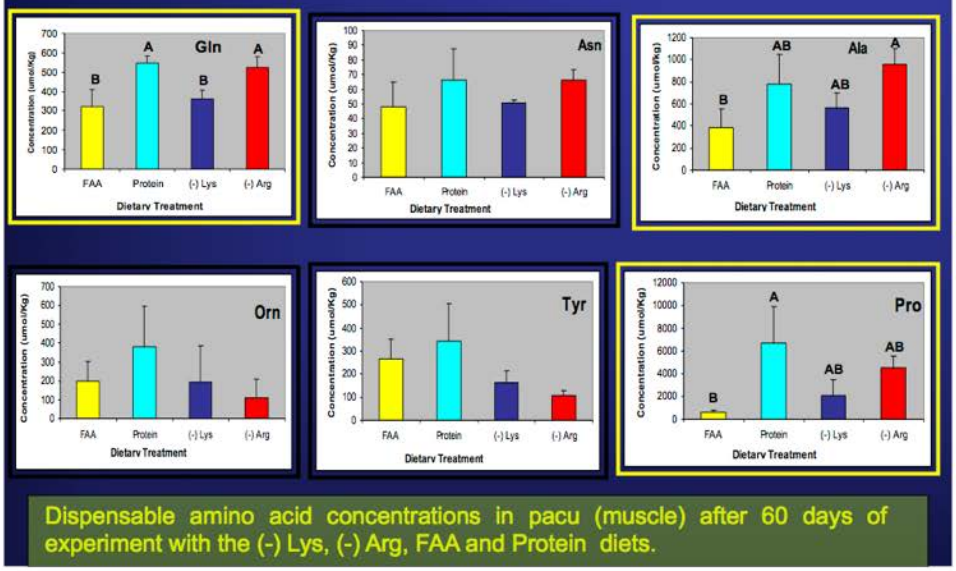


Indispensable amino acid concentrations in pacu (muscle) after 60 days of experiment with the (-) Lys, (-) Arg, FAA and Protein diets.

DISPENSABLE AMINO ACID

Results

60th experimental day – 3 h after meal

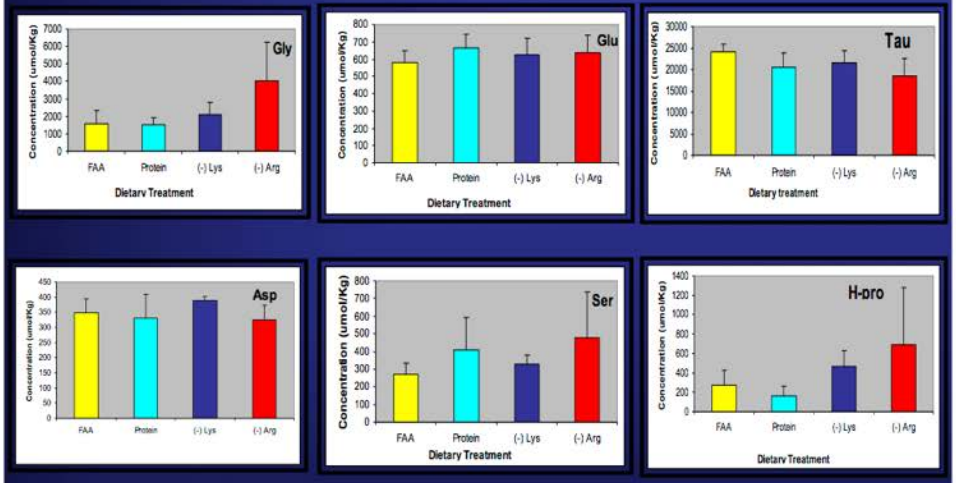


Dispensable amino acid concentrations in pacu (muscle) after 60 days of experiment with the (-) Lys, (-) Arg, FAA and Protein diets.

DISPENSABLE AMINO ACID

Results

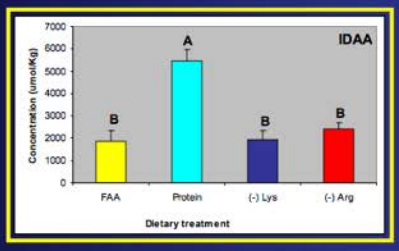
60th experimental day – 3h after meal

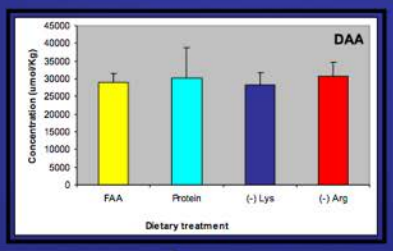


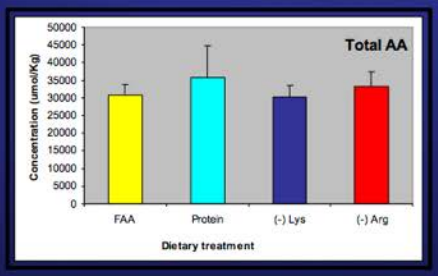
Dispensable amino acid concentrations in pacu (muscle) after 60 days of experiment with the (-) Lys, (-) Arg, FAA and Protein diets.

Results

AMOUNT OF AMINO ACID
60th experimental day 3 h after meal







Free amino acid concentrations in pacu (muscle) after 60 days of experiment with the (-) Lys, (-) Arg, FAA and Protein diets.

Conclusions

- Imbalanced and balanced diets in free amino acid are inferior to protein-based diet for juvenile pacu;
- After 60 days, pacu' survival was not affected by amino acid imbalanced diets;
- Pacu juveniles do not reject the diets devoid of IDAA, confirming previous results obtained with Midas fish, and have shown continuous growth over the time;
- The biomass gain and individual weight of the fish were directly affected by the nitrogen source (free amino acid and protein-based). Better growth was achieved with protein-based source;
- After 20 days, similar growth was found with the balanced and imbalanced diets in free amino acid;
- At the end of 60 days of experiment (unrestricted phase) fish that had received the (-) Arg diet (deficient in Arg, Thr, Val, Leu and Met) have shown similar growth as the fish fed with balanced FAA diet;

- The imbalanced and balanced diets in free amino acid and protein-based diet do not affect the pacu amino acid profile in postprandial analysis (compared before and 30 minutes after feeding);
- After 60 days, the IDAA Ile, Lys, Val and Leu were directly affected by the different diets; all of them have shown higher concentrations in the protein group;
- The DAA Gln, Ala and Pro, in general, increased their concentrations in fish from protein, (-)Lys and (-)Arg groups.

Muito obrigada!

Limnological characteristics and cage culture practice in Indrasarobar Reservoir of Nepal

Madhav K. Shrestha[1] and Narayan P. Pandit[2]

[1]Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal
[2]Fisheries Development Centre, Hetauda, Nepal
madhav_shrestha2003@yahoo.com

Indrasarobar Reservoir is situated at an altitude of 1430 msl in the mid hill region of Nepal and made for hydroelectricity generation. The water level in the reservoir fluctuates seasonally making water surface area from 220 ha at highest level to 75 ha at the lowest level. This reservoir is well known for the growth of planktivorous fish silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). Cage and open water stocking of planktivorous fish in this reservoir has been started since 1988 and significant amount of fish harvest has been obtaining each year. At present, 230 households have been involved for cage culture operation in 1600 cages of 75000 m3 volumes and production is 164 MT/year. The recent studies have shown that there is possibility of cage culture of high value rainbow trout (*Oncorhynchus mykiss*).

A limnological study of this Reservoir was carried out during 20 May 2006 to 26 March 2007. Different limnological parameters were measured monthly at four fixed sampling stations at 0700 to 0900 h following the procedure of APHA (1985). Results showed that most of the parameters fluctuated seasonally. Monthly mean, minimum and maximum limnological parameters are presented in Table 1. Temperature ranged from 12.20C during February to 24.90C in July. Chlorophyll-a concentration was lowest at May and highest during September. Water depth was lowest 60 m during May and highest in November 95.8 m. Increasing cages has affected fish growth. It needs to assess the carrying capacity for extensive cage culture as natural food limitation might have affected the growth of fish.

LIMNOLOGICAL CHARACTERISTICS AND CAGE CULTURE PRACTICE IN INDRASAROBAR RESERVOIR OF NEPAL

Madhav K. Shrestha (IAAS, Chitwan, Nepal)
Narayan P. Pandit (FDC, Hetauda, Nepal)

World Aquaculture Conference 2008, 19-23 May, Busan, South Korea

ACKNOWLEDGEMENTS

This research was funded by National Agricultural Research and Development Fund (NARDF), Nepal.

All the funding for attending WAS '08 was made by AquaFish CRSP.

AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

Introduction

- Indrasarobar reservoir was built by damming in small river valley of mountain region of central Nepal.
- Established in 1982 for hydropower generation.
- Lies at the altitude of 1430 m from mean sea level (msl) at bottom.

Introduction….

- When filled it reaches 1530 m msl.
- Reservoir is 7 km in length and 50-500 m in width.
- Total area is 216 ha when filled and 55 ha when dry.

Introduction….

- Water depth lowest 60 m and highest 100 m .
- Reservoir is fed with seven small streams.
- Filled during rainy season and draw down continuously till next rainy season occurs.

Cage culture

- Started in 1988
- Technology copied from Phewa lake (Extensive system)
- Fish Species:
  - Silver carp
  - Bighead carp

Cage culture .....

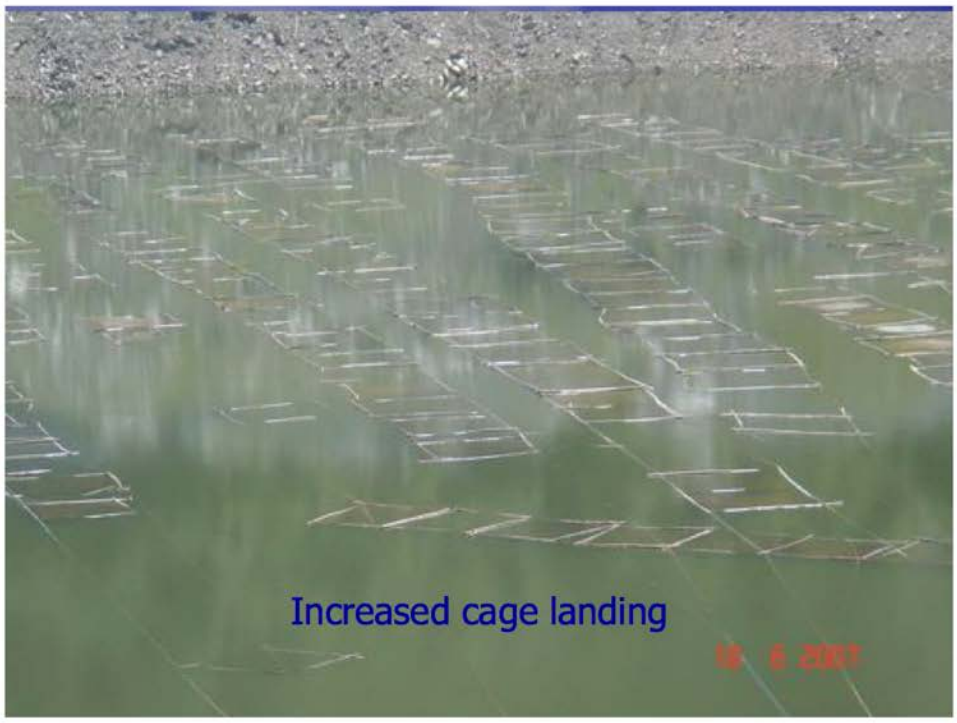
- Cage size:
  - 5 m x 5 m x 2 m
  - 50 m^3 (in general)
- Stocking density:
  - 10 fish/m^3
- Feeding:
  - None

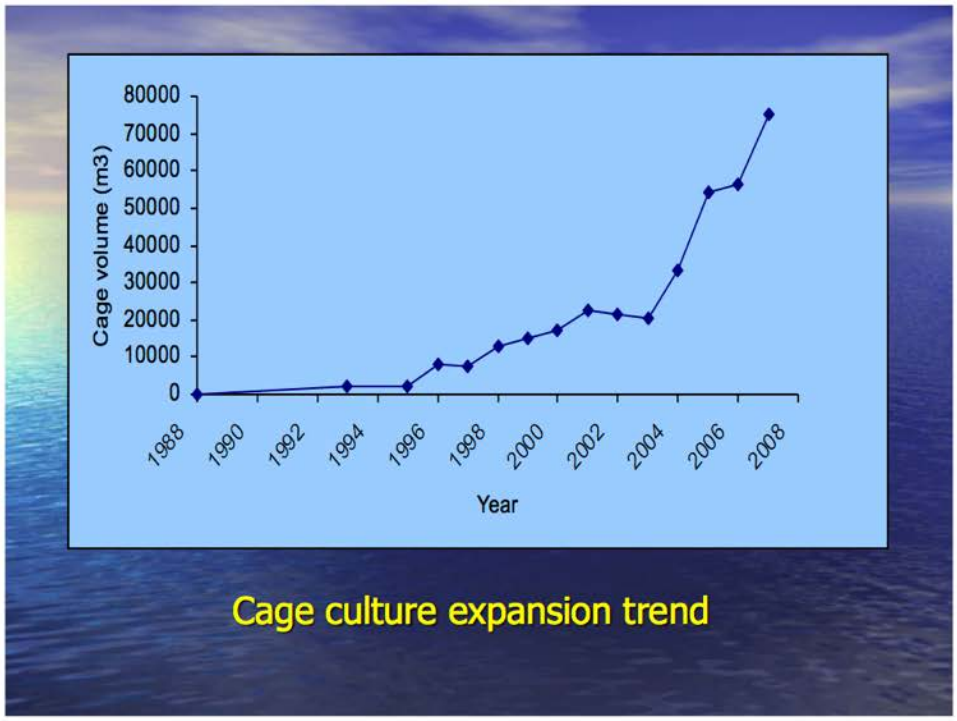
Cage Materials

- Bamboo poles for frame as well as for float
- Recently plastic drum as floats
- Nylon net Cage:
  - Nursery cage – 10 mm mesh
  - Grow out cage - 20 – 50 mm mesh

Cage culture expansion and production trend

Year	House-hold (no)	Cage (no)	Cage Volume (m^3)	Total prod^n (mt)
1988	5	5	125	-
1993	47	78	1974	-
1998	86	346	12875	47
2003	178	576	20279	71
2004	189	719	33173	116
2005	193	1155	54187	103
2006	205	1195	56197	107
2007	230	1600	75000	134

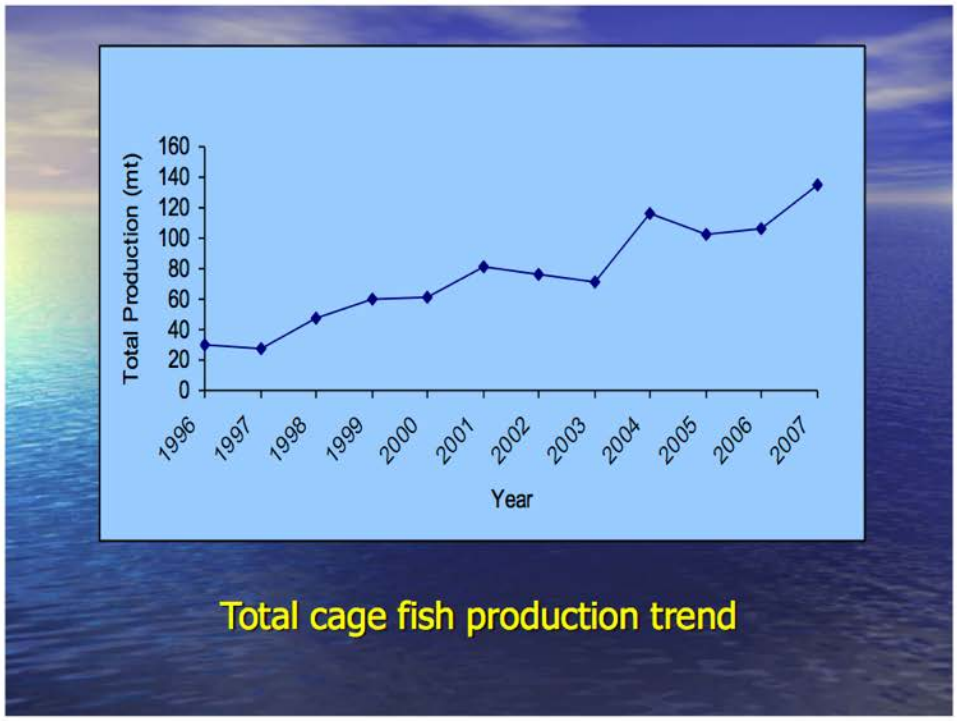




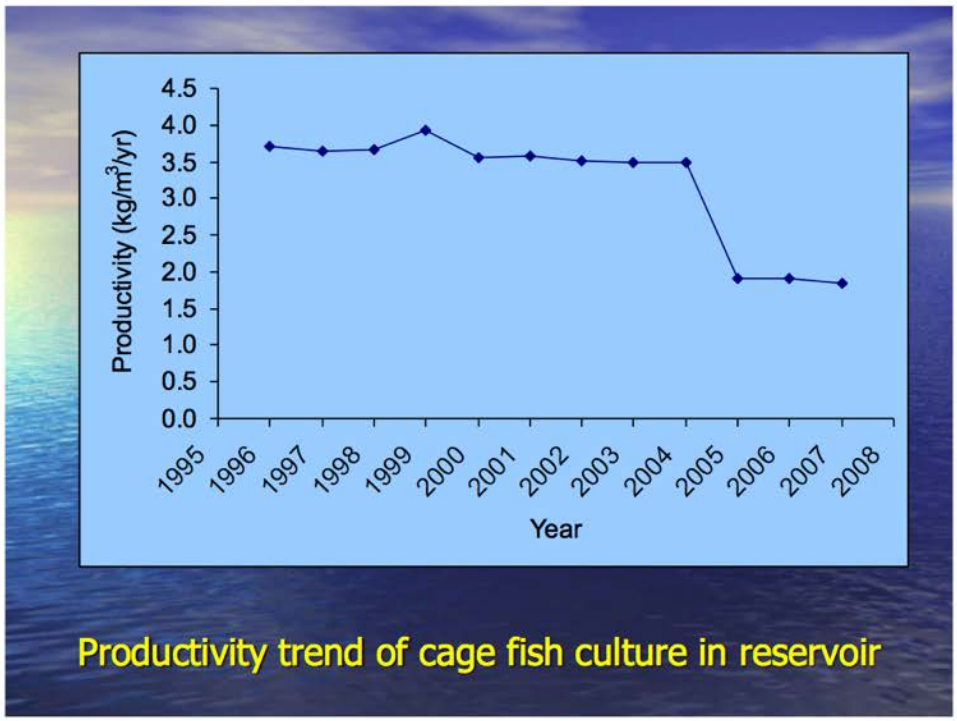
Cage culture expansion trend

Cage productivity and Open water catch of reservoir

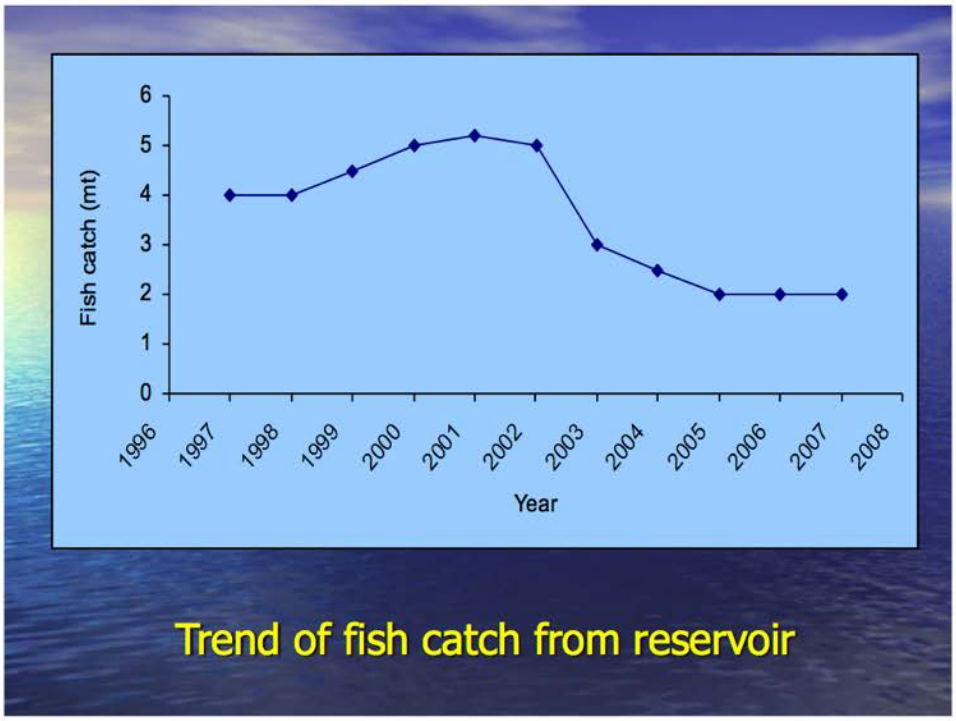
Year	Productivity in cage (kg/m^3)	Open water Catch (mt)
1996	3.70	-
1997	3.65	4.0
1999	3.92	4.5
2001	3.57	5.2
2003	3.50	3.0
2005	1.90	2.0
2007	1.84	2.0



Total cage fish production trend



Productivity trend of cage fish culture in reservoir

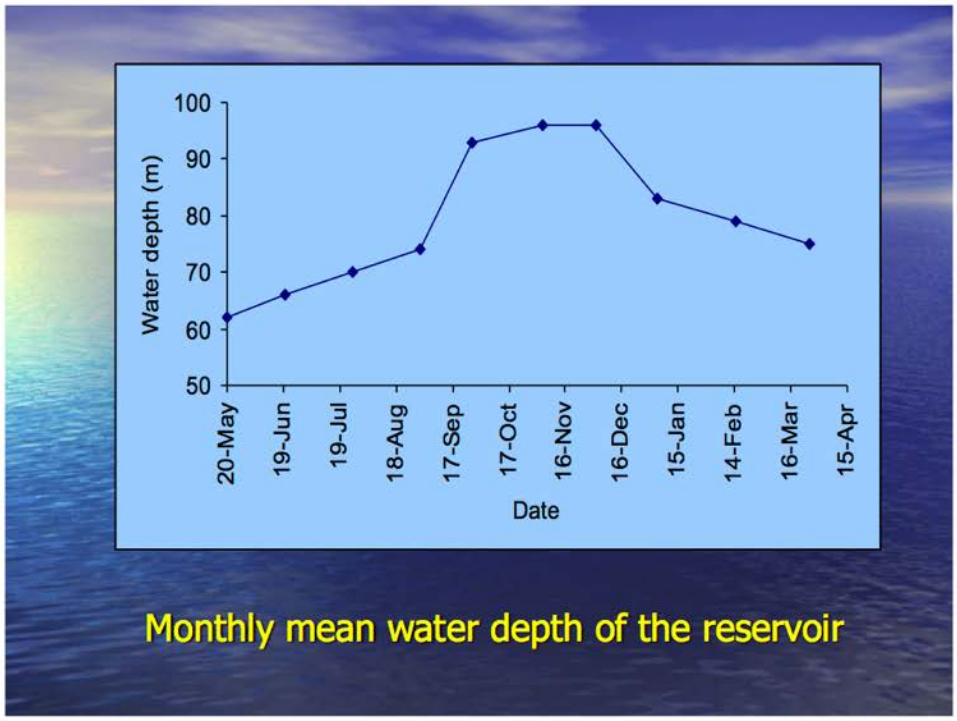


Trend of fish catch from reservoir

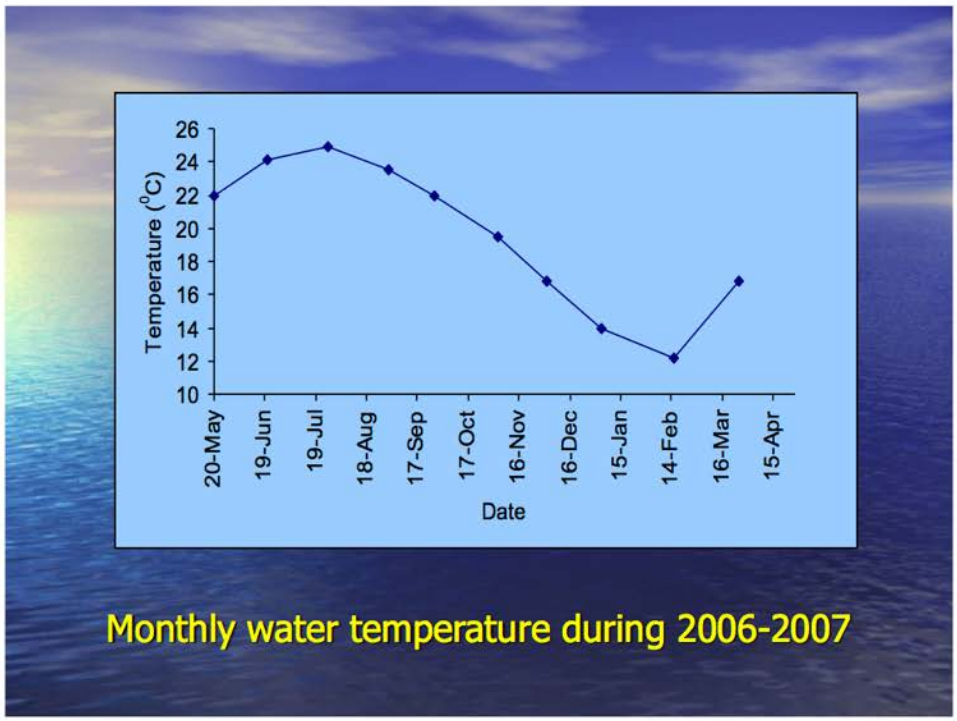
Limnological Characteristics of Reservoir

Monthly mean and range of water quality parameters of reservoir during May 2006 to April 2007

Parameter	Mean	Max	Min
Depth (m)	80	96 (Oct - Dec)	62 (May)
Temperature (^{0}C)	19.6	24.9 (Jul)	12.2 (Feb)
Dissolved oxygen (mg/L)	8.4	11.7 (Jun)	5.9 (Dec)
pH	8.4	9.5 (Mar)	7.6 (Dec/Jan)
Conductivity (µmhos/cm)	96	126 (May)	59 (Feb)
Transparency (m)	4.4	6 (Feb)	3 (Nov)



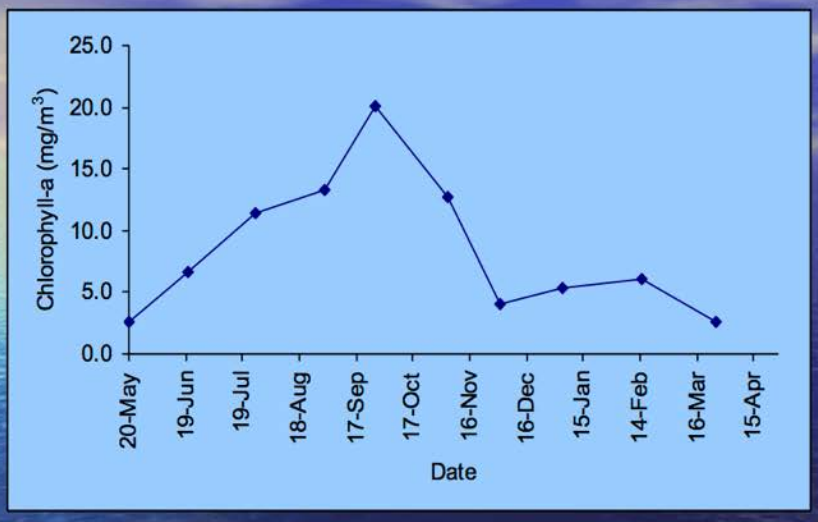
Monthly mean water depth of the reservoir



Monthly water temperature during 2006-2007

Limnological Characteristics.....

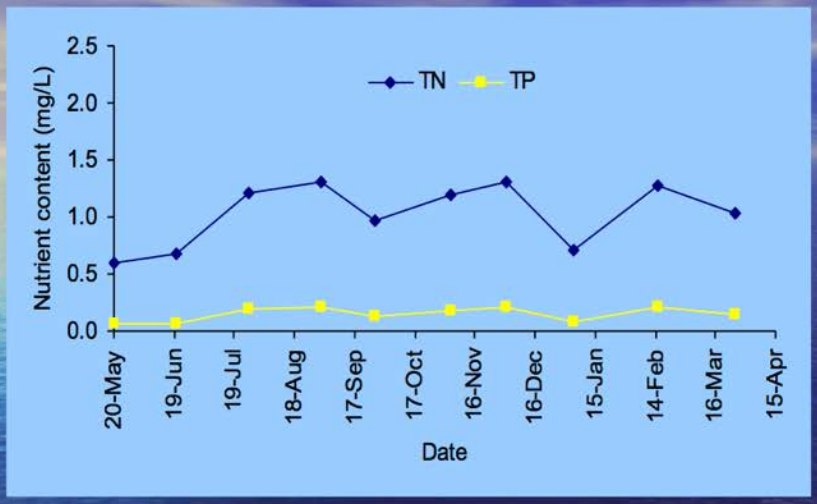
Parameter	Mean	Max	Min
Total alkalinity (mg/L)	57	65 (Mar)	50 (Sep)
Total suspended solids (mg/L)	146	980 (Aug)	48 (Sep)
Total dissolved solids (mg/L)	266	382 (Aug)	45 (Sep)
BOD_5 (mg/L)	4.8	5.6 (Dec)	3.7 (Aug)
Chlorophyll-a (mg/m^3)	8.5	20.7 (Sep)	2.7 (May)



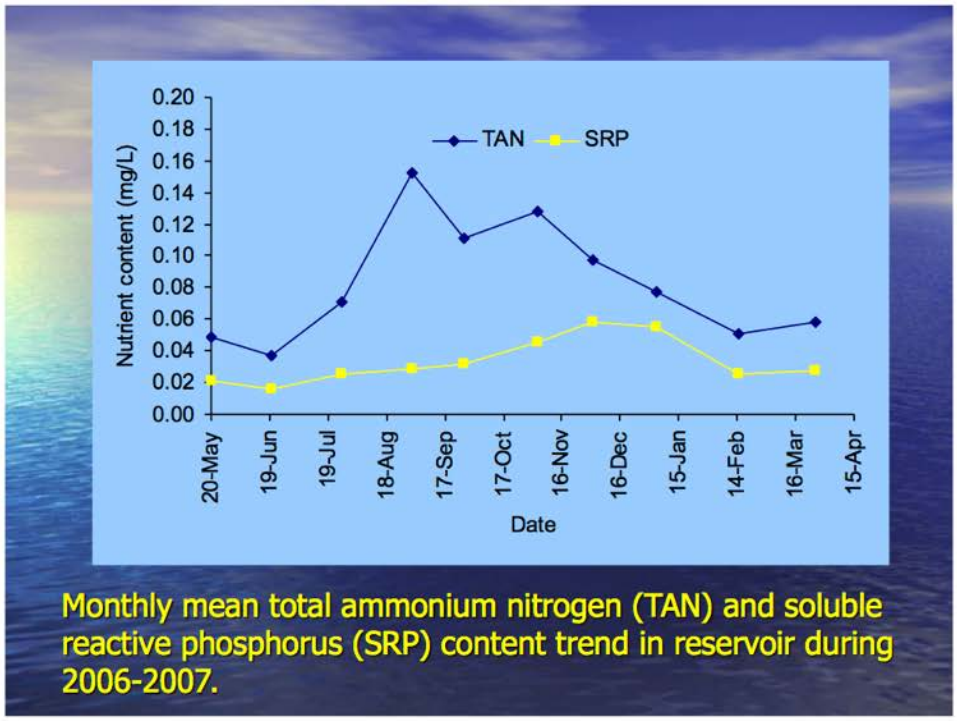
Monthly mean chlorophyll-a during 2006-2007

Limnological Characteristics.....

Parameter	Mean	Max	Min
Total Nitrogen (mg/L)	1.03	1.33 (Aug)	0.56 (May)
Total ammonium-N (mg/L)	0.08	0.15 (Aug)	0.04 (Feb)
NO_2-N (mg/L)	0.025	0.051	0.008 (Jun)
NO_2+NO_3-N (mg/L)	0.065	(Sep)	0.014 (May)
Total Phosphorus (mg/L)	0.15	0.106 (Jan)	0.06 (May)
SRP (mg/L)	0.03	0.22 (Dec)	0.02 (Feb)
		0.06 (Dec)	



Monthly mean total nitrogen (TN) and total phosphorus (TP) trend in reservoir during 2006-2007



Monthly mean total ammonium nitrogen (TAN) and soluble reactive phosphorus (SRP) content trend in reservoir during 2006-2007.

Conclusion

- Increased cage landing has decreased productivity – Plankton limitation
- Mean fish harvest size has been decreased
- Current cage culture is about 4 ha which is about 2% area of the reservoir when fully filled and about 7% of the reservoir when lowest water level.

Recommendation

Options

- Decrease cage culture area
- Decrease stocking density
- Introduce feeding system which will support non feeding cage culture system too.

➢ Currently Government has chosen recommendation # 2 and has asked cage culture farmer to reduce stocking density by 50% (10 to 5/m^3)

Enhancing local capacities on tilapia seed production through education in Honduras

Suyapa Triminio Meyer*, Daniel E. Meyer and Joseph Molnar

Aguaculture Outreach Program
Panamerican Agriculture School, Zamorano
P.O. Box 93 Tegucigalpa, Honduras
smeyer@zamorano.edu

The Panamerican Agriculture School (PAS) and the ACRSP are supporting the Luis Landa Technical Agriculture School (LLAS) in southern Honduras to become a source of tilapia fingerlings for distribution to local farmers. This region has varied water resources and is close to markets for tilapia in El Salvador. LLAS is a vocational high school with a program of study in aquaculture, but their on-campus ponds and other infrastructure are in disrepair due to minimum funding for maintenance and operations. Fingerling production on campus will provide practical training opportunities for students to complement their theoretical training and income to support aquaculture activities. LLAS is located in a socio-economically depressed area with potential for tilapia culture. This activity can have a positive impact on income generation and poverty alleviation for local farmers. After a visit to the LLAS campus and an initial meeting between LLAS and PAS aquaculture outreach staff, we established to following goals:

- inventory of the LLAS aquaculture infrastructure
- analyze the required resources for beginning fingerling production at LLAS
- define the training needs for the LLAS aquaculture teachers
- identify potential collaborators and possible donors to support our efforts
- establish a timeline and strategy for commencing fingerling production

This report details the situation of the LLAS aquaculture infrastructure and describes the ongoing strategy to train their teachers and implement a tilapia fingerling production unit on campus.

Enhancing local capacities on tilapia seed production through education in Honduras

Suyapa Triminio Meyer* and Daniel E. Meyer
Aquaculture Outreach Program
Panamerican Agriculture School, Zamorano
P.O. Box 93 Tegucigalpa, Honduras
smeyer@zamorano.edu

PD/A CRSP ZAMORANO The University of Georgia USAID

Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP)

The PD/A CRSP is funded in part by United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00 and by participating institutions.

Zamorano aquaculture station for teaching, outreach, training and research

The Luis Landa Technical Agriculture School (LLAS):

- **technical high school**
- **graduates are agricultural technicians**
- **option to pursue university education**
- **aquaculture technician program (unique in Honduras)**

High demand for tilapia fingerlings in the area

Training event for producers, students, government and NGO agents, Escuela Luis Landa, Nacaome, Honduras

Fingerling demand in the area

- Shrimp ponds dedicated to tilapia culture
- Small scale farmer inland interested in tilapia culture
- Cage culture in estuaries

Harvest at a cooperative production site in hydroelectric dam

Objective

- To support the Luis Landa Technical Agriculture School (LLAS) in southern Honduras to improve their institutional competence for teaching aquaculture and become a source of tilapia fingerlings for distribution to local farmers

Expected impacts:

- Production of tilapia fingerlings will serve as an important component for their practical training.
- Income from fingerling sales will support student's educational activities, buy feed, equipment and materials.
- Local farmers will have better access to fingerlings.

Methodology

- Establish communication between LLAS and Zamorano
- Arrange meeting on LLAS campus

Methodology

- Inventory and diagnosis of the situation of the LLAS aquaculture facilities and infrastructure
- Offer training opportunities for LLAS staff
- Identify strategies to establish the fingerling production
- Involve other stakeholders in this activity
- Collaborate with LLAS to develope capabilities to produce and distribute the fingerlings

Participants in the first meeting in LLAS in August 2007

Staff from the UNAH, PAS, and staff and students of LLAS

Inventory and diagnosis

Some strengths of the LLAS to become a fingerling provider for the region are:

- a good water source (quality and quantity)
- excellent climate (temps 25 to 40 C)
- good location on Pan-American highway
- existing aquaculture installations
- Trained aquaculture teachers
- Interested students
- Excellent facilities for offering short courses on campus

Inventory and diagnosis

Some weaknesses found at LLAS were:

- lack of equipment to manage fish
- Water pumping system is nonfunctional
- lack of capital for operations (feed, repairs)
- lack of staff training opportunities
- administrative problems (government dependency, very political institution)

Conclusions:

- LLAS has basic resources to become tilapia fingerling provider for the southern region
- Capability to train local farmers and technicians
- LLAS requires support from the government to obtain administrative stability and financial support
- Develop collaboration with stake holders to finance this endeavour

Diagnosis

Four concrete tanks for managing fry

Total of four fish ponds
800 to 1200 m2 each

Water pump
75 HP

Actions

Training of LLAS staff at Zamorano:

- Course on Tilapia production (September 2007):
  - reproduction, marketing, record keeping and cost calculation
  - two LLAS staff members
  - 4 day event
- Practical course tilapia reproduction (September 2007):
  - one staff member from LLAS
  - 7 day long event
  - broodstook management, fingerling management, sex reversal, packing and transport
- We have developed of a list of materials and tools to implement the production of fingerling in LLAS
- Donation of manuals developed by Zamorano and CRSP for their library

Second meeting in April 2008

Participants:

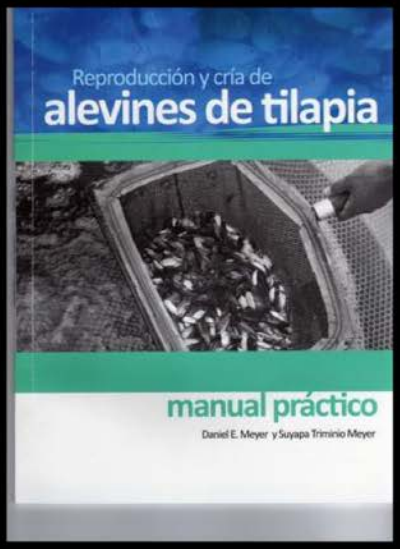
- Director of Education Ministry
- representative of ANDAH
- LLAS director
- LLAS aquaculture staff
- Zamorano aquaculture extension staff

Objective: to discuss options to reactivate and support the technical program in aquaculture at LLAS

Second meeting in April 2008

Results:

- A list of technical short and long term options
- Decision to prepare a project proposal to finance a sustainable aquaculture program for LLAS
- Meet with the Minister of Education and the Director for Fisheries and Aquaculture (GOH)
- Developed a list possible donations from each participating institution to begin fingerling production at LLAS



Gracias

Thank You

Dynamics of increase in Insulin-like Growth Factor-I mRNA expression in Nile tilapia, *Oreochromis niloticus*, in response to elevated temperature

Emmanuel M. Vera Cruz*, Christopher L. Brown

Freshwater Aquaculture Center/College of Fisheries
Central Luzon State University
Science City of Mu 3120
Philippines
bongbee15@hotmail.com

Insulin-like growth factor-I (IGF-I) is a physiological mediator and a potentially important growth indicator candidate in teleost fishes. In this study, the effects of increased temperature on the growth and hepatic IGF-I gene expression in *O. niloticus* were evaluated. Twenty all-male fish were reared separately at temperatures below 24 for 12 days and then water temperature in 15 aquaria was gradually raised to 30 within a day. Growth and hepatic IGF-I gene expression in five fish were obtained after 2, 5 and 7 days of rearing at warm temperature. The growth rate of the fish reared in the warmer temperature was significantly increased in a time dependent manner (r = 0.93). Mean hepatic IGF-I mRNA levels in fish reared at warm temperature for 2, 5 and 7 days were elevated 1.6-fold, 2.5-fold, and 3.6-fold, respectively compared to that of fish reared at cold temperature. The IGF-I levels were significantly elevated after at least 5 days of exposure to warm temperature, which is comparable with the idea that hepatic IGF-I gene expression can be used as a short-term growth rate indicator for *O. niloticus*. A significant positive correlation was observed between days of rearing at warm temperature and hepatic IGF-I levels (r = 0.92); between specific growth rate (length) and IGF-I levels (r =0.92); and between condition factor and IGF-I levels (r = 0.55). The high positive association between IGF-I mRNA and growth rate validated the assertion that hepatic IGF-I levels are sufficiently sensitive to be used as instantaneous growth rate indicator in this species of fish.

African Aquaculture Session
Abstracts and Presentations

Small-scale aquaculture and credit use in Ghana

S. Amisah[1] and K. Quagrainie[2]

[1]Department of Fisheries and Watershed Management
Kwame Nkrumah University of Science & Technology
Kumasi, Ghana
Steveamisah1@yahoo.co.uk

[2]Dept. of Agricultural Economics
Purdue University
West Lafayette, IN 47906, USA
kquagrai@purdue.edu

Fish farming remains a significant alternative farming activity in southern Ghana and also represents a potential income earner for farmers in northern Ghana. The contribution of fisheries aquaculture to the fish requirements of Ghana is limited; meanwhile harvest from capture fisheries is on the decline. Ghana's fish imports amounts to about US$200 million per annum, an amount that can be reallocated to other socioeconomic development of the country with a developed aquaculture industry supplying the fish requirements needed in the country. Growing the aquaculture industry therefore, will reduce fish imports into the country to save the country's hard-earned foreign exchange. Recent studies in Ghana by the Aquaculture Collaborative Research Support Program (ACRSP) have generated information and data on the economic profitability of fish farming (particularly, Tilapia *Oreochromis niloticus*) and catfish *Clarias gariepinus*) and preliminary studies show that it is a profitable farm enterprise. Any growth in the aquaculture industry would require additional capital investment. This study examines farmers use of credit in fish farming.

The study uses data from a survey of farmers in the Ashanti and Bring-Ahafo regions of Ghana in 2005. The data is part of a larger data set collected with funding from ACRSP. The data included demographic information, general farming information and information specific to fish farming. To examine factors affecting the use of credit in fish farming in Ghana, we use a Tobit model to analyze the effects of socio-economic factors, farm characteristics, infrastructure, and fish sales on use of credit by farmers in a given region. The Tobit model is a censored normal regression model, and its estimation is related to the estimation of a censored and truncated normal distribution. In this study, the credit use function is estimated from a censored sample where the sample population consists of both credit users and non-credit users in fish farming.

STUDY OF FISH FARMERS SOURCE OF AQUACULTURE INFORMATION IN GHANA

Kwamena Quagrainie
Purdue University, USA

Steve Amisah
Kwame Nkrumah University of Science & Technology, Ghana

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Aquaculture in Ghana

- Main species are tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*)
- Total production about 950 mt/year
- Farms concentrated in Ashanti region
- Majority of farmers are small-scale operators using extensive fish farming practices

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Purpose of Study

- In 2005 Government took steps to support and accelerate aquaculture development by providing
  - extension services
  - training in fish farming techniques
  - training in basic bookkeeping & business plan preparation
  - fingerlings for sale to fish farmers

KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Other agencies involved in aquaculture

- Government parastatals
- Non-Governmental Organizations
  - GTZ, FAO, WorldFish Center, etc
- Universities
- Farmer groups

KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Questions?

- Where do farmers obtain information about fish farming?
- What is the source of technical assistantship on fish farming?
- What factors influence farmers' choice of information source?

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Methodology

- Questionnaire solicited information on:
  - Source of information for aquaculture
  - Demographics
  - General Farm operations
  - Fish Farm operations

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Results – *Demographics*

- Regional responses
  - Ashanti = 72%
  - Brong-Ahafo = 28%
- Males = 91%
- Average age = 53yrs
- Years farming = 20yrs
- Literacy rate = 84%
  - Primary = 13%
  - Secondary = 35%
  - Adult Ed = 11%
  - Post-Sec = 33%

KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Results – *Demographics*

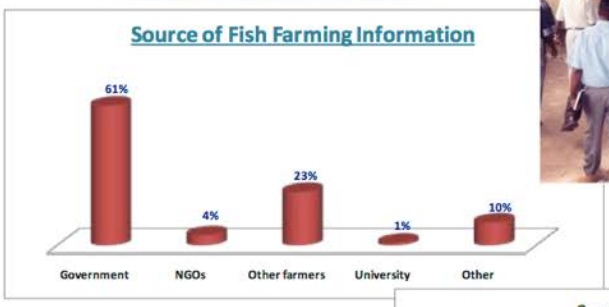
- Average agricultural farm size = 44 acres
- Average pond acreage = 1 acre

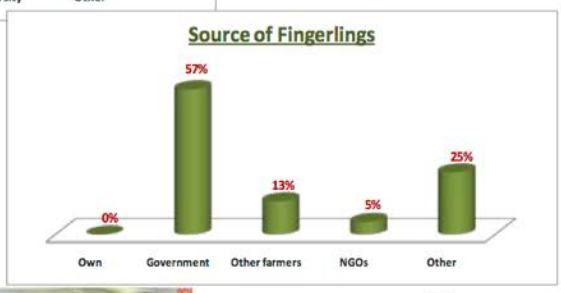
KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Results



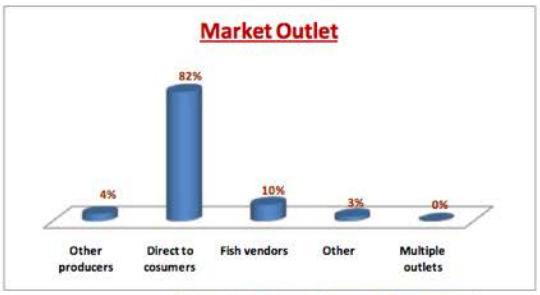


KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Results



KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Multinomial Logit Analysis

Dependent Variable (Aqua Info Source)

Y0 = Government
Y1 = NGOs
Y2 = Other Farmers
Y3 = University
Y4 = Other

Explanatory variables

- Region; Ashanti=1, otherwise=0
- Number of years farming
- Educational level; Read/write=1, otherwise=0
- Total pond acreage

KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Marginal Effects Averaged Over Individuals

Variable	Y=00 Gov	Y=01 NGO	Y=02 OFM	Y=03 UNI	Y=04 OTH
Ashanti region	-0.269	0.067	-0.081	0.018	0.265
Farming years	0.082	-0.054	0.099	-0.006	-0.121
Literacy	0.434	-0.103	-0.062	-0.016	-0.253
Pond acreage	0.338	0.026	0.004	-0.413	0.046
Actual	61%	4%	23%	1%	10%
Predicted Prob	59%	5%	25%	1%	10%

Overall % Correct Prediction	61%
Pseudo R-squared	0.14

KNUST

USAID Aquaculture & Fisheries CRSP

PURDUE UNIVERSITY

Interpretation of Results

- Ashanti Region farmers
  - Farmers have increasing probability of sourcing information from NGOs (7%), university (2%) and other sources (27%)
  - But a declining probability of obtaining aquaculture information from the government (-27%) and other farmers (-8%)

(Relative to Brong-Ahafo farmers)

PURDUE UNIVERSITY

Interpretation of Results

- Number of years farming
  - With more experience in farming, there is increasing probability of farmers seeking aquaculture information from the government (8%) and other farmers (10%)
  - But a decreasing probability of sourcing information from NGOs (-5%), university (-1%) and other sources (-12%)

PURDUE UNIVERSITY

Interpretation of Results

- Literacy (Relative to Illiterates)
  - Literate farmers have an increasing probability of obtaining aquaculture information from the government (43%)
  - But a decreasing probability of sourcing information from all others

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Interpretation of Results

- Pond Acreage
  - With larger pond acreage, there is increasing probability of farmers seeking aquaculture information from the government (34%), NGOs (3%), other farmers (0.4%), and other sources (5%).
  - But a decreasing probability of sourcing information from university (-41%).

KNUST USAID Aquaculture & Fisheries CRSP PURDUE UNIVERSITY

Implications of Results

- Government support for aquaculture development is critical since most factors indicated a positive effect on probability of sourcing from government (literacy 48%; Acreage 34%).
- Ashanti region farmers have a 27% probability of obtaining aquaculture information from other sources (Previous knowledge from schooling, self-education, etc). Reveals the impact of aquaculture curriculum in schools.
- Except for being in Ashanti region (2%), literacy (-2%), experience (-1%) and farmers with larger acreages (-41%) have a decreasing probability of seeking aquaculture information from the university. Suggest that outreach activities of universities, e.g. KNUST should go beyond Ashanti region.

KNUST USAID Aquaculture & Fisheries PURDUE UNIVERSITY

Implications of Results

- The impact of factors indicated a low effect on probability of sourcing from NGOs (Ashanti 7%; Experience -5%; literacy -10%; Acreage 2%). NGOs usually provide one-time assistance during the project period and do not have field personnel to offer continuous assistance.
- The impact of factors indicated a low effect on probability of seeking information from other farmers (Ashanti -8%; Experience -6%; literacy -6%; Acreage 0.4%).

KNUST USAID Aquaculture & Fisheries PURDUE UNIVERSITY

Acknowledgement

This study was sponsored by the Aquaculture & Fisheries Collaborative Research Support Program (AquaFish CRSP) funded under USAID Grant No. EPP-A-00-06-00012-00 and by Purdue University, USA and Kwame Nkrumah University of Science and Technology, Ghana.

PURDUE
UNIVERSITY

THANK YOU

Questions?

PURDUE
UNIVERSITY

The use of credit in fish production in Kenya

Kwamena Quagrainie*, Charles Ngugi and John Makambo

Dept. of Agricultural Economics
Purdue University
West Lafayette, IN 47906
kquagrai@purdue.edu

Aquaculture is a slow growing industry in Kenya, and contributes less that 1% of the total national protein requirements. Kenya's aquaculture industry consists of several small-scale and few medium-scale production of tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*). Total production is estimated to be about 1,000 metric tons annually. The artisanal fishery of Lake Victoria, the largest commercial fishery in Kenya, has been degraded by environmental deterioration (water hyacinth) resulting in increasing harvest of smaller foodfish species. Aquaculture therefore provides the best alternative of freshwater food fish for consumers to meet protein needs. This can be achieved through investments into fish farming and moving from subsistence production into commercial medium- to large-scale production. However, Kenyan farmers are generally poor and any expansion in the aquaculture industry would require additional capital injection. This study therefore examined farmers use of credit in aquaculture.

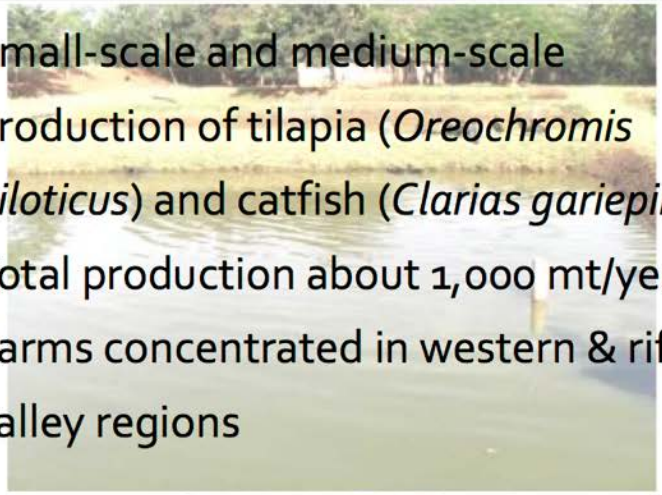
Data for the study was obtained from a survey of farmers in Kenya in 2005. Fish farmers in Western, Central, Eastern and the Rift Valley regions were surveyed. The study solicited demographic information, general farming information and information specific to fish farming. The survey results showed 70% of respondents were from Western Kenya. Respondents ranged in ages from 30 to 76 years and had farming experience ranging from 2 to 56 years. The average size of pond water acres was 604m2. To examine factors affecting the use of credit in fish farming, we used a Tobit model to analyze the effects of region, age, farming experience, education, pond acres, fish sales and source of aquaculture information on use of credit. The results show that fish farmers in Western Kenya were likely to use credit compared to farmers from other regions. High farm sales of tilapia and catfish were positive and important determinants of the probability of using credit in the fish farming business. Age, farming experience, and education had no significant effect on the probability of using credit. Results also showed that farmers who utilized only one source of information on fish farming were less likely to use credit. Sources of fish farming information available to respondents were government, NGOs, other farmers, universities, and other sources.

THE USE OF CREDIT IN FISH PRODUCTION IN KENYA

Kwamena Quagrainie
Purdue University, USA

Charles Ngugi and John Makambo
Moi University, Kenya

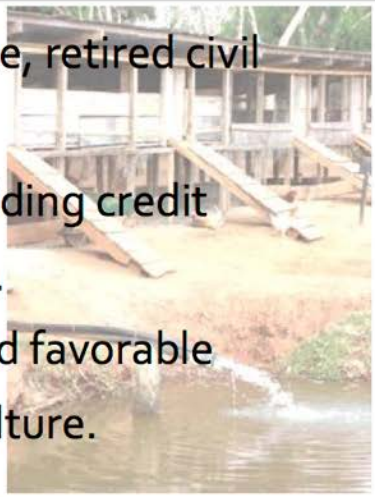
Kenya Aquaculture



- Small-scale and medium-scale production of tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*)
- Total production about 1,000 mt/year
- Farms concentrated in western & rift valley regions

Background

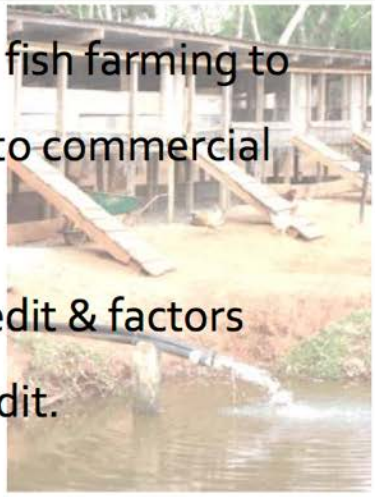
- Many farmers are literate, retired civil servants, etc.
- Commercial banks providing credit services for fish farming.
- Government support and favorable policies towards aquaculture.



PURDUE
UNIVERSITY

Purpose of Study

- Need for investments in fish farming to move from subsistence to commercial production.
- Examine attitudes to credit & factors that influence use of credit.

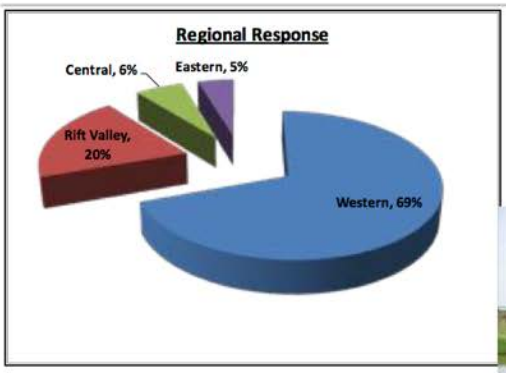


PURDUE
UNIVERSITY

Survey

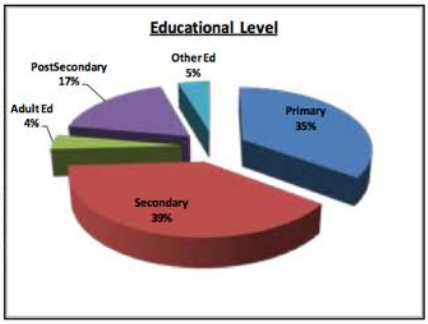
- Questionnaire solicited information on:
  - Demographics
  - General Farm operations
  - Fish Farm operations

Results - *Demographics*



- Responses = 131
- Males – 85%
- Average age 50yrs

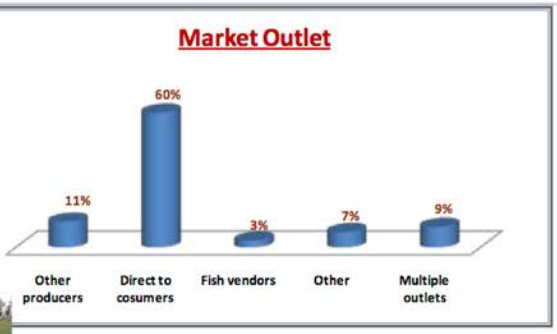
Results - *Demographics*



- Average # of ponds = 6
- Average acreage = 616m^2

PURDUE UNIVERSITY

Results



PURDUE UNIVERSITY

Economic Analysis of Credit Use

- Simple Binary Probit Analysis
- Dependent variable: Whether or not credit is used to purchase inputs

Explanatory variables

- Region; western=1, otherwise=0
- Age
- Educational level; primary, secondary or adult=1, otherwise=0
- Total pond acreage
- Value of tilapia sales in past 6 months (KSH)
- Value of catfish sales in past 6 months (KSH)
- Type of market outlets; multiple=1, otherwise=0
- Fulltime labor cost per day (KSH)

PURDUE UNIVERSITY

Parameter Estimates

Variable	Coefficient	*p*-value
Constant	-1.089	0.089
Western region	0.871	0.013
Age	0.005	0.664
Some education	-0.318	0.366
Pond acreage	-0.001	0.042
Tilapia sales	0.261	0.006
Catfish sales	0.086	0.080
Direct to Customers	-0.082	0.782
Fulltime labor cost	-0.006	0.009
Pseudo R-squared	0.20	
% Correct Predicted	78.62	

PURDUE UNIVERSITY

Marginal Effects

- How do variables affect the probability of credit use?

Variable	Coefficient	*p*-value
Western region	0.190	0.003
Age	0.001	0.662
Some education	-0.088	0.396
Pond acreage	0.000	0.022
Tilapia sales	0.067	0.005
Catfish sales	0.022	0.083
Direct to Customers	-0.021	0.783
Fulltime labor cost	-0.001	0.006

PURDUE UNIVERSITY

Interpretation of Results

- Farmers in the Western region have 19% higher probability to use credit than farmers from other regions.
- A m^3 increase in pond acreage and a KSH increase in fulltime labor cost, increase the probability of credit use by 0.02% and 0.14% respectively.
- A KSH increase in tilapia and catfish sales increase the probability of credit use by 7% and 2% respectively.

PURDUE UNIVERSITY

Implications of Results

- In general, there is a low probability of credit use by fish farmers.
- More education is needed about the use of credit to expand aquaculture operations and improve commercialization.
- Focus should be in the Western region where there is a greater % of aquaculture operations.

PURDUE
UNIVERSITY

Acknowledgement

This study was sponsored by the Aquaculture & Fisheries Collaborative Research Support Program (AquaFish CRSP) funded under USAID Grant No. EPP-A-00-06-00012-00 and by Purdue University, USA and Moi University, Kenya.

PURDUE
UNIVERSITY

THANK YOU

Questions?

PURDUE
UNIVERSITY

Improved principles and practices for small-scale cage culture on irrigation reservoirs in South Africa

Khalid Salie*, Monika Cermak and Dorette du Plessis

Division of Aquaculture, University of Stellenbosch, Matieland, 7602
Republic of South Africa
ks1@sun.ac.za

Aquaculture in open water systems, such as in irrigation reservoirs, is a relatively new development in Southern Africa. In most irrigation areas in South Africa there are opportunities to use on-farm and distribution systems for fish production in floating net cage systems. The primary use of the water is for irrigation of high value agricultural crops for the local and export market. A small-scale fish farming programme focusing on the production of fish in net cages in irrigation reservoirs has been running successfully for the last decade. Recent results have actually indicated that water and fish quality were compromised through incorrect procedures and non-compliance with proposed good management practices as taught and discussed in training and extension programmes. This could have a negative influence on the ecological integrity of the reservoir and larger watershed as well as the sustainability of production output. A preliminary investigation has revealed that site selection and feed management were the two aspects directly associated with product and water quality maintenance. Inadequate attention to these aspects not only has caused water quality deterioration but also financial losses due to inefficient feed utilisation. Projects were farming with rainbow trout (*Oncorhynchus mykiss*) in cages from May to November. The objective of the study was to evaluate to what extent proposed practices were implemented. General perceptions among farmers were also recorded as to the comprehension of these practices and the reward per unit effort experienced.

Research sites were commissioned at the farms that were implemented during 2006/7 and only those ones farming with trout were evaluated. Six small-scale fish farming projects were frequently visited. All six projects are situated in close proximity within a 100 km radius. As part of the capacity building of the farmers, participants have attended a three-day training programme, complemented by follow up training conducted on-site by the extension officers during the production cycle. The programme manager and representatives from the community forum conducted the site selection. Parameters that were discussed as to their nature and influence were: a) physical criteria (i.e. temperature profile, turbidity, replacing tempo), b) chemical criteria (i.e. dissolved oxygen levels, ammonia, pH) as well as c) historical data (age of reservoir, fertilisation and spraying regimes and plant and animal establishment). The forum was trained on the interpretation of measurements and instrument readings and to extrapolate recorded data to management. Much time was also conveyed to feed management such as feeding programme, methods of feeding, storage and record keeping. Focus group discussions were continually held throughout the production season and the progress was recorded.

Recorded data was banked in booklet form and has been included in the training packages for forthcoming programmes. An expert consultation workshop has been held where good management practices for cage culture and sediment quality were discussed. Further dissemination was conducted via seminars, meetings, and newsletters to the extension officers, processors as well as government officials. Comments and suggestions were tabled to improve future implementation and compliance.

Integration of GIS, remote sensing, and USLE to predict soil erosion distribution in Nzoia Basin (Kenya)

Herbert Ssegane[1], Tommy Jordan[2], and E. W. Tollner[1]

[1]Biological & Agricultural Engineering Department
[2]Geography Department
University of Georgia
Athens, GA 30602

btollner@engr.uga.edu

The study describes the application of the universal soil loss equation (USLE) model, to quantify soil erosion in Nzoia basin located entirely on the Kenyan side of Lake Victoria basin using the geographic information service, remote sensing, and global positioning service technologies. The approach adopted involved calculation of six universal soil loss equation factors inform of distributed remote sensing and geographic information service data using arcGIS / arcMap software. The USLE is comprised of six factors, rainfall, soils, slope length, slope steepness, vegetal cover and conservation practice. The data included spatial raster layers of soil, land cover, rainfall and digital elevation models ranging from 30 m to 1000 m spatial resolutions to adequately represent the surface characteristics. A GIS layer was created to model each of the six factors. The soil erosion distribution map was generated as a product of the six raster layers using the spatial analyst tool in arcMap. Even with continental scale spatial resolutions, the predicted erosion levels had the same order of magnitude as predictions made with site specific parameters utilizing Google Earth Pro. For a site at Moores bridge along the Moiben sub-watershed the predicted erosion levels ranged between 31 - 51 tons/ha-yr compared to the value of 97.2 tons/ha-yr obtained using USLE and Google Earth Pro. To improve the accuracy levels, use of recent land cover and land use data plus use of smaller variation of the data spatial resolution was recommended. The erosion potential for the Nzoia basin is shown in Figure 1. Erosion analyses enables one to rapidly assess the threats to fisheries from agricultural or urban development activity within a basin. The effects of sewerage on receiving waters is not shown in this analyses but could be inferred with additional analyses.

Integration of GIS, Remote Sensing and USLE to Estimate Soil Erosion Potential in Nzoia River Basin (Kenya).

Ernest W. Tollner
Herbert Ssegane
Tommy Jordan

The process…

- Define the relationship
- Assemble the data sources
- Process the data and assemble a GIS layer
- Compute the composite layer
- Remap based on an index


The setting…
UGANDA
KENYA
Nzoia
Basin
TANZANIA
AFRICA


The setting…
LAKE VICTORIA BASIN
30°0'0"E
32°0'0"E
34°0'0"E
UGANDA
KENYA
NZOIA
SIO
YALA
NYANDO
SONDU-MIRIU
LAKE VICTORIA
GUCHA
MARA
KAGERA
GURUMETI
MOARTADET
SIMIYU-DUMA
MAGOGA-MUAME
ISANGA
BURUNDI
TANZANIA
0°0'0"N
2°0'0"S
4°0'0"S

The Universal Soil Loss Equation

$$A = RKSLCP$$

Where
A = Average Annual Soil loss in (Mg ha^{-1})

R = Rainfall –Runoff erosive index factor in (MJ mm $ha^{-1}hr^{-1}yr^{-1}$)
K = Soil erodibility factor (ton ha hr $ha^{-1}MJ^{-1}mm^{-1}$)
S = Slope steepness factor (dimensionless)
L = Slope Length factor (dimensionless)
C = Crop-Management factor (dimensionless)
P = Conservation Practice factor (dimensionless)

Data Sources…

- The annual rainfall data for Kenya was obtained from flood early warning system (FEWS) dataset, a program developed jointly by the USGS and USAID. The data was accessed at USGS (2007).
- The soils data used was obtained in a binary format from the north oceanic and atmospheric administration (NOAA) at a spatial resolution of 0.0833 arc degrees. The data was accessed at national geophysical data center (NGDC, 2007).

Data Sources, continued…

- The Digital Elevation Model (DEM) data was obtained from the Global land covers facility (GLCF) under the shuttle radar and thematic mapper (SRTM) data category. Resolution was 90 m.
- The US Geological Survey (USGS) provides global land use and land cover data on continental scale with a spatial resolution of one kilometer.

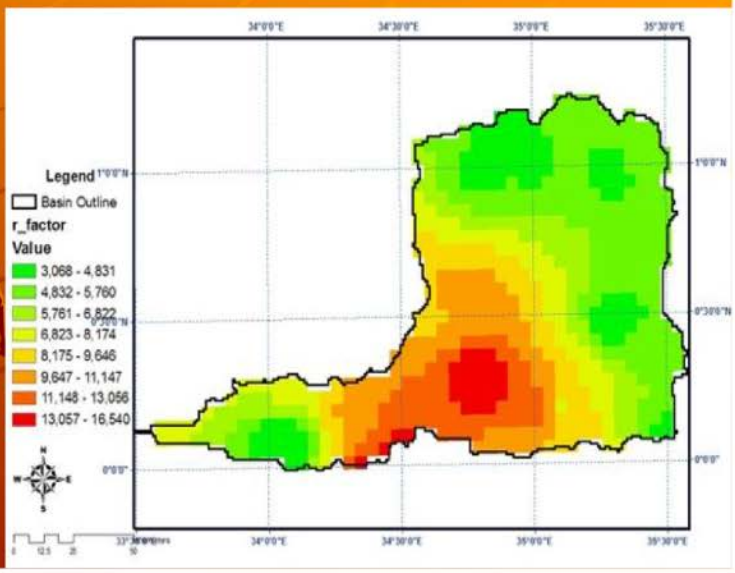
Data Processing – Precip

Renard and Freimund (1994)

$$R = \begin{cases} 0.0483P^{1.610}, & P \le 850mm \\ 587.8 - 1.249P + 0.004105P^2, & P > 850mm \end{cases}$$

Where R = rainfall erosivity (MJ mm ha^{-1} hr^{-1} yr^{-1}), and
P = annual precipitation (mm)

Rainfall factor

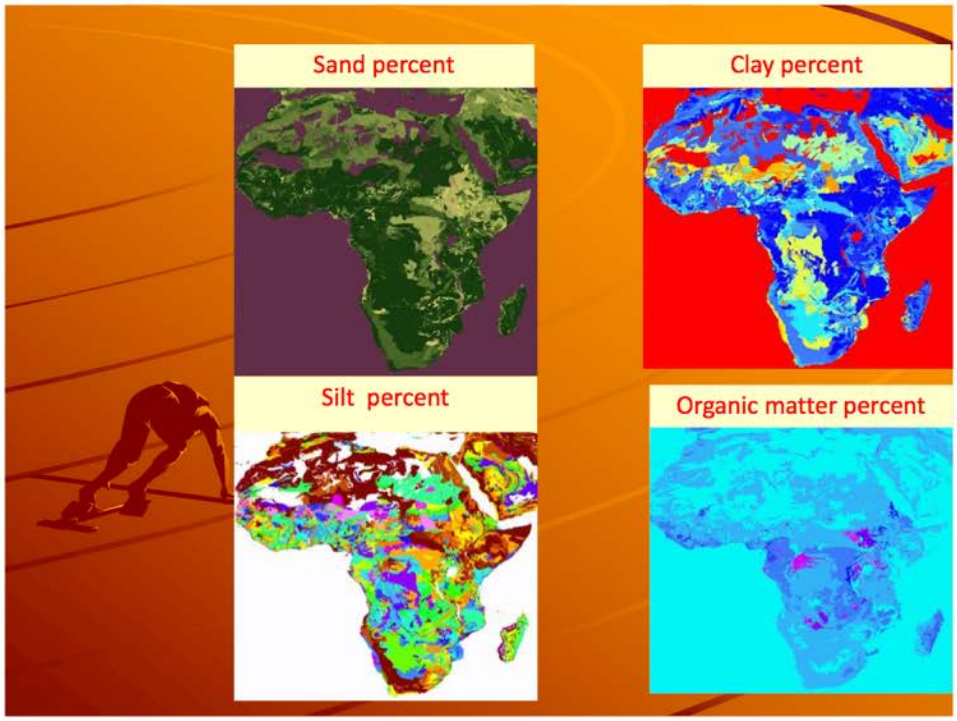


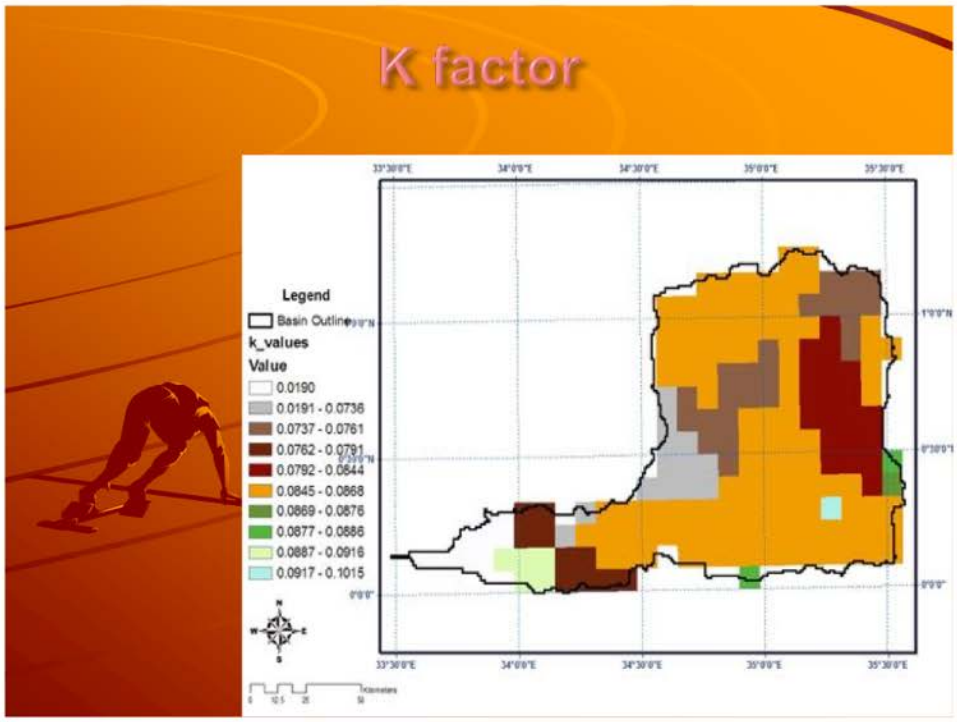
Data Processing – soils
(Torri, et al 1997)

$$K = 0.0293\left(0.65 - D_g + 0.24 D_g^{\,2}\right)\exp\left\{-0.0021\frac{OM}{f_{clay}} - 0.00037\left(\frac{OM}{f_{clay}}\right)^2 - 4.02 f_{clay} + 1.72 f_{clay}^{\,2}\right\}$$

$$D_g = -3.5 f_{clay} - 2.0 f_{silt} - 0.5 f_{sand}$$

Where K = soil erodibility (ton ha hr $ha^{-1}MJ^{-1}mm^{-1}$)
OM = percent organic matter
f_{sand} = sand fraction
f_{silt} = silt fraction
f_{clay} = clay fraction
D_g = the geometric mean of particle size


Sand percent
Clay percent
Silt percent
Organic matter percent


K factor
Legend
Basin Outline
k_values
Value
0.0190
0.0191 - 0.0736
0.0737 - 0.0761
0.0762 - 0.0791
0.0792 - 0.0844
0.0845 - 0.0868
0.0869 - 0.0876
0.0877 - 0.0886
0.0887 - 0.0916
0.0917 - 0.1015

Length Factor

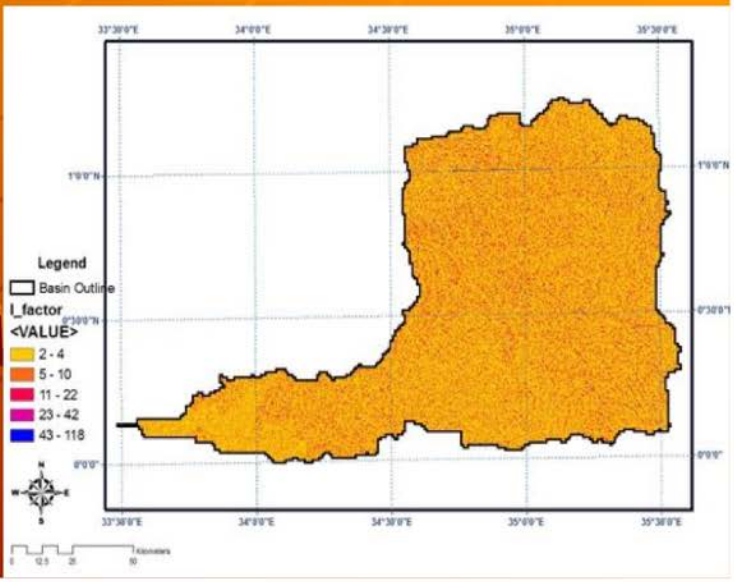
Desmet and Govers (1996)

$$\lambda = FlowAccumulation * CellSize$$

$$\lambda^{\otimes} = \lambda + CellSize$$

$$L = \frac{\left(\lambda^{\otimes}\right)^{1.4} - \lambda^{1.4}}{CellSize * 22.13^{0.4}}$$

Slope length



Slope

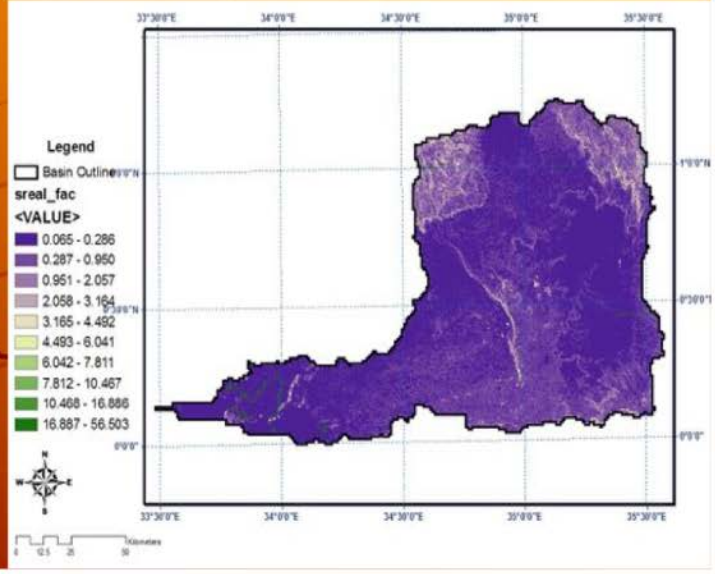
Smith and Wischmeier (1957)

$$S = \frac{\left[0.43 + 0.30s + 0.043s^2\right]}{6.613}$$

Where

s = the percent slope

Slope

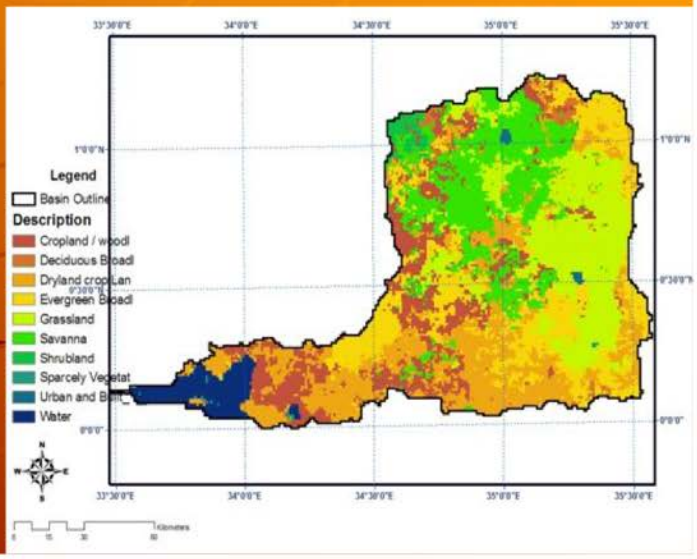


Cover and Practice

Renard et al (1994,1997)

Land classes	Crop management factor, C	Management practice Factor, P
Urban and built-up land	0.01	1
Dryland cropland and pasture	0.013	0.1
Irrigated cropland and pasture	0.013	0.1
Cropland / grassland mosaic	0.3	0.12
Cropland / woodland mosaic	0.3	0.12
Grassland	0.04	0.12
Shrubland	0.036	0.12
Savanna	0.039	0.12
Deciduous broadleaf forest	0.006	0.8
Evergreen broadleaf forest	0.006	0.8
Herbaceous wetland	0	1
Wooden wetland	0	1
Barren and sparsely vegetated	0.4	1

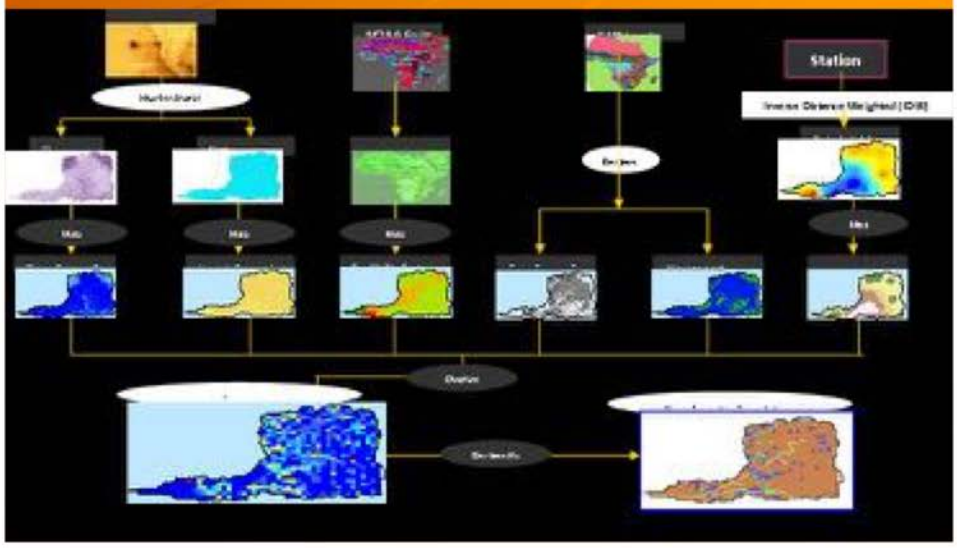
Land use



Erosion Index

Soil Erosion (tons $ha^{-1}yr^{-1}$)	Soil Erosion class	Erosion Map Index
0 - 5	Slight	1
5 - 10	Moderate	2
10 - 20	High	3
20 - 80	Very High	4
> 80	Severe	5

Data Layer handling to compute erosion and ratings…

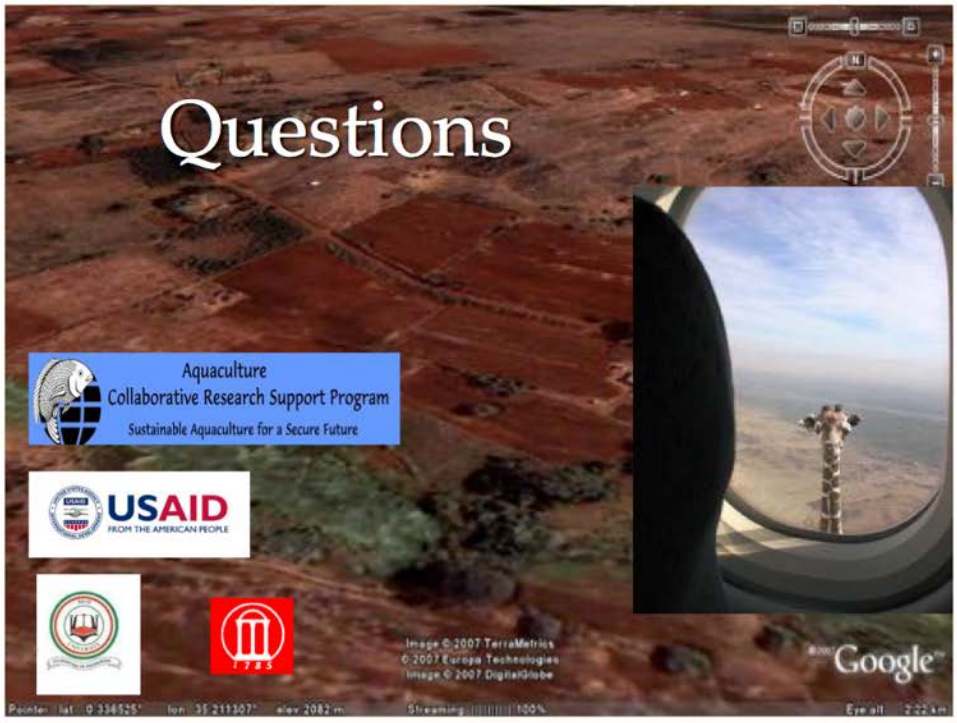


Conclusions

- Since most of Nzoia River basin (84.2 %) is under slight agricultural erosion hazard, other erosion sources such as unpaved and poorly graded roads plus point sources should be investigated.
- Results from the erosion estimation showed on average, potential erosion highest in the cropland and woodland mosaic (180 Mg ha^{-1} yr^{-1}) and lower in deciduous broadleaf forest (23 Mg ha^{-1} yr^{-1}) and shrubland (22.5 Mg ha^{-1} yr^{-1}).

Conclusions

- Data resolution is critical, and this problem is being addressed as more data is collected or one pays more.
- GIS and remote sensing platforms provide fast and robust tools for spatial modeling and are useful for strategic land use management and environmental monitoring.
- GIS related tools offer tremendous educational opportunities for engaging students.


Questions
Aquaculture
Collaborative Research Support Program
Sustainable Aquaculture for a Secure Future
USAID
FROM THE AMERICAN PEOPLE
Image © 2007 TerraMetrics
© 2007 Europa Technologies
Image © 2007 DigitalGlobe
Google

Adding value to weeds *Tithonia diversifolia* and *Chromolaena odorata* through their use as fishpond fertilizer

Rodrigue Yossa Nouaga*, Victor Pouomogne, Randall Eddie Brummett and James Gockowski

Department of Animal Sciences
Laval University
P O Box: 2425
Rue de Agriculture, QuG1K7P4, CANADA
Telephone: 1 418 656 2131 Ext 8823; Fax: 1418 6563766
E-mail: rodyossa@yahoo.fr

Two common introduced weeds of the rain forest, *Tithonia diversifolia* and *Chromolaena odorata*, were evaluated as fish pond organic fertilizers in Southern Cameroon. The aim of the study was to determine their effect on growth and survival of Nile Tilapia (*Oreochromis niloticus*) and to assess the systems financial profitability. Three treatments, *Tithonia diversifolia*, *Chromolaena odorata* and a mixture at equal proportion of these two weeds were applied at a rate of 2 kg dry matter/100 m^2 pond/day, in four replicates. Three of those replicates were done at Melen fish farming station and one on-farm using established protocols for farmer-participatory research (Farmer Scientist Research Partnership).

After rearing for 150 days (May-October 2003), no significant differences among treatments ($p<0.05$) were observed in terms of survival rate (from 82.27to 91.17%), average weight at harvest (from 86.87to 101.26g), daily individual growth (from 0.5458to 0.6519g/day), specific growth rate (from 1.89 to 1.99%/day) and production (2.1261.711and 1.689t/ha/year for *Tithonia diversifolia*, the mixture of the two weeds and *Chromolaena odorata*, respectively).

Financial analysis showed that, *Chromolaena odorata*, which generally grows close by the fish ponds, generates an income of 2.73 USD/day, which is greater than basic minimum salary (BMS) of a Cameroonian working in the agricultural sector (2.5 USD/day). However, when both of these weeds grow close by the fish ponds, fertilizing with *Tithonia diversifolia* generates an income of 4.22 USD/day, which is 54.66% more than that generated by *Chromolaena odorata*. In the situation where *Tithonia diversifolia* is situated at 350 m from the fish pond, the salary generated is equal to the BMS. Above this distance, it is then recommended to fertilize the ponds with *Chromolaena odorata*.

AquaFish-Supported
Abstracts and Presentations

Supporting the technical and economic viability of small and medium-scale tilapia producers through training and technical assistance in Central America: How governmental and nongovernmental organizations sustain the process

Joseph J. Molnar, Suyapa Triminio Meyer, Daniel Meyer, E. William Tollner, Pablo Martinez, Patricio Paz

Auburn University
International Center for Aquaculture and Aquatic Environments
292 Comer Hall
Auburn University
Auburn, AL 36849-5406 USA

The Aquaculture Collaborative Research Support Program recently completed a sustained series of research, training, and technical assistance events and activities in each Central American nation over the past two decades. First focused on Honduras, the shrimp industry, and water quality issues, the project shifted its scope to small and medium-scale tilapia producers and the governmental and nongovernmental organizations that provide technical and material support to the industry. Decision assistance software for pond design and construction was designed and disseminated. Practical research on tilapia production, fingerling production, and seed stock distribution identified the need for widespread fingerling production across the industry to sustain the industry among small and medium-scale producers. The Panamerican Agricultural School (Zamorano) became the center for research and training for the larger project. Research findings made clear that Nile tilapia have several advantages over red tilapia and are the fish of choice for farmers interested in beginning the commercial production and sale of fingerlings. Taking into account the significantly greater numbers of Nile fry produced both in the concrete tanks and in the earthen ponds and their better survival rate during sex reversal, the costs for reproducing the Nile fish is lower than for the red tilapia. Under market conditions for Zamorano, the economic benefits are much higher for producing and distributing Nile fingerlings compared to red fish. Production trials and economic analyses were complemented by a series of training events in Guatemala, Salvador, Dominican Republic, Nicaragua, Mexico, Panama, Costa Rica, and Belize that presented fundamental principles of tilapia reproduction, grow-out production, and fish handling to over 50 producers in each country. An intensives session on pond design and construction analysis was presented to interested producers in each locale. The two-day sessions also featured dialogue over production problems, management practices, and other practical aspects of tilapia culture in Central America. The presentation reviews the impacts, consequences, and prospects for the local and domestic tilapia industry in the region.

Bivalve Market Study in Pacific Mexico

Francisco Javier Martinez-Cordero, Q.S.W. Fond, M.C. Haws, E. Gaxiola-Camacho, and G. Rodriguez-Dominguez

Center for Food and Development Research
AP.711, Mazatlan, Mexico
Sinaloa, M82000
cordero@ciad.mx

A market study for oysters grown by social (cooperatives) groups of farmers, in Santa Maria de la Reforma Bay, Mwas carried out as part of a multi-component effort conducted with the beneficiaries in order to help them to successfully produce and commercialize their oyster production. This study focused on information from the demand side (including consumer preferences) specifically the market segment of local buyers, since this is considered the most feasible option for marketing given the small-scale nature of the stakeholders aquaculture operations. The work was conducted by the Research Center for Food and Development/Mazatlan (CIAD), Fishery Industrial Technology Center/University of Alaska Fairbanks-Kodiak, Autonomous University of Sinaloa and the Pacific Aquaculture and Coastal Resources Center/University of Hawaii Hilo, sponsored by the AquaFish Collaborative Research and Support Program (CRSP) of Oregon State University with funding from the United States Agency for International Development (USAID).

The results show that selling directly to local buyers (restaurants and owners of mobile point of sales) is the best marketing strategy to follow for the stakeholders, considering their current low production capacities. Analysis of the characteristics of this local market revealed preferences for the local regional oyster (*Crassostrea cortesiensis*), and a market window for product with consistent year-round supply that is high-quality (larger sizes), and safe. The stakeholders are advised to take advantage of a possible 0.50-1.00 peso increase in price per piece that buyers will pay when these desired characteristics are met. Stakeholders from this project may consider taking a price premium offer by survey respondents from local markets by delivering a high quality, larger sized oyster with safety guarantees. With products that include the said characteristics, a long-term commercial relationship that is based on trust and personal communications can then be established with buyers.

The timing may be right for the stakeholders to develop markets and buyer-seller relationships in the markets surveyed based on the results of the one-on-one interviews, which guarantees the price premium offered by the buyers. In a few years, there will be more products in the market, and the price elasticity of demand may turn negative. Finally, wholesale markets are not recommended to the stakeholders, since the local market is significant enough to absorb current production, but also due to a reduced margin profit in La Viga and Zapopan wholesale markets. The stakeholders would find it very difficult to sustain a high-volume supply of oyster, which is what these markets demand.

Transferring Market Information to Oyster Growing Stakeholders in Bahia Santa Maria: Synopsis of the Oyster Market in Guamuchil, Mexico

Martínez-Cordero*, F.J., Q.S.W Fong, M.C. Haws, E. Gaxiola-Camacho, and G. Rodríguez-Domínguez

Francisco Javier Martínez-Cordero, Ph.D.
Center for Food and Development Research (CIAD)
Mazatlan, Mexico

Quentin S.W Fong, Ph.D.
Fisheries Industry Technology Center
University of Alaska Fairbanks
Kodiak, Alaska, USA

Maria C. Haws, Ph.D.
Pacific Aquaculture and Coastal Resources Center (PACRC)
University of Hawaii Hilo
Hilo, Hawaii, USA

Eladio Gaxiola Camacho and Guillermo Rodríguez Domínguez, Ph.D.
Autonomous University of Sinaloa
Culiacan and Mazatlan, Sinaloa, Mexico

Background

- Bivalves are an important fishery and aquaculture resource in Mexico
- Most oyster culture on the Pacific Coast consists of *Crassostrea gigas* and *C. cortesiensis* (native species)
- Bivalves, along with tilapia, have been prioritized by Mexico Government for diversification of aquaculture
- Since 2003, this CRSP project team has worked to expand and improve bivalve culture as an alternative for poor coastal communities

Oyster Production

Mexico, 1993-2003

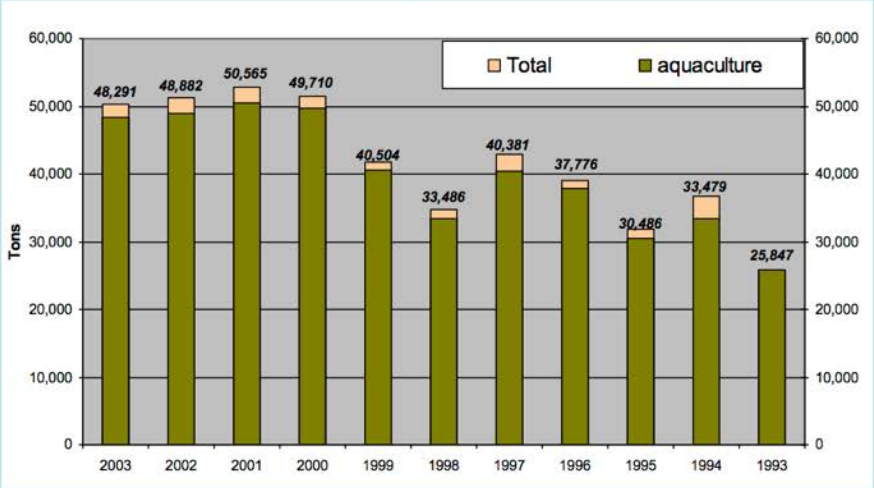


Figure on top of each bar is annual aquaculture oyster production

Oyster species

Crassostrea cortesiensis

(Pleasure Oyster)

Crassostrea gigas

(Japanese Oyster)

Improvement of bivalve culture requires market information

For example:

- What size of oyster should be grown?
- What prices would the buyer would pay?
- What is the preferred minimum shelf-life for the products?
- Is sanitation an important attribute?
- Does harvest location matter?

This information will help farmers in market identification and development of market penetration strategies

Objective

Overall:

- Assist oyster growing cooperatives in BSM to identify opportunities for marketing of oysters within the state of Sinaloa

Specific:

- Elicit the preference structure of managers/owners of seafood restaurants for oysters
- Recommend steps to be taken by growers for market penetration strategies based on the findings
- Use the process to educate Mexican small-scale oyster growing stakeholders in market research techniques and application of results.

Research Process

- Assess stakeholder's production capabilities, market knowledge, and market informational needs.
- Survey prepared that consists of structured and open ended questions.
- Survey administered to 15 restaurant managers and/or owners that serve oysters as part of their menu.
- Open-ended questions to consumers
- Feed back research results to producers

Type of restaurants and vendors

Results:
Importance of Oyster Attributes

Attributes	Average Score
Consistency in Supply	10.00
Uniformity in Size	10.00
Water Quality at Product Origin	10.00
Price	9.73
Mode of Transportation	9.64
Meat Fill	9.45
Oyster Size	9.36
Product Origin	9.00
Shape of Oyster	8.18

Results:
Highest Quality Oyster

Attribute with Highest Response vs Other	Percent
Live Shell-on Oyster vs. Other Products	84% vs. 16%
Native Oyster vs. Japanese Oyster	82% vs. 18%
Year Round vs. Intermittent Supply	70% vs. 30%
3 days vs. 1day and 10 days Shelf-Life	66.6%
Large vs. Medium and Small Size	61.5% vs. 39.5%
Wild Harvest vs. Cultured	57% vs. 43%
Northern Sinaloa State vs. Other Region in Origin	54% vs. 46%

Results:
Relationship with Supplier

Duration	Main Supplier	Secondary Supplier
> 3 Years	46%	18%
1 to 3 Years	27%	27%
6 to 12 Months	18%	37%
< 6 Months	9%	18%

Results:

Consumer's Preference for Oysters

Attributes	Strongly Agree	Agree	Disagree	Strongly Disagree
Prefer local oysters	37%	9%	18%	36%
Price is Important	91%	9%	0%	0%
Prefer Live, Shell-on Oyster	100%	0%	0%	0%
Harvest Date Important	73%	9%	18%	0%

Conclusions

BSM producers should:

- **Focus on producing large, live shell-on oysters as product form**
- **Adopt best sanitation management practices to ensure minimum preferred shelf-life**
- **Develop 3rd party water quality certification**
- **Develop relationship with potential buyer early**
- **Ensure year-round consistent supply**

Note: If the above attributes are met, all respondents are willing to pay higher prices)


Funding for this research was provided by the
AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM
USAID
FROM THE AMERICAN PEOPLE
OSU
Oregon State
AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM
The AquaFish CRSP is funded in part by United States Agency for International Development (USAID)
Cooperative Agreement No. EPP-A-00-06-00012-00 and by US and Host Country partners.
The contents of this presentation do not necessarily represent an official position or policy of the United States Agency for International Development
(USAID). Mention of trade names or commercial products in this presentation does not constitute endorsement or recommendation
for use on the part of USAID or the AquaFish Collaborative Research Support Program. The accuracy, reliability,
and originality of the work presented are the responsibility of the individual authors.

Biofilm Start-up on different filter media

Min Jiang*, Weiwei Miao, Chunfang Luo

College of Aqua-Science and Technology
Shanghai Fisheries University
Shanghai 200090, China
e-mail: mjiang@shfu.edu.cn

The process of biofilm start-up was studied in aquarium recycling system. Five kinds of filter media were used to establish stable bio-filters which were bio-fiber fill, netlike plastic fill, plastic ball, coral sand and porcelain ring respectively. Waste water from brocaded carp (*Cyprinus carprio*) culturing ponds was used to stimulate the formation of biofilm. The experiments were carried out under 30for 22 days.

Concentrations of TAN, NO2-N and NO3-N in the biofilter with coral sand media changed regularly (Fig.1). Nitrite increased corresponding to the rapidly decreasing of TAN during the first phase of start-up and kept growing up for 6 days after TAN had reached a steadily low level. While nitrate increased during all the experimental period. Same situation happened in biofilters with the other four media while the days needed to reach the turning points of NO2-N were different (Fig.2).

During the start-up period, coral sand media showed the best efficiency of turning TAN to NO2-N and then NO3-N. NO2-N was less than 1.0 mg/L after 12 days. Netlike plastic fill and porcelain ring were the worst. The intervenient were plastic ball and bio-fibre fill.

Other water quality parameters were analyzed and the results were as follows: pH and Total alkalinity decreased rapidly during first several days and then increased slowly to stable values. The biofilter with coral sand media had higher pH and alkalinity than others. Hardness in five filters nearly doubled after 22 days experiment. Hardness in filters with coral sand, bio-fibre fill and porcelain ring were higher than the other two. Phosphate-P was consumed in the course of nitrification. Biofilters with coral sand and porcelain ring had less phosphate-P than others. Different ability of COD Removal was approved in 5 biofilters.

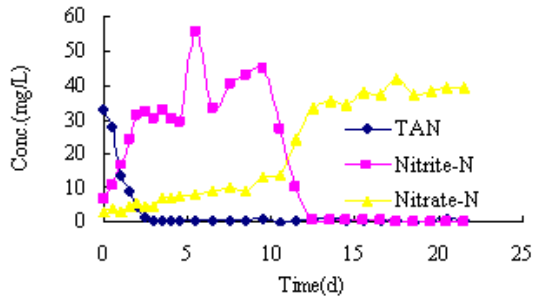


Fig.1 Concentrations of TAN,NO_2-N and NO_3-N in the biofilter with coral sand media

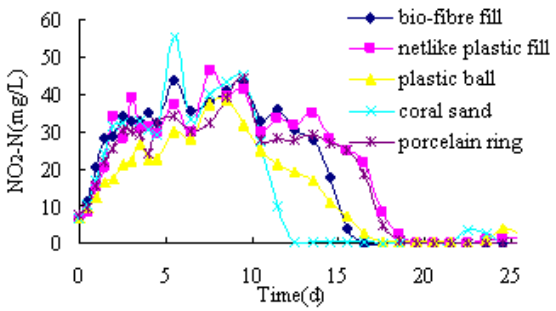


Fig.2 Nitrite-N concentrations in the biofilters with different media

BIOFILM START-UP ON DIFFERENT FILTER MEDIA

Min Jiang, Weiwei Miao, Chunfang Luo

Shanghai Ocean University

20-Apr-17

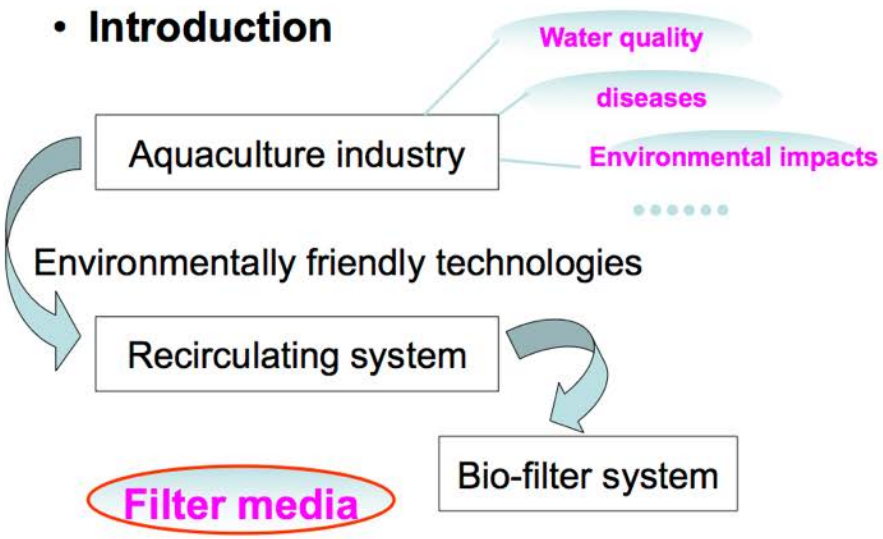
Travel funding for this presentation was provided by

AquaFish Collaborative Research Support Program

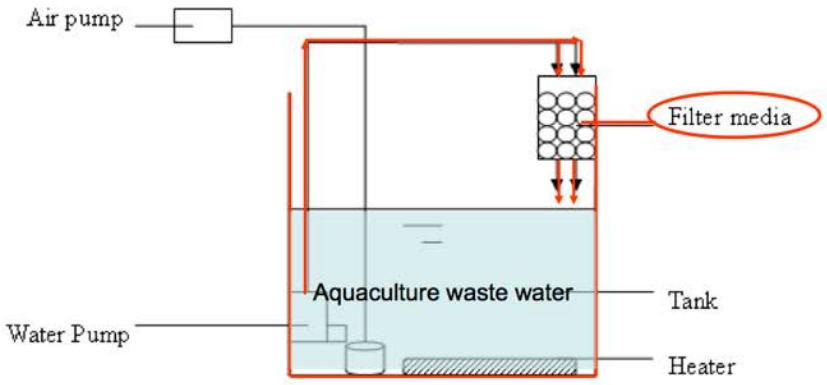
AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

- **Introduction**
- **Materials and methods**
- **Results and discussion**
- **Conclusions**



- **Materials and methods**



Schematic diagram of experimental system

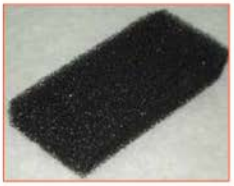
General conditions of the recirculating system

Volume of Waste water: 10L
Volume of Filter: 220mL
Velocity of flow: 63.3L/h
DO: 7.5±0.23mg/L

Water quality of the initial aquaculture waste water for biofilm start-up

pH	8.12
TAN-N (mg/L)	32.95
NO_2-N (mg/L)	6.69
NO_3-N (mg/L)	2.81
PO_4-P (mg/L)	5.60
COD_{Mn} (mg/L)	82.37
Alk (mmol/L)	2.62
H_T (mmol/L)	4.92

Bio-fiber fill



Netlike plastic fill

plastic ball

coral sand

Five kinds of filter media

porcelain ring

During the first 108h, water samples for quality analyses were collected from the tank every 12h. (8:30 & 20:30)

After that, samples were collected every 24h. (8:30)

The whole experiment lasted for 22days.

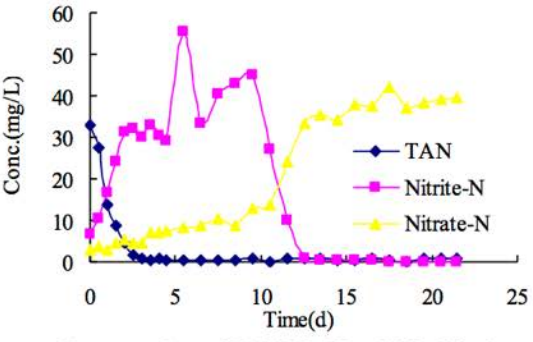
pH
TAN-N
NO_2-N
NO_3-N
PO_4-P
COD_{Mn}
Alk
H_T

Environmental quality standards for surface water

GB 3838—2002

People's Republic of China

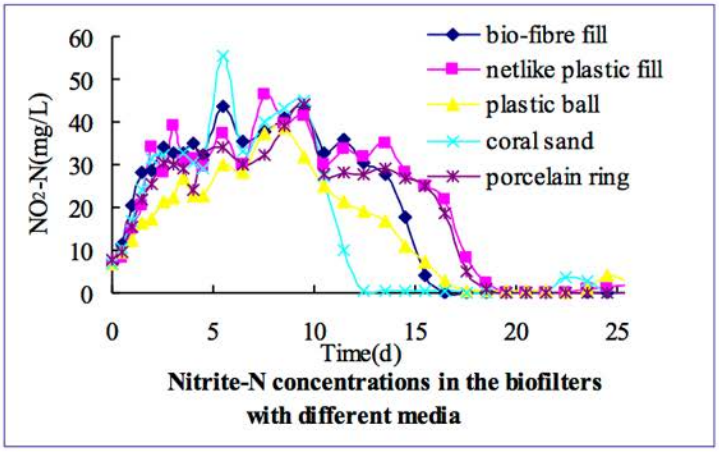
• Results and discussion



Concentrations of TAN,NO_2-N and NO_3-N in the biofilter with coral sand media

Nitrite increased corresponding to the rapidly decreasing of TAN during the first phase of start-up and kept growing up for 6 days after TAN had reached a steadily low level. While nitrate increased during all the experimental period.

Concentrations of TAN, NO_2-N and NO_3-N in the biofilter with coral sand media changed regularly.



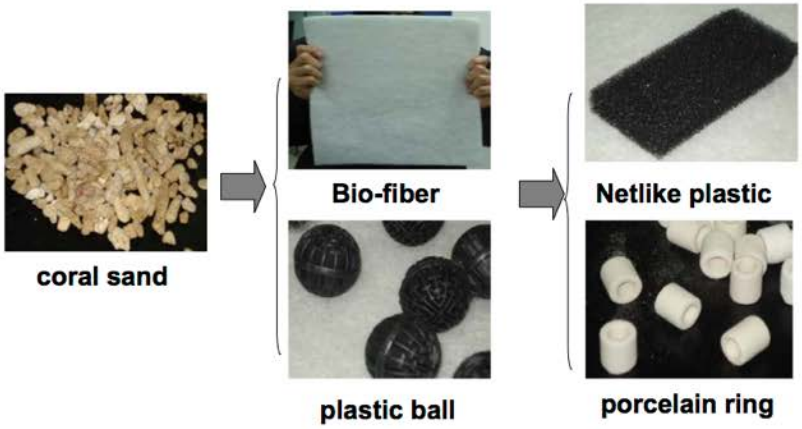
Nitrite-N concentrations in the biofilters with different media

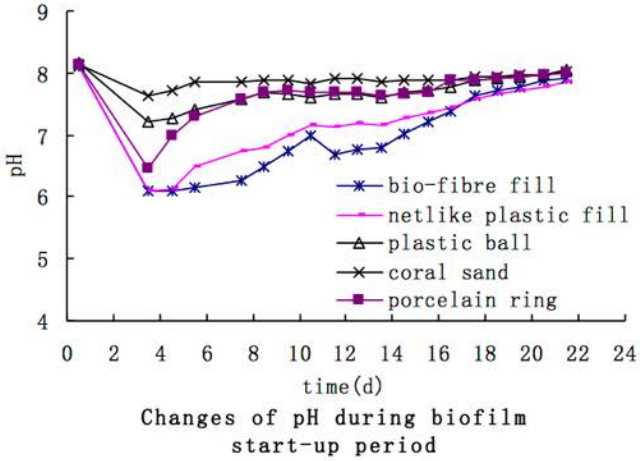
Same situation happened in biofilters with the other four media while the days needed to reach the stable low concentration of NO_2-N were different.

During the start-up period, coral sand media showed the best efficiency of turning TAN to NO_2-N and then NO_3-N. NO_2-N was less than 1.0 mg/L after 12 days.

Netlike plastic fill and porcelain ring were the worst.

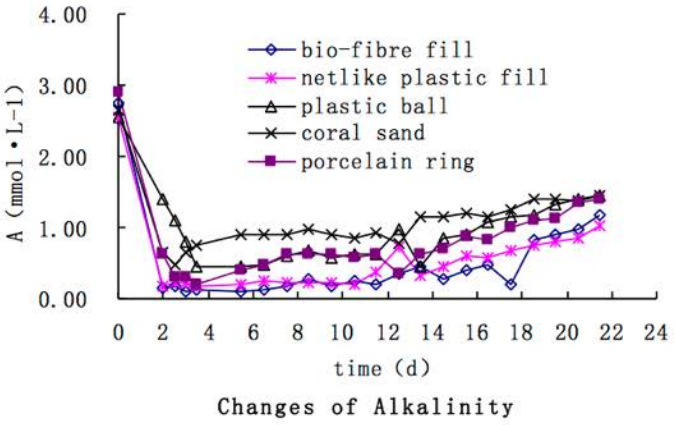
The intervenient were plastic ball and bio-fibre fill.





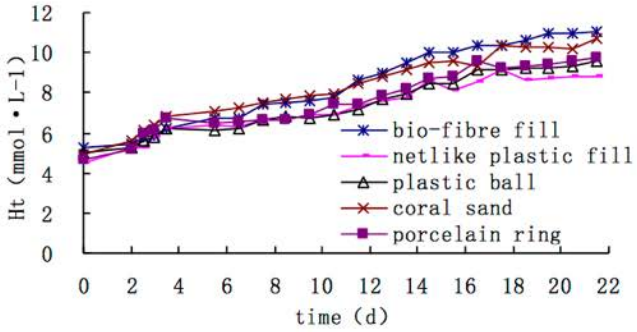
Changes of pH during biofilm start-up period

pH and Total alkalinity decreased rapidly during first several days and then increased slowly to stable values. The biofilter with coral sand media had higher pH and alkalinity than others.



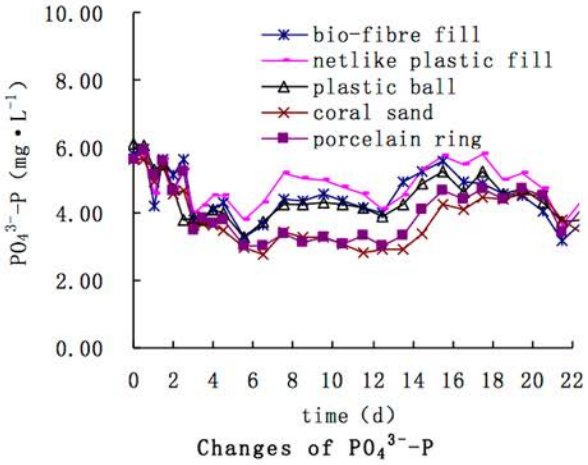
Changes of Alkalinity

pH and Total alkalinity decreased rapidly during first several days and then increased slowly to stable values. The biofilter with coral sand media had higher pH and alkalinity than others.



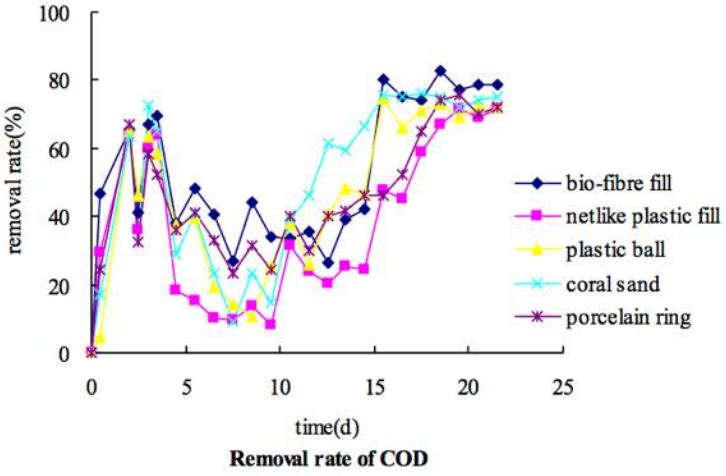
Changes of Hardness

Hardness in five filters nearly doubled after 22 days. Hardness in filters with coral sand, bio-fibre fill and porcelain ring were higher than the other two.



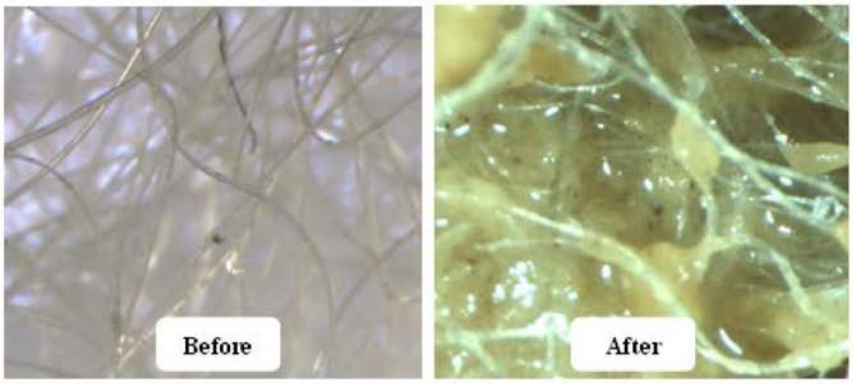
Changes of PO_4^{3-}-P

Phosphate-P was consumed.
Biofilters with coral sand and porcelain ring had less phosphate-P than others.

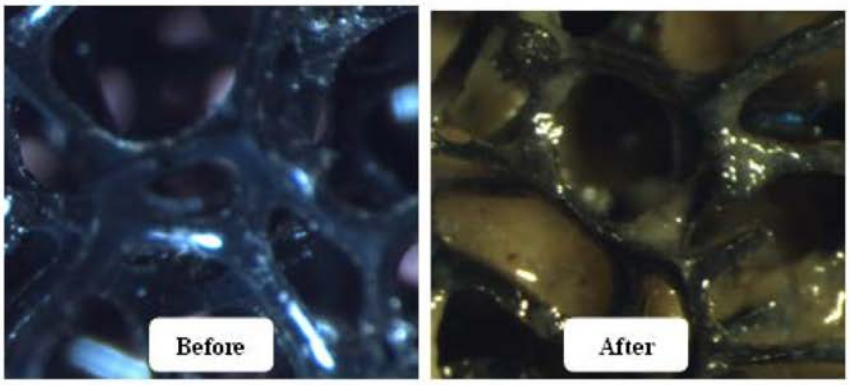


Removal rate of COD

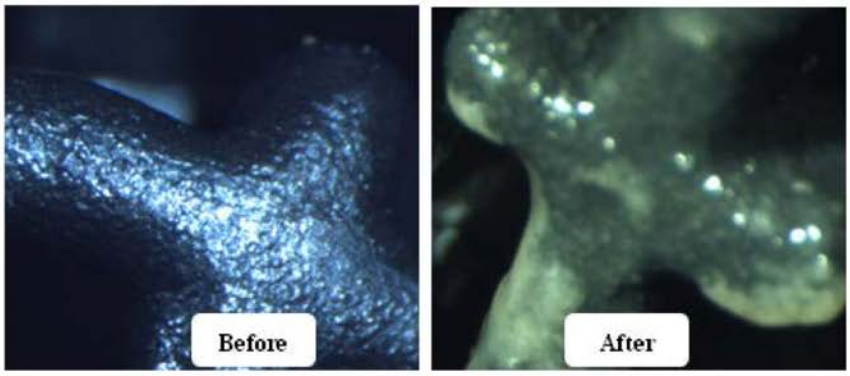
Different ability of COD removal was approved in 5 biofilters.



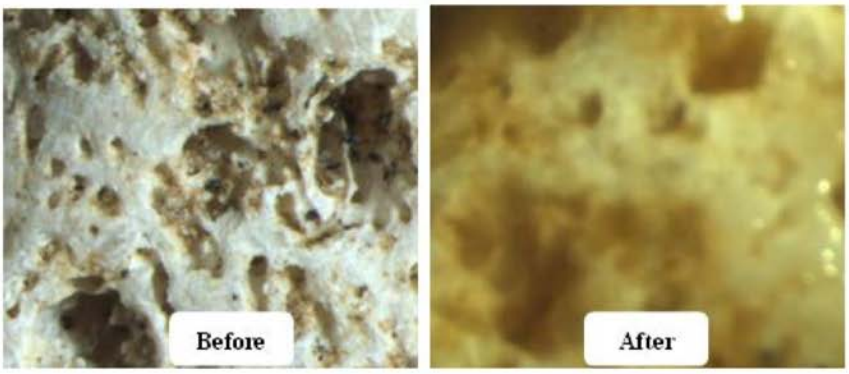
bio-fiber fill



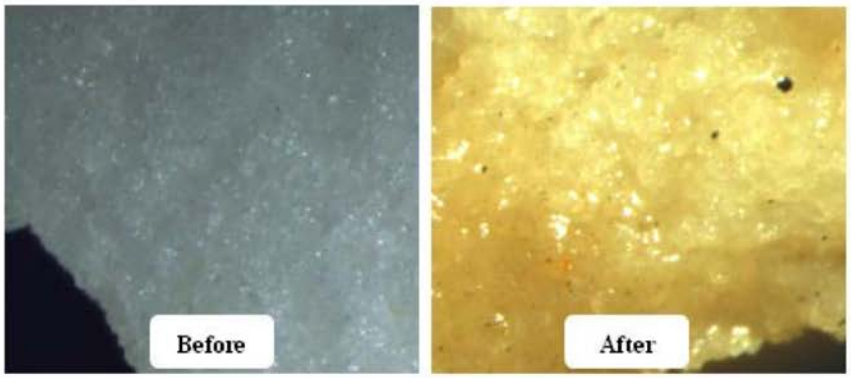
netlike plastic fill



plastic ball



coral sand



porcelain ring

- **conclusions**

Change of water quality: regularity

Biofilm start-up: coral sand media> plastic ball≈ bio-fibre fill > Netlike ≈ porcelain ring

coral sand

Advantage	Disadvantage
(1)Biofilm forming rapidly (2)Alkalinity and pH buffer (3)Higher consumption of phosphate (4)Relatively higher COD removal	(1)Increasing hardness (2)Heavy

Thanks for your attention

Microcystins in aquaculture systems – Their endangerment and research progress in detection and elimination methods

Liping Liu*, Taoying Chen and Yi Yang

Key Open Laboratory of Aquatic Genetic Resources Excavation and Utilization of Shanghai City and the Ministry of Education
College of Aqua-Life Science and Technology
Shanghai Fisheries University
334 Jungong Road, Shanghai 200090, China
lp-liu@shfu.edu.cn

Microcystins (MCs) are cyanobacterial (blue-green algal) metabolites found world-wide in freshwater, brackish and marine environments, and are produced by toxic cyanobacterial blooms. Microcystins are considered to be the most common and dangerous group of cyanotoxins. The occurrence of toxic cyanobacterial blooms producing microcystins in aquaculture ponds could represent a risk to the quality of fish flesh to consumers. The eutrophication of water ecosystems normally results in frequent cyanobacterial algal blooms producing microcystins, and as a result aquatic crops from these systems may be contaminated by microcystins and potentially hazardous to human health.

This paper reviewed potential hazards of MC-producing blooms in a variety of aquaculture systems including ponds and lakes as well as the ways of detecting MCs in fishes. In recent years, appearance of complete antigen of MCs and toxin standards together with a series of detecting techniques such as HPLC, ELISA, LC/MS and the protein phosphatase assay have made the detection and quantification of total and individual toxins possible. Removal methods and depuration strategies of MCs from aquaculture systems were also discussed in this paper.

MICROCYSTINS IN AQUACULTURE SYSTEMS THEIR ENDANGERMENT AND RESEARCH PROGRESS IN DETECTION METHODS

Liu Li-ping

Ph.D, Associate professor

Shanghai Ocean University

Travel funding for this presentation was provided by

AquaFish
Collaborative Research Support Program

AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

- The eutrophication of water ecosystems normally results in frequent cyanobacterial algal blooms
- The occurrence of toxic cyanobacterial blooms producing microcystins in aquaculture ponds could represent a risk to the quality of fish flesh to consumers.

- Microcystins (MCs) are cyanobacterial (blue-green algal) metabolites found world-wide in freshwater, brackish and marine environments, and are produced by toxic cyanobacterial blooms.

Microcystins are considered to be the most common and dangerous group of cyanotoxins.

- The presence of mirocystins in water bodies had led to fatalities in wild and domestic animal worldwide, and the toxins have also been associated with episodes of human illness (Kuiper-Goodman et al, 1999).
  - Consumption of contaminated drinking water;
  - Recreational activities such as swimming;
  - The most serious incident of human intoxication occurred in 1996, when the deaths of over 50 patients at a hemodialysis clinic in Brazil were attributed to microcystin, which were later identified in the clinic's water supply (Azevedo et al, 2002)

- A further exposure route may be through the consumption of contaminated foods.
  - The toxin have been shown to accumulate in certain species of freshwater mussel;
  - There is some evidence for the concentration of microcystins in fish;
  - Some edible plants may be present an additional route for exposure to microcystins.

These findings are significant since food crops may be exposed to high level of cyanobacterial toxins in water used for irrigation.

- The toxicity of microcystins is associated with the inhibition of serine/threonine protein phosphatases 1 and 2A, which can lead to hepatocytes necrosis and hemorrhage.
  - Acute hepatotoxicity;
  - Exposure to low concentration of microcystins in drinking water can cause chronic effects in mammals due to their potent tumor promoting activity (such as the increased rates of primary liver cancer in some areas of China have been attributed to the contamination of drinking water with microcystins).

- To minimize the risk to human health through exposure to microcystins, there is a requirement for sensitive and reliable method capable of detection this class of toxin in a wide range of sample matrices.
  - Mouse bioassay;
  - Enzyme-linked immunosorbent assay;
  - The protein phophatase assay;
  - Reversed-phase high performance liquid chromatographic methods combined with ultra-violet(UV) detection;
  - Mass spectrometry

- Identification of microsystins using analytical techniques
- Biological screening methods for microcystins

- Identification of microsystins using analytical techniques

  The influenced factors:

  - the method used to extract the toxins from sample

    5% acetic acid;
    methanol;
    acidified methanol;
    butanol/ methanol/water (5:20:75);
    aqueous methanol (50-80%).

  - another important factor is the requirement for sample concentration and clean-up process

- Identification of microsystins using analytical techniques

  Liquid chromatographic methods

- Biological screening methods for microcystins
  - Whole organism bioassays

    Mouse bioassay: lacks sensitivity and specificity, and has suffered from increasing public opposition to the use of animals in toxicity testing

    Several invertebrates (including *Daphnia* spp, *Drosophila melanogaster*, and *mosquitoe larvae*), none has been fully validated for use in routine monitoring.

  - Biochemical assays
  - Immunological assays
  - Artificial receptors

Table 1 Comparison of biological detection methods for microcystins

Method	Sensitivity (MCYST-LR)	Specificity for MCYSTS	Cross reactivity	Cost	Comments
Mouse bioassay	LD_{50}: 25-150µg/kg	Non-specific	All microcystins	M	• Requires animal license • Being phased out in most coutries
Brine shrimp assay	LD_{50}: 5-10µg/L	Non-specific	All microcystins	L	• Inter-laboratory variation • May be affected by sample matrix interferences
Protein phosphata-se	Radiometric: 0.1µg/L Colorimetric: 0.25-2.5µg/L Fluorometri: 0.1µg/L	Non-specific	All microcystins	M-H	• Radiometric assay require specialized facilities • Unable to distinguish between PPase inhibitors • Purified enzyme can be expensive
Polyclonal antibodies	Anti-MCYST-LR:2.5µg/L Anti-Adda: 0.6µg/L	Specific	Variable Below 1µg/L for tested variants	M-H	• Dependent on laboratory animals • Difficult to maintain a reproducible source

Table 1 Comparison of biological detection methods for microcystins

Method	Sensitivity (MCYST-LR)	Specificity for MCYSTS	Cross reactivity	Cost	Comments
Monoclonal antibodies	Anti-MCYST-LR:0.1µg/L Anti-MCYST-LR:0.06µg/L (4-arginine specific)	Very specific	Below 1µg/L for tested variants Only detects 4-arginine	H	• Cross-reactivity depends on conjugation method • Hybridoma techniques are labor intensive
Recombinant antibody fragments	4µg/L	Specific	Variable	L	• Requires facilities for bacterial expression • Sensitivity/cross reactivity can be modified
Molecularly imprinted polymers	Approx: 0.2µg/L	Very specific	Specific for microcystin-LR	L	• Different polymers required for each variant • Very stable; suitable for biosensor format

Our goals:

- Determine the content of microcystins in aquaculture ponds/tanks at different levels of intensification and different culture environments;
- Evaluate body burdens of microcystins in the flesh of tilapia cultured under different systems
- Develop possible depuration strategies to eliminate microcystins from the flesh of cultured tilapias.

- Thanks for your attention.

Use of fresh bull and hog testis in sex reversal of Nile tilapia fry

Daniel Meyer*, Marco Guevara, Willie Chan and Claudio Castillo

Escuela Agricola Panamericana
P.O. Box 93
Tegucigalpa, Honduras
dmeyer@zamorano.edu

An important criticism of modern tilapia production technology is the use of a synthetic hormone in the sex reversal procedure. We tested fresh ground bull and hog testis as natural sources of testosterone in the sex reversal of Nile tilapia fry.

Fresh testis were manually cut into small pieces and then minced in an electric meat grinder. Eggs were removed from the mouth cavities of females and artificially incubated. Nile tilapia fry of approximately 8-10 mm total length were stocked 50 in each of twelve 20 L plastic buckets placed inside of our wet lab. The water in each bucket was continuously aerated with a 10 cm long silica diffuser connected to a 2.5 HP blower and was exchanged (100%) every third day. The experiment consisted of three treatments (bull testis, hog testis and meat meal = control) with four replicates each (buckets). Any dead fry were removed daily. After 35 days of feeding with the testis, all fry were placed in cages made of 3 mm mesh plastic netting and fed a 32% CP tilapia diet for 36 days. The cages were placed in a tank with green water inside a greenhouse. All fish were then sacrificed and dissected for microscopic examination of their gonads.

Laboratory analysis showed a concentration of 18.8 and 20.4 ppm of testosterone in fresh bull and hog testis, respectively. Overall survival of fry was low (40%) during the 35-day treatment period. Fish survival was > 90% during the growth phase. The treatment with bull and ram testis resulted in 87 and 83% male fish, respectively, significantly greater than the 58% of males in the control group.

Use of fresh bull and hog testis in sex reversal of Nile tilapia fry

Daniel Meyer, Marco Guevara
Willie Chan and Claudio Castillo

Funding for this research was provided by the

Aquaculture Collaborative Research Support Program

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00 and by participating institutions.

Location:

Aquaculture Station at the Escuela Agrícola Panamericana, Honduras

Fish

- Fertile eggs collected from incubating females
- Artificial incubation

Fish

- Fry (± 8 mm) were treated in 20 L plastic buckets in lab
- Continuous aeration, daily cleaning and water exchange
- After 36 days fry transferred to cylindrical net enclosures (3 mm mesh) located in a greenhouse for ongrowing to > 50 mm

Preparation of meat products

- fresh testis cut and processed with grinder
- salt added
- dried at 32° C
- stored frozen
- purchased meat meal from local supplier

Water quality

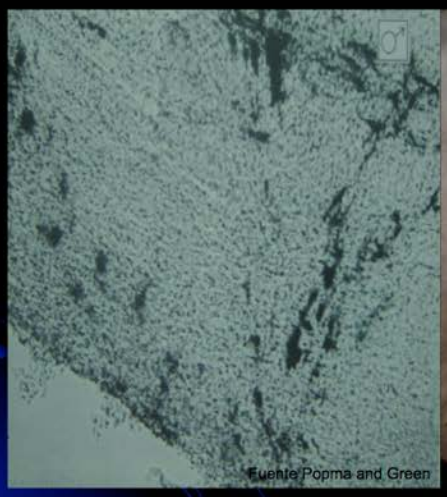
- Dissolved oxygen and water temp a.m. and p.m.
- Experimental units cleaned with syphon every other day

Sex determination thru microscopy

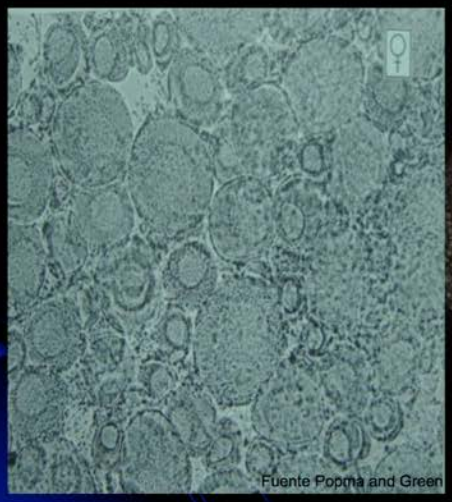
- Fry grown to a total length ± 50 mm
- Gonads removed and observed 50X

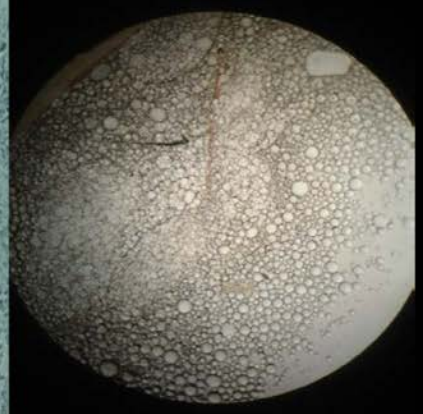
Sex determination via microscopy

Male fry = No presence of oocytes, granular texture of the gonad



Female fry = oocytes observed at varios stages of development





Experimental design:

- Three treatments:
  - bull testis
  - hog testis
  - meat meal
- Four repetitions of each
- Chi^2 for evaluating treatment effectiveness
- ANOVA with survival results
- Statistical Analysis System (SAS® 2003)

Results and discussion

Water quality: dissolved oxygen

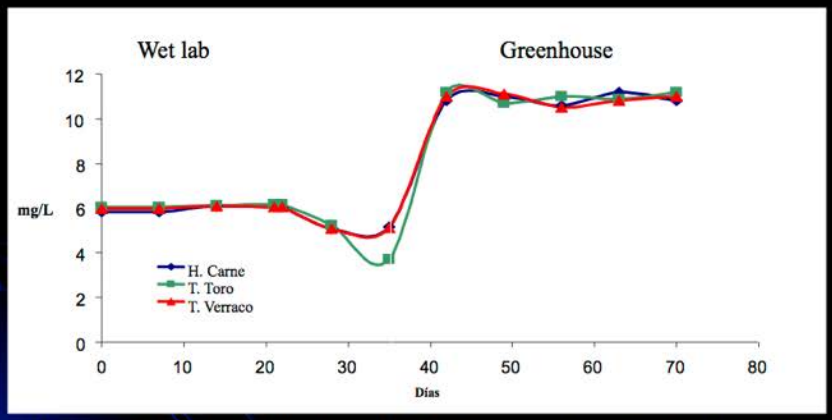


Figure 1. Average daily dissolved oxygen concentration, Zamorano, Honduras. Water in all containers was aerated continuously.

Water quality: temperature

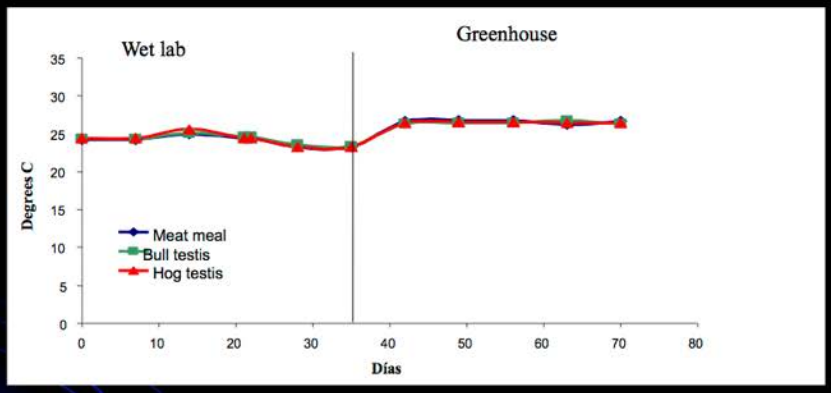


Figure 2. Average daily water temperature for 20 L buckets and net enclosures during a 71-day feeding trial, Zamorano, Honduras.

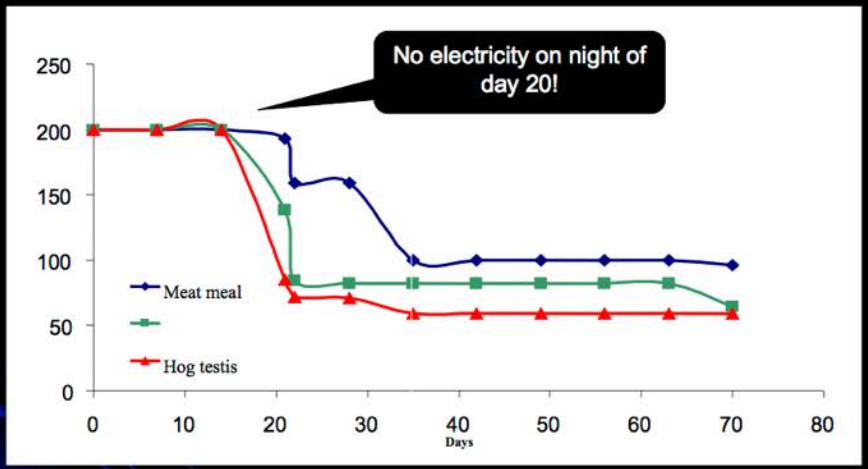


Figure 3. Number of fry surviving based on original 200 fish for each treatment.

Fry survival by environment

Culture environment	Culture days	Initial population	Final population	% survival
Wet lab (20 L buckets)	1 thru 36	600	241	40.2%
Greenhouse (net enclosures)	36 thru 71	241	219	90.9%

Overall fry survival for 71-day long experiment was 36.5%.

Fry survival by environment

- Low DO detected after electrical black-out (on day 20 of experiment)
- 100% meat based feeding regime (tilapia is primarily an herbivore)
- Small initial size of fish (mortality observed 20 days after stocking)
- Low survival rates reported in previous studies
  - Haylor, G. S.; A. B. Pascual. 1991. Effect of using ram testis in a fry diet for Oreochromis niloticus (L.) on growth, survival and resultant phenotypic sex ratio. Aquaculture and Fisheries Management 22: 265-268.
  - Phelps, R.P. 2001. Sex Reversal: the directed control of gonodal development un tilapia, pages 35-60. En D.E.Meyer (editor). Memoria 6to simposio Centroamericano de Acuacultura. Asociación Nacional de Acuicultores de Honduras y PD/A CRSP, Honduras.

Fry survival by treatment

Treatment	Initial number fry/repetition	Final number fry/repetition	% fry survival
Meat meal	50	24	48[a]
Bull testis	50	16	32[b]
Hog testis	50	15	30[b]

Unable to explain observed difference in survival by treatment!

Growth of the fry during 71 days

	Average body weight (g)		Average body length (cm)	
Treatment	Initial	Final	Initial	Final
Meat meal	0.01	3.48 ± 0.60	0.8	4.8 ± 0.5
Bull testis	0.01	4.13 ± 0.48	0.8	5.7 ± 0.3
Hog testis	0.01	3.88 ± 0.16	0.8	5.5 ± 0.2

Percent of male fish

Treatment	% male fish
Meat meal	58 ± 4.9
Bull testis	87 ± 5.6
Hog testis	83 ± 7.7

Chemical analysis of testis

Processed meat product	Testosterone level
Fresh ground bull testis	18.8 ppm
Fresh ground hog testis	20.4 ppm

Testosterone levels in dried testis would be > 60 ppm.

Conclusions

- Bull and hog testis were effective to produce mostly male fry of tilapia beginning sex reversal at a small total body length (± 8 mm)
- Low survival of fry attributed to water quality problems and possible nutritional deficiency.
- In green water survial of fry was > 90%!

Recomendations

- Attempt to use testis for sex reversal in outdoor tanks with green water.
- Use freeze-drying of testis for better preparation and stroage
- Develop a methodology for extracting hormone from fresh testis

Fin

Effects of stocking density to growth and survival rate of giant snakehead (*Channa micropeltes*) larvae

Nguyen Thanh Phyong and Bui Minh Tam

College of Aquaculture and Fisheries
Cantho University
Cantho City, Vietnam

Giant snakehead (*Channa micropeltes*) has been cultured commonly in the Mekong delta. This species is carnivorous fish so the cannibalism is very high. During larval stage, the bigger size always bite and eat small size. These research focus on the effects of the density to growth and survival rate of the fry. In the first 30 days, the weight and survival rate of fish in concrete tanks at the stocking densities of 600, 900 and 1200 larval/m2 were not differed significantly at p>0,05. Similarly, the same stocking densities in hapa did not give significantly differences at p>0,05. From 31-60 days, the weight and survival rate of fish rearing at 600, 900 and 1200 larval/m2 in concrete tanks were not significant different at p>0,05.

The morphology, histology and mucin histochemistry of the digestive tract in yellow catfish, *Pelteobagrus fulvidraco*

Cao xiaojuan[1], Wang weimin[1], Yi Yang[2], Li Su[1]

[1]College of Fishery
Key Laboratory of Agricultural Animal Genetics
Breeding and Reproduction of Ministry of Education
Huazhong Agricultural University
Wuhan, 430070, China
E-mail: wangwm@mail.hzau.edu.cn

[2]College of Aqua-Life Science and Technology
Shanghai Fisheries University
Shanghai, 200090, China

Pelteobagrus fulvidraco is an important small freshwater commercial fish in China. It has a promising market potential in Japan, South Korea, East and South Asia. Due to its high market value, the demand of this species has increased rapidly in recent years. However, at present no reports can be found in the literature on the structure and histochemistry of the digestive tract in *P. fulvidraco*. Therefore, the purpose of our study is to establish the normal morphological and histological structures of digestive tract in *P. fulvidraco* and to identify the general types and distribution of mucous cells in the digestive tract. The information gathered can offer baseline knowledge for future studies on the digestive tract in *P. fulvidraco*, as well as conduce to better understanding of nutritional physiology and disease prevention.

The alimentary tract was composed of an oral cavity, pharynx, esophagus, U-shaped stomach (including a cardiac, fundic and pyloric portion) and intestine (with a fore, middle and posterior intestine). The estimated mean intestinal coefficient of *P. fulvidraco* was 0.64 0.02. Histologically, the wall of the digestive tract was composed of a mucosa, submucosa, muscularis and serosa. The epithelial lining of the pharynx and esophagus was of the stratified type, whereas that of the stomach and intestine was simple columnar. Two types of goblet cells were observed along the alimentary canal, except in the stomach. A plenty of gastric glands was present in the cardiac and fundic stomach. In the intestine, the number of mucosal folds where well-developed microvilli were observed decreased from foreintestine to posterior intestine. The mucous cells were distributed in whole digestive tract, and mostly existed in epidermis. The majority of the epithelial mucous cells contained neutral mucins although there were small amounts of two mixtures of acid and neutral mucins. The statistic results showed that the mucous cells had different densities in different parts of the digestive tract.

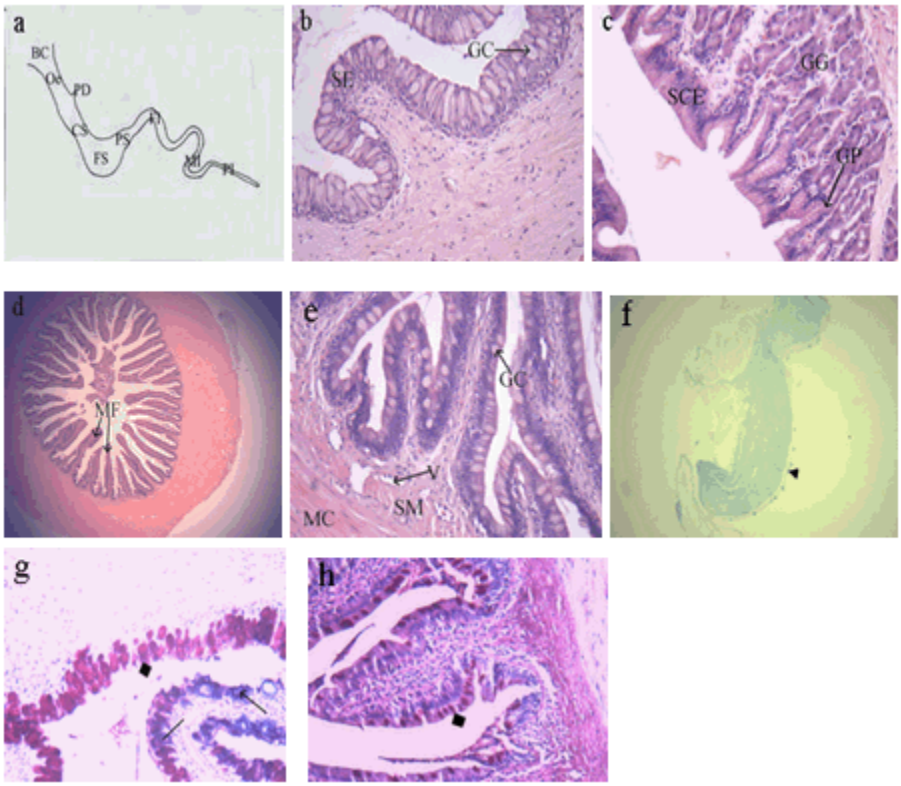


Fig a. Schematic drawing of the structure of the digestive tract in P. fulvidraco; Fig b. Oesophagus. HE. ×400; Fig c. Cardiac stomach. HE. ×400; Fig d. Foreintestine. HE. ×40; Fig e. Middle intestine. HE. ×400; Fig f. Oral cavity. AB (pH1.0). ×100; Fig g. Oesophagus. AB-PAS (AB pH2.5). ×400; Fig h. Foreintestine. AB-PAS (AB pH2.5). ×400.

The morphology, histology and mucin histochemistry of the digestive tract in yellow catfish, *Pelteobagrus fulvidraco*

教育部直属国家211工程重点大学

華中農業大學

HUAZHONG AGRICULTURAL UNIVERSITY

Cao Xiaojun Wang Weimin Yang Yi
College of Fishery, Huazhong Agricultural University, 430070 Wuhan, PR China
crescent1985@yahoo.cn

Research funding for this presentation was provided by

AquaFish Collaborative Research Support Program

AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

Contents

- Introduction of *Pelteobagrus fulvidraco*
- Purpose and significance of this study
- Morphological and histological structures of digestive tract in *P. fulvidraco*
- Mucous cells in the digestive tract of *P. fulvidraco*
- Conclusion

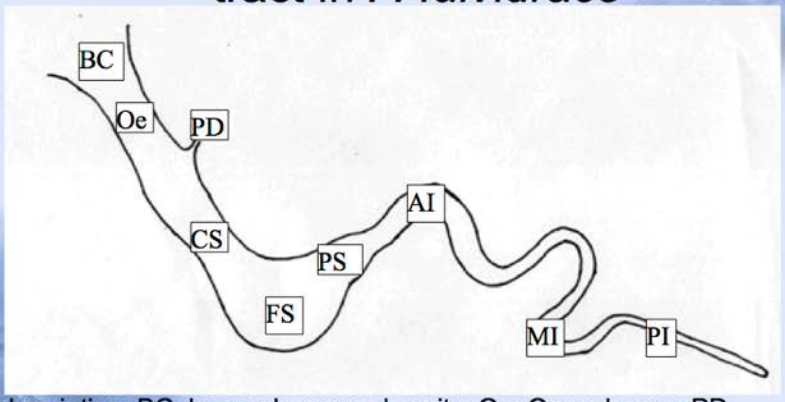
Pelteobagrus fulvidraco

- an very important small freshwater commercial fish in China.
- It has a promising market potential in South Korea, Japan, East and South Asia.
- In recent years, the cultivation of this species has developed rapidly for its high market value

Purpose and significance of this study

- To establish the normal morphological and histological structures of digestive tract in *P. fulvidraco*
- To identify the general types and distribution of mucous cells in the digestive tract of *P. fulvidraco*
- Knowledge of structure and histochemistry of fish digestive tract is essential to the understanding of digestive physiology and pathological alterations

Morphological structure of the digestive tract in *P. fulvidraco*



Abbreviation: BC: buccopharyngeal cavity; Oe: Oesophagus; PD: Pneumatic duct; CS: cardiac stomach; FS: fundic stomach; PS: pyloric stomach; AI: anterior intestine; MI: middle intestine; PI: posterior intestine
The estimated mean intestinal coefficient (IC) was 0.64±0.02

The histology of digestive tract of *P.fulvidraco*

- Wall of the digestive tract was composed of the mucosa, submucosa, muscularis and serosa.
- The epithelial lining of pharynx and oesophagus was of stratified type, whereas that of the stomach and intestine was simple columnar.
- A large number of goblet cells (two types) were observed along the alimentary canal, except in the stomach.

The histology of digestive tract of *P.fulvidraco*

- A plenty of gastric glands was present in the cardiac and fundic stomach.
- Abundant collagenous fibers were found in the lamina propria and submucosa of stomach. Figure 2. The histological structure of digestive tract o...
- The muscularis of pyloric stomach was thicker than any other parts of the digestive tract.幻灯片 17 Histological features of digestive tract in P. fulvidraco...
- In the intestine, the number and height of mucosal folds decreased from anterior to posterior intestine.

The mucous cells of digestive tract of *P. fulvidraco*

- The mucous cells were distributed in whole digestive tract, and mostly existed in epidermis.
- The majority of the epithelial mucous cells contained neutral mucins although there were small amounts of two mixtures of acid and neutral mucins.
- In the stomach, anterior and middle intestine, only neutral mucins were observed.

The mucous cells of digestive tract of *P. fulvidraco*

- The sulphated mucins content was more than carbonic mucins' in the oral cavity and pharynx, whereas it was opposite in the oesophagus and posterior intestine.
- The number of mucous cells in the stomach was the highest.
- The quantity of mucous cells varied in different parts of the digestive tract. Figure 3. The mucous cells of digestive tract of P. fulvi...

conclusions

- As an omnivorous fish, the digestive tract of *P. fulvidraco* consisted of an oral cavity, pharynx, oesophagus, U-shaped stomach and intestine.
- Wall of the digestive tract of *P. fulvidraco* was composed of the mucosa, submucosa, muscularis and serosa.
- The stomach and intestine could be subdivided from the perspective of the morphological and histological structure.

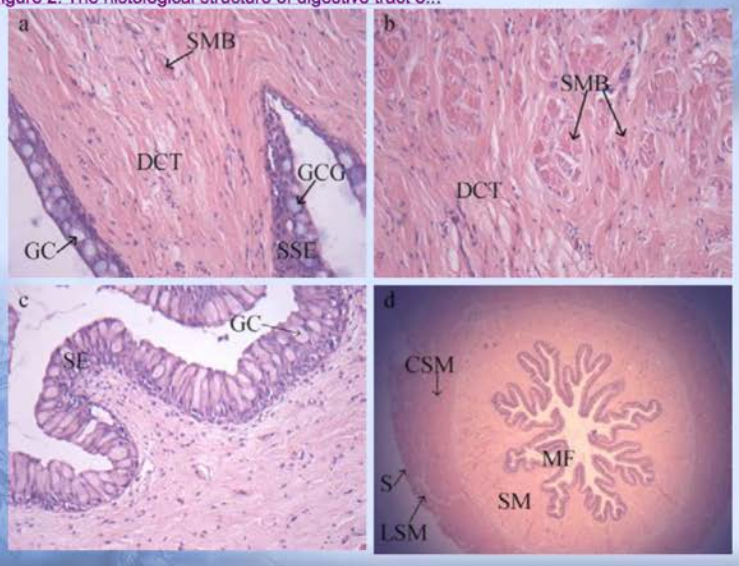
conclusions

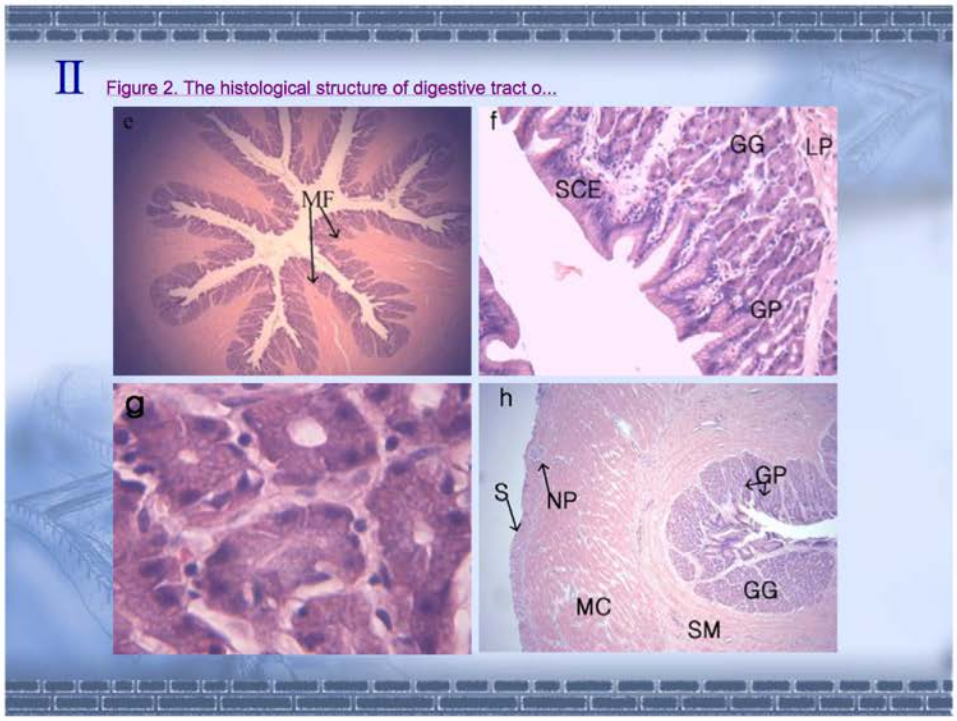
- The mucous cells were distributed in whole digestive tract.
- The majority of mucins in the digestive tract were neutral.
- All of these characteristics were suitable for its feeding habits.

Anytime, you are welcome to our country and our university

I

Figure 2. The histological structure of digestive tract o...




II
Figure 2. The histological structure of digestive tract o...
e
MF
f
GG
LP
SCE
GP
g
h
S
NP
GP
GG
MC
SM

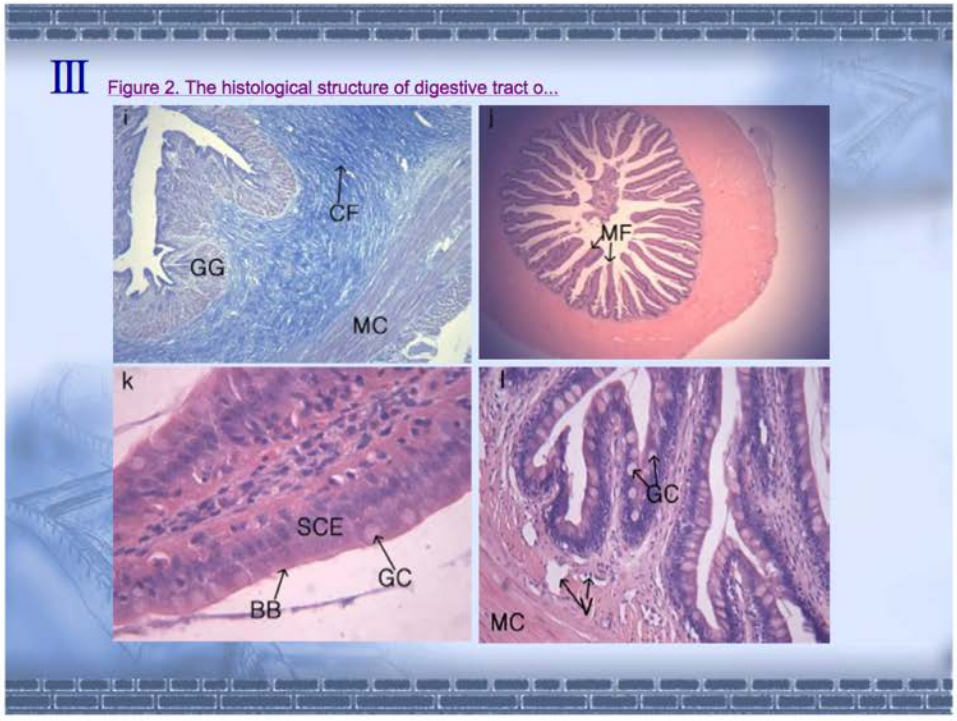

III
Figure 2. The histological structure of digestive tract o...
i
CF
GG
MC
l
MF
k
SCE
GC
BB
l
GC
V
MC

Figure 2. The histological structure of digestive tract of *P. fulvidraco* The histology of digestive tract of P.fulvidraco

- (a) Pharynx. H.E. (×400); (b) Pharynx. H.E. (×400); (c) Oesophagus. H.E. (×400); (d) Oesophagus. H.E. (×40); Ⅰ
- (e) Cardiac stomach. H.E. (×40); (f) Cardiac stomach. H.E. (×400); (g) Gastric glands in cardiac stomach. H.E. (×1000); (h) Fundic stomach. H.E. (×100); Ⅱ
- (i) Cardiac stomach. Masson trichrome stain. (×100); (j) Panoramic anterior intestine. H.E. (×40); (k) Anterior intestine. H.E. (×1000); (l) Middle intestine. H.E. (×400). Ⅲ

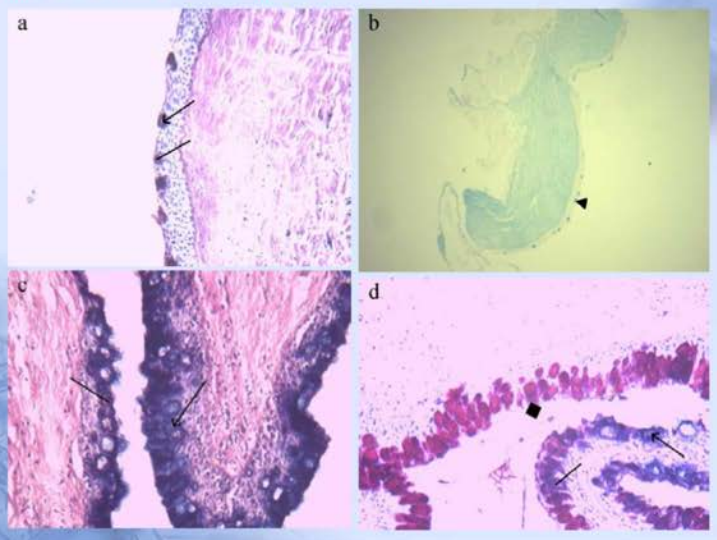
Histological features of digestive tract in *P. fulvidraco* (X±SD) (一) The histology of digestive tract of P.fulvidraco

Items	Ph	Oe	CS	FS
Number of mucosal folds (fold number/ transverse section)	23.47 ± 1.09	11.80 ± 0.98	10.73 ± 0.77	11.53 ± 1.09
Height of mucosal folds (μm)	453.25 ± 67.50	525.01 ± 120.15	1108.05 ± 248.82	905.34 ± 144.76
Width of mucosal folds (μm)	311.90 ± 72.98	492.23 ± 151.60	903.82 ± 130.00	983.55 ± 123.06
Thickness of muscularis (μm)	452.57 ± 32.91	501.46 ± 28.66	371.55 ± 25.67	506.10 ± 26.37
Thickness of CM (μm)	452.57 ± 32.91	383.01 ± 47.22	284.17 ± 33.69	456.02 ± 63.00
Thickness of LM (μm)	–	120.97 ± 22.21	87.30 ± 17.61	50.09 ± 3.36
Number of goblet cell (cell number/100μm)	11.80 ± 4.11	11.67 ± 2.06	–	–

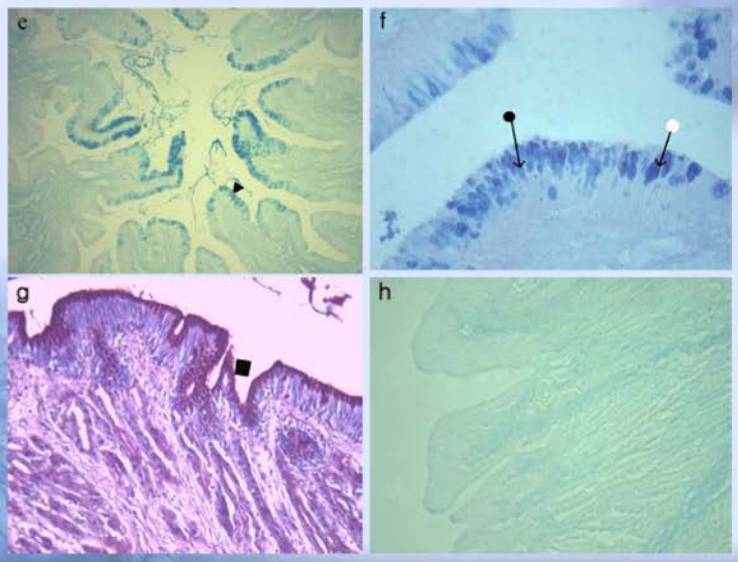
Histological features of digestive tract in *P. fulvidraco* (X±SD) (二)The histology of digestive tract of P.fulvidraco

Items	PS	AI	MI	PI
Number of mucosal folds (fold number/ transverse section)	11.87 ± 0.81	39.47 ± 1.15	27.53 ± 0.72	12.27 ± 0.77
Height of mucosal folds (μm)	853.60 ± 255.60	607.76 ± 93.11	353.76 ± 71.67	348.25 ± 74.75
Width of mucosal folds (μm)	854.05 ± 221.96	118.55 ± 15.11	89.86 ± 8.26	183.76 ± 31.34
Thickness of muscularis (μm)	1208.92 ± 55.66	529.37 ± 66.44	383.11 ± 16.46	83.13 ± 2.45
Thickness of CM (μm)	1015.59 ± 175.47	506.21 ± 66.44	318.40 ± 44.69	49.27 ± 4.05
Thickness of LM (μm)	193.46 ± 44.50	23.71 ± 2.49	64.79 ± 8.26	34.02 ± 2.15
Number of goblet cell (cell number/100μm)	–	8.07 ± 2.09	7.53 ± 1.13	6.60 ± 1.40

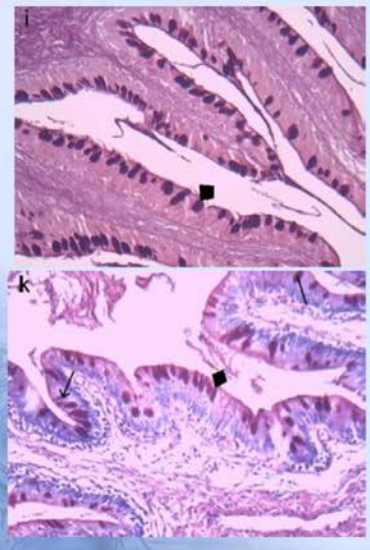
i Figure 3. The mucous cells of digestive tract of P. fulvi...



ii Figure 3. The mucous cells of digestive tract of P. fulvi...



iii Figure 3. The mucous cells of digestive tract of P. fulvi...



Note: ◆(neutral mucous cell);▲(the mucous cell contained acid mucins); ○(the mucous cell contained sulphated mucins);●(the mucous cell contained carbonic mucins);| (mixed mucous cell stained with reddish purple color); ↑ (mixed mucous cell stained with bluish purple color)

Figure 3. The mucous cells of digestive tract of *P. fulvidraco* The mucous cells of digestive tract of P. fulvidraco

- (a) Oral cavity stained with AB-PAS (AB pH1.0). (×400); (b) Oral cavity stained with AB (pH1.0). (×100); (c) Pharynx cavity stained with AB-PAS (AB pH2.5). (×400); (d) Oesophagus stained with AB-PAS (AB pH2.5). (×400); i
- (e) Oesophagus stained with AB (pH2.5). (×100); (f) Oesophagus stained with AF-AB (AB pH2.5). (×400); (g) Cardiac stomach stained with AB-PAS (AB pH2.5). (×400); (h) Cardiac stomach stained with AB (pH2.5). (×400); ii
- (i) Middle intestine stained with AB-PAS (AB pH2.5). (×400); (j) Middle intestine stained with AB (pH1.0). (×100); (k) Posterior intestine stained with AB-PAS (AB pH2.5). (×400). iii

Effects on growth and survival of loach (*Misgurnus anguillicaudatus*) larvae when co-fed on live and microparticle diets

Youji Wang[1], Weimin Wang[1], Yi Yang[2]

[1]College of Fishery
Key Laboratory of Agricultural Animal Genetics
Breeding and Reproduction of Ministry of Education
Huazhong Agricultural University
Wuhan, 430070, China
E-mail: wangwm@mail.hzau.edu.cn

[2]College of Aqua-Life Science and Technology
Shanghai Fisheries University
Shanghai, 200090, China

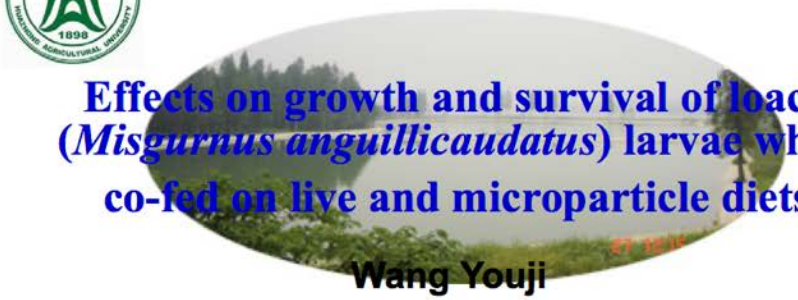
The effectiveness of co-feeding with live and microparticle diets on weaning performance of *Misgurnus anguillicaudatus* was described. Dry weight, total length, length and weight-specific growth rate (SGR) and survivals were monitored from the 4 day post-hatching (dph) to 60 dph in following diet regimes: microparticle diets (A), live daphnia (B), enriched daphnia (C), half microparticle diets plus half live daphnia (D) and half microparticle diets plus half enriched daphnia (E). The highest survival achieved on 60 dph was in treatment E (67.15% 4.2%). The SGR (W and L) of fish in treatment B and C were similar but lower than in treatment A, D and E respectively. However, dry weight (mg) and total length (mm) in treatment A were significantly lower than in treatment D and E. It is suggested that weaning of loach from early development would appear to be feasible by co-feeding and that larval co-feeding improves the growth and the survival.

Loach (*Misgurnus anguillicaudatus*) is an autochthonous fish of Asia, there is a need for higher production of it, especially in Japan and Korea. Expanded and consistent requirement of loach production will therefore ultimately require development of all kinds of culture systems. The important aspect of loach culture is larval rearing. Appropriate first feeding diet is the major problem for successful larval loach rearing. This study aims to evaluate the effects of different treatments using live and microparticle diets on the growth, survival and weaning of *M. anguillicaudatus* during the early phase of larval culture. The information is needed to determine the influence of larval co-feeding on weaning of *M. anguillicaudatus*.

Five different feeding regimes were supplied from 4 to 30 dph. Weaning commenced on day 31 and during the first 7 days both live and inert feeds were fed. The transition from initial regime to only dry feed was performed progressively, with a 12.5% decrease of the daphnia rations per day. From day 38 onwards, in all treatments, only dry feed were supplied. Samplings were performed at day 4, 12, 20 and then every 10 days. The individual dry weight and total length were calculated
.
The results indicate that larvae of *M. anguillicaudatus* had capacity to complete metamorphosis when fed live and dry feed (co-feeding), achieving better growth and survival than larvae fed live feed or dry diets alone (Table 1). That reduces the dependence on live foods, makes weaning easy and would mean a hatchery cost reduction in total live feeds requirement and tank facilities as well as help advance the commercial aquaculture of this species.

	SGR (L, %)			SGR (W, %)			TL(mm)	DW (mg)	SR (%)
	4 - 20	20 - 30	30 - 60	4 - 20	20 - 30	30 - 60	4 - 60	4 - 60	4 - 60
A	6.79±0.5^{a}	2.51±0.3^{a}	1.14±0.2^{a}	21.57±1.7^{a}	9.26±0.9^{a}	8.94±0.7^{a}	29.88± 3.60^{a}	305.95± 21.48^{a}	20.87± 3.9^{a}
B	8.53±0.7^{b}	3.06±0.6^{b}	0.72±0.1^{b}	23.65±2.2^{b}	12.40±1.5^{b}	6.78±1.1^{a}	35.02± 3.15^{b}	370.36± 25.84^{b}	33.44± 5.0^{b}
C	8.68±0.8^{b}	3.05±0.5^{b}	0.76±0.2^{b}	24.13±2.4^{b}	11.96±1.4^{b}	6.86±0.6^{a}	35.18± 3.42^{b}	382.22± 23.78^{b}	45.96± 4.1^{c}
D	8.78±0.5^{b}	2.97±0.4^{b}	1.21±0.3^{a}	23.72±2.5^{b}	11.95±1.1^{b}	9.30±0.9^{b}	42.21± 3.22^{c}	650.98± 27.90^{c}	48.33± 5.5^{c}
E	8.81±0.6^{b}	3.12±0.3^{b}	1.34±0.2^{a}	23.97±2.4^{b}	11.63±1.2^{b}	9.47±0.8^{b}	43.78± 3.18^{c}	670.60± 25.66^{c}	67.15± 4.2^{d}

Table 1 Specific growth rate (SGR, %), Total length (TL, mm), dry weight (DW, mg) and survival rate (SR, %) of different treatments from 4 to 60 dph (different superscript letters within a column indicate significant differences at $p < 0.05$)



Effects on growth and survival of loach (*Misgurnus anguillicaudatus*) larvae when co-fed on live and microparticle diets

Wang Youji

Wang Weimin

Yang Yi

College of Fishery

Huazhong Agricultural University

Wuhan, Hubei, China

Travel funding for this presentation was provided by

AquaFish Collaborative Research Support Program

AquaFish CRSP

USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.

The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

Outline of Talk

- 1.Background information
- 2.Materials and Methods
- 3.Results
- 4.Discussion
- 5.Conclusion
- 6.Future Work

1.Background information

loach culture has been developed in China

1.Background information

All kinds of culture systems of loach

1.Background information

Loach culture in Jiangsu province, China

1.Background information

The value of loach

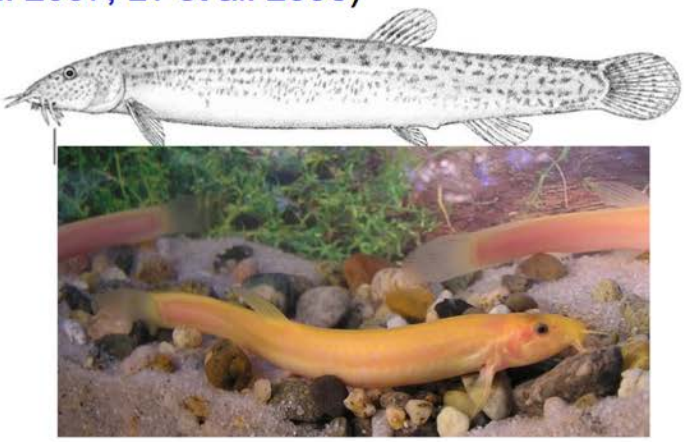
1.Background information

The medical value of loach

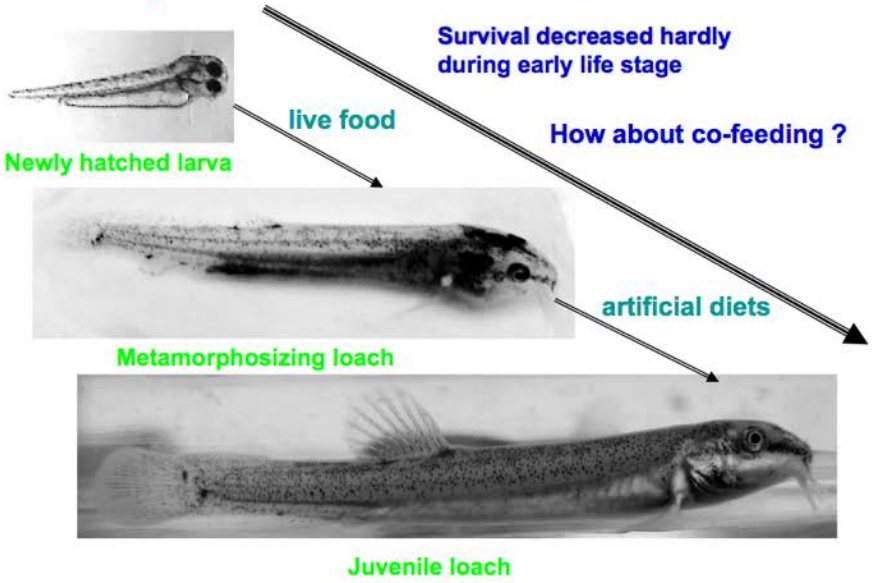
1. A novel antimicrobial peptide from loach: misgurin (Park et al.,1997)
2. A novel oligosaccharide from the mucus of the loach (Qin et al., 2002)
3. A novel polysaccharide from the mucus of the loach (Zhang and Huang,2005)
4. A novel antimicrobial polypeptide from loach (Dong et al.,2003)

1.Background information

An ideal model animal in genetics and toxicology (Khan and Arai 2000; Xie et al. 2003; Shao et al. 2005; Lv et al. 2006; Morishima et al. 2008; Lv et al. 2007; Lv et al. 2008)



However, poor survival has been recorded during weaning from live food to artificial diets



Purpose and significance of this study

- The important aspect of loach culture is larval rearing, providing, enough high quality larval fish is insured before large-scale culture is developed. Appropriate first feeding diet is the major problem for successful larval loach rearing
- The present study aims to evaluate the effects that different treatments of live and microparticle diets were on the growth, survival and weaning of loach during the early phase of larval culture.

2.Materials and methods

- **Fish larvae**

2.Materials and methods

Experimental design

The larvae were reared in a set of 15 fiberglass tanks of 100 L capacity. Each tank was stocked with 5000 larvae with the environmental conditions as follow: dissolved oxygen 8.0 - 10.0 mg L^{-1}, water temperature 23 - 25 °C, pH 7.0 - 7.3.

2. Materials and methods

Regime	Food	Larval age (days)		
		4 --12	13 - 20	21 - 30
A	Algae	0.3	-	-
	Rotifer	10	-	-
	Dry feed	12	16	24
B	Algae	0.3	-	-
	Rotifer	10	-	-
	Daphnia	10	12	18
C	Algae	0.3	-	-
	Rotifer	10	-	-
	Enriched daphnia	10	12	18
D	Algae	0.3	-	-
	Rotifer	10	-	-
	Dry feed	6	8	12
	Daphnia	5	6	9
E	Algae	0.3	-	-
	Rotifer	10	-	-
	Dry feed	6	8	12
	Enriched Daphnia	5	6	9

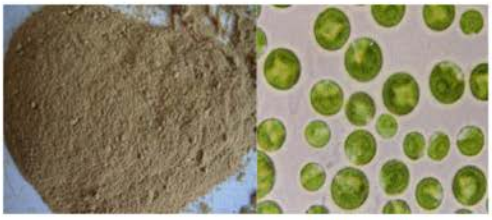
Daily amounts of microalgae (10^6 cells mL^{-1}), rotifers (individual mL^{-1}), daphnia (individual mL^{-1}) and dry feed (mg dry weight mL^{-1}) given to *Misgurnus anguillicaudatus* larvae under five different feeding regimes

Rotifer

Brachionus calyciflorus

Daphnia

Moina micrura



Microparticle diets

Microalgae (*Chlorella* sp.)

2.Materials and methods

2.Materials and methods

Sampling

Samplings were performed at day 4, 12, 20 and then every 10 days. On each sampling day, 15 larvae from each tank were sampled and dried at 70 ºC to constant weight.

The individual dry weight was calculated with an electronic balance. Larvae less than 10 mm TL were measured under a dissecting microscope (×20) by a micrometer, while larger fish were directly measured on a measuring board.

2.Materials and methods

Data and statistical analysis

- The growth rate of larvae was determined using the length and weight - specific growth rate (SGR) equation respectively.
- $SGR=([\ln(Lt) - \ln(L0)] / t) \times 100$ (1)
- $SGR=([\ln(Wt) - \ln(W0)] / t) \times 100$ (2)
- Survival (%) = Number of survivors at the end of the experiment / (Number of larvae at the start – number of larvae sampled) × 100% (3)

3.Results: Growth of weight

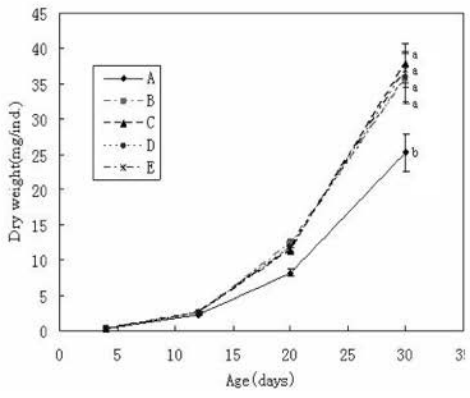


Figure 1 Changes in dry weight (mean ± SD of three replicates) before weaning of loach larvae with different feeding regimes. See Table 1 for details of the feeding regimes. Points sharing a common lowercase letter are not significantly different ($P > 0.05$).

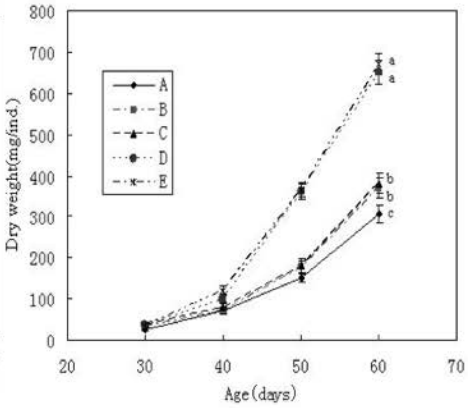


Figure 2 Changes in dry weight after weaning of loach larvae with different feeding regimes. See Table 1 for details of the feeding regimes until day 30. From day 30 to day 60, all fish were fed dry feed. Points sharing a common lowercase letter are not significantly different ($P > 0.05$).

3.Results: Growth of total length

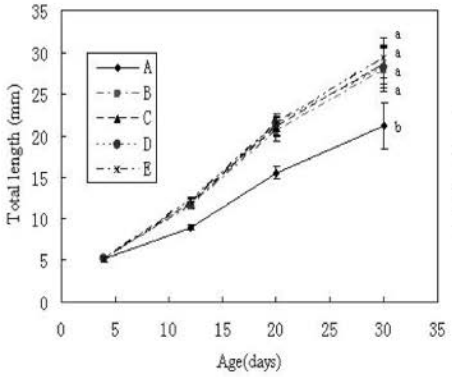


Figure 3 Changes in total length (mean ± SD of three replicates) before weaning of loach larvae with different feeding regimes. See Table 1 for details of the feeding regimes. Points sharing a common lowercase letter are not significantly different ($P > 0.05$).

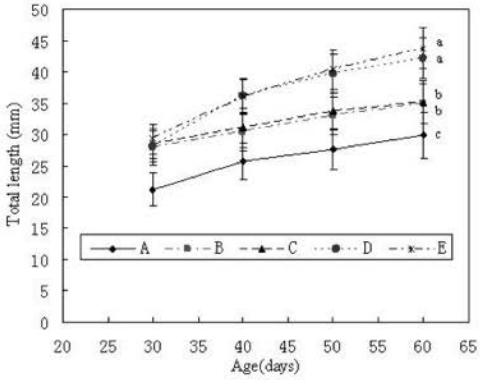


Figure 4 Changes in total length after weaning of loach larvae with different feeding regimes. See Table 1 for details of the feeding regimes until day 30. From day 30 to day 60, all fish were fed dry feed. Points sharing a common lowercase letter are not significantly different ($P > 0.05$).

3.Results

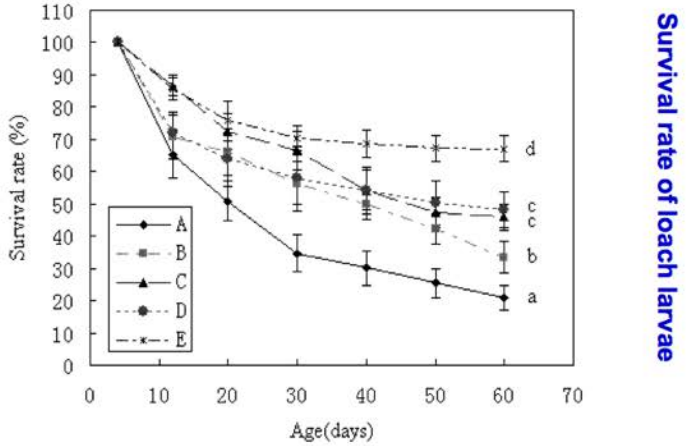
Weight and length - specific growth rate (SGR)

Table 2 Influence of diet on specific growth rate (% SGR) (mean ± SD) before and after weaning in *Misgurnus anguillicaudatus*

Feeding	SGR (W, %)			SGR (L, %)		
regime	4 -- 20	20 - 30	30 - 60	4 -- 20	20 - 30	30 - 60
A	21.57 ± 1.7^{a}	9.26 ± 0.9^{a}	8.94 ± 0.7^{b}	6.79 ± 0.5^{a}	2.51 ± 0.3^{a}	1.14 ± 0.2^{a}
B	23.65 ± 2.2^{b}	12.4 ± 1.5^{b}	6.78 ± 1.1^{a}	8.53 ± 0.7^{b}	3.06 ± 0.6^{b}	0.72 ± 0.1^{b}
C	24.13 ± 2.4^{b}	11.96 ± 1.4^{b}	6.86 ± 0.6^{a}	8.68 ± 0.8^{b}	3.05 ± 0.5^{b}	0.76 ± 0.2^{b}
D	23.72 ± 2.5^{b}	11.95 ± 1.1^{b}	9.3 ± 0.9^{b}	8.78 ± 0.5^{b}	2.97 ± 0.4^{b}	1.21 ± 0.3^{a}
E	23.97 ± 2.4^{b}	11.63 ± 1.2^{b}	9.47 ± 0.8^{b}	8.81 ± 0.6^{b}	3.12 ± 0.3^{b}	1.34 ± 0.2^{a}

Different superscript letters within a column indicate significant differences at $p < 0.05$

3.Results



Survival rate of loach larvae

Figure 5 Mean survival of *M. anguillicaudatus* larvae in day 12, 20, 30, 40, 50 and 60 with different feeding regimes. See Table 1 for details of the feeding regimes until day 30. From day 30 to day 60, all fish were fed dry feed. Points sharing a common lowercase letter are not significantly different ($P > 0.05$).

4.Discussion

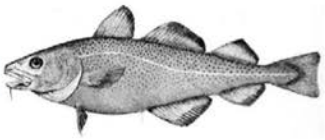
- live food in combination with microdiets from first feeding or early development has been proved better than live feed or dry diets alone. Application in the following species:

- coregonids  (Champigneuille 1988)

- sea bream  (Kanazawa et al. 1989)

- Ayu (sweet fish)  (Kanazawa et al. 1989)
- Asian sea bass  (Walford et al. 1991)
- Philippine freshwater catfish  (Fermin 1991)
- snakehead  (Qin et al. 1997)
- milkfish  (Borlongan et al. 2000)

4.Discussion

- barramundi  (Curnow et al. 2006)
- Atlantic cod  (Fletcher Jr et al. 2007)

- In this study, loach larvae eat dry feed in the presence of daphnia, and can utilize dry feed from an earlier developmental stage.

4.Discussion

Successful weaning by co-feeding in loach

- The present study indicated that the post-hatchlings of loach successfully weaned on to microdiets on an experimental scale.
- Enriched *M. micrura* plus dry feed was tested for the first time as diet of loach which interestingly proved to be highly useful rearing of the post-hatchlings.
- Dry feed might have stimulated the digestive system of loach larvae in co-feeding treatments and increased the acceptance of formulated feed after 30 dph.
- It has an important implication for the commercial culture of this species, as the use of these formulated feed can reduce material and labour costs.

4.Discussion

Co-feeding affected digestive physiology

- It was shown that the type of food consumed by larval fish affected indefinitely the digestive physiology (Segner, Rosch, Verreth & Witt 1993), which implied that co-feeding would improve the digestive capacity of larval loach to suit dry diets.

4.Discussion

Co-feeding affected weaning time and survival

- It was shown that loach only feeding live feeds before weaning suffered higher mortality than did co-feeding fish.

- It is suggested that co-feeding preconditions the larvae to accept the microdiet when live feed is withdrawn, resulting in a shorter weaning period (Watanabe & Kiron 1994; Rosenlund et al. 1997)

4.Discussion

The defect of dry feed alone

- The poor growth rate and survival of loach larvae fed with dry feed could be due to low digestibility and incomplete nutriment of the feed as well as impaired feeding of the larvae.

- The deterioration of water quality and tank cleanliness due to the use of formulated feeds probably also affected the growth and survival of larvae in their early stage.

5.Conclusions

- Our results indicate the reliability of the protocols for larval loach rearing by co-feeding with live and dry diets.
- It reduces the dependence on live foods, makes weaning easy and would mean a hatchery cost reduction in total live feeds requirement.
- This study will help aquaculturists and researchers in improving loach larvae survival as well as help advance the commercial aquaculture of this species.
- Based on results, loach can successfully convert to formulated feeds using the following approach: feed larval loach with enriched daphnia plus formulated feed; after 30 days, gradually eliminate the live food over 7-10 days; then switch completely to formulated feed.

6.Future Work

Though the experiment ended at day 60, a prolonged rearing period was needed to detect nutritional problems and observe remote effect of co-feeding on weaning in the future.

The potential areas for further investigation to facilitate progress towards aquaculture of this species:

- ✓ optimization of stocking density
- ✓ nutritional requirement of dry feed
- ✓ feeding frequency
- ✓ genetic improvement


Thanks
EMMA TURNER 2008

Application of microbial phytase in fish feed

Ling Cao*, Weimin Wang, James Diana, Yi Yang

School of Natural Resources and Environment
University of Michigan, Ann Arbor, MI 48109, USA
caoling.8348@hotmail.com

Phytate is the main storage form of phosphorus (P) in many plants, but phytate-bound P is not available to monogastric or agastric fish animals. Phytase, an enzyme specific to hydrolyze indigestible phytate, has been increasingly used in fish feed during the past two decades, mainly in response to heightened concerns over P pollution to the aquatic environment. Since global phosphate reserves are not renewable, phytate-P as an alternative and economical P source can be effectively converted to available-P by phytase. The capability of this enzyme to enhance bioavailability of P and reduce P load is well documented. Phytase supplementation also leads to improved availability of other minerals and trace elements. Nevertheless, there is still no consistent conclusion that phytase could enhance protein and energy utilization. Studies in amino acid digestibility after phytase supplement are mutative and the underlying mechanisms have not been fully understood. Because phytase is very sensitive to pH and temperature, the utilization of phytase in fish feed is still on its first stage compared with that of in poultry and swine feed. A wide variety of phytases were discovered and characterized in order to find the optimum enzyme which is stable in application, resistant against high temperatures, dust-free, and easy to handle. Initial steps to produce phytase in transgenic plants and fish animals are also undertaken. In this review, the authors focus on comparing properties of phytase from different sources, examining the effects of phytase on P utilization and aquatic environment pollution, meanwhile providing commercial potentiality and impact factors of phytase utilization in fish feed.

Company	Country	Phytase source	Production strain	Trademark
AB Enzymes	Germany	*Aspergillus awamori*	*Trichoderma reesei*	Finase
Alko Biotechnology	Finland	*Aspergillus oryzae*	*Aspergillus oryzae*	SP, TP, SF
Alltech	USA	*Aspergillus niger*	*Aspergillus niger*	Allzyme phytase
BASF	Germany	*Aspergillus niger*	*Aspergillus niger*	Natuphos
BioZyme	USA	*Aspergillus Oryzae*	*Aspergillus Oryzae*	AMAFERM
DSM	USA	*Peniophora lycii*	*Aspergillus oryzae*	Bio-Feed Phytase
Fermic	Mexico	*Aspergillus Oryzae*	*Aspergillus oryzae*	Phyzyme
Finnfeeds International	Finland	*Aspergillus awamori*	*Trichoderma reesei*	Avizyme
Genencor International	USA	*Penicillium simplicissimum*	*Penicillium funiculosum*	ROVABIO
Roal	Finland	*Aspergillus awamori*	*Trichoderma reesei*	Finase
Novozymes	Denmark	*Aspergillus Oryzae*	*Aspergillus Oryzae*	Ronozyme® Roxazyme®

TABLE 1. Commercial production information of microbial phytases

Effects of pretreatment in all-plant feedstuff with microbial phytase on phosphorous utilization and growth performance of Nile tilapia

Cao, Ling and James Diana
University of Michigan

Wang Weimin
Huazhong Agricultural University
Wuhan China

Yang Yi
Shanghai Ocean University
Shanghai China

Travel funding for this presentation was provided by

AquaFish Collaborative Research Support Program

AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.

The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

Outline

- Background
- Experimental design
- Results
- Conclusions
- Acknowledgement

1. Background

- Decrease of annual fishmeal production
- Fishmeal substitution by plant proteins
- High concentration of phytate in plant proteins

- Phytate and phytase enzyme

Phytate = phytin = phytic acid

Up to 80% of the total P content in plants may be present in the form of phytate and is practically not available for monogastric or agastric aquatic animals.

Table 1. Phytate contents in plants or plant products (NRC, 1993)

	Total P (g/kg)	Phytate-P (g/kg)	Proportion (%)
Cereals			
Wheat grain	3.07	2.19	71.6
Oat	3.60	2.10	59.0
Corn grain	2.62	1.88	71.6
Barley grain	3.21	1.96	61.0
Sorghum grain	3.01	2.18	72.6
Rye	3.05	1.95	63.9
Oilseed meals			
Canola meal	9.72	6.45	66.4
Cottonseed meal	10.02	7.72	77.1
Corn glutton meal	4.24	2.67	63.0
Rapeseed meal	9.60	6.34	66.0
Soybean meal	6.49	3.88	59.9
By-products			
Rice bran	17.82	14.17	79.5
Wheat bran	10.96	8.36	76.3

- **Phytase:**

  An enzyme specific to hydrolyze phytate, naturally found in animals, plants and microorganisms

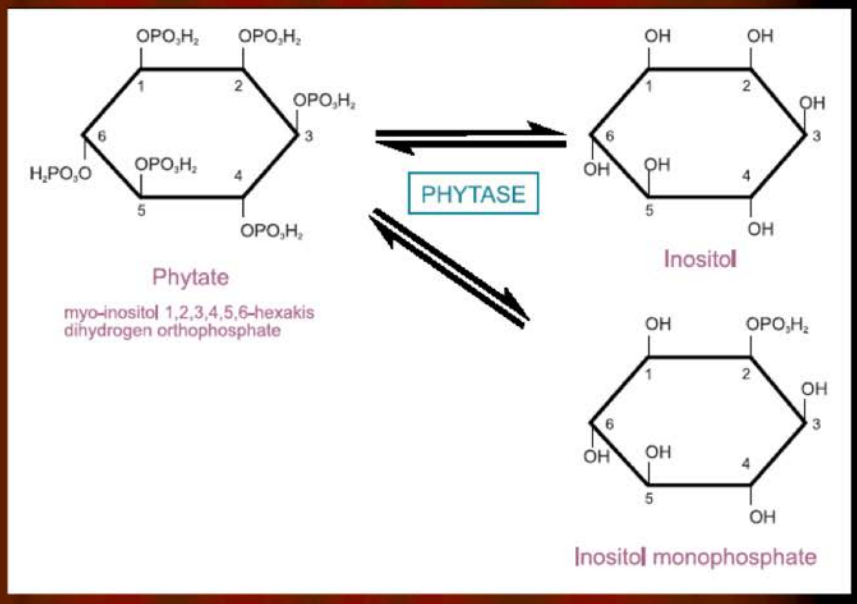


Figure 1. Action of phytase (Baruah *et al.* 2004)

Table 2
Comparison of microbial phytases from different sources[a]

Phytase source	Phytase activity (U/mg) (37 °C)	PH optimum	Temperature optimum (°C)
Fungi			
Aspergillus cæspitosus	NA[b]	5.5	80
Aspergillus fumigatus	23–28	5.0–6.0	60
Aspergillus niger	50–103	5.0–5.5	55–58
Aspergillus oryzae	11	5.5	50
Aspergillus terreus	142–196	5.0–5.5	70
Penicillium simplicissimum	3	4	55
Peniophora lycii	1080	5.5	58
Thermomyces lanuginosus	110	6	65
Bacteria			
Bacillus amyloliquefaciens	20	7.0–8.0	70
Bacillus subtilis	9.0–15	6.5–7.5	55–60
Citrobacter braakii	3457	4	50
Escherichia coli	811–1800	4.5	55–60
Klebsiella terrigena	205	5	58
Lactobacillus sanfranciscensis	NA	4	50
Pantoea agglomerans	23	4.5	60
Pseudomonas syringae	769	5.5	40
Yeasts			
Candida krusei	1210	4.6	40
Pichia anomala	NA[b]	4	60

[a] *Source*: [31].
[b] NA: not available.

Table 3. Commercial information of microbial phytase (Cao *et al.* 2007)

Company	Country	Phytase source	Production strain	Trademark
AB Enzymes	Germany	*Aspergillus awamori*	*Trichoderma reesei*	Finase
Alko Biotechnology	Finland	*A. oryzae*	*A. oryzae*	SP, TP, SF
Alltech	USA	*A. niger*	*A. niger*	Allzyme phytase
BASF	Germany	*A. niger*	*A. niger*	Natuphos
BioZyme	USA	*A. oryzae*	*A. oryzae*	AMAFERM
DSM	USA	*P. lycii*	*A. oryzae*	Bio-Feed Phytase
Fermic	Mexico	*A. oryzae*	*A. oryzae*	Phyzyme
Finnfeeds International	Finland	*A. awamori*	*T. reesei*	Avizyme
Genencor International	USA	*P. simplicissimum*	*Penicillium funiculosum*	ROVABIO
Roal	Finland	*Aspergillus awamori*	*T. reesei*	Finase
Novozymes	Denmark	*A. oryzae*	*A. oryzae*	Ronozyme® Roxazyme®

Table 4. Optimum dose of phytase addition in diets of different fish species (Cao *et al.* 2007)

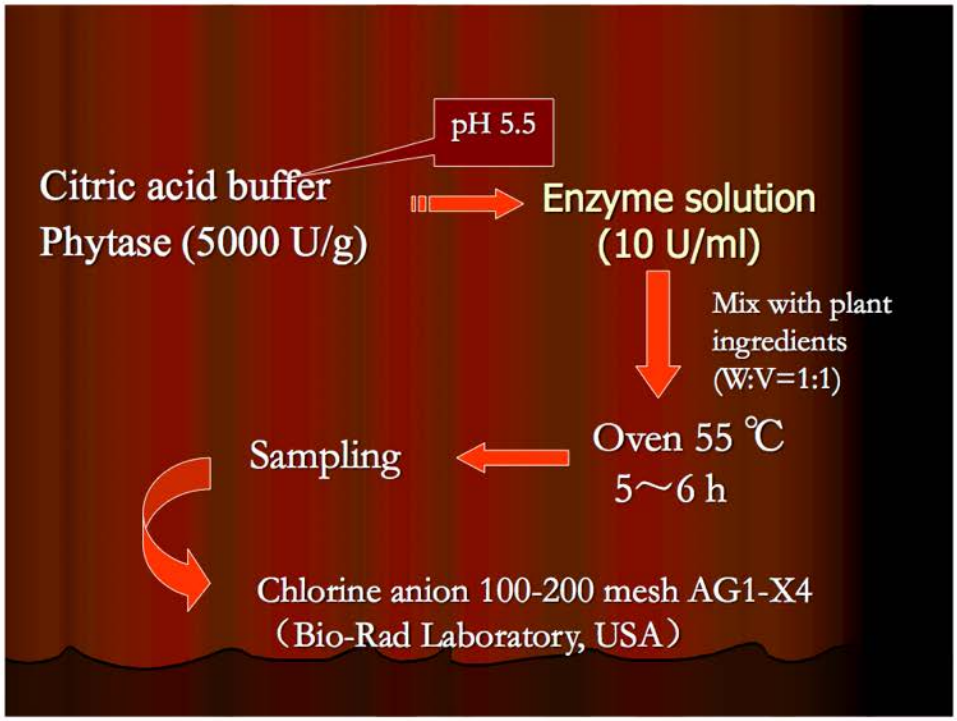
Fish species	Optimam dose of phytase addition (U/kg)
Channel catfish	250–500
African catfish	250–500
Stripped bass	1000
Nile tilapia	500–1500
Crucian carp	500
Common carp	800–1000
Korean rockfish	1000
Pangasius pangasius	500

2. Experimental design

- 2.1 Pretreatment trial

Objectives:

* Find out the most efficient phytase dose for treating plant ingredients



- **2.2 Growth trial**

Objectives:

* Find out the optimum addition dosage for growth performance of tilapia juveniles.
* Determine the best replacing amount for inorganic phosphorous by phytase.

Table 1 Composition of the basal diet for Nile tilapia juveniles.

Ingredients	% (as-fed basis)
Soybean meal	53.65
Wheat	10.00
Corn gluten meal	20.00
Cassava	10.35
Mineral premix [a]	1.00
Fish oil	3.50
Soy lecithin	1.00
Vitamin premix [b]	0.50
TOTAL	100.00
Proximate analysis	**% (Dry matter basis)**
Moisture	10.91
Crude protein	36.38
Crude fat	5.71
Fibre	2.82
Ash	5.10
Nitrogen-free extract	49.99
Calcium	0.20
Total P	0.45
Phytate P	0.30
Available P [c]	0.15

[a] Mineral premix (per kg of diet): $MnSO_4$, 54 mg; Ferric citrate, 142 mg; $CuSO_4$, 10 mg; $ZnCO_3$, 29 mg; NaCl, 3.3 mg; KI, 0.9 mg; K_2SO_4, 90 mg; CoCl, 0.21 mg; MgO, 10mg.
[b] Vitamin premix Roche 2118 (Hoffman-La Roche, Inc., Nutley, NJ, USA) (per kg of diet): Vitamin A, 12000 UI; Vitamin D_3, 5000 UI; Vitamin E, 30 mg; Vitamin K_3, 3 mg; Vitamin B_1, 2.2 mg; Vitamin B_2, 8 mg; Vitamin B_6, 5 mg; Vitamin B_{12}, 11 mg; Folic acid, 1.5 mg; Biotin, 150 mg; Pantothenic acid: 25 mg; inositol, 65 mg.
[c] Calculated as: available P = total P - phytate P (Sajjadi and Carter 2004)

Table 2 Experimental diets for Nile tilapia juveniles

Ingredients (%, as-fed basis)	Phytase control (diet 1)	Inorganic-P control (diet 2)	2.5% MCP supplement (diet 3)	1.875% MCP supplement (diet 4)	1.25% MCP supplement (diet 5)	0.625% MCP supplement (diet 6)	0% MCP supplement (diet 7)	Pretreatment control (diet 8)
Soybean meal	53.65	53.65	53.65	53.65	53.65	53.65	53.65	53.65
Corn gluten meal	20	20	20	20	20	20	20	20
Wheat	10	10	10	10	10	10	10	10
Cassava	10.350	7.850	7.850	8.475	9.100	9.725	10.350	10.350
Fish oil	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Soy lecithin	1	1	1	1	1	1	1	1
Mineral premix [a]	1	1	1	1	1	1	1	1
Vitamin premix [b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	0	2.500	2.500	1.875	1.250	0.625	0	0
Total	100	100	100	100	100	100	100	100
Phytase [c] (U kg^{-1})	0	0	1,000 [d]	1,000 [d]	1,000 [d]	1,000 [d]	1,000 [d]	1,000 [e]
Proximate composition (%, dry matter basis)								
Calcium	0.20	0.62	0.61	0.52	0.41	0.31	0.19	0.20
Total-P	0.45	1.11	1.11	0.96	0.78	0.62	0.45	0.44
Phytate-P	0.30	0.33	0.09	0.08	0.06	0.10	0.07	0.20
Available-P [f]	0.15	0.78	1.02	0.88	0.72	0.52	0.38	0.24

[a] Mineral premix (per kg of diet): the same as table 1 stated.
[b] Vitamin premix Roche 2118 (Hoffman-La Roche, Inc., Nutley, NJ, USA) (per kg of diet): the same as table 1 stated.
[c] Phytase: provided by Sichuan Habio Bioengineering Co. Ltd., Chengdu, Sichuan, China. Enzyme activity: 5000 U/g
[d] Plant ingredients were pretreated with phytase at 1000 U kg^{-1} before mixed with other feed ingredients.
[e] Phytase mixed directly with other ingredients without pretreatment before pelleted
[f] Calculated as: available P = total P - phytate P (Sajjadi and Carter 2004)

3. Results

3.1 Pretreatment

- Soybean meal
- Mix plant ingredients

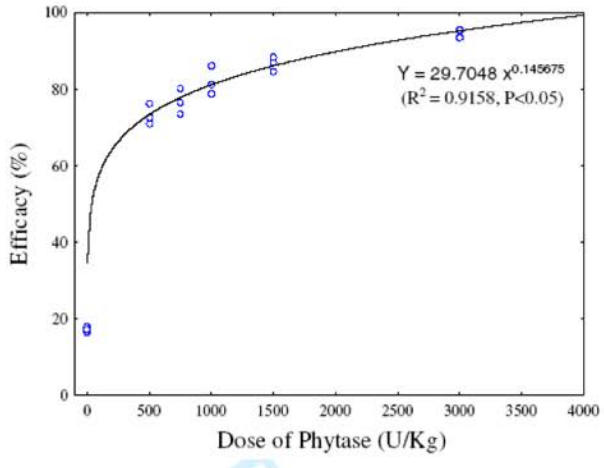


Figure 1 Efficacy of converting phytate P to available P by the pretreatment of soybean meal using phytase at different doses

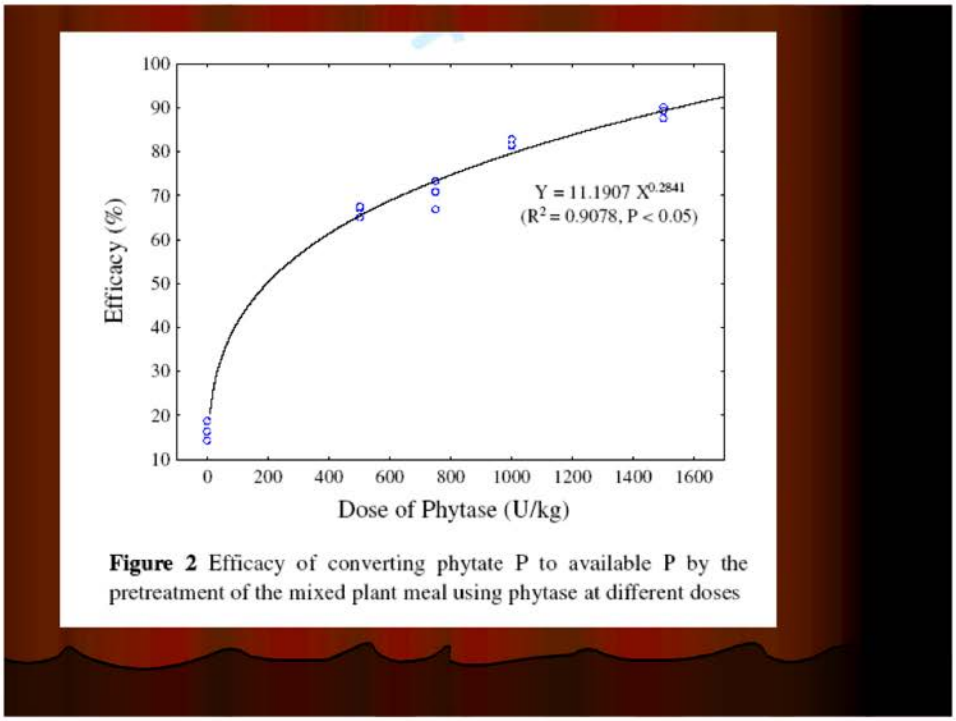


Figure 2 Efficacy of converting phytate P to available P by the pretreatment of the mixed plant meal using phytase at different doses

3.2 Growth performance

Table 5 Growth performance of Nile tilapia juveniles fed the experimental diets for 60 days

Parameter	Unit	Experimental Diets							
		Phytase control (diet 1)	Inorganic P control (diet 2)	2.5% MCP supplement (diet 3)	1.875% MCP supplement (diet 4)	1.25% MCP supplement (diet 5)	0.625% MCP supplement (diet 6)	0% MCP supplement (diet 7)	Pretreatment control (diet 8)
Initial mean weight	g	0.74±0.09	0.74±0.09	0.73±0.00	0.74±0.09	0.70±0.04	0.77±0.05	0.80±0.00	0.74±0.09
Final mean weight	g	19.1±0.60^{e}	34.6±0.76^{a}	34.9±1.23^{a}	34.6±0.56^{a}	35.8±1.21^{a}	30.8±0.74^{b}	26.8±0.45^{c}	23.0±1.07^{d}
Weight gain	g	18.4±0.51^{e}	33.9±0.86^{a}	34.2±1.23^{a}	33.9±0.47^{a}	35.1±1.15^{a}	30.0±0.79^{b}	26.0±0.45^{c}	22.3±0.98^{d}
Daily weight gain	g/fish/d	0.31±0.01^{e}	0.57±0.01^{a}	0.57±0.01^{a}	0.56±0.01^{a}	0.59±0.02^{a}	0.50±0.01^{b}	0.44±0.01^{c}	0.37±0.01^{d}
Survival	%	95.00±2.36	96.67±0.00	91.67±2.35	93.34±4.27	91.67±2.35	93.34±4.27	90.00±0.00	96.67±0.00
FCR[1]		1.85±0.03^{e}	1.12±0.07^{a}	1.14±0.00^{a}	1.16±0.01^{ab}	1.10±0.06^{a}	1.24±0.04^{b}	1.35±0.02^{c}	1.64±0.04^{d}
SGR[2]	%	5.45±0.16^{e}	6.43±0.25^{ab}	6.44±0.06^{ab}	6.43±0.18^{ab}	6.56±0.06^{a}	6.16±0.14^{bc}	5.85±0.03^{cd}	5.75±0.14^{de}
PER[3]		1.49±0.02^{e}	2.59±0.04^{a}	2.54±0.08^{a}	2.61±0.04^{a}	2.66±0.08^{a}	2.36±0.06^{b}	2.16±0.04^{c}	1.68±0.04^{d}

[1] Feed conversion ratio (FCR) = dry feed weight given (g)/wet fish weight gained (g).
[2] Specific growth rate (SGR) (%) = 100 × [(ln final mean weight − ln initial mean weight)/days of the experiment]
[3] Protein efficiency ratio PER = fish weight gained (g)/protein given (g)
Values are means ± SD (n=3), values with different superscript letters in the same row are significantly different (P<0.05)

Table 6 Whole-body chemical composition (%, dry matter basis) of Nile tilapia juveniles fed the experimental diets for 60 days

Parameter	Experimental Diets							
	Phytase control (diet 1)	Inorganic P control (diet 2)	2.5% MCP supplement (diet 3)	1.875% MCP supplement (diet 4)	1.25% MCP supplement (diet 5)	0.625% MCP supplement (diet 6)	0% MCP supplement (diet 7)	Pretreatment control (diet 8)
Dry matter (%)	25.93±0.04^{a}	23.10±0.03^{e}	23.05±0.01^{e}	23.09±0.06^{e}	23.13±0.04^{e}	23.29±0.10^{d}	23.78±0.11^{c}	25.36±0.04^{b}
Ash (%)	8.80±0.01^{d}	10.61±0.03^{a}	10.61±0.10^{a}	10.55±0.07^{a}	10.53±0.04^{ab}	10.42±0.06^{b}	9.65±0.07^{c}	8.90±0.01^{d}
Lipid (%)	18.84±0.01^{a}	13.38±0.03^{cd}	13.29±0.07^{d}	13.40±0.06^{cd}	13.31±0.04^{d}	13.48±0.24^{cd}	13.59±0.11^{c}	17.53±0.09^{b}
Crude protein (%)	50.76±0.65^{c}	55.34±0.40^{a}	54.97±0.39^{a}	54.81±0.27^{a}	55.00±0.34^{a}	53.14±0.60^{b}	52.72±0.63^{b}	51.37±0.97^{c}
Phosphorous (%)	1.33±0.05^{d}	1.83±0.04^{a}	1.83±0.05^{a}	1.79±0.04^{a}	1.81±0.05^{a}	1.70±0.02^{b}	1.68±0.03^{b}	1.43±0.03^{c}

Values are means ± SD (n=3); values with different superscript letters in the same rows are significantly different (P<0.05)

Table 7 Apparent digestibility coefficients of crude protein (ADC_{CP}) and P (ADC_{P}) for the experimental diets fed to Nile tilapia juveniles.

Diet	ADC_{CP} (%)	ADC_{P} (%)
Phytase control (diet 1)	84.40±1.47	28.23±1.15^{f}
Inorganic P control (diet 2)	84.37±0.83	52.37±2.31^{c}
2.5% MCP supplement (diet 3)	84.43±0.56	47.10±0.65^{d}
1.875% MCP supplement (diet 4)	84.57±0.61	55.33±1.68^{b}
1.25% MCP supplement (diet 5)	85.27±0.65	69.03±2.00^{a}
0.625% MCP supplement (diet 6)	83.63±0.47	69.47±0.38^{a}
0% MCP supplement (diet 7)	83.10±0.91	67.83±1.84^{a}
Pretreatment control (diet 8)	83.40±1.17	36.03±0.97^{e}

Values are means ± SD (n=3); values in the same column with different superscript letters are significantly different (P<0.05).

4. Conclusion

- The optimal dose of phytase for the pretreatment of plant ingredients is 1,000 U kg^{-1}, which can efficiently convert phytate P to available P and replace 50% of the inorganic P supplementation to the diets for Nile tilapia juveniles without affecting their growth performance, apparent digestibility coefficients of both crude protein and phosphorous.
- The addition of phytase in the diets can reduce the use of inorganic P, enhance the utilization of P in plant ingredients, and thus minimize the P discharge to the environment.

Optimization of the stocking density and size of red tilapia in intensive polyculture of white shrimp (*Litopenaeus vannamei*) and red tilapia (*Oreochromis spp.*)

Derun Yuan*[1] and Yang Yi[1,2]

[1]Aquaculture and Aquatic Resources Management
School of Environment, Resources and Development
Asian Institute of Technology, Pathumthani 12120, Thailand
Email yuand@ait.ac.th

[2]College of Aqua-Life Science and Technology
Shanghai Fisheries University, Shanghai 200090, China
yiyang@shfu.edu.cn

An experiment was conducted in 21 cement tanks (2 x 2.5 x 1 m) at the Asian Institute of Technology, Thailand, from 8 December 2005 to 3 March 2006, to determine the optimal stocking density and size of red tilapia (*Oreochromis spp.*) polycultured intensively with white shrimp (*Litopenaeus vannamei*). Shrimp postlarvae of 0.06 g were stocked into the tanks at the density of 70 postlarvae m-1. Red tilapia fingerlings of either small (g) or large (g) size were stocked into the shrimp tanks half month later at the density of 0.4, 0.8, or 1.2 fish m-2. The experiment followed a 2 (sizes) x 3 (densities) factorial design, and all treatment combinations were randomly allocated to the tanks in triplicate with three shrimp monoculture tanks as the control. Shrimp were fed three times a day with commercial pellets at the same feeding rate as the control. There was no water exchange throughout the experimental period.

Mean survival rate of shrimp, ranging from 49.56% to 66.78%, was highest in the tanks with 2 small fish and lowest in the tanks with 6 large fish ($P<0.05$). There seemed to be a tendency that with the increase of either stocking size or stocking density of tilapia, shrimp survival was negatively affected. However, compared with the control, all treatments had higher shrimp survival rates except the treatment with 6 large fish. Shrimp grew to 6.71 - 9.39 g in 84 days. The control tanks had the faster growth than the treatments with 2 large fish, 4 large fish, 6 small fish or 6 large fish ($P<0.05$), but not significantly different from those in the treatments with 2 or 4 small fish. The highest yield of shrimp was obtained from the treatments with 2 small fish, followed by the control, which were significantly higher than those in other treatments ($P<0.05$). The treatments with 2 small fish had a similar FCR to that of the control but significantly better than that in the treatments with larger fish or with fish at higher stocking densities ($P<0.05$). Water quality analyses revealed significant increase of nutrient recover with the increase of tilapia stocking size and density. The results also showed that the treatments with 2 small fish had better economic performance than the control and other treatments.

The experiment concluded that stocking tilapia into shrimp monoculture improved water quality and nutrient utilization, and possibly the overall system production performances. However stocking large fish with high stocking density in early growth period of shrimp might negatively affect shrimp survival and growth. The experiment showed that a tilapia stocking density about 0.4 fish m2 at the size about 12 - 13 g might be suitable for such a polyculture system to ensure a superiority to shrimp monoculture in terms of economic performances and environmental concerns.

OPTIMIZATION OF THE STOCKING DENSITY AND SIZE OF RED TILAPIA IN INTENSIVE POLYCULTURE OF WHITE SHRIMP *Litopenaeus vannamei* AND RED TILAPIA *Oreochromis spp.*

Yuan Derun[1], Yang Yi[2], Amararatne Yakupitiyage[1]

1. AARM, SERD, AIT
2. SHFU

4/21/17

Travel funding for this presentation was provided by

Aquaculture Collaborative Research Support Program

Aquaculture CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00 and by participating institutions. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

17/4 2

Introduction

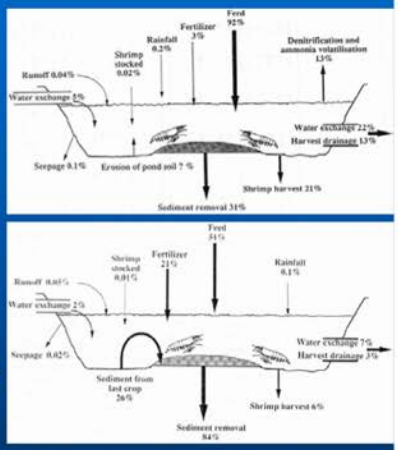
- Shrimp culture has been one of the most active and important sector in aquaculture in past two to three decades.
- Production increased from 87,831 metric tons (MT) in 1981 to about 2 million MT in 2005 (FAO, 2006).

Introduction

- Despite the benefits, it has long been associated with environmental issues (Pruder, 1992; Phillips *et al.*, 1993; Lin, 1995; Boyd and Clay, 1998; Fast and Menasveta, 2000; Lin, 2000).

Introduction

- Semi-intensive farms in Honduras: 72% of the N entering the ponds was discharged to the environment as a result of water exchange (Teichert-Coddington *et al.*, 2000).
- Jackson *et al.* (2003).
  - Intensive tropical shrimp farm in Australia
  - A 10-month period observation.
  - 90% N entered the farm ponds as formulated shrimp food, and
  - within the ponds only 22% of the input N was converted to harvested shrimp.



(N. P, Funge-Smith and Briggs, 1994)

Introduction – the Problem

- Negative environmental impacts
- Economic loss of costly nutrients, thereby reducing farm profitability (Burford *et al.*, 2001).

Introduction

- Need to develop culture technology/systems with increased waste assimilating capacity:
  - to transfer the excessive nutrients into harvestable aquatic products and
  - to avoid uncontrolled effluent discharge.

Introduction

- Polyculture: Centuries old (Lin, 1969; Lin, 1982); Worldwide practice (Hepher and Milstein, 1989).
- The rationale:
  - complementary to each other,
  - more efficient utilization of food available in the pond.
- A possible solution/alternative?

Introduction

- Shrimp polyculture: old practice
- Been cultured with fish (milk fish, mullet, tilapias, other shrimp, Glacilaria seaweed, bivalves etc.
- Purposes:
  - To increase overall production
  - To earn extra income
  - To control water quality, and
  - To spread culture risks.

Introduction

- The researches and practices were however mainly based on extensive and semi-intensive systems.
- Few attempts have been made to polyculture shrimp intensively.

Introduction

- Akiyama and Anggawati (1999) observed two cycles of shrimp production in ponds in Ecuador and found that yields of shrimp increased when red tilapia (*Oreochromis spp*.) were stocked into existing shrimp ponds.
- It was believed that red tilapia assisted shrimp performance by improving and stabilizing the water quality, by foraging and cleaning the pond bottom and by having a probiotic type effect in the pond environment.

Introduction

- A preliminary study of intensive shrimp/tilapia polyculture conducted by Yang Yi *et al.* (2002) in Thailand demonstrated positive specific interaction and mutual benefit between two co-cultured species. *P. monodon* in such an intensive polyculture system seemed to have the similar survival rates and FCR to those in monoculture controls.

Introduction

- However, questions still remain:
  - how shrimp would respond to the interaction of tilapia socking density and size
  - at what stoking density and size tilapia should best benefit shrimp production.
- Furthermore, few studies done on polyculture aspect of *L. vannamei,* - the dominant species in shrimp culture worldwide.

Introduction

- The objective was:
  - To assess the effects of addition of red tilapia *Oreochromis spp.* at different densities and sizes on:
    - shrimp growth,
    - water quality and
    - nutrient recovery

  in intensive culture of white shrimp *Litopenaeus vannamei*.

MATERIALS AND METHODS

- Site: the Asian Institute of Technology, Thailand
- 8 December 2005 to 3 March 2006
- Cement tanks (2.5 x 2 x 1.3 m)
- Water: 20 ppt, 1 m deep, weekly add-up.
- Aeration: 9 spherical air-stones in each tank suspended 10 cm above tank bottoms. Aeration was supported by a 2 HP air blower.

MATERIALS AND METHODS

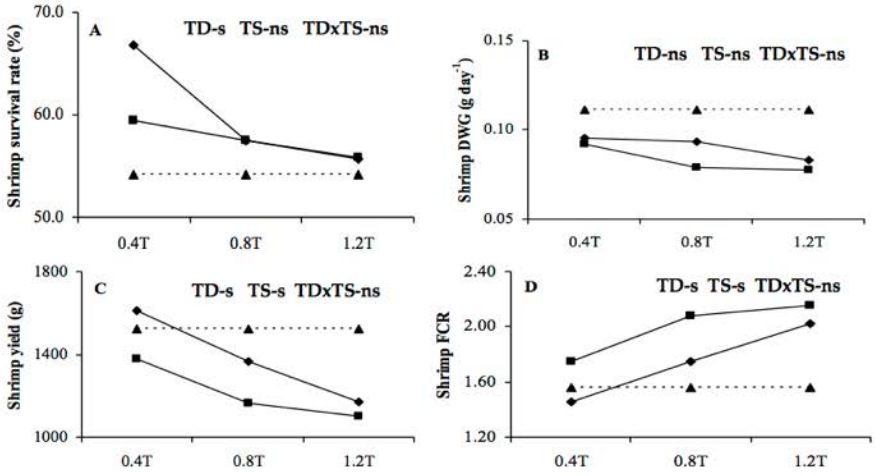
- *L. Vannamei* post-larvae: 0.06 g, 60 pcs m^{-2}.
- 2x3 factorial design: Two different sizes of red tilapia (*small at 13.8 ±0.2 and large at 41.9 ±0.3 g respectively*) were added to the shrimp tanks at three different densities (0.4, 0.8 or 1.2 fish m^{-2}) two weeks after shrimp were stocked.
- Shrimp were fed with commercial shrimp pellets of different sizes following a fixed feeding scheme.

RESULTS: Shrimp growth performances

	Treatment (combination of tilapia stocking size and density)						
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	Control
STOCKING							
Biomass (g $tank^{-1}$)	17.75	17.75	17.75	17.75	17.75	17.75	17.75
Number (shrimp $tank^{-1}$)	300	300	300	300	300	300	300
Density (shrimp m^{-2})	60	60	60	60	60	60	60
Mean weight (g $shrimp^{-1}$)	0.06	0.06	0.06	0.06	0.06	0.06	0.06
HARVESTING							
Number (shrimp $tank^{-1}$)	200^{a}	178^{b}	172^{b}	173^{b}	167^{b}	167^{b}	162^{b}
Biomass (g $tank^{-1}$)	$1,614^{a}$	$1,381^{ab}$	$1,368^{ab}$	$1,165^{b}$	$1,169^{b}$	$1,101^{b}$	$1,525^{a}$
Mean weight (g $shrimp^{-1}$)	8.06^{b}	7.82^{bc}	7.94^{bc}	6.71^{bc}	7.01^{bc}	6.58^{c}	9.39^{a}
GROWTH PERFORMANCES							
Daily weight gain (g $shrimp^{-1}$ day^{-1})	0.10^{b}	0.09^{bc}	0.09^{bc}	0.08^{c}	0.08^{c}	0.08^{c}	0.11^{a}
Survival rate (%)	66.8^{a}	59.4^{b}	57.4^{b}	57.6^{b}	55.7^{b}	55.8^{b}	54.1^{b}
FCR	1.46^{a}	1.74^{ab}	1.75^{abc}	2.08^{bc}	2.02^{bc}	2.15^{c}	1.56^{a}

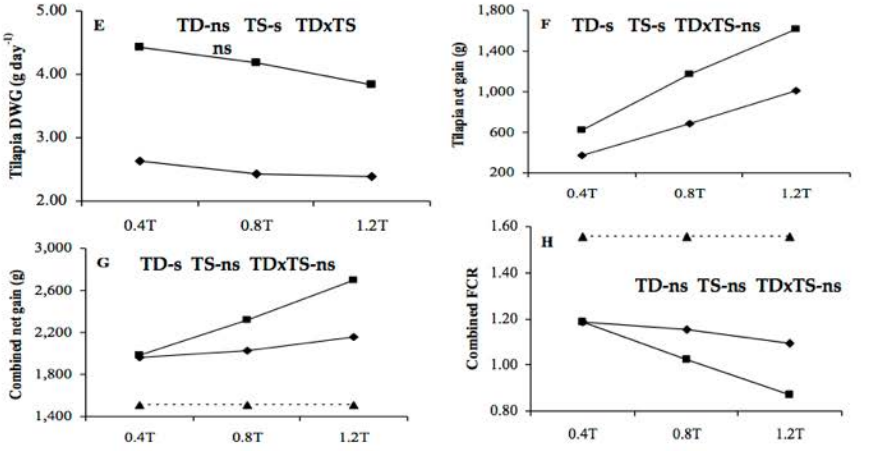
RESULTS: Growth performances of tilapia and combined

	Treatment (combination of tilapia stocking size and density)						
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	Control
TILAPIA							
Stocking							
Biomass (g $tank^{-1}$)	27	83	55	169	84	250	
Number (fish $tank^{-1}$)	2	2	4	4	6	6	
Density (fish m^{-2})	0.4	0.4	0.8	0.8	1.2	1.2	
Mean weight (g $fish^{1}$)	13.67	41.67	13.67	42.33	13.94	41.61	
Harvesting							
Number (fish. $tank^{-1}$)	2	2	4	4	6	6	
Biomass (g $tank^{-1}$)	397^{a}	704^{ab}	736^{b}	$1,342^{c}$	$1,091^{c}$	$1,863^{d}$	
Mean weight (g $fish^{-1}$)	198^{a}	352^{b}	184^{a}	335^{b}	182^{a}	310^{b}	
Growth performances							
Daily weight gain (g $fish^{-1}$ day^{-1})	2.64^{a}	4.43^{b}	2.43^{a}	4.19^{b}	2.40^{a}	3.84^{b}	
COMBINED							
Total biomass (g $tank^{-1}$)	$2,011^{b}$	$2,085^{b}$	$2,105^{b}$	$2,507^{c}$	$2,260^{bc}$	$2,963^{d}$	$1,525^{a}$
Total net gain (g $tank^{-1}$)	$1,961^{b}$	$1,984^{b}$	$2,032^{b}$	$2,320^{c}$	$2,159^{bc}$	$2,696^{d}$	$1,508^{a}$
FCR	1.18^{b}	1.19^{b}	1.15^{b}	1.02^{c}	1.09^{bc}	0.87^{a}	1.56^{a}



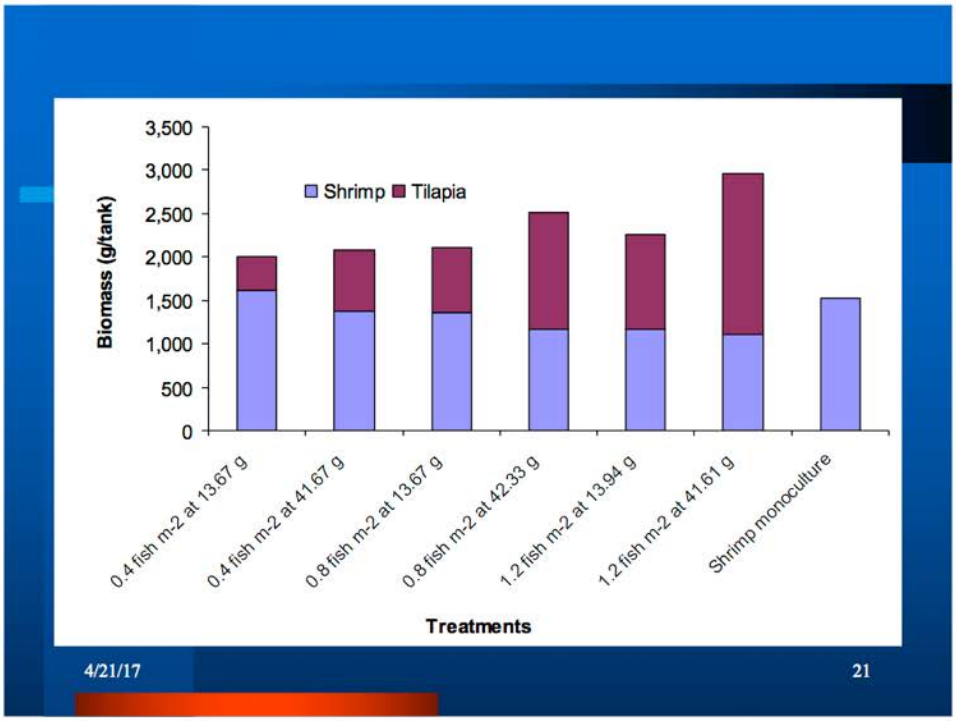
Interaction effects of stocking density and size of red tilapia added to intensive shrimp culture tanks on shrimp survival rate (A), shrimp daily weight gain (B), shrimp biomass at harvest (C), shrimp FCR (D). 0.4T, 0.8T and 1.2T stand for treatments with tilapia at stocking density levels of 0.4, 0.8, and 1.2 fish m-2 respectively. Solid lines with diamond and square marks indicate treatments with small (13.7 – 13.9 g fish-1) and large (41.6 – 42.3 g fish-1) tilapia respectively. Dotted lines indicate the shrimp monoculture control.

4/21/17 19



Interaction effects of stocking density and size of red tilapia added to intensive shrimp culture tanks on tilapia daily weigh gain (E), tilapia net gain (F), combined weight gain of shrimp and tilapia (G) and combined FCR (H). 0.4T, 0.8T and 1.2T stand for treatments with tilapia at stocking density levels of 0.4, 0.8, and 1.2 fish m-2 respectively. Solid lines with diamond and square marks indicate treatments with small (13.7 – 13.9 g fish-1) and large (41.6 – 42.3 g fish-1) tilapia respectively. Dotted lines indicate the shrimp monoculture control.

4/21/17 20



Overall values of water quality parameters in polyculture treatments measured during the experiment in comparisons with monoculture control.

Parameters	Treatments (shrimp/tilapia polyculture with tilapia stocked at two sizes and three densities)						Control
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	
DO (mg L^{-1}) at dawn	6.18^{a}	6.13^{a}	6.12^{a}	6.09^{ab}	6.04^{b}	6.01^{c}	6.10^{ab}
pH	7.76	7.70	7.75	7.75	7.73	7.78	7.76
Temp. (°C) at dawn	24.5	24.6	24.5	24.6	24.6	24.6	24.6
Alkalinity (mg L^{-1})	107.6	106.7	106.7	105.2	107.0	108.9	109.3
TAN (mg L^{-1})	0.38	0.38	0.48	0.42	0.46	0.37	0.42
NO_3-N (mg L^{-1})	0.2	0.17	0.21	0.20	0.2	0.18	0.18
NO_2-N (mg L^{-1})	0.05	0.04	0.04	0.04	0.04	0.04	0.05
TKN (mg L^{-1})	9.31^{a}	8.32^{b}	7.95^{bc}	7.38^{cd}	7.54^{bcd}	7.16^{d}	9.70^{a}
TP (mg L^{-1})	1.54^{ab}	1.43^{bcd}	1.47^{bc}	1.45^{bc}	1.40^{cd}	1.30^{d}	1.63^{a}
SRP (mg L^{-1})	0.34	0.24	0.42	0.26	0.30	0.26	0.31
Chlorophyll *a* (μg L^{-1})	137.2^{b}	120.8^{b}	119.6^{b}	137.2^{b}	121.3^{b}	135.1^{b}	164.0^{a}
TSS (mg L^{-1})	86.5^{ab}	83.3^{ab}	84.6^{ab}	81.0^{b}	81.9^{b}	72.3^{c}	89.4^{a}
TVSS (mg L^{-1})	65.3^{ab}	62.7^{ab}	63.9^{ab}	61.9^{b}	60.7^{b}	53.7^{c}	68.6^{a}

4/21/17 22

Values of water quality parameters in polyculture treatments measured during the experiment in comparisons with monoculture control at the end of the experiment.

Parameters	Treatments (shrimp/tilapia polyculture with tilapia stocked at two sizes and three densities)						Control
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	
DO (mg L^{-1}) at dawn	5.63^{a}	5.50^{a}	5.33^{b}	5.27^{bc}	5.23^{bc}	5.13^{c}	5.30^{b}
pH	7.41	7.20	7.29	7.24	7.33	7.46	7.35
Temp. (°C) at dawn	26.1	26.4	26.2	26.2	26.2	26.2	26.5
Alkalinity (mg L^{-1})	118.8	114.7	109.3	108.0	111.4	122.2	130.9
TAN (mg L^{-1})	1.08	0.96	1.15	1.13	1.17	0.80	1.04
NO_3-N (mg L^{-1})	0.45	0.29	0.46	0.48	0.45	0.26	0.44
NO_2-N (mg L^{-1})	0.21^{b}	0.16^{b}	0.15^{b}	0.16^{b}	0.19^{b}	0.14^{b}	0.28^{a}
TKN (mg L^{-1})	16.27^{b}	15.90^{b}	15.54^{bc}	14.17^{c}	15.08^{bc}	12.49^{d}	18.24^{a}
TP (mg L^{-1})	3.65^{b}	3.56^{bc}	3.44^{bc}	3.32^{c}	3.39^{bc}	2.97^{d}	4.05^{a}
SRP (mg L^{-1})	1.22	0.85	1.28	1.00	0.96	0.74	1.08
Chlorophyll *a* (μg L^{-1})	147.7^{b}	124.2^{b}	136.6^{b}	158.2^{b}	145.3^{b}	170.8^{b}	327.6^{a}
TSS (mg L^{-1})	178.4	164.9	182.0	156.0	168.0	128.1	196.3
TVSS (mg L^{-1})	140.5^{ab}	124.0^{bc}	136.7^{ab}	122.0^{bc}	130.3^{ab}	98.1^{c}	155.0^{a}

4/21/17 23

RESULTS N Recovery

	Treatments						Control
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	
Total N input (g)	140.0	141.4	140.7	143.4	141.5	145.3	139.4
Recovered by:							
Shrimp (g)	40.91^{a}	35.29^{abc}	36.58^{ab}	28.32^{c}	29.65^{bc}	28.85^{c}	37.72^{a}
(%)	29.2^{a}	25.0^{abc}	26.0^{ab}	19.7^{c}	21.0^{bc}	19.8^{c}	27.1^{a}
Fish (g)	9.50^{a}	16.68^{a}	17.59^{ab}	31.53^{c}	25.41^{bc}	43.14^{d}	
(%)	6.8^{a}	11.8^{a}	12.5^{ab}	22.0^{c}	18.0^{bc}	29.7^{d}	
Total recovery:							
Amount (g)	50.41^{b}	51.96^{b}	54.17^{b}	59.85^{b}	55.06^{b}	71.99^{c}	37.72^{a}
(%)	36.0^{b}	36.8^{b}	38.5^{b}	41.7^{b}	38.9^{b}	49.5^{c}	27.1^{a}

4/21/17 24

RESULTS P Recovery

	Treatments						Control
	0.4 fish m^{-2} 13.67 g	0.4 fish m^{-2} 41.67 g	0.8 fish m^{-2} 13.67 g	0.8 fish m^{-2} 42.33 g	1.2 fish m^{-2} 13.94 g	1.2 fish m^{-2} 41.61 g	
Total N input (g)	34.69	34.96	34.82	35.30	34.98	35.65	34.64
Recovered by:							
Shrimp (g)	3.42^{a}	2.82^{bc}	2.82^{bc}	2.37^{c}	2.34^{c}	2.31^{c}	3.07^{ab}
(%)	9.85^{a}	8.06^{bc}	8.09^{bc}	6.72^{c}	6.70^{c}	6.49^{c}	8.85^{ab}
Fish (g)	1.52^{a}	2.66^{a}	2.86^{ab}	5.03^{c}	4.22^{bc}	7.14^{d}	
(%)	4.37^{a}	7.61^{a}	8.21^{ab}	14.24^{c}	12.07^{bc}	20.04^{d}	
Total recovery:							
Amount (g)	4.94^{b}	5.48^{bc}	5.68^{bc}	7.40^{d}	6.57^{cd}	9.46^{e}	3.07^{a}
(%)	14.22^{b}	15.67^{bc}	16.30^{bc}	20.96^{d}	18.77^{cd}	26.53^{e}	8.85^{a}

CONCLUSIVE SUMMARY

- No significant reduction of shrimp production occurred in the polyculture tanks with red tilapia of 13.8 g stocked at 0.4 to 0.8 fish m^{-2}, or with red tilapia of 41.9 g stocked at 0.4 fish m^{-2}.
- Synergistic effect in terms of improved shrimp survival rate happened in shrimp tanks with red tilapia at 0.4 fish m^{-2} with the stocking size at 13.8 g. The effects nearly diminished in the treatments with larger tilapia or at higher socking density as compared with the control.

CONCLUSIVE SUMMARY

- Increasing tilapia stocking density from 0.4 to 1.2 fish m^{-2} and stocking size from 13.8 to 41.9 g in polyculture negatively affected shrimp production performances, but remarkably increased overall nutrient utilization and total production.
- The study demonstrated that white shrimp could be cultured intensively with red tilapia in a polyculture system. With proper stocking size and density of red tilapia, the polyculture system could achieve a similar shrimp production level comparable to that of monoculture without extra feed inputs, and produce tilapia as an additional crop.

Thank You !!!

Phylogenetics and population pattern of *Elopichthys bambusa* (Richardson, 1845) from the middle reaches of Yangtze River as inferred from *Cytochrome b* sequences

Khalid Abbas[1], Wang Weimin[1]*, Yi Yang[2]

[1]College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, 430070, China
[2]College of Aqua-Life Science and Technology, Shanghai Fisheries University, Shanghai, 200090, China
*Author for correspondence (Phone: +86-027-87282113; Fax: +86-027-87282114; E-mail: wangwm@mail.hzau.edu.cn)

The phylogenetic configuration and population structure of cyprinid *Elopichthys bambusa* from the middle reaches of the Yangtze River were examined. In order to accomplish the project, complete mitochondrial *Cytochrome b* (*Cyt b*) genes were sequenced. Total 20 sequences were employed to represent five different hydrographic localities for the analyses of phylogenetic relationship and genetic population patterns. The combined analyses revealed significantly low nucleotide divergence (0.00085) for Tajima and Nei substitution. Overall, there were 5 haplotypes (I-V) found with a low to moderate haplotype diversity (0.60). The phylogenetic tree constructed by the Neighbor Joining depicted neither distinct genealogical branches nor identifiable geographical clades among 5 haplotypes. The haplotypes were unevenly distributed among the localities. Analysis of molecular variance (AMOVA) indicates that most of the variance exists within the sampling localities (72.74%) rather than among the localities (27.26%) implying a frequent gene flow among the localities. The results of nucleotide divergence, phylogenetic and population analyses reveal that *E. bambusa* sampled from all the localities constitute a single population. Being the ever first study for *E. bambusa* at molecular level, it can contribute a lot to the further understanding of molecular ecology of the fish and hence, can help adopting the accurate strategies for its conservation and management.

The cyprinid *E. bambusa* is the only species of genus *Elopichthys* that is confined to the aquatic ecosystems of China, Vietnam and Russia. Recently, because of isolation of rivers and lakes, construction of irrigation works and environmental depravation of aquatic ecosystems, the natural populations of *E. bambusa* has declined rapidly. Now, it is largely restricted to the Yangtze River and the lakes connected with the river. It can scarcely be found from great mass of rivers and lakes. To investigate the phylogeographic characteristics and population patterns of the species, we sequenced *Cyt b* gene (accession # EU287772-EU287790) and used 20 sequences x 1140 nucleotide data set for the sequence analysis. The nucleotide frequencies are 0.308 (A), 0.315 (T), 0.248 (C), and 0.129 (G). Out of data set of 1140 nucleotides, 5 sites were found to be polymorphic. The genetic distance found within the populations ranged from 0.000 to 0.00087 (Table I). The mitochondrial phylogeny of *E. bambusa* was reconstructed using Neighbor Joining, Minimum Evolution and UPGMA methods with Kamura 2-parameter model. We could observe neither the distinct genealogical branches for 5 haplotypes nor clusters representing specific sampling localities. The nonsignificant *Fst* and negative Tajima-D (-0.957297) values point out that the current populations may have evolved from a small number of founders followed by demographic expansion The current sequence divergence estimates can hardly distinguish the individuals from different localities as identifiable populations. The findings of present research endeavor reveal that *E. bambusa* from different localities around the middle reaches of the Yangtze River belongs to a single population. Exhaustive sampling from other drainages of the Yangtze River and other river systems of China may provide better evidence on molecular phylogeny and

population genetics of the species. The present study can appreciably boost up molecular investigation of the fish with more reliable genetic markers.

Population	Samples	Haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)
Dongting lake	4	1	0.000	0.000
Poyang lake	5	3	0.800	0.00087
Wuhan	5	2	0.600	0.00052
Dan river	5	2	0.600	0.00053
Danjiang reservoir	1	1	0.000	0.000
Overall	20	5	0.600	0.00085

Table I: Haplotype and nucleotide diversities from 5 localities

Mitochondrial phylogeny and population pattern of *Elopichthys bambusa* (Richardson 1845) in Yangtze River as inferred from *Cytochrome b* sequences

by

Khalid Abbas
Wang Weimin
Yang Yi

Huazhong Agricultural University, Wuhan, 430070, Hubei, PR China

Research funding for this presentation was provided by

AquaFish Collaborative Research Support Program

AquaFish CRSP USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

Elopichthys bambusa (Richardson 1845)

Yellowcheek fish: an introduction

- *E. bambusa* is the only one species of genus *Elopichthys* ever reported
- a large carnivorous pelagic fish of high meat quality
- gains maximum size of two meters length and weight over 40 kg
- The female fish grows more rapidly than the male
- The male gets sexual maturity one year earlier than female

Why to study its population genetics!

- Confined to Asia, specially Chinese mainland, Russia and Vietnam
- In China, *E. bambusa* is widely distributed from north to south especially in the Yangtze, Pearl and Heilong River
- Rapid decline in populations due to anthropogenic interventions and environmental depravation
- largely restricted to the Yangtze River and the connected lakes

Major Focus of the study

1. **Construction of molecular-based phylogeny**
2. **Disclose the genetic population patterns of the species among different localities in the Yangtze River**
3. **Provide sound basis for further genetic studies and conservation strategies**

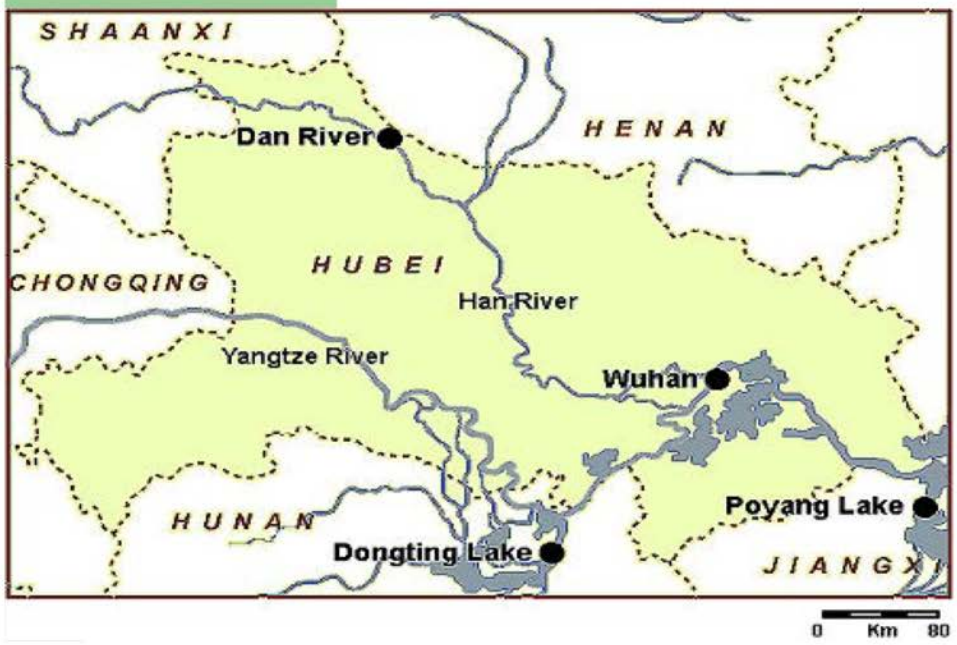
MATERIALS AND METHODS

Fish sampling

***Localities*:**

1. Dongting Lake (DTL) (29° 18′N, 112° 57′E) Hunan Province
2. Poyang lake (PYL) (29° 00′N, 115° 30′E) in Jiangxi province
3. East Lake Wuhan (WHN) (30° 41′N, 114° 28′E) in Hubei province
4. Dan river (DNR) (32° 32′N, 111° 30′E) at the junction of Hubei and Henan province

MATERIALS AND METHODS



MATERIALS AND METHODS

DNA Extraction and gene amplification

Total genomic DNA was extracted from small amounts (~0.2g) of frozen dorsal muscle tissues by using standard phenol/chloroform techniques

Primers for PCR

L14724 (5'-GACTTGAAAAACCACCGTTG-3') and

H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3')

MATERIALS AND METHODS

Amplification conditions

- 50 µl reaction **mixtures** (5 µl 10×buffer, 4 µl Mg2, 3 µl of each primer, 0.5 µl dNTP of each nucleotide, 31 µl H2O, 2.5 µl template DNA and 1 µl *Taq* DNA polymerase (Invitrogen)) under following conditions:
- The **thermal cycling** profile started with 94° C for 180s followed by 35 cycles of denaturation at 94° C for 30s, annealing at 54° C for 45s, extension at 72° C for 6s, with a final extension at 72° C for 10min. The amplified DNA fragments were checked in 0.8% agarose gel

MATERIALS AND METHODS

Sequencing and accession

Sample #	Sampling locality	Code	Sample	Genbank accession #
1	Dongting Lake	DTL	DTL1	EU287772
2	Dongting Lake	DTL	DTL2	EU287773
3	Dongting Lake	DTL	DTL3	EU287774
4	Dongting Lake	DTL	DTL4	EU287775
5	Poyang Lake	PYL	PYL1	EU287776
6	Poyang Lake	PYL	PYL2	EU287777
7	Poyang Lake	PYL	PYL3	EU287778
8	Poyang Lake	PYL	PYL4	EU287779
9	Poyang Lake	PYL	PYL5	EU287780
10	East Lak Wuhan	WHN	WHN1	EU287781
11	East Lak Wuhan	WHN	WHN2	EU287782
12	East Lak Wuhan	WHN	WHN3	EU287783
13	East Lak Wuhan	WHN	WHN4	EU287784
14	East Lak Wuhan	WHN	WHN5	EU287785
15	Dan river	DNR	DNR1	EU287786
16	Dan river	DNR	DNR2	EU287787
17	Dan river	DNR	DNR3	EU287788
18	Dan river	DNR	DNR4	EU287789
19	Dan river	DNR	DNR5	EU287790
20	Danjiang Reservoir	DRV		AY744501

MATERIALS AND METHODS

Analytical tools

- Multiple sequence alignment using Clustal W
- Nucleotide composition and tree construction with MEGA 3
- Phylogenetic history by NJ, MP, ME and UPGMA approaches
- The hypothesis for neutral mutation was tested by conducting the Tajima neutrality test

MATERIALS AND METHODS

Population analysis

- Haplotypic and genetic diversity at both within and among populations revealed by DnaSP
- Characterization of genetic population structure and genetic variation by Arlequin
- AMOVA applied to know the geographical patters of population variation

RESULTS

Nucleotide composition and divergence

- 1140 nucleotides for 380 amino acids
- Gene , exceptionally terminated with thymine deviating from other cyprinids
- GC content stunted as compared to TA
- The transition/transversion rate ratios are *k1* = 11.783 (purines) and *k2* = 21.454 (pyrimidines) while overall transition/transversion bias is *R* = 6.888
- Five variable sites with only two being parsimony informative

RESULTS

PATTERN OF NUCLEOTIDE SUBSTITUTION

	A	T	C	G
A	-	*1.64*	*1.29*	**7.9**
T	*1.6*	-	**27.66**	*0.67*
C	*1.6*	**35.17**	-	*0.67*
G	**18.87**	*1.64*	*1.29*	-

RESULTS

Nucleotide composition and divergence

- The genetic distance within the populations ranged from 0.000 to 0.00087 while between localities varied with a rage from 0.00035 to 0.0030
- Tajima's D value found to be -0.957297. inferring that sequences were influenced by neutral mutation

RESULTS

Divergence between the populations (Tajima and Nei, 1983)

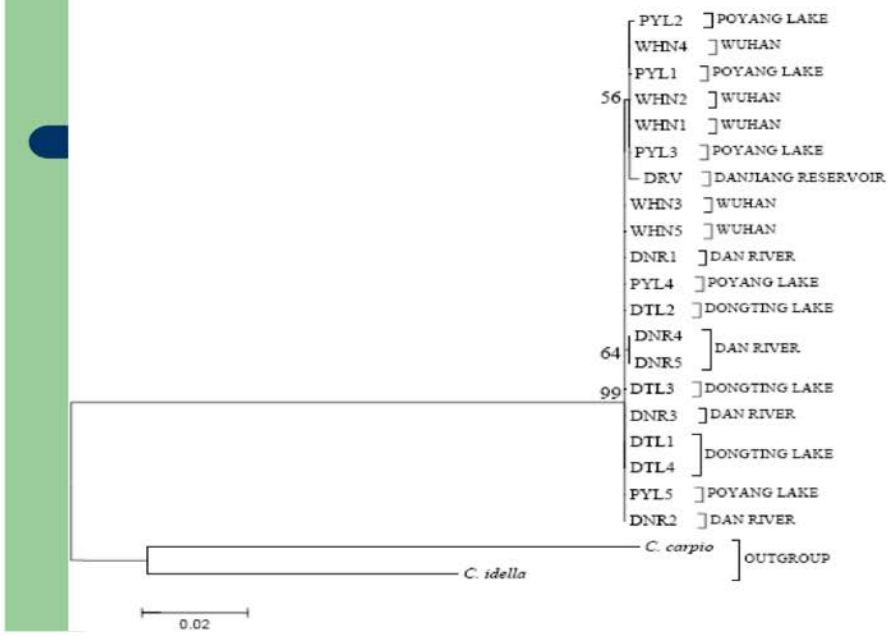
	Dongting lake	Poyang lake	Wuhan	Dan river
Dongting lake				
Poyang lake	0.0007026772			
Wuhan	0.0005268387	0.0005971698		
Dan river	0.0003512592	0.0010543698	0.0008784491	
Danjiang Reservoir	0.0026394440	0.0022866060	0.0021105040	0.0029923547

RESULTS

Phylogenetic analysis

- Congruent phylogenies with almost similar tree topologies and bootstrap values
- Dandrogram clustering showed one basal clade with two derivative clades
- The distribution of taxa at terminal nodes depicted no specific pattern

RESULTS



RESULTS

Haplotype Structure and AMOVA

- The 20 taxa came out into 5 haplotypes
- The distribution of individuals in haplotypes is remarkably uneven
- As a whole, all the individuals had a haplotype diversity of 0.6526 and a nucleotide diversity of 0.0085
- The maximum haplotype diversity was among the individuals from Poyang Lake (0.80) where as the same was zero within populations of Dongting Lake and Wuhan.

RESULTS

Analysis of Molecular Variance

Source of variation	d.f.	Sum of squares	Variance components	Variance Percentage
Among populations	4	2.442	0.10993 Va	27.26
Within populations	19	4.400	0.29333 Vb	72.74
Total	23	6.842	0.40326	100

Fixation Index *Fst*: 0.27259

P-value = 0.03519

RESULTS

The inter-population variance was 27.26%, significantly lower ($P>0.05$) than that of among the individuals at intra-population level (72.74%) as shown in Table

Source of variation	d.f.	Sum of squares	Variance components	Variance Percentage
Among populations	4	2.442	0.10993 Va	27.26
Within populations	19	4.400	0.29333 Vb	72.74
Total	23	6.842	0.40326	100

Fixation Index *Fst*: 0.27259

P-value = 0.03519

RESULTS

Haplotype and nucleotide diversities from 5 localities

Population	Samples	Haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)
Dongting lake	4	1	0.000	0.000
Poyang lake	5	3	0.800	0.00087
Wuhan	5	2	0.600	0.00052
Dan river	5	2	0.600	0.00053
Danjiang reservoir	1	1	0.000	0.000
Overall	20	5	0.600	0.00085

FINDINGS

- Nucleotide composition almost same to cyprinid confamilials except for gene termination with Thymine
- The nucleotide diversity level is much lower than that reported for most of other cyprinids
- The maximum genetic distance was found between Danjiang reservoir to all other localities probably due to barriers provided by reservoir to the flux of gene flow.
- Despite significant geographical distance, the current sequence divergence estimates can hardly distinguish different localities as identifiable populations.

FINDINGS

- The phylogenetic tree constructed by the Neighbor Joining approach using Kimura 2-paramete model depicted neither distinct genealogical branches nor identifiable geographical clades among 5 haplotypes.
- Nonsignificant *Fst* and negative Tajima-D values point out that the current populations may have evolved from a small number of founders
- The sharing of haplotypes suggested substantial gene flow among the specimens from different localities

FINDINGS

- It can be attributed to the breeding habits, dispersal capability, egg characteristics of the fish and absence of reproductive barriers among the localities under investigation.
- The findings of present research endeavor reveal that *E. bambusa* from different localities around the middle reaches of the Yangtze River belongs to a single population.

Conclusion & Remarks

- The rapid decline in Yellowcheek populations and its vulnerability demands a major concern over its conservation and management
- The low genetic diversity might be the outcome of restricted sampling, so, exhaustive sampling would provide better evidence over the subject
- Due to unavailability of genetic data on the species, the present study may serve as a ground for further molecular studies

Thanks for your patience

Application of water purification technology in intensive shrimp culture

Lai Qiuming[1]*, Yang Yi[2,3], and Qiu Yunhao[1]

[1]College of Ocean, Hainan University, Haikou 570228, Hainan province, China
lqming815@163.com

[2]College of Aqua-Life Science and Technology, Shanghai Fisheries University, Shanghai 200090, China

[3]Sichuan Aquacultural Engineering and Technology Research Center, Chengdu 610081, Sichuan province, China

With the rapid development of shrimp culture in China, it has been a concern that wastewater discharged from shrimp ponds leads to water pollution in coastal area. To improve water quality of shrimp ponds and protect the coastal environment, an experiment was conducted in a shrimp pond of 2,000 m2 in surface area connecting to a wastewater treatment unit consisting of a drum filtration tank and a foam separation tank. In the pond, there was a central drainage system from which pond wastewater flowed into the filtration tank passing through a 250 mesh filter, then pumped continuously at a rate of 100 m3/hr to the foam separation tank which was installed with 4 sets of skimmers. Then the treated water flowed back to the shrimp pond. Water quality parameters were monitored for consecutive 5 days by taking water samples at 0700 h at four locations. Results indicated that the shrimp feces and suspend organic particles were removed effectively from the filtration and foam separation tanks. The drum filtration reduced chemical oxygen demand (COD) by 1.88% - 4.57% and total ammonia nitrogen by 15.02% - 22.73%, while the foam separation reduced COD by 1.78% - 3.60% and increased dissolved oxygen by 39.3% - 60.6%. The application of wastewater treatment system not only improves the water quality and bottom in the shrimp pond but also reduces wastewater discharge to the environment.

APPLICATION OF WATER PURIFICATION TECHNOLOGY IN INTENSIVE SHRIMP CULTURE

Lai Qiuming [1]*, Yang Yi [2,3], and Qiu Yunhao [1]

1. College of Ocean, Hainan University, Haikou 570228, Hainan province, China
2. College of Aqua-Life Science and Technology, Shanghai Fisheries University, Shanghai 200090, China
3. Sichuan Aquacultural Engineering and Technology Research Center, Chengdu 610081, Sichuan province, China

Travel funding for this presentation was provided by

AquaFish Collaborative Research Support Program

AquaFish CRSP
USAID

The Aquaculture CRSP is funded in part by United States Agency for International Development (USAID) Grant No. EPP-A-00-06-00012-00.
The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development.

1. Background

Shrimp farming has been a fast-growing industry especially in southern China. However, along with its development, waste water from shrimp farms has continuous contribution of nutrients that adds to eutrophication at different levels.

Due to high stocking density, water quality is more and more difficult to be controlled in intensive shrimp ponds. In latter period, white-leg shrimp *Penaeus vannamei* often die because of poor water quality, which has seriously affected the farming efficiency of *Penaeus vannamei*.

2. Purpose of Study

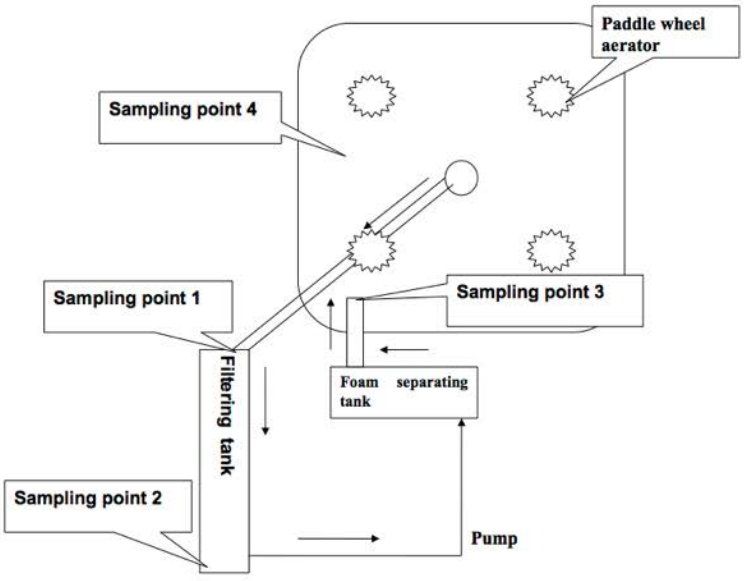
1）Use filtration and foam separation techniques to enhance the water purification capacity of shrimp pond; To improve water quality and reduce the discharge of waste water; And to protect the environment of coastal area.

2）By recycling flow in the closed pond system, water mixes better than before, which leads to the improvement of water quality and less death of shrimp. Thus, the economic returns of shrimp culture is enhanced.

3. Parameters of water quality monitoring

（1） DO
（2） COD
（3） NO_2^-- N
（4） NO_3^-- N
（5） Total ammonia
（6） PO_4^-- P

Figures 1. The work flow chart of water purification



5. Research Design

1) **Experimental site: Wanning Biotechnology Co., Ltd. Hainan province, China.**
2) **Experimental period: Oct 2, 2006 ~ Oct 6, 2006.**
3) **Experimental pond: size-2000 m^2, water depth - 1.5 m; with plastic membrane in the bottom and four sides; central drainage located at pond's bottom; four paddle wheel aerators located evenly in the pond; white-leg shrimp** *Penaeus vannamei* (average body weight: 5.2 g/piece); **stocking density about 200 piece/ m^2.**
4) **During the experiment period, no water exchange. Water purification treatment systems work 24 hours per day. Water samples are collected once at 7:00 am every day.**

6. Results and analysis

Table 1 DO Concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
• 10.1	1.50	（Before the system operation）		6.02
10.2	2.45	2.77	4.25	3.68
10.3	2.61	2.92	4.69	3.13
10.4	2.90	2.98	4.32	3.74
10.5	2.56	2.95	4.21	4.65
10.6	3.08	3.13	4.36	4.55

+39.3~+60.6%

Table 2 COD concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
10.1	32.26 （Before the system operation）			9.29
10.2	12.25	11.69	11.43	11.72
10.3	12.09	11.81	11.52	11.26
10.4	12.22	11.99	11.59	11.64
10.5	12.14	11.82	11.61	11.80
10.6	12.36	11.93	11.50	11.65
		-1.88 ~ -4.57%	-1.78 ~ -3.60%	

Table 3 NO_2^--N concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
• 10.1	1.21 （Before the system operation）			0.41
10.2	0.56	0.55	0.56	0.55
10.3	0.65	0.65	0.64	0.63
10.4	0.67	0.67	0.66	0.65
10.5	0.78	0.79	0.77	0.78
10.6	0.88	0.88	0.88	0.85

Table 4 NO_3^--N concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
10.1	3.25 （Before the system operation）			2.03
10.2	2.81	2.70	2.72	2.50
10.3	2.65	2.66	2.68	2.65
10.4	2.47	2.45	2.46	2.34
10.5	2.58	2.50	2.56	2.22
10.6	2.56	2.58	2.56	2.51

Table 5 ammonia concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
• 10.1	1.65 （Before the system operation）			0.42
10.2	0.93	0.92	0.75	0.58
10.3	1.05	0.98	0.79	0.56
10.4	1.18	1.20	1.02	0.74
10.5	1.48	1.50	1.16	0.82
10.6	1.13	1.11	0.87	0.66
			-15.0~22.7%	

Table 6 PO_4^+-P concentration of different water sample Unit: mg/L

Date	Before filtration	After filtration	After foam separation	Pond side
• 10.1	0.28 （Before the system operation）			0.08
10.2	0.15	0.13	0.14	0.11
10.3	0.14	0.13	0.12	0.12
10.4	0.17	0.18	0.16	0.13
10.5	0.20	0.21	0.20	0.16
10.6	0.18	0.17	0.16	0.18

Conclusions

- Results indicated that the shrimp feces and suspending organic particles were removed effectively by filtration and foam separation.
- The drum filtration reduced COD by 1.88% - 4.57% and total ammonia nitrogen by 15.02% - 22.73%, while the foam separation reduced COD by 1.78% - 3.60% and increased dissolved oxygen by 39.3% - 60.6%.

- The application of wastewater treatment system not only improves the water quality, but also reduces wastewater discharge to the environment.
- Results also suggested that NO_2^-- N, NO_3^-- N and PO_4^-- P can not be removed from the filtration and foam separation tanks.
- The purification system is simple, and easy to operate. It is useful for water mixing and is effective to reduce the death of shrimp in the pond's bottom.