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Aquaculture & Fisheries Collaborative Research Support Program

AquaFish CRSP Air Breathing Fishes Symposium
Shanghai, China
18 April 2011

Proceedings

Assembled by Stephanie Ichien
Edited by Hillary Egna

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AQUAFISH COLLABORATIVE RESEARCH SUPPORT PROGRAM

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.

Acknowledgments
The Management Entity of the AquaFish CRSP gratefully acknowledges the contributions of CRSP researchers and the support provided by participating US and Host Country institutions.

This publication may be cited as:
AquaFish Collaborative Research Support Program. August 2011. Air Breathing Fishes Symposium Proceedings. AquaFish CRSP, Oregon State University, Corvallis, Oregon, 50 pp

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PREAMBLE

AquaFish CRSP Air Breathing Fishes Symposium
Shanghai, China
18 April 2011

Workshop Organizer
Dr. Hillary Egna
Organizing Committee
Ms. Stephanie Ichien
Dr. Liping Liu

Introduction
While most fish rely on dissolved oxygen in the water to survive, air-breathing fish are capable of surviving on oxygen from the air. This characteristic allows them to cope with a variety of environmental conditions and makes them more resilient to environmental change. As aquatic habitats become degraded and climate change shifts global temperatures, the value of these fish will become apparent as they can survive low water quality conditions and even extended droughts. Whether for human consumption, the aquarium trade, or for biodiversity enhancement, the culture of a species that is resilient to the global changes that are occurring will be pivotal for developing sustainable solutions in aquaculture.

On 18 April 2011, nearly 20 researchers assembled in Shanghai, China for the AquaFish CRSP Air-Breathing Fishes Symposium organized by AquaFish CRSP Director Dr. Hillary Egna. Ten presentations were made in the morning followed by a mid-day discussion of the merits of each concept. During the working lunch, each presenter addressed the work that has already been done and the research needs for each fish group, engaging the whole group in a brief conversation regarding the benefits and the hurdles of future work. The final hour of the symposium provided time for further discussion among the presenters and the other workshop attendees, ranking and combining the concepts and species groups to continue forward with prospectus work in seven funded investigations.

Monday, 18 April 2011
0830-1400

AIR BREATHING FISHES SYMPOSIUM AGENDA
Duplex Suite: Upper Floor
Shanghai Marriott Hotel Hongqiao

Dr. Hillary Egna, Workshop Organizer
Ms. Stephanie Ichien, Organizing Committee
Dr. Liping Liu, Organizing Committee

Workshop Presenters
Dr. Hillary Egna, USA
Dr. So Nam, Cambodia
Dr. Felix Ayson, Philippines
Dr. Remedios Bolivar, Philippines
Dr. Jim Diana, USA
Dr. Phu Hoa, Vietnam
Dr. Charles Ngugi, Kenya
Dr. Joe Molnar, USA (Uganda)
Dr. Maria Haws, USA (Ecuador)
Dr. Maria Portella, Brazil

Other Workshop Attendees
Dr. Russell Borski
Dr. Madhav Shrestha
Dr. Wilfrido Contreras-Sanchez
Dr. Christine Crawford
Dr. Rafael Matinez-Garcia

Agenda

0830-0845	Hillary Egna – Welcome
0845-0900	Hillary Egna – Air Breathing Fishes Introduction
0900-0915	So Nam – snakehead
0915-0930	Felix Ayson – gouramis
0930-0945	Remedios Bolivar –gouramis
0945-1000	Jim Diana – gars
1000-1030	Coffee break
1030-1045	Maria Portella – Gars and Arapaima
1045-1100	Charles Ngugi –African lungfish
1100-1115	Maria Haws – chame
1115-1130	Phu Hoa – marble goby
1130-1145	Joe Molnar – lungfish
1145-1300	Lunch together in restaurant; discuss merits of the concepts presented
1300-1400	Ranking and combining ideas for future work (Hillary Egna)
1400	Workshop adjourns
1400	Afternoon tea & coffee in Ballroom meeting area

AQUAFISH
COLLABORATIVE RESEARCH
SUPPORT PROGRAM

INTRODUCTION: WHY ARE AIR-BREATHING FISHES OF SUCH GREAT INTEREST & APPEAL?

Hillary Egna: Workshop Organizer

Background

- Air-breathing has evolved in fish to cope with hypoxic water
- At least 374 species in 49 families of fish (i.e. it has evolved many times)
- Occurs in both freshwater and saltwater (especially intertidal)

Organs used for air-breathing:

- Modified gills (e.g. Clarias, monkeyface prickleback, sculpins)
- Skin (e.g. European eel, mudskipper)
- Mouth (e.g. electric eel, gouramis)
- Gut (e.g. tropical catfishes like Hoplosternum & Plecostomus)
- Lungs and swim bladders (e.g. lungfishes)

Air-breathing fishes can be categorized as obligate (e.g. S. American and African lungfish) or facultative (eg. gars, bowfin, bichirs) air breathers. **Amphibious fish** are fish that are able to leave water for extended periods of time. About 11 distantly related genera of fish are considered amphibious.

Examples of obligate air breathers:

Lung breathers

Lungfish (Dipnoi): Six species, have limb like fins, and can breathe air. Some are obligate air breathers. Some species will bury in the mud when the body of water they live in dries up, surviving up to two years until water returns.

Gill or skin breathers

Rockskippers: These blennies are found in Panama and elsewhere on the western coastline of the Americas. These fish come onto land to catch prey and escape aquatic predators. They often come out of water for up to 20 minutes. Leaping blennies (*Alticus arnoldorum*) are able to jump over land using their tails.

Wooly sculpin: Found in tide pools along the Pacific coast, these sculpins will leave water if the oxygen levels get low and can breathe air for 24 hours.

Mudskippers (*Oxudercinae*): This subfamily of gobies is probably the most land adapted of fish. Mudskippers are found in mangrove swamps in Africa and the Indo-Pacific, they frequently come onto land and can survive in air for up to three and a half days. Mudskippers breathe through their skin and also through the lining of the mouth (the mucosa) and throat (the pharynx). This requires the mudskipper to be wet, limiting mudskippers to humid habitats. This mode of breathing, similar to that employed by amphibians, is known as cutaneous breathing. They propel themselves over land on their sturdy forefins.

Eels: Some eels, such as the European eel and the American eel, can live for an extended time out of water and can crawl on land if the soil is moist.

Electric eel. The electric eel is an obligate air-breather.

Snakehead fish (Channidae): This family of fish are obligate air breathers, breathing air using their suprabranchial organ, which is a primitive labyrinth organ. The Northern Snakehead of Southeast Asia can "walk" on land by wriggling and using its pectoral fins, which allows it to move between the slow-moving, and often stagnant and temporary bodies of water in which it lives. Another amphibious species of this family is the Eel catfish (*Channallabes apus*), which lives in swamps in Africa, and known to hunt beetles on land.

Labyrinth fish (*Anabantoidei*). This suborder of fish also use a labyrinth organ to breathe air. Some species from this group can move on land. An amphibious fish from this family is the Climbing gourami, an African and Southeast Asian fish that is capable of moving from pool to pool over land by using its pectoral fins, caudal peduncle and gill covers as a means of locomotion. It is said that climbing gourami move at night in groups.

The many aspects of interest include:

- Biodiversity
- Food fish
- Ornamental fish
- Medicinal & other

Major points from PB Moyle & JJ Chech. (2000) Fishes: An Introduction to Ichthyology, 4th Editions, Prentice Hall, Upper Saddle River, NJ 07458

CONCEPT PAPERS OR POWERPOINT PRESENTATION TEXT FROM THE 19 APRIL 2011 SYMPOSIUM

The presenters were asked to provide concept papers on their topic, which can be found in the following section of this Symposium Prceedings. The topics for which no concept paper was provided include text from the associated PowerPoint presentation in place of the concept paper.

SUSTAINABLE SNAKEHEAD (*CHANNA STRIATA*) AQUACULTURE DEVELOPMENT IN CAMBODIA

So Nam

Inland Fisheries Research and Development Institute (IFReDI), Fisheries Administration, Phnom Penh, Cambodia. Email: so_nam@hotmail.com

Significance

In Cambodia, over 70% of freshwater aquaculture production comes from floating cage culture (total: 4,492 fish cages) operated in Mekong basin, including the Tonle Sap Great Lake (43%), Tonle Sap River (17%), upper stretch of the Mekong River (19%), lower stretch of the Mekong River (14%) and Bassac River (7%) (So & Thuok, 1999). Snakehead aquaculture production represented more than 75% of total freshwater cage aquaculture production. It is entirely dependent on wild fish both as seed and feed (So et al., 2005; So et al., 2010). Feed represents more than 70% of the total operational cost and the main type of feed for wild snakehead culture is small-sized or small fish, including juvenile of commercially important fish species, which represent 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers (So et al., 2005). During the dry season (October to May), the most important source of feed is freshwater small-sized fish, while more marine small-sized or low value fish species are used during the rainy season (June to September) (So et al., 2005).

The government of Cambodia put a ban on snakehead farming in May 2005 and the reasons for this was the potential negative impacts on wild fish populations from wasteful snakehead seed collection and on other fish species diversity, and also potential negative effects on poor consumer groups from decreased availability of small-sized/low valued fish (So et al, 2007). After the ban on snakehead culture in Cambodia, snakeheads have illegally been imported from the neighboring countries, particularly from Vietnam, to supply high local market demands in Cambodia. Furthermore, the study showed that freshwater small-sized fish have illegally been exported to Vietnam for feeding the significantly and commercially developed snakehead aquaculture in Vietnam. The first phase study indicated that the incentives for choosing snakehead before other fish species by tens of thousands of fish farmers are strong as it generates more than 10 times higher profits than other fish species (So et al., 2010). Therefore, the ban does not only result in positive impacts on poor consumer groups from increased availability of freshwater small-sized fish in Cambodia, but also providing negative effects on livelihood of tens of thousands of snakehead farmers who depend on this livelihood for generating household income. In other words, these snakehead fish farmers have lost their important livelihoods and household income. Moreover, the ban also does not provide positive impacts on snakehead wild stocks as fishing pressure on wild snakehead using illegal and destructive fishing gear, particularly the use of electro-shockers has increased in recent years in order to supply local and external markets (So et al., 2010). During the second phase (2009-2011) of the AquaFish CRSP, wild broodstock of the snakehead has been developed and managed at the hatchery of Freshwater Aquaculture Research and Development Center (FARDeC) of IFReDI, and the first generation (F1) of snakehead fish are being grown from larvae to adult fish by feeding them with formulated pelleted diets adopted from Can Tho University (AquaFish CRSP snakehead formulated feed). F1 mature adult snakehead will be developed by the end of AquaFish CRSP Phase 2. The intention is to develop F4 or F5 snakehead broodstock for snakehead seed mass production, which can be fed with formulated diets, in order to lift the ban on snakehead aquaculture and to sustain this aquaculture industry in Cambodia.

Objectives
The specific objectives of this investigation are as follows.

- To review bio-ecological characteristics of the snakehead *Channa striata* for sustainable management and aquaculture development in Cambodia
- To domesticate wild snakehead to address the snakehead banning issue in Cambodia in order to lift the ban on snakehead culture in Cambodia; and
- To develop methods for weaning snakehead larvae to adults from low-value fish to formulated or pelleted diets.

Quantified Anticipated Benefits
This research will provide information on domestication breeding, feeding and weaning of snakehead fish, especially development of Cambodia's captive snakehead broodstocks in order to lift the ban on snakehead culture in Cambodia.

- At least 20,000 farmers in Cambodia will benefit from this research by restarting their snakehead culture.
- 200 scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staff, and private sector working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries will be better informed, and have better recommended policies and strategies for sustainable snakehead aquaculture.
- Two (under)graduate students will be supported and trained through their B.Sc./M.Sc. thesis research.
- At least 1,000,000 indirect beneficiaries in Cambodian who consume snakehead fish in their protein diets.

Research Designs or Activity Plan
(1) Location of work
All domestication breeding, feeding and weaning trials will be conducted at Freshwater Aquaculture Research and Development Center (FARDeC), Prey Veng province, Cambodia, which has sufficient facility such as broodstock, breeding and weaning earthen ponds, and a small fish feed mill for fish pellet production.

(2) Research methods
The domestication experimental study of the snakehead *Channa striata* will comprise four interrelated parts:

(a) Broodstock collection and fattening: Wild snakehead fish will be collected from different natural water bodies to fatten at FARDeC hatchery, Cambodia.
  Experiment 1: Effects of different diets to spawning performance
(b) Optimization of hormone injection:
  Experiment 2: Effects of different dosages of HCG to spawning performances
  Experiment 3: Effects of different injecting methods to spawning performances
(c) Larvae rearing and weaning to fingerling stage
  Experiment 4: Effects of different feeds (moina, small fish, formulated diets)
(d) Growing fingerling to broodstock
  Experiment 5: Effects of different protein levels of formulated pelleted diets to growth and survival rate of snakehead

Project period
3 years (2011-2014)

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DEVELOPMENT OF CULTURE TECHNIQUES OF SNAKESKIN GOURAMI (*TRICHOGASTER PECTORALIS*) FOR SMALL-SCALE RURAL FRESHWATER AQUACULTURE AND STOCK ENHANCEMENT IN THE PHILIPPINES (PPT Text)

*Felix Ayson and Evelyn Grace de Jesus Ayson

Aquaculture Department Southeast Asian Fisheries Development Center Tigbuauan, Iloilo, Philippines

General Objective:
To improve the breeding and seed production techniques, and develop nursery and grow-out culture techniques of snakeskin gourami (*Trichogaster pectoralis*) to support small-scale rural freshwater aquaculture and for stock enhancement of natural freshwater bodies in inland areas in the Philippines

Specific Objectives:
1. To determine location of freshwater bodies (lakes, rivers, reservoirs, impoundments, rice paddies) in Panay Island, Central Philippines where snakeskin gourami can still be found.
2. To document the annual reproductive cycle of snakeskin gourami present in the area.
3. To improve the breeding and seed production techniques of snakeskin gourami and come up with reliable techniques to ensure steady supply of fry in large quantities.
4. To develop appropriate technologies for nursery and grow-out culture of snakeskin gourami in inland freshwater bodies, small water impoundments, and in combination with traditional method of rice farming.
5. To develop practical diets for broodstock, nursery and grow-out culture of snakeskin gourami using indigenous materials or ingredients produced on-farm.
6. To replenish natural stocks of snakeskin gourami in inland freshwater bodies by restocking.

Expected Outcomes:
1. Information on the presence of snakeskin gourami in inland waters, rice paddies and other inland water impoundments in Panay Island, central Philippines will be available.
2. Information as to the probable cause of the disappearance or reduction in the natural population of gourami in Panay Island will be known.
3. Information on the annual reproductive cycle of snakeskin gourami in Panay Island will be known.
4. Improved captive breeding technology for snakeskin gourami will be developed.
5. Improved technology for the mass production of snakeskin gourami fry in the hatchery will be developed.
6. Technology for nursery and grow-out culture of snakeskin gourami will be established.
7. Practical diets using on-farm ingredients for all phases of culture of snakeskin gourami will be developed.
8. Natural population of snakeskin gourami in inland waters in Panay Island will be restored.

Initially, the promotion of snakeskin gourami for small-scale freshwater aquaculture in inland areas and the restocking of natural bodies of water with the species will be done in Panay Island, central Philippines, and in the future in the whole country.

PROPAGATION AND CULTURE OF SNAKE-SKINNED AND GIANT GOURAMIS IN THE PHILIPPINES

***Remedios B. Bolivar and Tereso A. Abella**

Central Luzon Sate University-Freshwater Aquaculture Center, Science City of Munoz, Nueva Ecija, Philippines

Background

The air-breathing fishes (ABFs) represent a unique group of fishes belonging to diverse genera, which have developed the ability to obtain oxygen from the air allowing them to survive in waters under anoxic conditions. These fishes represent over 40 genera under 14 orders belonging to either facultative air breathers (only supplementing gill respiration when necessary) or obligate air-breathers (utilizing atmospheric and water oxygen simultaneously) (Helman et al., 1997). These air-breathing fishes are distributed in the tropical areas of South America, Africa and Asia (Kramer and McClure, 1982).

The Philippines, as one of the tropical countries in Asia is home to a number of air-breathing fishes such as snake-skinned gourami, (*Trichogaster pectoralis*), climbing perch (*Anabas testudineus*), mudfish/snakehead fish (*Channa striata*), giant gourami (*Osphronemus goramy*), catfishes (*Clarias macrocephalus, C. batrachus,* and *C. garipeneus*). The potential for freshwater aquaculture of these ABFs in the Philippines is something to reckon with. While tilapia is the dominant cultured fish species in freshwater environment, the culture of ABFs can be popularized to exploit the vast swampy areas for immediate benefit of the people. The introduction of air-breathing fishes for culture will diversify the freshwater aquaculture in the Philippines. As they tolerate water bodies with low levels of oxygen, they are suitable for profitable culture in swamps, marshes and wetlands, which at present are not fully utilized.

The supply of these ABFs for food is generally sourced out from the wild with the exception of the *Clarias* species and that the continuous collection of these fish species from the wild is declining every year. The status of the ABFs in the Philippines necessitates a sustainable breeding and propagation of the species to support the demand for fingerlings to provide impetus for the culture of these ABFs particularly snake-skinned gourami, giant gourami, climbing perch and mudfish. The propagation of these species will also ensure the ex-situ conservation of the declining population of the aforementioned ABFs in the wild. This will also afford fish farmers to diversify their cultured fish in freshwater aside from the dominant tilapia thereby increasing the contribution of ABFs in the country's freshwater fish production. To achieve this, a concerted effort in terms of research and development with strong financial support must be provided.

Snake-skinned and giant gouramis will be the focal fish species in this proposed research because of its greater potential for culture in freshwater in the country and what is needed is an inventory of their populations in the different geographical regions and the availability of fingerlings to sustain the culture of these two ABFs.

Although the snake-skinned gourami is an introduced species, it has successfully established its population in swampy areas (i.e. Candaba Swamp) and became an important species in inland water fisheries. It is considered as the largest species among the genus *Trichogaster* found in the Philippines. This species has an appeal to Filipino fish consumers because of its good taste although the supply is seasonal because it is solely sourced out from the wild. On the other hand, giant gourami which is also an exotic species, has generated interest among fish farmers because its firm, straw-colored flesh and easy to digest. It also has a superior taste. On top of its palatability, this species also possesses the virtues for aquaculture. In the Philippines, giant gourami is highly esteemed and has been considered to compare favorably with that of the cultivated sea bass, *Lates calcalifer*. However the enthusiasm to grow this fish has waned because of the introductions of more favored cultured species like the popular Nile tilapia.

In the light of diversifying the fish species for culture, there is a need to look for alternative species for culture in freshwater environments. The ABFs, particularly the snake-skinned and giant gouramis that have already adapted to Philippine waters, are good candidates for freshwater aquaculture. Their entry to the aquaculture industry will allow fish farmers to diversify their products. What is needed is the development of technologies (hatchery and culture) or even adoption of existing technologies in relation to the propagation and culture of these species and thereby increasing more fish production from freshwater aquaculture.

The Freshwater Aquaculture Center at the Central Luzon State University in the Philippines has made some research endeavors in the study of ABFs as follows:

1. Artificial Propagation of Giant Gourami (*Osphronemus goramy, Lacepede*) Using Suprefact with Domperidone
2. Artificial Propagation of Snake-skinned Gourami (*Trichogaster pectoralis*)
3. Rice-Loach (*Misgurnus anguillicaudatus*) Culture in Sagada, Mountain Province
4. Induced Spawning of Climbing Perch (*Anabas testudineus*) using Carp Pituitary Extract (CPE) Hormone Under Laboratory Condition
5. Investigation on the Rearing/Nursing of *Anabas testudineu*s Fry
6. Induced Spawning of Climbing Perch using Suprefact in Conjunction with Domperidone under Philippine Condition
7. A Preliminary Study on the Egg Hatchability of the Climbing Perch Incubated Under Various Levels of Salt Concentration
8. Induced Spawning of Three-Spotted Gourami Using Single Dose and Double Injection of Carp Pituitary Extract (CPE)
9. Indoor and Outdoor Rearing/Nursing of Climbing Perch

For the proposed research, the objective is to develop and establish hatchery and culture techniques for snake-skinned gourami (SSG) and giant gourami (GG) under Philippine setting. The specific objectives are to:

1. Biologically characterize SSG and GG from different populations and from different hatchery sources;
2. Evaluate the growth performance of SSG and GG in different culture systems;
3. Demonstrate the potential of SSG and GG culture

Proposed Activities

1. Collection of SSG and GG samples from different populations
    - 1.1 Survey of areas in Central Luzon with abundant populations of SSG particularly in Candaba Swamp
    - 1.2 Biological marking of the collected SSG and GG samples for DNA identification which will make use of the bar coding technique
2. Breeding and propagation of SSG and GG
    - 2.1 Conditioning/rearing of fish breeders
    - 2.2 Natural and induced spawning of fish breeders
    - 2.3 Establishing hatchery techniques for each fish species
    - 2.4 Larval rearing techniques for each fish species
3. Culture of SSG and GG
    - 3.1 Monoculture (at different stocking densities)
        - o Organic farming
        - o Commercial system
    - 3.2 Polyculture
        - o Polyculture with tilapia and other species
        - o Culture in rice paddies
    - 3.3 Cage culture (at different stocking densities)

Expected Outcome

1. Increase on fish production from aquaculture
2. Significant contribution of SSG and GG in freshwater fish production
3. Increase in the number of fish farmers engaging in SSG and GG aquaculture
4. Development of technoguides for SSG and GG aquaculture in the Philippines

SUSTAINABLE FEED AND IMPROVED STOCKING DENSITIES FOR GAR (*ATRACTOSTEUS* SPP.) CULTURE

***James Diana[1], Solomon David[1], and Wilfrido Contreras[2]**

[1]University of Michigan, [2]UJAT

Introduction

Gars are a group of ancient air-breathing fishes that make up the family *Lepisosteidae*. The family consists of two genera, *Atractosteus* and *Lepisosteus*, and seven extant species. The genus Lepisosteus consists of the longnose gar (*L. osseus)*, shortnose gar (*L. platostomus*), spotted gar (*L. oculatus*), and Florida gar (*L. platyrhincus*); Atractosteus consists of the tropical gar (*A. tropicus*), Cuban gar (*A. tristoechus*), and alligator gar (*A. spatula*). Although the fossil record for gars exhibits a Pangeaic distribution, extant species are relegated to North & Central America and Cuba, and range from southern Canada (longnose gar) to Costa Rica (tropical gar).

Gars are top-level predators in their native ecosystems and are characterized by their elongate jaws, cylindrical bodies, and diamond-shaped ganoid scales. Their maximum size and age varies with species from approximately 80 cm and 10 years (shortnose gar) to 300 cm and over 70 years (alligator gar). Gars are generally polyandrous in reproductive strategy, with multiple male individuals spawning with 1-2 females. Gars spawn during late spring and early summer in temperate regions, and during the rainy season in tropical regions. Growth is extremely rapid, with all species capable of reaching 30 cm or more in their first growing season (young–of-the-year alligator gar can reach over 30 cm, 250 g in 3 months).

Aquaculture

Gars are excellent candidates for aquaculture as they exhibit rapid growth to large sizes, are highly resistant to disease, can be maintained at high densities, readily adapt to artificial feed at early life stages, and are highly tolerant of low water quality conditions due to their air-breathing abilities. Their tolerance of low water quality via aerial respiration also allows for a less complicated technological system for aquaculture, as opposed to other fishes, which may require considerable aeration and water turnover. Gars are therefore well suited for culture in developing regions.

Much progress has already been made in the aquaculture of *Atractosteus* gars (tropical, Cuban, alligator), primarily in regions of Mexico, Cuba, and the southern United States. Broodstock for all three species have been established and are currently maintained in their native regions, and juveniles have been released to help restock diminishing wild populations. Further efforts are being made in the southern US to protect alligator gar populations and manage them as a viable sport fishery, as well as increase its potential as a food fish. Gars are already popular food fish in various regions of Mexico and Cuba.

Due to their unique appearance and predatory nature, gars are becoming increasingly popular in the ornamental fish trade. Gars have been sought-after aquarium fish in Southeast Asia for many years, and are growing in popularity in the United States and other countries. The Florida gar, native to only a small portion of the southeastern United States, is the most popular aquarium species of gar in the US (and usually wild-caught) and most readily available abroad. Prices in the United States range from $15-$40 USD for 20-35 cm individuals. Other gar species at similar sizes command a much higher price largely due to their rarity in the aquarium hobby, such as $200 USD for an individual tropical gar and over $300 USD for a Cuban gar (in the United States). Tropical and Cuban gars are also highly valued overseas; in Singapore 15 cm tropical gars average $150 USD and Cuban gars $400 USD. Ironically, tropical and Cuban gars are among the most commonly cultured gar species. Specimens exhibiting genetic mutations in pattern or coloration (i.e. melanistic, xanthochroic, leucistic) command an even higher price, ranging from $1000 to over $5000 USD. Hybrid gars, although rare in the trade, are also much sought-after.

Research Concepts

In its efforts to successfully culture tropical gars for food and restocking of wild populations, Mexico has greatly increased the body of knowledge surrounding gar biology, ecology, and aquaculture. In contrast, little information is available on the culture of Cuban gars, and few papers on either species have been published in scientific literature. Even with the progress over the past two decades, there still remains much to be learned and developed for successful and sustainable gar culture.

We propose to investigate 3 major aspects of gar aquaculture with the goal of applying our findings to present and new operations in developing countries. Our studies will involve tropical and Cuban gars and will focus on further developing their (1) potential as food fish, (2) value and availability in the ornamental fish trade, and (3) better understanding their roles in native biodiversity.

Culture of tropical and Cuban gars is directly beneficial to their respective developing regions as a local source of protein, additional revenue to farms from sales to the ornamental fish trade, and restocking local wild populations to help conserve biodiversity.

With our current stocks of both species, we will be able to develop growth models based on different feeding, temperature, and stocking regimes using common environment experiments. Our primary experiments will investigate the following:

1. Determine fishmeal substitution using by-products (treatments 25, 50, 75 and 100% substitution).
2. Determine optimal densities for rearing tropical gars (treatments: 25, 50 and 100 fish/m3)

From these trials we hope to develop low-cost and environmentally friendly methods (such as using lower-fishmeal content feeds), for culture of tropical and Cuban gars in developing regions so they can be applied to all three major aspects listed above.

Research projects for tropical gars will be carried out by Wilfrido Contreras at UJAT in Tabasco, Mexico, and projects for Cuban gars will be carried out by James Diana and Solomon David at The University of Michigan in the United States. We will be using the same feed types and methodology for our gar culture projects. This preliminary research will allow for further development of similar studies on other gar species in the future.

Further Aquaculture Potential & Role as Food Fish

Because gars are air-breathers they may perform well in completely closed recirculating systems, potentially using less water for culture. Gars may also be cultured in systems with reduced or no additional aeration, further reducing energy consumption. Furthermore, gars from different latitudes may exhibit different growth rates (latitudinal variation) therefore specific populations may be better candidates for culture than others. By comparing our growth models with those from other regions (specifically with the wide-ranging tropical gar) we may determine the populations with the highest capacity for growth and therefore production in culture. There is also potential in culturing hybrid gars to take advantage of the faster growth of one species (i.e. Cuban gar), but managing fewer and/or younger broodstock of another species (tropical). These practices could be incorporated into existing operations to potentially increase efficiency, sustainability, and production, as well as making the technology for gar culture more accessible to developing regions.

Tropical* and Cuban** gars are already popular food fish in their respective regions, therefore demand already exists. Because of their fast growth rates to large sizes, individuals can reach market size after a single growing season. Increased productivity based on new research could also enhance potential export of these fishes as a food source, whether to neighboring regions or beyond.

Ornamental Fish Trade

In the ornamental fish trade, tropical and Cuban gars are the most expensive and sought-after species, and they are seldom available in the United States (where gars are becoming increasingly popular in the hobby). Ironically, these two species (along with the alligator gar) are cultured in greater numbers than any *Lepisosteus* gars, yet wild-caught Florida gars are the most abundant gar species in the US aquarium trade. Increased networking between aquaculture operations and ornamental fish suppliers could lead to additional revenue for gar farms as well as decrease pressure on potentially sensitive wild populations. Increased popularity and availability of gars in the ornamental fish trade would also lead to better public awareness of gars in general, potentially decreasing their needless extermination by anglers and others considering them merely trash fish. Increased public awareness by ornamental fish trade on a local level may also help develop further interest in sustainable culture practices as well as conservation efforts.

Role in Biodiversity

Successful and sustainable aquaculture of gars is also valuable from a biodiversity perspective. Culturing tropical, Cuban, and alligator gars has been useful in replenishing depleted wild stocks which have suffered due to overfishing and habitat loss. A tropical gar program in Mexico further involved the public by allowing elementary school children to raise juveniles to fingerlings and release them into native waters, therefore helping to conserve native biodiversity. Continued research on various aspects of gar biology and ecology provides a better understanding of their role in native ecosystems and can better inform conservation efforts. Few scientific papers have been published on Cuban and tropical gars, our studies would help fill a major void in the existing body of knowledge on gar ecology and culture.

Our proposed research would address all 3 of these major aspects of air-breathing fishes aquaculture, providing useful results for the culture of these fishes in developing regions as well as benefits in a global context. We currently have access to broodstock and juveniles needed for the proposed experiments, as well as the facilities (closed recirculating and flow-through systems) and experience to carry them out starting immediately.

*Tropical gar (*Atractosteus tropicus*) ranges from Mexico through Central America to Costa Rica.
**Cuban gar (*Atractosteus tristoechus*) is found in Cuba and the Isle of Pines.

EFFECTS OF EPIGENETIC PLASTICITY OF GILLS AND GAS BLADDER ON BIMODAL-BREATHERS, GARS (*LEPISOSTEUS* SP. AND/OR *ATRACTOSTEUS* SP.) AND PIRARUKU (*ARAPAIMA GIGAS*) INDUCED WITH HYPOXIA AND HYPEROXIA

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The long-term goal of this proposed research is to increase our understanding of what constitutes in aquatic environment phenotypic adaptations to changes in oxygen concentration, beyond sustaining growth. The growth response is implicitly related to the controlled aquaculture conditions, however the predictive value of oxygen saturation on growth can be extended to the interpretation of the results in the wild (ponds, estuaries) where variation in oxygen saturation are diurnal and seasonal. The design of the experimental matrix will allow us to distinguish between several components of the response, basic morphological changes, behavioral adjustments, and extended ecological consequences on survival of gars and piraruku.

We will address the following questions/hypotheses:

1. How oxygen stress caused by hypoxia or hyperoxia affects the development of gills and respiratory gas (swim) bladder (two respiratory organs in lepisosteid and osteoglossid fishes)? We further hypothesize that either organ can fulfill oxygen requirement in these species, however, major changes in differentiation of alternative respiratory epithelia will occur depend on the aerial (hypoxia) or water respiration (hyperoxia) predominance during early development, i.e. tissue differentiation.
2. We hypothesize that a significant change in oxygen level will enhance or limit the "scope for growth". Consequently, we will be able to estimate "metabolic efficiency" of two means of respiration, loss or gain due to conditions different than normoxia.
3. We hypothesize that fish is protected from oxygen stress by the integrated action of defense mechanisms on multiple levels, molecular-cellular-organ-organismal. Through a series of morphological studies at the tissue (light microscopy), cellular level (electron microscopy), and organ level (respiration) we will be able to couple growth (including muscle fibers) and metabolism to these responses at the organ, cellular, and subcellular levels.

This project will be performed with spotted gar (*Lepisosteus oculatus*) from Louisiana (Allyse Ferrara, Nicholls State University, Thibodaux, LA) USA, or tropical gar (*Atractosteus* sp.). Fish will be hatched and raised in laboratory of the School of Environment and Natural Resources, Ohio State University, Columbus. Alternatively, longnose gar (Ohio) may be used after an induced reproduction will be performed in (April-May) using matured females injected with LHRHa (Sigma Chem., St Louis, MO) (Jaroszewska et al. 2010). Embryos will be incubated in original tanks prepared for hatching (aeration, without flow), and hatched larvae (2-3 days at 26-28oC) will remain in the tanks for 4-5 days until yolk sac absorption is completed. By that time larvae achieve 10-12 mm in length and they will be divided and transferred to experimental rearing units.

Arapaima juveniles at the earliest stage of development will be obtained from the Só Peixe Fish Farm, Rondônia, Brazil, as described by Halverson (2008). Fish will be preserved and shipped to the Aquaculture Center, Jaboticabal, SP, where they will be maintained in open water flow tank system in normoxic conditions and fed with live prey (fish). We will sample fish during early ontogeny when gill respiration is dominant, at the older stages when aerial respiration becomes frequent (transitory phase), and later when it becomes obligatory (pirarauku). Behavioral observations (frequency of air gulping) will be recorded in order to relate those to morphological changes.

In the case of garfish, three major treatments will include hypoxic (30% oxygen saturation), normoxic and hyperoxic (180%) conditions. One oxygen regime will be assigned to 4 replicate tanks of fish. Normoxia conditions will be provided by simply running dechlorinated, aerated city water through a degassing column. Hyperoxia will be achieved by running fresh water through

an open, packed column supplied with pure oxygen. The ratio of water flow and oxygen volume will be established experimentally to secure continuous oxygen level at the outlet at 180% saturation at 26-28°C. Oxygen stripped water will be produced in a similar fashion, by supplying pure nitrogen gas into a third, identical packed column. In our experience the use of this type of gas exchange columns insures that the dissolved gas levels can be altered without creating total gas supersaturation, and thus prevent oxygen or nitrogen induced gas bubble disease. Oxygen concentrations will be monitored in each tank.
At the end of the feeding trial, growth performance will be evaluated in terms of individual body weight, survival, specific growth rate and weight gain as described earlier (Jaroszewska et al. 2010). Fish from each dietary treatment will be sampled for histological analysis. Respiratory organs, gills and gas bladder (GB) development and differentiation will be determined by histological analysis at approximately 30, 60 and 90 days (Jaroszewska and Dabrowski, 2008; 2009). The participation of hyperplasia and hypertrophy in muscle fiber development (Leitão et al. 2011) will be monitored in fish from the different oxygen regimes.

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AIR BREATHING FISHES OF KENYA: THE CASE OF MARBLED AFRICAN LUNGFISH (*PROTOPTERUS ETHIOPICUS*) (PPT Text)

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Goals:
1. Gain understanding of the biology of the lungfish in Lake Baringo
2. Provide baseline data for fishery management

Specific objectives:
1. To describe its life history characteristics
2. To elucidate its movement and space

Finding from Culture Trials:
1. Results showed that lungfish realized growth increments of 2.7 and 14.5 cm over time periods ranging from 70 to 238 days.
2. The mean absolute growth rate was 0.049 (±0.008 SE) cm day-1, whereas specific growth rates ranged from 0.048 to 0.140% day-1.
3. This study demonstrated that marbled lungfish can be raised in earthen ponds and we recommend that further research be carried out on their culture

IMPROVEMENT OF GROWTH AND SURVIVAL IN HATCHERY-PRODUCED LARVAE OF PACIFIC FAT SLEEPER DORMITATOR LATIFRONS

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Overview:
The Pacific Fat Sleeper, "chame" or "puyeque" (*Dormitor latrifons*) is a facultative air-breathing eleotrid fish with a wide geographical distribution in the tropical Eastern Pacific, extending from Mexico to Ecuador. Despite its relative abundance and potential for aquaculture, the species has limited aquaculture production in the region, although it is a fisheries species in some areas. Chame is particularly widely consumed and valued in Ecuador, where it has the status of a cultural icon. An important aspect of the Pacific Fat Sleeper is that has low market price at about US$1.6/kg, thus representing a potentially inexpensive source of animal protein in coastal rural areas. This fish provides an opportunity to diversify coastal aquaculture activities in most countries in the region, which heavily depend on marine shrimp as their most important aquaculture species. Recent devastating shrimp disease problems and the capital-intensive nature of the industry has accentuated the need to diversify the aquaculture. In contrast, the local government Manabi, Ecuador, has estimated that an investment of US$3,000 to $4,000 per hectacre of chame pond can produce a profit of $16,000 over a culture period of five months and disease are not prevalent.

Efforts to cultivate *D. latrifons* under controlled conditions in Ecuador (EcoCostas, 2006), showed promising results. It was demonstrated that this fish can be grown in earth ponds and fed on formulated aquaculture diets, either in monoculture or in polyculture with shrimp. These trials depended on stocking wild caught juveniles. A bottleneck was encountered when attempting to scale up culture activities in response to wide spread interest and demand by local farmers. The number of wild juveniles was insufficient to support expanded efforts, particularly as its wetland habitats had greatly diminished due to human activities. Future aquaculture efforts therefore depend on developing methods for induced spawning and larval rearing. Despite sporadic efforts in this direction by Ecuadoran researchers for twenty years, there was little success.

Thus recognizing the tremendous potential to develop this native specie for the Latin America region, developing reliable hatchery methods was a goal of Mexico AquaFish CRSP program 2009-2010 (AquaFish, 2010). Broodstock management under hatchery conditions allowed the production of thousands of viable eggs and larvae of *D. latrifons*. Diverse aspects on the larval feeding with live food are currently under study. However, constraints still exist to achieving optimal growth and survival of larvae. Total larval mortality occurs at day 7 post-hatch, as he fish apparently do not readily accept the live food offered at first-feeding (Rodriguez-Montes de Oca et al., unpublished). The project continues to study the most important feeding and nutrition factors affecting larval growth and survival of this for the optimization of larval and juvenile fish production in the hatchery. Simultaneously, studies on the local fisheries and possible need of fisheries management guidelines are also underway.

Background:
The Pacific Fat Sleeper, "chame" or "puyeque" (*Dormitor latrifons*) is a fish with a life cycle that includes a freshwater phase during its adult life, and a brackish water phase during the larval and early juvenile development (Bonifaz et al, 1985). This fish inhabits estuaries, stagnant ditches or creeks of low current velocity between the sea and an elevation of 30 m. It is benthic and most abundant on sandy and muddy bottoms. It ingests mud and detritus, although it also filters plankton (Bussing, 1998). It is found in shallow inshore areas; typically in freshwater but moves freely into the sea. Although it is not a true air-breathing species, *D. latrifons* can withstand long periods outside the water (Bonifaz et al, 1985).

The commercial fishery of the Pacific Fat sleeper in Mexico is mostly practiced in the southern part of the country, including the Guerrero, Oaxaca and Chiapas states. Official statistics on the fisheries of date back to 1985, with better capture records that provide an idea of the extent of consumption as recent as 2005, when it was reported an estimate of 350MT captured and commercialization at Guerrero state and Mexico City (CONAPESCA, 2005). Its market price at the largest seafood market at Mexico City was reported to be stable, with a price of US$6.00 per kilogram throughout 2007 and 2008 (PROFECO, 2008). It is noteworthy that the Mexican National Institute of Ecology does not include Pacific Fat Sleeper as an endangered species on its official norm NOM-059-ECOL-2001 (INE, 2008). Although it is not considered a threatened species despite an increasing capture effort over the last few years, there is concern in Ecuador at least, that wetland destruction has led to local extinctions of this species.

FAO reports aquaculture production for *D. latrifons* exclusively for Ecuador since 1987 until 2009, in terms of total production. Such values show an interesting trend of a remarkable high production back in 1993, close to 2,000 MT. However, the last data available indicates that reported production stabilized at 1000MT in 2008 and 2009. Local observations attribute the decline to the decimation of its wetland habitats. It is noticeable that the only country that appears in these statistics is Ecuador. For Mexico, this organization does not report statistics for the species either for aquaculture or fisheries (CONAPESCA, 2010).

Early research on this species focused on describing aspects such the distribution of the species in various locations in the Mexican Pacific coast (Yanes-Arancibia and Diaz-Gonzales 1977), its relevance as a temporary component of fish larval assemblages in several locations on the central-northern coast of the Mexican Pacific ocean (Franco-Gordo et al., 2002; Navarro-Rodriguez et al., 2004; Navarro-Rodriguez et al., 2006), the description of exploitation in a particular location near Acapulco, Guerrero (Gil Guerrero and Rojas Herrera, 2005), the depiction of the species as a component of associated macrofauna on shrimp farms in Sinaloa, Mexico (Hendrickx et al., 1996) and small scare growth-out experiments to evaluate weight gain under aquaculture conditions (Larumbe-Moran, 2002; Castro Rivera et al., 2005). In one of the first reports on *D. latrifons* culture in Mexico, it was concluded that the poor fish growth obtained was due to the limited acceptance of tilapia feed by the species when cultivated in freshwater (Larumbe-Moran, 2002). In a similar work, it was grown in concrete tanks and fed a 30% protein tilapia feed; mixed results were obtained and no sound conclusion could be made on the fish growth under adequate culture conditions (Castro Rivera et al., 2005). Other works have been published with the species in Ecuador (where efforts to cultivate fat sleeper have been done in the past) describing some technical aspects of its culture in brackish water pond (Velasco, 2001; Blacio-Game and Alvarez-Noboa, 2002; CORPEI, 2008). Due to the lack of hatchery-produced fish to supply fish farms in the region, *D. latrifons* culture has not yet filled the expectations as to its aquaculture potential.

In order to reduce the dependence on wild juveniles to stock fish farms, recent work sponsored by CRSP at the Autonomous University of Sinaloa (UAS), Mexico has focused on the induction of gonad and oocyte maturation under culture conditions (Rodriguez-Montes de Oca et al., 2009), and on larval feeding trials with diverse live food organisms (Medina-Hernandez, 2011). In these studies, the first feeding of the larvae after yolk sac absorption and the subsequent days were identified as the critical phase in the larviculture of the species because high fish mortality occurs, possibly due to inability of the fish the ingest the food because of its size, the relative abundance of food in the tanks, and/or the nutritional quality of the live food offered.

Proposed Research Activities

As follow-up to the CRSP-funded project "induced Spawning and larval rearing of the "chame" *Dormitor latrifons* in laboratory conditions," we aim to optimize the fish larvae growth and survival by improving the nutritional profile of live food though fatty acid enrichment protocols. In addition, with the use of brackish water copepods with a superiors nutritional profile compared to traditional live food organisms used in aquaculture (rotifers and *Artemia*), we expect to significantly improve larval fish survival, and reduce the time of weaning the fish larvae onto artificial microdiets.

Experimental and analytical work with fat sleeper larvae will be carried out in Mexico at the UAS under the supervision of Dr. Gustavo Rodriguez-Montes de Oca. Protocols for live food nutritional improvements, fish weaning, formulation and preparation of experimental artificial microdiets will be developed at UHH under the supervision of Dr. Armando Garcia-Ortega.

Its is expected that in a two year period after at least four experimental trials, we will be able to improve current larviculture protocols for the species, and obtain at least 15% fish survival at the post-weaning stages.

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OVERVIEW OF MARBLE GOBY (OXYELEOTRIS MARMORATA BLEEKER) CULTURE IN ASIA (PPT Text)

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Challenges In Marble Goby Culture

- The supply of fingerlings (about 50-100g) depends on the wild collection
- Lack of inputs, inappropriate feed, lack of appropriated culture techniques, poor feed formulation, feeding and poor management of cages and pond
- Disease is also one of the main factors causing the reduction in culture of this high valued fish

Prospect For Marble Goby Culture

- Marble goby fetch the high price:
- In Vietnam: about 200,000VND ~ 14 USD/kg
- In Thailand: 400 B – 600 B/kg ~ 10 – 15 USD/kg
- In Malaysia: 70 – 100 RM/kg
- Successful in seed production and larval rearing
- Basic study on feeding behavior of marble goby

Future Concerns

- Overview all studies on marble goby to produce a book:
- “Marble goby – biology and culture in Asia” (dedicate to our beloved Yang Yi)
- Reduce the collection of marble goby fingerlings from wild through improve nursing method
- Disease study

MARBLED AFRICA LUNGFISH (PPT Text)

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Why the African Lungfish?
- High-value fish that is native to East African lakes, rivers and wetlands
- Endangered fish in Uganda;
- Natural stocks rapidly declining mainly due to overexploitation, environmental degradation and the large-scale conversion of wetlands to agricultural land
- Biological control against bilharzias or schistosomiasis
- has other “medicinal” and cultural functions for some tribes
- Most literature examines lungfish ecology, fishery, biology, and physiology,
- Few studies treat its use as a food fish in aquaculture
- Study will apply what is known about this fish
- To explore potentials in improving food security and livelihoods in Uganda and Sub-Saharan Africa.

Research Questions
- How do we propagate or develop culture techniques for the African lungfish
- While addressing handling and harvesting issues
- Associated with a fish that has a “beak” like a snapping turtle, and,
- Has a tendency to burrow in the soil when a pond is drained?

Objectives
- Identify socio-economic conditions shaping the culture African Lungfish including prices, demand, and public perceptions,
- Conduct performance trial of collected “nestlings” of African lungfish cultured in ponds, cages and/ tanks,
- Develop harvest and handling procedures that ensure worker safety and protect yield,
- Identify simple fingerling production techniques for African lungfish.

IMPLEMENTATION PLANS FOR AIR BREATHING FISHES PILOT STUDIES (POST SYMPOSIUM)

After the 18 April Symposium, seven air breathing fishes pilot studies were funded by AquaFish CRSP for further investigation. The Implementation plans for these seven pilot studies are included in the following section.

SUSTAINABLE SNAKEHEAD (*CHANNA STRIATA*) AQUACULTURE DEVELOPMENT IN CAMBODIA

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Introduction

The striped snakehead *Channa striata* is an obligatory air breathing fish, which can survive dry season by burrowing in bottom mud of lakes, canals and swamps as long as skin and air-breathing apparatus remain moist and subsists on the stored fat. The snakehead inhabits ponds, streams and rivers, preferring stagnant and muddy water of plains and is found mainly in swamps, but also occurs in the lowland rivers, more common in relatively deep (1-2 m), still water. It feeds on fish, frogs, snakes, insects, earthworms, tadpoles and crustaceans. It undertakes lateral migration from the Mekong mainstream or other permanent water bodies, to flooded areas during the flood season and returns to the permanent water bodies at the onset of the dry season. The striped snakehead is commonly used for processing into pra-hoc, mam-ruot, and mam-ca-loc (varieties of fish paste) in Cambodia. It is very economic important on both cultures and captures throughout southern and southeastern Asia. The maximal total length published is 100 cm or maximal weight 3,000 g, but commonly found 60 cm (Vidthayanon 2002; Sokheng et al., 1999; Menon, 1999).

Significance

In Cambodia, over 70% of freshwater aquaculture production comes from floating cage culture (total: 4,492 fish cages) operated in Mekong basin, including the Tonle Sap Great Lake (43%), Tonle Sap River (17%), upper stretch of the Mekong River (19%), lower stretch of the Mekong River (14%) and Bassac River (7%) (So & Thuok, 1999). Snakehead aquaculture production represented more than 75% of total freshwater cage aquaculture production. It is entirely dependent on wild fish both as seed and feed (So et al., 2005; So et al., 2010). Feed represents more than 70% of the total operational cost and the main type of feed for wild snakehead culture is small-sized or small fish, including juvenile of commercially important fish species, which represent 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers (So et al., 2005). During the dry season (October to May), the most important source of feed is freshwater small-sized fish, while more marine small-sized or low value fish species are used during the rainy season (June to September) (So et al., 2005).

The government of Cambodia put a ban on snakehead farming in May 2005 and the reasons for this was the potential negative impacts on wild fish populations from wasteful snakehead seed collection and on other fish species diversity, and also potential negative effects on poor consumer groups from decreased availability of small-sized/low valued fish (So et al, 2007). After the ban on snakehead culture in Cambodia, snakeheads have illegally been imported from the neighboring countries, particularly from Vietnam, to supply high local market demands in Cambodia. Furthermore, the study showed that freshwater small-sized fish have illegally been exported to Vietnam for feeding the significantly and commercially developed snakehead aquaculture in Vietnam. The first phase study indicated that the incentives for choosing snakehead before other fish species by tens of thousands of fish farmers are strong as it generates more than 10 times higher profits than other fish species (So et al., 2010). Therefore, the ban does not only result in positive impacts on poor consumer groups from increased availability of freshwater small-sized fish in Cambodia, but also providing negative effects on livelihood of tens of thousands of snakehead farmers who depend on this livelihood for generating household income. In other words, these snakehead fish farmers have lost their important livelihoods and household income. Moreover, the ban also does not provide positive impacts on snakehead wild stocks as fishing pressure on wild snakehead using illegal and destructive fishing gears particularly electro-shockers has been increased for the recent years in order to supply local and external markets (So et al., 2010). During the second phase (2009-2011) of the AquaFish CRSP, wild broodstock of the snakehead has been developed and managed at the hatchery of Freshwater Aquaculture Research and Development Center (FARDeC) of IFReDI, and the first generation (F1) of snakehead fish are being grown from larvae to adult fish by feeding them with formulated pelleted diets adopted from Can Tho University (AquaFish CRSP snakehead formulated feed). F1 mature adult snakehead will be developed by the end of AquaFish CRSP Phase 2. The intention is to develop F4 or F5 snakehead broodstock for snakehead seed mass production, which can be fed with formulated diets, in order to lift the ban on snakehead aquaculture and to sustain this aquaculture industry in Cambodia without negatively affecting the small-fish populations. In order to successfully domesticate breeding and to develop sustainable snakehead aquaculture in Cambodia, genetic status of the wild snakehead *Channa striata* should be available.

Objectives

The overall objective of this Project is to develop a sustainable snakehead aquaculture industry in order to lift the ban on snakehead aquaculture by the Royal Government of Cambodia and to improve livelihoods of fish farmers in Cambodia.

The specific objectives of this investigation are as follows.

- To biologically characterize the snakehead *Channa striata* from different populations within Cambodia freshwater bodies (i.e. Tonle Sap Great Lake, upper and lower stretch of Mekong River and Bassac River, their associated floodplains) for determining good or favorable traits for aquaculture development.
- To assess genetic diversity and populations of snakehead collected from different locations within Cambodia for maintaining diversity of wild stocks and overall conservation of this species, and for enhancing the diversity of snakehead breeders when conducting domestication/breeding program for this fish.
- To domesticate breeding of wild snakehead to address the snakehead banning issue in Cambodia in order to lift the ban on snakehead culture in Cambodia.
- To develop practical formulated diets for broodstock, nursery and grow-out culture of snakehead to replace small-sized fish from captured fisheries.
- To evaluate the growth performance of snakehead in different culture systems by using practical diets.

Quantified Anticipated Benefits

- At least 20,000 farmers in Cambodia will benefit from this research by restarting their snakehead culture.
- 200 scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staff, and private sector working on the issues of snakehead aquaculture in Cambodia as well as in other Mekong riparian countries will be better informed, and have

better recommended policies and strategies for sustainable snakehead aquaculture.
- One graduate student will be supported and trained through his/her M.Sc. thesis research.
- At least 1,000,000 indirect beneficiaries in Cambodian who consume snakehead fish in their protein diets.

Activities

1. Identifying areas with existing population of the snakehead *Channa striata*;
2. Collecting wild breeders and fin clips of snakehead from different populations and from different freshwater water bodies within Cambodia bodies (i.e. Tonle Sap Great Lake, upper and lower stretch of Mekong River and Bassac River, their associated floodplains);
3. Studying good and favorable traits (e.g. growth rate, survival rate, disease resistance, higher tolerance to deterioration of water quality, etc.) for snakehead aquaculture development;
4. Assessing genetic variation of snakehead for management of genetic diversity of wild stocks and overall conservation of this species, and for enhancement of the diversity of snakehead breeders when conducting domestication/breeding program for this fish.
5. Generating information on the annual reproductive cycle of the species in the locality by making year-round monthly collection of snakehead from the field or from local markets;
6. Conducting domestication breeding and weaning of snakehead;
7. Studying the nutritional and environmental conditions for optimum reproductive performance of breeders, growth and survival of juvenile and adult snakehead;
8. Studying nutrition aspects for the development of practical formulated diets for broodstock, nursery and growth out culture locally available plant proteins to replace small-sized fish from captured fisheries;
9. Studying the growth performance of snakehead in different culture systems by using practical diets; and
10. Analyzing economic aspects of snakehead culture in different culture systems by using practical diets.

Project period

15 June – 30 September 2011

Note

The US$ 10,000 grant from AquaFish CRSP will be used initially for the following activities from June 15 to September 30 2011:

1. Identification of areas with existing populations of the snakehead *Channa striata*; and
2. Collection of breeders and collection of fin clips from different populations and from the five provinces in the Tonle Sap Great Lake: Kampong Chnang, Pursat, Battambang, Siem Reap and Kampong Thom for favorable traits studies (e.g. growth rate, survival rate, disease resistance, higher tolerance to deterioration of water quality, etc.) and for genetic characterization.

All the other activities listed in this proposal will be done once additional funds from AquaFish CRSP or/and other donors will be available in the future.

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DEVELOPMENT OF IMPROVED CULTURE TECHNIQUES ON GOURAMIS (*TRICHOGASTER PECTORALIS, T. TRICHOPTERUS* AND *OSPHRONEMUS GORAMY*) FOR SMALL-SCALE RURAL FRESHWATER AQUACULTURE, AQUARIUM INDUSTRY, AND STOCK ENHANCEMENT IN THE PHILIPPINES

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Objectives

The general objective of this proposal is to develop improved techniques for breeding, seed production, nursery and grow-out culture of snakeskin gourami (*Trichogaster pectoralis*), three-spot gourami (*T. trichopterus*) and giant gourami (*Osphronemus goramy*) to support small-scale rural freshwater aquaculture and aquarium industry in the Philippines.

The objectives of this study are to identify areas with existing populations of gouramis and to biologically characterize the collected fish samples using DNA bar coding.

The specific objectives are as follows:

1. To biologically characterize snakeskin gourami (*Trichogaster pectoralis*), three-spot gourami (*T. trichopterus*) and giant gourami (*Osphronemus goramy*) from different populations in the three islands in the country, namely; Luzon, Visayas and Mindanao
2. To develop appropriate technologies for nursery and grow-out culture of snakeskin, three-spot and giant gouramis in inland freshwater bodies, small water impoundments, and in combination with traditional and organic method of rice farming;
3. To evaluate the growth performance of snakeskin, three-spot and giant gouramis in different culture systems;
4. To develop practical diets for broodstock, nursery and grow-out culture of snakeskin, three-spot and giant gouramis using indigenous feed ingredients.

Significance

The air-breathing fishes represent a unique group of fishes belonging to diverse genera which have developed the ability to obtain oxygen from the air allowing them to survive in waters under anoxic conditions. These fishes represent over 40 genera under 14 orders belonging to either facultative air-breathers (only supplementing gill respiration when necessary) or obligate air-breathers (utilizing atmospheric and water oxygen simultaneously. These air-breathing fishes are distributed in the tropical areas of South America, Africa and Asia.

The Philippines, as one of the tropical countries in Asia, is home to a number of air-breathing fishes such as gouramis, (*Trichogaster pectoralis, T. trichopterus* and *Osphronemus goramy*), climbing perch (*Anabas testudineus*), snakehead fish (*Channa striata*), catfish (*Clarias macrocephalus*) among others. While the gouramis were introduced species, they have already established populations in the country and being herbivores, they are not considered threat to the indigenous species. However, the population of gouramis in the country is dwindling as claimed by many farmers which we need to confirm through this proposed study. There are reports that these fishes that were commonly present in freshwater bodies are now difficult to find in the Philippines. The introduction and proliferation of non-native and carnivorous freshwater fishes like *Clarias batrachus* and *C. gareipinus* and the use of chemicals like pesticides and weedicides in rice farming and other agricultural activities are implicated as the probable causes for the disappearance or the reduction in the population of such fish species in natural freshwater bodies.

People in inland areas find the gouramis an esteemed food fish because of their delicious flavor. For instance, the snakeskin gourami has an appeal to Filipino fish consumers because of its good taste although the supply is seasonal because it is presently only sourced out from the wild. The three-spot gourami is famous for the aquarium industry. However, their disappearance from the rice paddies and other habitats is unfortunate especially to the sustenance rice farmers. Mass production of these species under captive condition will help restore the population of these important resources. Natural freshwater bodies, small water impoundments and reservoirs in inland areas may be re-stocked with this species to re-populate them. Rice farming areas employing the traditional method and organic way of growing rice will be very important in this regard since chemicals are not used in them. On the other hand, giant gourami has generated interest among fish farmers because its superior taste, firmness and straw-colored flesh.

The Philippines has about 750,000 ha of inland resources, almost half of this (370,859 ha) are freshwater areas (Philippine Fisheries Profile, 2005). These areas can be maximally utilized for aquaculture. Snakeskin, three-spot and giant gouramis are very good candidate species for rural freshwater aquaculture since aside from being food fish, they are also important for the aquarium trade industry. They are hardy, relatively easy to breed and omnivores. The culture of gouramis can be popularized to exploit the vast swampy areas for immediate benefit of the people. The introduction of gouramis will diversify the freshwater aquaculture in the Philippines. Since they can tolerate freshwater bodies with low levels of oxygen, they are suitable for culture in swamps, marshes and wetlands which at are not fully utilized at present.

Methodology
In the identification of areas with existing population of gouramis, field survey and interviews with local farmers will be done.
Collection of gourami samples will be done in the three big islands of the Philippines (Luzon, Visayas and Mindanao)

Activities
1. Identification of areas with existing populations of gouramis
2. Biological marking of the collected gourami fish samples from different populations and from the three island in the country, namely, Luzon, Visayas and Mindanao for DNA identification using the bar coding technique
3. Generation of information on the annual reproductive cycle of the species in the locality by making a year-round monthly collection of gouramis from the field or from local fish markets
4. Collection of live gourami breeders to start breeding and seed production activities in captivity
5. Induced spawning on gouramis will be done when applicable and then seed production trials will be done
6. Studies to determine the nutritional and environmental conditions for optimum reproductive performance of breeders, growth and survival of juveniles and adult gouramis
7. Nutritional studies for the development of practical diets for broodstock, nursery and grow-out culture using locally available feed ingredients
8. Polyculture of gouramis with tilapia
9. Culture of gouramis in rice paddies

Expected Outcome
1. Significant contribution gouramis in freshwater fish production
2. Increase in the number of fish farmers engaging in gourami aquaculture
3. Information on the availability/presence of gouramis in inland waters, rice paddies and other inland water impoundments in the three islands in the country, namely, Luzon, Visayas and Mindanao
4. Information on the probable cause of the disappearance or reduction of the natural population of gouramis will be known
5. Information on the annual reproductive cycle of gouramis will be known

6. Improved technology on the captive breeding of gouramis will be developed
7. Improved technology for the mass production of gourami fry in the hatchery will be developed
8. Technology for nursery and grow-out culture of gouramis will be established
9. Practical diets using indigenous ingredients for all phases of culture of gouramis will be available
10. Information on the genetic characterization of selected population of gouramis in the Philippines will be available
11. Development of technoguides for gourami aquaculture in the Philippines

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SUSTAINABLE FEED AND IMPROVED STOCKING DENSITIES FOR GAR (*ATRACTOSTEUS* SPP.) CULTURE

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Objectives:

1. To determine the degree to which by-products can be substituted for fish meal (treatments 25, 50, 75 and 100% substitution) in culture of tropical (*Atractosteus tropicus*) and Cuban gars (*A. tristoechus*).
2. To determine optimal densities for rearing tropical gars (treatments 25, 50 and 100 fish/m3)

Significance:
Gars are a group of ancient air-breathing fishes that make up the family *Lepisosteidae*. The family consists of two genera, *Atractosteus* and *Lepisosteus*, and seven extant species. The genus *Lepisosteus* consists of the longnose gar (*L. osseus*), shortnose gar (*L. platostomus*), spotted gar (*L. oculatus*), and Florida gar (*L. platyrhincus*); *Atractosteus* consists of the tropical gar (*A. tropicus*), Cuban gar (*A. tristoechus*), and alligator gar (*A. spatula*). Although the fossil record for gars exhibits a Pangeaic distribution, extant species are relegated to North & Central America and Cuba, and range from southern Canada (longnose gar) to Costa Rica (tropical gar) (Suttkus 1963, Wiley 1976).

Gars are top-level predators in their native ecosystems and are characterized by their elongate jaws, cylindrical bodies, and diamond-shaped ganoid scales. Their maximum size and age varies with species from approximately 80 cm and 10 years (shortnose gar) to 300 cm and over 70 years (alligator gar). Gars are generally polyandrous in reproductive strategy, with multiple male individuals spawning with 1-2 females. Gars spawn during late spring and early summer in temperate regions, and during the rainy season in tropical regions. Growth is extremely rapid, with all species capable of reaching 30 cm or more in their first growing season (young–of-the-year alligator gar can reach over 30 cm, 250 g in 3 months).

Aquaculture
Gars are excellent candidates for aquaculture as they exhibit rapid growth to large sizes, are highly resistant to disease, can be maintained at high densities, readily adapt to artificial feed at early life stages, and are highly tolerant of low water quality conditions due to their air-breathing abilities (Alfaro et al. 2008). Their tolerance of low water quality via aerial respiration also allows for a less complicated technological system for aquaculture, as opposed to other fishes which may require considerable aeration and water turnover. Gars are therefore well-suited for culture in developing regions.

Much progress has already been made in the aquaculture of *Atractosteus* gars (tropical, Cuban, alligator), primarily in regions of Mexico, Cuba, and the southern United States. Broodstock for all three species have been established and are currently maintained in their native regions, and juveniles have been released to help restock diminishing wild populations. Further efforts are being made in the southern US to protect alligator gar populations and manage them as a viable sport fishery, as well as increase its potential as a food fish. Gars are already popular food fish in various regions of Mexico and Cuba.

Due to their unique appearance and predatory nature, gars are becoming increasingly popular in the ornamental fish trade. Gars have been sought-after aquarium fish in southeast Asia for many years, and are growing in popularity in the United States and other countries. The Florida gar, native to only a small portion of the southeastern United States, is the most popular aquarium species of gar in the US (and usually wild-caught) and most readily available abroad. Prices in the United States range from $15-$40 USD for 20-35 cm individuals. Other gar species at similar sizes command a much higher price largely due to their rarity in the aquarium hobby, such as $200 USD for an individual tropical gar and over $300 USD for a Cuban gar (in the United States). Tropical and Cuban gars are also highly valued overseas; in Singapore 15 cm tropical gars average $150 USD and Cuban gars $400 USD. Ironically, tropical and Cuban gars are among the most commonly cultured gar species. Specimens exhibiting genetic mutations in pattern or coloration (i.e. melanistic, xanthochroic, leucistic) command an even higher price, ranging from $1000 to over $5000 USD. Hybrid gars, although rare in the trade, are also much sought-after.

Research Concepts
In its efforts to successfully culture tropical gars for food and restocking of wild populations, Mexico has greatly increased the body of knowledge surrounding gar biology, ecology, and aquaculture. In contrast, little information is available on the culture of Cuban gars other than an outline of spawning methodology and very early-stage development (i.e. 0-28 days after hatch), and few papers on either species have been published in scientific literature (Comabella et al. 2006, Alfaro et al. 2008, Comabella et al. 2010, Comabella personal communication). Even with the progress over the past two decades, there still remains much to be learned and developed for successful and sustainable gar culture.

We propose to investigate 3 major aspects of gar aquaculture with the goal of applying our findings to present and new operations in developing countries. Our studies will involve tropical and Cuban gars and will focus on further developing their (1) potential as food fish, (2) value and availability in the ornamental fish trade, and (3) better understanding their roles in native biodiversity.

Culture of tropical and Cuban gars is directly beneficial to their respective developing regions (Mexico and Cuba) as a local source of protein, additional revenue to farms from sales to the ornamental fish trade, and restocking local wild populations to help conserve biodiversity.

Quantified Anticipated Benefits:
Further Aquaculture Potential & Role as Food Fish
Because gars are air-breathers they may perform well in completely closed recirculating systems, potentially using less water for culture. Gars may also be cultured in systems with reduced or no additional aeration, further reducing energy consumption. Furthermore, gars from different latitudes may exhibit different growth rates (latitudinal variation) therefore specific populations may be better candidates for culture than others. By comparing our growth models with those from other regions (specifically with the wide-ranging tropical gar) we may determine the populations with the highest capacity for growth and therefore production in culture. There is also potential in culturing hybrid gars to take advantage of the faster growth of one species (i.e. Cuban gar), but managing fewer and/or younger broodstock of another species (tropical). These practices could be incorporated into existing operations to potentially increase efficiency, sustainability, and production, as well as making the technology for gar culture more accessible to developing regions.

Tropical* and Cuban** gars are already popular food fish in their respective regions, therefore demand already exists. Because of their fast growth rates to large sizes, individuals can reach market size after a single growing season. Increased productivity based on new research could also enhance potential export of these fishes as a food source, whether to neighboring regions or beyond.

Ornamental Fish Trade
In the ornamental fish trade, tropical and Cuban gars are the most expensive and sought-after species, and they are seldom available in the United States (where gars are becoming increasingly popular in the hobby). Ironically, these two species (along with the alligator gar) are cultured in

greater numbers than any Lepisosteus gars, yet wild-caught Florida gars are the most abundant gar species in the US aquarium trade. Increased networking between aquaculture operations and ornamental fish suppliers could lead to additional revenue for gar farms as well as decrease pressure on potentially sensitive wild populations. Increased popularity and availability of gars in the ornamental fish trade would also lead to better public awareness of gars in general, potentially decreasing their needless extermination by anglers and others considering them merely trash fish. Increased public awareness by ornamental fish trade on a local level may also help develop further interest in sustainable culture practices as well as conservation efforts.

Role in Biodiversity
Successful and sustainable aquaculture of gars is also valuable from a biodiversity perspective. Culturing tropical, Cuban, and alligator gars has been useful in replenishing depleted wild stocks which have suffered due to overfishing and habitat loss. A tropical gar program in Mexico further involved the public by allowing elementary school children to raise juveniles to fingerlings and release them into native waters, therefore helping to conserve native biodiversity. Continued research on various aspects of gar biology and ecology provides a better understanding of their role in native ecosystems and can better inform conservation efforts. Few scientific papers have been published on Cuban and tropical gars, our studies would help fill a major void in the existing body of knowledge on gar ecology and culture.

Our proposed preliminary research would lay the groundwork to address all 3 of these major aspects of air-breathing fishes aquaculture, providing useful results for the culture of these fishes in developing regions as well as benefits in a global context. We currently have access to broodstock (tropical gars) and juveniles (tropical and Cuban gars) needed for the proposed experiments, as well as the facilities (closed recirculating and flow-through systems) and experience to carry them out starting immediately.

Deliverables on this preliminary research will include at least two peer-reviewed papers as well as at least one article for Aquanews to present results to a more general audience. The resulting growth models based on different feed types will also provide the basis for further experiments on other gar species and gar culture in general.

*Tropical gar (*Atractosteus tropicus*) ranges from Mexico through Central America to Costa Rica.
**Cuban gar *(Atractosteus tristoechus*) is found in Cuba and the Isle of Pines.

Research Design:
With our current access to stocks of both species, we will be able to develop growth models based on different feeding, temperature, and stocking regimes using common environment experiments. Our primary experiments will investigate the following:

1. Determine the degree to which by-products can be substituted for fish meal in feed (treatments 25, 50, 75 and 100% substitution; we have already determined approximate 0% substitution levels for Cuban gars using live fish as feed.) Cuban gar treatments will consist of approximately 3 replicates per treatment using 4-5 fish per replicate*.
2. Determine optimal densities for rearing tropical gars (treatments: 25, 50 and 100 fish/m3)

Fishes used in both experiments will be approximately 20-30 cm in length (fingerling size). Both species of gars will be maintained in recirculating system environments for experiments. Fish length and weight will be measured weekly to determine growth over the course of the experiments. From these growth data we will determine optimal feed types and stocking densities. From these trials we hope to develop low-cost and environmentally-friendly methods (such as using lower-fishmeal content feeds), for culture of tropical and Cuban gars in developing regions so they can be applied to all three major aspects listed above. Cuban gars will be maintained at The University of Michigan for further research projects as well as for establishing broodstock for future work.

Research projects for tropical gars will be carried out by Wilfrido Contreras-Sanchez at UJAT in Tabasco, Mexico, and projects for Cuban gars will be carried out by James Diana and Solomon David at The University of Michigan in the United States. We will be using the same feed types and methodology for our gar culture projects. This preliminary research will allow for further development of similar studies on other gar species in the future.

*We recognize that these are relatively low numbers for replication, but such is the cost of working with this rare (and expensive) fish for which little information on juvenile and sub-adult growth exists in current literature. Furthermore, what little is available on sub-adult and adult Cuban gars in the literature is based on very few specimens (Comabella, personal communication). We believe even with low replication we will produce useful preliminary data on Cuban gar growth at these stages under varying feeding regimes. All resulting information from this research will significantly contribute to filling in the many and large gaps in the literature regarding this species.

Schedule

1 June 2011 to 30 August 2011
Report submission: no later than 29 September 2011.

Schedule includes:

- Acquire and pellet-train juvenile gars – June 1 – June 21 2011
- Initial feed/growth measurements for conventional feed – June 1- June 21 2011
- Begin growth trials using alternative feed types – July 1-August 1 2011
- Begin growth trials using different stocking densities – June 14- August 1 2011 (UJAT)
- Develop growth models based on alternative feed types – through September 2011
- Preparation of final reports – through 29 September 2011

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EFFECTS OF ENVIRONMENTAL CONDITIONS ON GILLS AND GAS BLADDER DEVELOPMENT IN BIMODAL-BREATHERS, GAR (*LEPISOSTEUS* SP.), PIRARUCU (*ARAPAIMA GIGAS*) AND BOWFIN (*AMIA CALVA*)

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Introduction

Response to hypoxia or hyperoxia in tropical and temperate fish and production

At the organismal level, environmental hypoxia or hyperoxia will involve similar interaction of chemoreceptors, the respiratory control in brain, general metabolism, growth, behavior, and most importantly morphological adaptations. However, at the tissue level, it is uncertain if respiratory epithelium in fish (gills and gas bladder) in response to hypoxic or hyperoxic conditions will differ. At the cellular level, oxygen-sensing system by mitochondria was well documented in mammals, however, debate continues among fish physiologists due to the diverse responses in aquatic animals. In this context, information obtained from fish models will advance basic knowledge in general biology and integrated biology of aquatic animals with diverse respiratory options. Much can be learned about the metabolic state, and scope of growth when coupled with studies on effect of oxygen level in relation to development (Roesner et al., 2006) and tissue differentiation (Matschak et al., 1997). This aspect of oxygen availability can be linked to changes in fish growth in aquaculture (Dabrowski et al., 2003).

In contrast to terrestrial animals, aquatic animals face an ever changing availability of environmental oxygen, resulting from the dynamics of temperature changes, surface agitation, primary production by plants and algae, and consumption by plants, animals and chemical processes. There is not only a lack of sufficient oxygen in large ecosystems such as parts of the Amazon River (MacCormack et al., 2003) or Andean, high altitude rivers and lakes (Villafane et al., 2004), but also oxygen supersaturation can result in sudden fish mortalities in natural environments. Oxygen levels exhibit a diurnal cycle of depletion during the night, and a supersaturation levels up to 300% during the day (Boyd et al., 1994). To maximize fish growth by manipulating oxygen concentrations, supersaturation may be used to increase fish production (Dabrowski et al. 1994). Aquatic hypoxia has been cited as the primary driving force in the evolution of air breathing in fish. Numerous studies have supported this hypothesis, by characterizing the capacity of air-breathing fishes to survive extreme aquatic hypoxia (see review by Dabrowski and Guderley, 2002; Burleson at al., 1998). Air breathing may confer a high degree of independence from water quality to the metabolic scope for activity and the ability to recover. Thus, air-breathing fish may not simply survive aquatic hypoxia but may also maintain normal levels of activity when branchial O2 uptake is limited. Phenotypic plasticity results in morphological diversification, expansion of the species to new habitats starting an “evolutionary cascade” of reproductive genetic isolation. Morphological plasticity can be directly linked to genetic isolation in different habitats, normoxic versus hypoxic, and this consequently contributes to the speciation rate (Barluenga et al., 2006). We argue that phenotypic plasticity will be less constrained in early life history, during morphogenesis, metamorphosis of organs and systems, and consequently enhance variation (plasticity). Irrespective of the results of our experimentation, observation of respiratory system development will be directly related to the conditions in natural habitats of these species (gars and pirarucu) and possibly explain inter annual variation/resistance to hypoxia and growth rates in the wild.

Why arapaima, garfish and bowfin?

Garfish

The garfish is a unique model for investigating responses to hypoxia and hyperoxia. This highly tolerant fish can survive a wide range of stressful conditions, from anoxia to hyperoxia because of the presence of gills and respiratory gas bladder (RGB) (Graham, 1997). A. tropicus, the tropical garfish, L. oculatus, the spotted gar, and the longnose gar L. osseus are facultative air-breathing fish. For this reason, it would be possible to use any one of these species in order to address question of different oxygen conditions (hypoxia, hyperoxia) in the environment as well

as to examine adaptation of the gas bladder and gills to effectively exchange gas and most energetically efficient means of respiratory cost/benefit.
In normally aerated water, at 200C, gar accounted for 42% of their oxygen metabolism from their gas bladder. Aerial ventilation increased 1150% and was accompanied by an elevation of pulmonary perfusion in hypoxia. It has been shown that L. oculatus actually loses oxygen from their gills in hypoxic water. The gills are ineffective as oxygen uptake organs when severe levels of hypoxia were tested (Burleson et al., 1998). The exhaustive activity in hypoxic condition determined the primary role of the air-breathing organ in supporting active metabolism and recovery. With a gas bladder that can support the metabolic scope equivalent to both, gas bladder and gills, gar can maintain activity under hypoxic condition that incapacitate virtually every other temperate climate fish that relies on aquatic respiration (Burleson at al., 1998).

Pirarucu
Arapaima as adults are an obligate air-breathing teleost (Stevens and Holeton, 1978). Adult pirarucu of 2-3 kg ventilates their gills 16-24 times per minute and replaces air in their lung every 1-2 min (Stevens and Holeton, 1978). Despite that, about 75% of their oxygen consumption comes from air and 63% of carbon dioxide is excreted via gills. Brauner et al. (2004) and Gonzalez et al. (2010) analyzed gill structure in pirarucu in the size range of 10-1,000 g and concluded that the secondary lamellae respiratory surface disappear between 67 and 110 g body weight. However, in none of those studies was the respiratory gas bladder examined during the transition from water to air breathing. The effect of environmental conditions (oxygen saturation) on the morphological changes and related growth rate (scope for metabolism and activity) was not analyzed in pirarucu ontogeny. Impact of this ontogenetic change from "aquatic" to "terrestrial" respiration mode is critical to aquaculture production capacity and fish growth rate.

Bowfin
In Amia exposed to temperatures below 10oC, air breathing becomes negligible whereas at 32.4oC, 20-30 breaths per hour were recorded (Horn and Riggs, 1973). At 20oC, gills contribute to approximately 65% of the total oxygen uptake, whereas at 30oC, bowfin becomes an obligate air breather with diminished gill ventilation and proportion of the air oxygen fulfils over 70% of oxygen demand (Johansen et al., 1970). Aquatic hypoxia significantly increases air breathing frequency (Hendrick and Jones, 1993).

Therefore, we conclude that bowfin when maintained at water temperatures of 26-30oC would be an ideal surrogate species for pirarucu in order to study responses of respiratory tissues to changes in environmental conditions. No studies were performed to our knowledge on the ontogenetic changes in gill and gas bladder morphology in this species despite extensive research of evolutionary morphologists and embryologists in the 1900s (Ballard, 1984).

Research Concept

The long-term goal of this proposed research is to increase our understanding of what constitutes in aquatic environment as phenotypic adaptations to changes in oxygen concentration, beyond sustaining growth. The growth response is implicitly related to the controlled aquaculture conditions, however the predictive value of oxygen saturation on growth can be extended to the interpretation of the results in the wild (ponds, estuaries) where variation in oxygen saturation are diurnal and seasonal. The design of the experimental matrix will allow us to distinguish between several components of the response, basic morphological changes, behavioral adjustments, and extended ecological consequences on survival of gars and pirarucu.

AquaFish CRSP Topic Areas of Study - Indigenous Species Development
We will address the following questions/hypotheses:
(1) How oxygen stress caused by hypoxia or hyperoxia affects the development of gills and respiratory gas (swim) bladder (the two respiratory organs in lepisosteid, amiidae and osteoglossid fishes)? We further hypothesize that either organ can fulfill oxygen requirement in these species,

however, major changes in differentiation of alternative respiratory epithelia will occur depend on the aerial (hypoxia) or water respiration (hyperoxia) predominance during early development, i.e. tissue differentiation.
(2) We hypothesize that a significant change in oxygen level will enhance or limit the "scope for growth". Consequently, we will be able to estimate "metabolic efficiency" of two means of respiration, loss or gain due to conditions different than normoxia.
(3) We hypothesize that fish are protected from oxygen stress by the integrated action of defense mechanisms on multiple levels, molecular-cellular-organ-organismal. In further studies, beyond the scope of the current proposal, through a series of morphological studies at the tissue (light microscopy), cellular level (electron microscopy), and organ level (respiration) we will be able to couple growth (including muscle fibers) and metabolism to these responses at the organ, cellular, and subcellular levels.

Our primary experiments will investigate the following:
1) Determine morphological structure of gills and respiratory bladder in young-of-year garfish and pirarucu .
2) Determine ontogenetic changes in the development of gills and respiratory bladder in bowfin larvae and juveniles in order to establish a model species for air-breathing fishes.

This project will be performed with spotted gar (Lepisosteus oculatus) from Louisiana (Allyse Ferrara, Nicholls State University, Thibodaux, LA, USA). Fish were hatched and raised in laboratory of the School of Environment and Natural Resources, Ohio State University, Columbus. Embryos were incubated in original tanks prepared for hatching (aeration), and hatched larvae (2-3 days at 26-28oC) remained in the tanks for 4-5 days until yolk sac absorption was completed. By that time larvae achieve 10-12 mm in length and swam-up they were divided and transferred to experimental rearing units.

Pirarucu juveniles at the earliest stage of development will be obtained from the Só Peixe Fish Farm, Rondônia, Brazil, as described by Halverson (2008). Fish will be shipped to the Aquaculture Center, Jaboticabal, SP, where they will be maintained in open water flow tank system in normoxic conditions and fed with live prey (fish). In the proposed study we will concentrate on sampling pirarucu of 3-6 months old. If available, in the follow up studies we will sample fish during early ontogeny when gill respiration is dominant, at the older stages when aerial respiration becomes frequent (transitory phase), and later when it becomes obligatory (pirarucu). Behavioral observations (frequency of air gulping) will be recorded in order to relate those to morphological changes.

In the case of bowfin, three major treatments will be used: hypoxic (40% oxygen saturation), normoxic and hyperoxic (180%) conditions. One oxygen regime will be assigned to 4 replicate tanks. Normoxia conditions will be provided by simply running dechlorinated, aerated city water through a degassing column. Hyperoxia will be achieved by running dechlorinated city water through an open, packed column supplied with pure oxygen. The ratio of water flow and oxygen volume will be established experimentally to secure continuous oxygen level in the tanks at 180% saturation at 18-20°C. Oxygen stripped water will be produced in a similar fashion, by supplying pure nitrogen gas into tanks equipped with identical packed column. In our experience, the use of this type of gas exchange columns insures that the dissolved gas levels can be altered without creating total gas supersaturation, and thus prevent oxygen or nitrogen induced gas bubble disease. Oxygen concentrations will be monitored daily in each tank.

At the end of the feeding trial, growth performance will be evaluated in terms of individual body weight, survival, specific growth rate and weight gain as described earlier for garfish (Jaroszewska et al., 2010). Fish from each dietary treatment will be sampled for histological analysis. Respiratory organs, gills and gas bladder (GB), development and differentiation will be determined by histological analysis at approximately 10-30 day intervals (Jaroszewska and Dabrowski, 2008; 2009;

Satora, 1998). The participation of hyperplasia and hypertrophy in muscle fiber development (Leitão et al., 2011) will be monitored in fish from the different oxygen regimes.

Further Aquaculture Potential and Role as Food Fish

Arapaima, commonly known in Peru as paiche (Spanish) and in Brazil as pirarucu, is the largest freshwater teleost fish in the world (measuring up to 1.8 m long and weighing up to 250 kg) and has been listed in the 2000 IUCN Red List of Threatened Species since 1996 (Hilton-Taylor, 2000). It is naturally found only in the Amazon and Essequibo River basins surrounding Peru, Guyana and Brazil. In the last 30 years, illegal fishing and poaching have drastically reduced populations of Arapaima in their local habitats. Studies on artificial propagation of this fish are important to alleviate the condition of poverty-stricken rural areas by providing food and a source of income for the Amazon people (Gram et al., 2001). Being at the top of the food web, maintaining pirarucu populations in the wild will also contribute toward ecological balance in the Amazon rainforest. As reported in January 2007 Christian Science Monitor, culture of paiche (pirarucu) in Peruvian Amazon may be a viable alternative for coca (processed for cocaine) farmers, and USAID has already contributed $250,000 and is partnering with local organizations in the Amazon-Ucayali region towards the costs of the program to "grow endangered fish rather than endangering drugs". The subsistence fishery is an important social activity, considering the high daily fish consumption in Amazon (550g/ per capita), and could represent about 60% of the total fisheries production in the region (Freitas and Rivas, 2006). In Brazil, a governmental program promotes conservation and management of Arapaima populations in Unites of Conservation in Amazon, where fisher families protect the lagoons containing breeding pairs of fish. The number of arapaima individuals is evaluated annually (possible because being an air-breathing fish, it comes periodically to the water´ surface) and families are authorized to capture up to 30% of the adult fish (>1.5 m). Recently the Ministry of Fisheries and Aquaculture in Brazil created the "Amazon Project Aquaculture and Fisheries: Sustainable Development Plan". The goal is to stimulate the production of fish in captivity and limited pay fishing activity so as to balance the capture of native species.

Role in Biodiversity

Considering that fish is a major part of the diet of Amazon communities (Brazil and Peru), studies on aquaculture of fishes will allow these communities to maintain their consumption without overfishing natural populations, and in effect, promote the utilization and conservation of wild stocks in the Amazon rainforest to maintain biodiversity. Controlled culture of pirarucu may also considerably limit illegal international trade of this and other osteoglossid species (Matsumura and Millikin, 1984).

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IMPROVEMENTS IN SPAWNING CONTROL, LARVAL GROWTH AND SURVIVAL OF PACIFIC FAT SLEEPER *DORMITATOR LATIFRONS*

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Justification

Dormitator latifrons ("chame" in Ecuador) is found extensively along the Pacific Coast of Latin America from northern Mexico to Peru. It has long been cultured in Ecuador, and is consumed in other countries, mainly by poor coastal or indigenous inhabitants with few resources. It is also the basis of a commercial fishery in Mexico. Chame is locally scarce in some areas due to overfishing. In areas where it is cultured, previous development efforts were stymied by a shortage of wild fingerlings as chame spawns naturally only 4-6 weeks per year. Moreover its natural wetland habitats are disappearing. The controlled hatchery production of eggs and larvae, and the poor growth and survival during the larviculture of *Dormitator latifrons* remain as the bottleneck for the expansion in the culture of this species. If reliable hatchery methods are developed, chame has excellent potential to rival tilapia as a common culture species in Latin America, and has the added advantage of being a native species and accepted food fish.

In this project we aim to test the manipulation of light and temperature under captive conditions, in combination with hormone treatment to improve fish spawning and egg quality. In addition, a histological description of the digestive tract ontogeny will be conducted. The nutritional improvement of live food organism used during larval rearing will be tested to establish optimal fish feeding protocols at the different stages of larval fish development.

Quantifiable benefits:

- Development of a new, native species for aquaculture;
- Improvements in current aquaculture technology;
- Benefits to poor coastal aquaculture farmers;
- May be an alternative to shrimp culture as it is euryhaline; and
- Participation of three graduate students in the research

Objectives

1. Induce the spawning of *D. latifrons* by the combined effects of environmental conditions manipulation and long term hormone treatment.
2. Study the ontogeny of the digestive tract during larval development of *D. latifrons*.
3. Determine the effect of nutritionally enriched live food on fish growth and survival during the larviculture of D. latifrons.

Methods

Spawning induction

D. latifron's natural spawning period in captivity has been found to be limited to two months (September-October) based on our previous findings. Thus, we propose to evaluate an earlier gonad maturation protocol, both for males and females, by long term hormonal treatments. This methodology is similar to that used for Japanese Eel (*Anguilla japonica*), in which weekly injections (for up to 10 to 12 weeks) of gonadotropins such as carp pituitary extract (CPE) or steroids such as 17α-methyltestoterone (a highly aromatizable synthetic steroid) as a potential source of endogenous estradiol biosynthesis were used (Otha et al., 1997; Hao-Ran et al., 1998; Tanaka et al., 2003; Kagawa et al., 2005). Maturation and gamete release were successfully induced using this approach. The proposed experiment will be carried out as follows.

This work will be conducted at the aquaculture lab of the Facultad de Ciencias del Mar UAS, located in Mazatlan, Mexico. Four groups of fish (4 females and 3 males per tank) per treatment will be kept in 250 L tanks connected to a recirculation unit made with 100% recycled filtered media (high density polyethylene soft-drink bottle caps). The fish will be acclimated for one week under controlled conditions at constant temperature (27°C) and photoperiod (14:10 hours light:dark). Fish will then be injected as follows: Control group (0.1 ml/kg saline solution), carp pituitary extract (15 mg fish-1 week-1 from a stock solution of 1000 mg of CPE in 5 ml of saline solution), human chorionic gonadotropin (1 UI g-1 week-1 from vials with 5000 UI per ml of saline solution), 17α-methyltestosterone 17α-MT (20 μg g-1 week from a stock solution of 1000 μg MT ml -1 diluted in DMSO) or DesGLy10-Ala6 LHRHa (Syndel® from a stock solution of 100 μg ml-1 in saline solution, for a period of 10 consecutive weeks. During the trial, enlargement of the abdominal area and changes in coloration of the genital papilla will be monitored on a weekly basis. After six weeks, all fish will be checked for sperm or oocytes release after abdominal pressure. Egg quality will be evaluated in terms of relative fecundity and oocyte size, and sperm quality as motility, activation time and sperm concentration. The fertilization rate will also be evaluated.

Histology analysis of digestive tract development

Larvae obtained from induced reproduction of captive adult *D. latifrons*, either from broodstock used in studies related to Objective 1, or from fully matured fish, will be used for the histology analysis. After hatching, fish will be collected at 12-24 h intervals until all larvae are dead, or for a longer period if initiation of exogenous feeding is achieved following the methods proposed for Objective 3. For each sample, 10 to 15 fish will be anesthetized with a solution of 0.01% benzocaine and immediately fixed in 10% buffered formalin for 24 h. After fixation, samples will be transferred into a solution of 75% ethanol and kept under refrigeration until further processing. Also, 8% paraformaldehyde and Karnovsky fixatives will be used in a similar fashion. For each sample, 10 individual fish will be selected at random and the nothochordal length will be measured under a stereoscopic microscope after optical analysis of digital images using Motic Image Plus 2.0 software. Fish larvae will be dehydrated in an ethanol series and embedded in HistoResin (Leica). Histological sections will be obtained from two fish of each sample with an automatic microtome. Histological sections will be stained with hematoxylin-eosin and toluidine blue. Samples will be photographed and analyzed in a compound microscope (Bertolucci et al., 2008). Samples fixed in 8% paraformaldehyde and Karnovsky are suitable for histochemical analysis with PAS, Sudan Black and Alcian Blue.

Live food nutritional enhancement

The rotifer *Brachiounus rotundiformis* and the brine shrimp Artemia spp. will be used during the larviculture of *D. latifrons* from day post-hatch (dph) 0 to 16 as the control diets to compare the effects of live food enrichment on fish larvae growth and survival. In one feeding trial, rotifers grown on microalgae will be compared to rotifers submitted to a protein and fatty acid enrichment treatment as food for *D. latifrons* larvae. In a similar way, un-enriched Artemia will be tested against enriched Artemia with omega-3 fatty acids and vitamins. Two commercially-available products for bioenrichment of rotifers and Artemia will be tested: Algamac and Super Selco, and these will be used according to the manufacturer specifications. Three feeding treatments will be tested in triplicate: one control with non-enriched live food (T1), a second one with enriched rotifers and *Artemia* with Algamac (T2), and a third with rotifers and *Artemia* enriched with Super Selco (T3). *Artemia nauplii* will be produced from decapsulated cysts according to Lavens and Sorgeloos (1996). Fish stocking density will depend on the availability of hatched larvae, but is expected to be at least 40 larvae per liter. A feeding schedule based on previous experiences with marine fish (García-Ortega et al., 2002; 2005; 2009) is to be applied at a density of rotifers of 5 per ml from 0 to 4 dph, then progressively increased to 15-20 rotifers per ml until dph 16. *Artemia nauplii* (non-enriched) will be provided at an initial density of 0.5 per ml in all treatments at dph14 to16, T1 will be fed un-enriched *Artemia* until weaning. For T2 and T3 enriched *Artemia* will be fed to the larvae at a gradually increased density of 1-5 metanauplii per ml from dph 17 to 25. Rotifers will be offered to the larvae twice a day and *Artemia* four times daily. Weaning the fish onto artificial microdiets will start at day 26 when 10% of the *Artemia* will be daily reduced from the feeding protocol until is completely replaced by the artificial microdiet at day 36. Fish growth and survival will be determined during the feeding trial every to two four days. Depending on the results of this trail, a second trial can be done with modifications in the feeding protocol in order to reduce the time of live food feeding and the weaning time.

Deliverables

- One final report on the results of the project with information on the reproduction, digestive tract ontogeny and larviculture of *D. latifrons*.
- One manuscript drafted for submission to a scientific journal.

Student involvement

Three graduate students at FACIMAR-UAS are actively involved in the project. Objective 1 will complement first year student Jeniffer Velazquez Sandoval´s Master´s thesis entitled, "Hormonal manipulation for induced reproduction of Puyeque *Dormitator latifrons* (Richardson, 1844)". Objective 2 will be conducted first with fixed larvae from second Year student Eva A. Medina Hernandez´s Master thesis entitled, "Evaluation of the survival and growth of puyeque larvae *Dormitator latifrons* using live and inert feeds". Also, Ms. Hernandez will be visiting UNESP in Brazil during July-August 2011 to participate in the histology analysis of previously fixated larvae. As third graduate participant, first year student Vanesa V. Lopez Lopez will process most of the larvae produced in 2010 and all of the larvae produced in 2011 for histological analysis as described in Objective 2, during an academic visit at the Aquaculture Lab of the School of Environment and Natural Resources of the Ohio State University in Columbus Ohio from July-December of 2011.

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EFFECT OF DIFFERENT TEMPERATURE AND SALINITY LEVELS ON STRESS RESPONSES OF MARBLE GOBY (*OXYELEOTRIS MARMORATA*) FINGERLING

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Objectives:

1. To determine the temperature levels causing stress on marble goby (control 28˚C , treatments 22 and 34˚C)
2. To determine the salinity levels causing stress on marble goby (control 0ppt, treatments 5, 10 and 15ppt)

Significance

Marble goby (Oxyeleotris marmorata Bleeker, 1852) belongs to the family *Eleotridae* of Order *Perciformes*. This fish is also called marbled sleeper goby (Keneth et al., 1996) and characterized by a large head, symmetrical patterning on the dorsal surface and by the rounded, outstretched pectoral fins. Marble goby is a native species in many parts of Asia including Thailand (Vidthayanon et al., 1997), Peninsular Malaysia (Department of Fisheries, 1987), Vietnam (Khoa et al., 1993), Brunei (Kottelat et al., 1993), Cambodia (Rainboth, 1996), Laos (Kottelat, 2001) and Indonesia (Kottelat et al., 1993). It may be the largest species of the goby-like fishes in the world with a recorded biggest size of 65 cm in standard length (Kottelat, 2001).

Marble goby is a large, solitary and slow-moving fish which rests undisturbed on the bottom of quiet streams, canals and lakes (Robert, 1993). It rarely moves, even when disturbed. It lives in freshwater and brackish water with temperatures of 22 – 28˚C, pH range of 6.5 – 7.5 and depth range of about 1- 10 m (Kottelat et al., 1993). It is believed that marble goby is a potamodromous species (Riede, 2004). Marble goby is a facultative air breather capable of surviving under terrestrial conditions for up to 7 days (Jow et al., 1999). In the natural environment, it may encounter an absence of water during voluntary emergence or habitat desiccation (Choo, 1974, cited by Jow et .al., 1999). Loc (2001) also reported that marble goby often burrows itself in bottom mud or a cave and even can live under 1 m mud depth for more than 10 hours. This fish also prefers to live in habitat with aquatic plants near the river bank (Loc, 2001). Menasveta (2000) reported that marble goby usually hides at the pond bottom during the day and becomes active during the night as it normally dislikes intense light.

Aquaculture

Marble goby is commonly cultured in cages in rivers and reservoirs, ponds, and coves in Thailand, Vietnam, Malaysia and Cambodia (Suwansart, 1979; Jee, 1980; Cheah, 1994; Menasveta, 1999; Luong et al., 2005; Annon, 2005; MAFF, 2005). In late 1980s and early 1990s, marble goby cage culture in Thailand expanded rapidly, and reached the peak in 1990 (Lin and Kaewpaitoon, 2000), however, marble goby culture has decreased since 1996 due mainly to lack of seed supply and disease outbreak. In recent years, marble goby culture in Malaysia has shown a tremendous potential (Liem, 2001). In Vietnam, marble goby culture has been developed in ponds and coves since early 2000s (Luong et al., 2005).

The major type of marble goby culture in Thailand is in floating cages in rivers, reservoirs and irrigation canals (Lin and Kaewpaitoon, 2000). Marble goby is cultured intensively in box shaped floating cages (from 10 to 30 m^3) made of wood or bamboo. Initial stocking density is 30 - 180 fish/m2 using fingerlings of 100 g size. Marble goby is fed with chopped marine fish once a day in late afternoon.

In Tri An reservoir, Viet Nam, marble goby were polycultured with common carp (31 g), silver carp (14 g), bighead carp (17 g) and grass carp (20 g), giving an overall stocking density of 2,540 fish/ha. Neither fertilizer nor feed was added so fish growth was dependent on natural food resources in the cove. After 7 months, marble goby attained an average size of 353 g with a survival rate of 73.7% and net yield of 172 kg ha-1crop. Farmers in Ca Mau province fattened marble goby fingerlings (>100 g/fish) in ponds for 5 to 12 months. Optimum pond size for marble goby culture is 300 – 400 m^2 of depth of 1.5 – 1.8 m. The stocking density in running ponds is 8 -10 fish/m2 and in tidal water exchange ponds is 4 -5 fish/m2. Fish were treated with 2 -3% salt before stocking. Trash fish were fed to marble goby in suitable rate (Hoa and Tam, 2009).

Research Concepts
Investigate the temperature and salinity levels that may cause stress on marble goby fingerling.

Quantified Anticipated Benefits:
Identification of the temperature and salinity levels that cause stress on marble goby will enhance the nursing conditions of this species.

Research Design:
Our activities are as follows:

1. To determine the temperature levels causing stress on marble goby fingerlings:
   Control: Marble goby fingerlings (24 fingerlings/aquaria) will be exposed to temperature of 28˚C (optimum temperature for marble goby culture)
   Treatment 1: Marble goby fingerlings (24 fingerlings/aquaria) will be exposed to temperature of 22˚C
   Treatment 2: Marble goby fingerlings (24 fingerlings/aquaria) will be exposed to temperature of 34˚C.
2. To determine the salinity levels causing stress on marble goby fingerlings:
   Control: marble goby fingerlings (24 fingerlings/aquaria) will be exposed to salinity of 0ppt
   Treatment 1: marble goby fingerlings (24 fingerlings/aquaria) will be exposed to salinity of 5ppt
   Treatment 2: marble goby fingerlings (24 fingerlings/aquaria) will be exposed to salinity of 10 ppt
   Treatment 3: marble goby fingerlings (24 fingerlings/aquaria) will be exposed to salinity of 15 ppt

Exposure and sampling:
Baseline blood samples were taken from the caudal vasculature of one fish from each aquarium (a total of 24 fish) with a heparinized syringe for blood glucose and plasma cortisol measurements (described below). After the baseline sampling, all fish from each aquarium were netted and quickly transferred to randomly assigned temperature and salinity treatments. Fish will be held in each exposure treatment for 10 minutes and then blood will be sampled from three fish, and the remaining fish will be at their aquarium. Three fish from each aquarium will be again sampled 30, 60,120, 240 and 480 minutes

Glucose and cortisol contents of marble goby fingerlings will be measured to identify the stress appearance.

Stress measurement:
Glucose content: One drop of blood will be used to measure blood glucose with the portable OneTouch ® Ultra ® blood glucose meter.

Cortisol content: Plasma, obtained from the remaining whole blood by centrifugation at 2500 x g, will be used for cortisol assays. Plasma cortisol concentrations will be measured by time-resolved fluoroimmunoassay.

Statistical analysis:
Data will be analyzed by one-way analysis of variance (ANOVA). Measured values from individual

fish will be averaged for each aquarium (experimental unit) for use in statistical analyses.

Schedule:
1 June 2011 to 15 September 2011 Report submission: no later than 29 September2011.

Schedule includes:

- Repair aquarium and order fingerlings – June 1 – June 30, 2011
- Carry out the experiment 1: temperature level causing stress on marble goby fingerling - June 30- July 30, 2011)
- Carry out the experiment 2: salinity level causing stress on marble goby – July 30 - August 30, 2011
- Preparation of final reports – through 29 September 2011

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PROSPECTS AND POTENTIAL OF THE AFRICAN LUNGFISH (*PROTOPTERUS* SPP): AN ALTERNATIVE SOURCE OF FISHING AND FISH FARMING LIVELIHOODS IN UGANDA AND KENYA

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Objectives:
1. Evaluate lungfish as a culture species in Uganda and Kenya.
2. Assess indigenous knowledge and practices associated with the culture and use of lungfish on farms in ponds, in natural water bodies and reservoirs.
3. Identify socio-economic conditions shaping the culture of African Lungfish including prices, demand, and public perceptions.
4. Develop harvest and handling procedures that ensure worker safety and protect yield.
5. Identify simple fingerling production and grow-out techniques for African lungfish.

Significance:
Climate changes manifested in shifting rainfall and temperature regimes are bringing new challenges to the management of water bodies and fish ponds in sub-Saharan Africa. The culture of species resilient to drought and stressed water quality conditions may be a significant part of the future of African aquaculture. Air breathing fishes such as the Marbled African Lungfish (*Protopterus aethiopicus*) are able to obtain and utilize oxygen from air to meet all or part of their metabolic demands. These fish are classified obligate air breathers based on anecdotal records and early scientific work concluding that adults of the species asphyxiated when denied access to air. They are carnivorous, eating crustaceans, aquatic insect larvae, and molluscs.

Lungfish are members of the taxonomy class: *Sarcopterygii*, they are lobe finned fishes (together with coelacanths). In nature, aestivating lungfish remain buried in cocoons relying solely on air to survive drought periods (lasting several months). Lungfish are locally important food fishes exploited from natural habitats in lakes and reservoirs using a variety of gear including gillnets, long lines, and other methods.

The African lungfish is cited as an example of how the evolutionary transition from breathing water to breathing air can happen (Wikipedia 2011). It is native to East African lakes, swamps, rivers and wetlands (Birt et al., 2006; Greenwood, 1958, 1986). It is an endangered fish in Uganda as its natural stocks are rapidly declining mainly due to overexploitation, environmental degradation and the large-scale conversion of wetlands to agricultural land (Balirwa et al., 2003; Goudswaard et al., 2002). In Kenya's Lake Baringo, however, they dominate catches with annual landings of up to ninety nine metric tons after being introduced in 1970s and the fishery emerged in 1984 (Mlewa et al., 2005; 2007; Mlewa & Green, 2004; 2006).

Local practice is to dig up lungfishes, burrow and all, and store it for later use when they want fresh fish to eat. It is reported that they have a strong taste such that "it is locally either highly appreciated or strongly disliked" (Wikipedia 2011). As technology advancements such as longlines and gillnets have been increasingly applied over the past fifty years, it is believed that lake and river lungfish populations there are decreasing. In Uganda, women reportedly do not eat the lungfish because they consider it a "sister fish," and therefore it is associated with men and manhood (Wikipedia, 2011; Bruton, 1998).

Air breathing fishes potentially have a role in low-management culture systems because dissolved oxygen is not a limiting factor. The African catfish (*Clarias gariepinus*) can tolerate low levels of dissolved oxygen but its flesh is often of less consumer interest. Concurrently, the Pangasius catfish's flesh is of high quality but is a species exotic to Uganda. Therefore, the African lungfish (*Protopterus aethiopicus* and *Protopterus amphibious*) is advantageous because it is an indigenous fish with good quality flesh, an air-breather, a biocontrol agent against schistosome vector snails (Daffalla et al., 1985), and has some existing level of indigenous culture (Greenwood, 1966). The food value of the lungfish is enhanced by its high muscle to bone ratio, and its bones and cartilage pose less danger of choking consumers.

Little is known about indigenous practices of culture, harvest, and marketing of Protopterus spp from farm ponds and water bodies. Anecdotal evidence suggests farmers gather wild nestlings of lungfish and stock small water bodies but with no documentation about management practices and yields (Mwatete et al., 2005). Preliminary attempts in Kenya to grow wild Marble lungfish (*Protopterus aethiopicus*) 'fry' in earthen pond encountered difficulties because most fish went into burrows and disappeared.

Mlewa et al. (2009) find indications of early breeding behavior, as the trial lungfish attained sexual maturity slightly earlier than those in wild populations since the lungfish that made burrows and were not accessible for harvest. Culture trial results showed that lungfish realized growth increments of 2.7 and 14.5 cm over time periods ranging from 70 to 238 days. The mean absolute growth rate was 0.049 (±0.008 SE) cm/ day, whereas specific growth rates ranged from 0.048 to 0.140% per day. This study demonstrated that marbled lungfish can be raised in earthen ponds and suggested that further research determine its potential in the aquaculture industry. Furthermore, efforts to develop culture techniques must also address handling and harvesting issues associated with a fish that has a "beak" like a snapping turtle and a tendency to burrow in the soil when a pond is drained (Mlewa et al. 2009).

The literature on African lungfish mainly examines lungfish ecology, fishery, biology, and physiology, but few studies treat its use as a food fish in aquaculture (Baer et al., 1992). Therefore, this study will apply what is known about this fish to explore its aquaculture potentials in improving food security and livelihoods in sub-Saharan Africa. The exploratory study will assess indigenous practice and understandings about production parameters and approaches. The field work will assess potential paths for producer adoption and training to use lungfish as a culture species and a managed water body resource.

Quantifiable Anticipated Benefits:
The report emanating from this assessment will address each of the six objectives in a way that identifies researchable issues, suggesting a path toward increasing food security, incomes, and nutritional improvement in local populations. Current practices and understandings about the culture and use of lungfish in Uganda and Kenya will be portrayed.

Activity Plan:
Evaluate lungfish as a culture species in Kenya and Uganda.
Understanding the current status of aquaculture potential for lungfish in Uganda and Kenya will provide a basis for planning and the future support to improve livelihoods. A preliminary assessment will be conducted in seven districts (Kampala, Wakiso, Kumi, Busia, Soroti, Pallisa and Jinja).
For Kenya, six districts along the shores of lake Victoria will be selected to focus on *Protopterus aethiopicus* while the Lower Tana River in the Tana Delta District is a region where *Protopterus amphibious* is reportedly popular.

A semi-structured checklist will be used to assess conditions and identify research questions. Key stakeholders during the study will include fish farmers, fisher folk communities, District extension officers, fish processors, fish traders, and consumers.

The information generated will be used as guidance for the subsequent data collection and experimentation. Primary field assessments will be supplemented by reports from government fisheries departments and other relevant institutes and bureaus. Sites visited will be recorded GIS to provide spatial relationships between aquaculture and socio-economic indicators.

Assess indigenous knowledge and practices associated with the culture and use of lungfish on farms in ponds, in natural water bodies and reservoirs.
Fish farmers growing lungfish in a district will be identified and rapid rural appraisal research techniques used to generate a preliminary assessment of farmer status, production practices and market options. The study will identify major constraints of production with the aim of improving productivity so that the producers are able to sustainably produce for the identified niches. Production practices will include pond designs, stocking rates, feeds and feeding and water quality management.

Identify socio-economic conditions shaping the culture of African Lungfish including prices, demand, and public perceptions.
Using rapid appraisal techniques information will be generated to profile the human capital, financial capital, social capital together with natural and physical capital associated with lungfish production. Semi-structured interviews and discussions will be conducted to generate information on factors that affect farmers' production at farm-level.

Develop harvest and handling procedures that ensure worker safety and protect yield.
African lungfish has a "beak" like that of a snapping turtle and has a tendency to burrow in the soil when a pond is drained. Its dentition is in the form of sharp occluding blades that make it dangerous to handle. Therefore, various harvesting and handling techniques will be tested at all stages of growth during the culture period. A combination of indigenous and disciplinary knowledge will be used to profile efficiency and survival effects.

Identify simple fingerling production and grow-out techniques for African lungfish.
We will assess the procedures and process used by fish farmers who raise lungfish in ponds or cages (if this exists). We seek to document the processes farmers have developed through trial and error process to cultivate these fish.

Regional and Global Integration
The results of this work have consequences for aquacultural development in Africa and in other places in the world. Future research must also address habitat restoration and conservation to ensured sustainable harvest of lungfish fingerlings from natural habitats. Subsequent work should promote farm-based business that grow fingerlings and grow out crops of lungfish as a source livelihood and to reduce pressure on habitats. New understandings about the culture and management of lungfish may provide suggestions for use of other species of air breathing fish with similar propagation and rearing issues.

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