AQUAFISH CRSP
IMPLEMENTATION PLAN 2009–2011
ADDENDUM II

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Aquaculture & Fisheries
Collaborative Research Support Program
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AQUAFISH CRSP IMPLEMENTATION PLAN 2009–2011, ADDENDUM

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Cover Photo
Aquaculture workers netting a catch of market-size tilapia from an earthen pond in the Philippines.

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INTRODUCTION

This second addendum to the AquaFish CRSP *Implementation Plan 2009-2011* includes three add-on research project investigations dealing with air-breathing fishes as well as a work plan change to the Auburn University Certificate of Aquaculture Professional training investigation included in the first addendum. Also included is the work plan for a new Program-Wide Project serving as the AquaFish CRSP component of a CRSP Council project dealing with knowledge and data management.

The new research investigations focus on promoting the extension of CRSP technologies through outreach, commercialization, and partnerships; assessing impacts; and communicating the importance of CRSP research. Summaries of these investigations are provided below.

AUBURN UNIVERSITY
This multi-component investigation is taking several initial steps to establish a low-management culture system for African lungfish (*Protopterus aethiopicus and P. amphibious*) in Uganda and Kenya. Lungfish is a promising aquaculture candidate due to its ability to survive in low-levels of dissolved oxygen. Work includes assessments of indigenous knowledge and practices in the culture and use of lungfish as well as the the socio-economic conditions shaping its current culture. CRSP researchers will also develop harvest and handling procedures and fingerling production and grow-out techniques. — 09IND07AU

UNIVERSITY OF HAWAI'I AT HILO
This experimental investigation focuses on phenotypic adaptations of the air-breathing fishes to changes in oxygen concentration in terms of basic morphological changes, behavioral adjustments, and extended ecological effects on survival. Investigators will study the morphological structure of gills and respiratory bladder in young-of-year garfish and pirarucu. For establishing a model species for air-breathing fishes, they will determine ontogenetic changes in the development of gills and the respiratory bladder in bowfin larvae and juveniles. — 09IND08UH

UNIVERSITY OF MICHIGAN
This investigation will build on the body of knowledge on culturing tropical and Cuban gars (*Atractosteus tropicus and A. tristoechus*) for food and restocking their wild populations. The work involves two production components: a sustainable feed technology study that will focus on testing levels at which plant and animal by-products can be used in gar feed to substitute for fishmeal; and stocking density trials in recirculating system aquaculture environments. — 09SFT07UM

RESEARCH PROJECT INVESTIGATIONS

TOPIC AREA
SUSTAINABLE FEED TECHNOLOGY

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

Sustainable Feed Technology/Experiment/09SFT07UM

Collaborating Institutions & Lead Investigators

University of Michigan (USA) James S. Diana
Universidad Juarez Autonoma de Tabasco (Mexico) Wilfrido M. Contreras-Sanchez

Objectives

1. To determine the degree to which plant and animal 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/m^3)

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.

Quantifiable 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[1] and Cuban[2] 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

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[1] Tropical gar (*Atractosteus tropicus*) ranges from Mexico through Central America to Costa Rica.

[2] Cuban gar (*Atractosteus tristoechus*) is found in Cuba and the Isle of Pines.

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.

Research Design & Activity Plan

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 plant and animal 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[3].
2) Determine optimal densities for rearing tropical gars (treatments: 25, 50 and 100 fish/m^3)

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.

[3] 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

Literature Cited

Alfaro, R.M., C.A. Gonzales, and A.M. Ferrara. 2008. Gar biology and culture: status and prospects. Aquaculture Research 39: 748-763.

Comabella, Y., R. Mendoza, C. Aguilera, O. Carrillo, A. Hurtado, and T. Garcia-Galano. 2006. Digestive enzyme activity during early development of the Cuban gar *Atractosteus tristoechus*. Fish Physiology and Biochemistry 32: 147-157.

Comabella, Y., A. Hurtado, and T. Garcia-Galano. 2010. Ontogenetic changes in the morphology and morphometry of Cuban gar (*Atractosteus tristoechus*). Zoological Science 27: 931-938.

Suttkus, R.D. 1963. Order Lepisostei. *In* Fishes of the Western Atlantic; Part Three, Soft-rayed fishes. Yale University Memoir Sears Foundation for Marine Research 1, eds. H.B. Bigelow et al., 61-88. New Haven, Connecticut.

Wiley, E.O. 1976. The phylogeny and biogeography of fossil and recent gars (Actinopterygii: Lepisosteidae). University of Kansas Museum of Natural History Miscellaneous Publication 64: 1-111.

TOPIC AREA
INDIGENOUS SPECIES DEVELOPMENT

❖

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

Indigenous Species Development /Study/09IND07AU

Collaborating Institutions & Lead Investigators

Auburn University (USA)	Joseph J. Molnar
National Fisheries Resources Research Institute (Uganda)	John Walakira
Kenyatta University (Kenya)	Charles C. Ngugi

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.[4] 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).[5] In nature, aestivating lungfish remain buried in cocoons relying solely on air

[4] Globally, rising food prices have shifted forty four million people into extreme poverty while Uganda has nine million people facing an acute food shortage and Kenya has similar problems (World Bank, 2010). Aquaculture provides alternative source to food security and livelihood improvement, in the Sub-Saharan region population (Brummet and Williams, 2000). Lake fisheries continue to decline, and aquaculture production (subsistence and commercial oriented) is less than 1% of total national production in each country (FAO, 2010; Isyagi et al., 2009).

[5] Order: Ceratodontiformes, Australian, S. American and African species Family: Protopteridae; Genus: Protopterus; there are at least four African lungfish species, Species: Protopterus aethiopicus (with three subspecies), Protopterus amphibious, Protopterus annectens, and Protopterus dolloi (Haeckel 1851)

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.

Research Design & Activity Plan

1. *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.

2. *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.

3. *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.

4. *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.

5. 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.

Literature Cited

Baer, S., Haller R. D., Freyvogel T. A. (1992). Growth of the African lungfish, Protopterus amphibious Peter, in aquaculture. Aquaculture Research 23, 2, 265-267.

Balirwa, J.S, Chapman C. A, Chapman L.J, Cowx I.G, Geheb K, Kaufman L, Lowe-McConnell R. H, Seehausen O, Wanink J.H, and Welocomme R.L and Witte F. (2003). Biodiversity and fishery sustainability in the Lake Victoria Basin: an unexpected marriage? BioScience, 53(8):703-716. 2003.

Birt, T.P, Mlewa. C. M, Green M, Seifert. A, and Friesen V. L. (2006). Genetic variation in the marbled lungfish *Protopterus aethiopicus* in Lake Victoria and introduction to Lake Baringo, Kenya. J of Fish Biology (2006) 69, 189-199.

Boyd, C. E., S. Soongsawang, E. W. Shell, and S. Fowler. In press. Small impoundment complexes as a possible method to increase water supply in Alabama. Proceedings of the 2009 Georgia Water Resources Conference, University of Georgia, Athens, Georgia, USA.

Brummett R.E and Williams M. J, (2000). The evolution of aquaculture in African rural and economic development. Ecological Economics 33 (2000) 193-203.

Bruton, Michael N. (1998). In Paxton, J.R. & Eschmeyer, W.N.. ed. Encyclopedia of Fishes. San Diego: Academic Press. pp. 70–72. ISBN 0-12-547665-5.

Daffalla, A.A., Elias E.E., Amin M.A. (1985). The lungfish Protopterus annectans (Owen) as a biocontrol agent against schistosome vector snails. J Trop Med Hyg. Apr 88(2):131-4.

Dunbrack, Green & Mlewa (2006) Lungfish growth - Journal of Fish Biology 68, 443-447.

FAO. (2010). The State of World Fisheries and Aquaculture. 2010. Rome Italy.

Garner, Birt, Mlewa et al. Population genetic structure - (2006) Journal of Fish Biology 69(b), 189-199.

Goudswaard, K.P.C., F. Witte, and L. J. Chapman. (2002) Decline of the African lungfish (*Protopterus aethiopicus*)in Lake Victoria (East Africa). Afr. J. Ecol., 40, 42-52.

Goudswaard, Kees, Frans Witte, Lauren J. Chapman. (2002). Decline of the African lungfish (*Protopterus aethiopicus*) in Lake Victoria (East Africa) East African Wild Life Society, African Journal of Ecology, 40, 42-52.

Greenwood, P. H. (1966) The Fishes of Uganda. The Uganda Society, Kampala.

Greenwood, P.H. (1958) Reproduction in the East African lung-fish *Protopterus aethiopicus* Heckel. Proc. Zool. Soc. London 130, 547-567.

Greenwood, P.H. (1986) The natural history of African lungfishes. J.Morph. Suppl.1,16 3-179.
Isyagi N, Atukunda G, Aliguma L, Ssebisubi M, Walakira J, Kubiriza G, Mbulameri E (2009). Assessment of national aquaculture policies and programmes in Uganda. Aquaculture Compendium. 87 pp.

Mlewa and J. Green (2004) Biology of the Kamongo African Journal of Ecology 42, 338-345.
Mlewa, C.M. & J. Green (2006) Introduction and Fishery - African Journal of Aquatic Science 31, 131-136.

Mlewa, C.M. C.M., Green & Dunbrack Lungfish Respiration in the wild - (2007) African Zoology 42, 131-134.

Mlewa, C.M., Green and Simms (2005) Movement and Space Use - Hydrobiologia 537, 229-238.

Mlewa, C.M., J. Green & Dunbrack, in Press) Natural history (Book Chapter: Jorgensen & Joss (Eds).

Mlewa, M. C., Ogola, D. W. and Ngugi, C.C. 2009. Aspects of the biology of marbled lungfish (Protopterus ethiopicus) raised in earthen ponds at the Chepkoilel fish farm, Kenya. In press: Journal of East African Resources Management JEARNARM 16: 231-34.

Moehl, J., M. Halwart, and R. Brummett. (2005). Report of the FAO-WorldFish Center Workshop on Small-scale Aquaculture in Sub-Saharan Africa: Revisiting the Aquaculture Target Group Paradigm. Limbé, Cameroon, 23-26 March 2004. CIFA Occasional Paper. No. 25. Rome, FAO. 2005. 54p. Retrieved 14 January, 2009: http://www.fao.org/docrep/008/a0038e/a0038e04.htm#TopOfPage.

Mwatete, M. C., Ogola, D. W., and Ngugi, C.C. (2005). Towards the recruitment of marbled lungfish (Protopterus ethiopicus) in aquaculture in the lake Victoria basin: some preliminary results of culture trials at the Chepkoilel fish farm, Kenya. 2nd National Scientific Conference, Lake Victoria Environmental Management Project. 17th to 19th October 2005. Tom Mboya College, Kisumu, Kenya.

Wikipedia. (2011). Protopterus. Retrieved May 14, 2011, http://en.wikipedia.org/wiki/Protopterus

World Bank. (2010). Global Economic Prospects 2010: Crisis, Finance, and Growth. Washington, DC (www-wds.worldbank.org).

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

Indigenous Species Development /Study/09IND08UH

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Objectives

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.

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 O_2 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.

Therefore, air breathing fishes represent great opportunity for aquaculture particularly in tropical and subtropical regions where aerial respiration allows to avoid aquatic hypoxia and associated with these conditions mortality. We argue that respiratory physiology of spotted gar as a surrogate species for tropical gar can be explored and becomes critical to the success of aquaculture of this latter species for conservation purposes in the USA (AR, TX, OK, and NM) as well as the food fish in Mexico (Tabasco) and Central American countries.

Why garfish, arapaima and bowfin?

<u>Garfish</u>

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 20^0C, 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. To develop a model species for generalization of physiological responses is certainly a challenging task. However, some aspects of fish response to variable oxygen levels such as respiratory organs morphology and particularly respiratory neuroepithelial cells responses are similar in teleosts and mammals (see reviews by Jonz and Nurse 2006; Jaroszewska and Dabrowski 2010b). 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 Design & Activity Plan

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 26-28^{o}C) 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.

In the case of garfish, the major experiment was already completed and that included fish exposed to normoxia (90% oxygen saturation), hypoxia (40%) and hyperoxia (180%). This study was carried out in 4 replicate tanks per treatment. Fish were sampled at early stages and at 73rd day when exposure from the larval swim-up stage was terminated and all fish were transferred to normoxic conditions. At least 6 individual fish per treatment were sampled at each time. These animals are kept until now and in the proposed study we will examine 18 months old garfish that were PIT-tagged and we will be able to distinguish their exposure to different oxygen conditions during early life history. Morphological analysis will include measurement of gill filament numbers on all gill arches, counting of gill lamellae and measuring of secondary lamellae surface. This will allow to compare the total gill respiratory surface area among different oxygen treatments. Histological analysis of respiratory surfaces will be the next step in the analysis where different type of epithelial cells can be described and quantified.

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, mature fish were acquired from Lake Erie's wetlands and progenies were obtained after artificial reproduction in OSU's laboratory. Fish were induced by hormonal injections and eggs fertilized and larvae raised with live food prior to transition to a formulated, commercial diet. Fish were subjected to the identical experimental design as used with garfish (3 oxygen treatments in 4 replicates) and samples of fish were collected several times during the exposure. Due to much faster growth rate of bowfin than garfish, experiment with exposure was terminated after 4 weeks and all fish were transferred to normoxic condition, PIT-tagged and are maintained until present. Gills and swim-bladder analysis by histological methods will follow.

Three major treatments were used: hypoxic (40% oxygen saturation), normoxic and hyperoxic (180%) conditions. One oxygen regime was assigned to 4 replicate tanks. Normoxia conditions was provided by simply running dechlorinated, aerated city water through a degassing column. Hyperoxia was achieved by running dechlorinated city water through an open, packed column supplied with pure oxygen. The ratio of water flow and oxygen volume was established experimentally to secure continuous oxygen level in the tanks at 180% saturation at 18-20ºC. Oxygen stripped water was 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 was monitored daily in each tank.

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

Significance

Further Aquaculture Potential and Role as Food Fish

Mendoza et al. (2006) have recently reviewed the "state-of-the-art" in tropical gar in Southeastern Mexico where aquaculture is concentrated. The authors emphasized practical value of garfish as food fish and cultural-historical value of the species in the region. The demand for garfish continues to increase and both intensive culture in cages and stocking of open waters is practiced.

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).

Literature Cited

Ballard, W.W., 1984: Morphogenetic movements in embryos of the Holestean fish, *Amia calva*: A progress report. *American Zool*. 24: 539-543.

Boyd, C.E., Watten, B.J., Goubier, V., Wu, R., 1994: Gas supersaturation in surface waters of aquaculture ponds. *Aquacult. Eng*. 13:31-39.

Burleson, M.L., Shipman, B.N., Smatresk, N.J., 1998: Ventilation and acid-base recovery following exhausting activity in an air-breathing fish. *J. Exp. Biol.* 201: 1359-1368.

Brauner, C.J., Matey, V., Wilson, J.M., Bernier, N.J. Val, A.L. 2004: Transition in organ function during the evolution of air-breathing: insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *J.exp.Biol*., 207: 1433-1438.

Dabrowski, K., Guderley, H., 2002: Intermediary metabolism. *In*: J.E. Halver and R. Hardy, (Eds.), Elsevier Science, USA, pp. 309-365.

Dabrowski, K., Lee, K.J., Guz, L., Verlhac, V., Gabaudan, J., 2003: Effects of dietary ascorbic acid on oxygen stress (hypoxia or hyperoxia), growth and tissue vitamin concentrations in juvenile rainbow trout. *Aquaculture* 233:383-392.

Freitas, C. E. C., Rivas, A. A. F., 2006: A pesca e os recursos pesqueiros na Amazônia ocidental. Ciên. Cult., São Paulo, 58: 30-32 Available at: http://cienciacultura.bvs.br/scieo.php? script=sci_arttext&pid=S0009-67252006000300014&lng=en&nrm=iso. Access in: March 05, 2009.

Gonzalez, R.J., Brauner, C.J., Wang, Y.X., Richards, J.G., Patrick, M.L., Xi, W., Matey, V., Val, A.L., 2010: Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. *Physiol.Biochem.Zool*., 83: 322-332.

Graham, J.B., 1997: Air-Breathing Fishes, Evolution, Diversity and Adaptation, Academic Press, San Diego, California; pp. 299

Halverson, M., 2008: Pirarucu o peixe gigante: novas descobertas e acertos. Panorama da Aquicultura, 18 (105): 36-41 (In Portuguese).

Hendrick, M.S., Jones, D.R., 1993: The effects of altered aquatic and aerial respiratory gas concentrations on air-breathing patterns in a primitive fish (*Amia calva*). *J. exp.Biol.,* 181: 81-94.

Horn, M.H., Riggs, C.D., 1973: Effect of temperature and light on the rate of air breathing of the bowfin, *Amia calva*. *Copeia* 1973: 653-657.

Jaroszewska, M., Dabrowski, K.,, 2008: Morphological analysis of the functional design of the connection between alimentary tract and the gas bladder in air-breathing lepisosteid fish. *Ann. Anat. –Anatomisher Anzeiger,* 190: 383-390.

Jaroszewska M. and Dabrowski, K., 2009: Early ontogeny of Semionotiformes and Amiiformes (Neopterygii: Actinopterygii). In: *Development of Non-Teleost Fish*, Y.W. Kunz, C.A. Luer, B.G. Kapoor [Eds.], *Science Publishers Inc*. pp. 231-275.

Jaroszewska, M., Dabrowski, K., Rodríguez, G., 2010a: Development of testis and digestive tract in longnose gar (*Lepisosteus osseus*) at the onset of exogenous feeding of larvae and in juveniles. *Aquaculture Research* 41: 1486-1497.

Jaroszewska, M., Dabrowski, K. 2010b. Does a fish with lungs exists? Morphological and physiological adaptations to aquatic hypoxia and hyperoxia. Kosmos 59: 479-496.

Johansen, K., Hanson, D., Lenfant, C. 1970: Respiration in a prmitive air breater, *Amia calva. Respiration Physiol*. 9: 162-174.

Jonz, M.G., Nurse, C.A. 2006. Ontogenesis of oxygen chemoreception in aquatic vertebrates. Resp.Physiol.Neurobiol. 154: 139-152.

Leitão, N. de J., Pai-Silva, M. D., Almeida, F. L. A., Portella, M. C., 2011: The influence of initial feeding on muscle development and growth in pacu *Piaractus mesopotamicus* larvae. *Aquaculture*. DOI:10.1016/j.aquaculture.2011.01.006

MacCormack, T.J., Treberq, J.R., Almeida-Val, V.M., Driedzic, W.R., 2003: Mitochondrial K(ATP) channels and sarcoplasmic reticulum influence cardiac force development under anoxia in the Amazonian armored catfish *Liposarcus pardalis*. *Comp. Biochem. Physiol., Part A Mol. Integr. Physiol.* 134:441-448.

Matschak, T.W., Stickland, N.C., Mason, P.S., Crook, A.R., 1997: Oxygen availability and temperature effect embryonic muscle development in Atlantic salmon (*Salmo salar* L.). *Differentiation* 61: 229-235.

Matsumura, S., Milliken, T. 1984: The Japanese trade in bonytongue and CITES-listed fish. *Traffic Bull*., 6: 42-50.

Mendoza, R.A., Gonzalez, C.A., Ferrara, A.M. 2006. Gar biology and culture: status and prospects. Aquaculture Res., 39: 748-763.

Roesner, A., Hankel, T., Burmester, T., 2006: Hypoxia induces a complex response of globin expression in zebrafish (*Danio rerio*). *J. Exp. Biol.* 209:2129-2137.

Satora, L., 1998: Histological and ultrastructural study of the stomach of the air-breathing *Ancistrus multispinnis* (Siluriformes, Teleostei). 1998: *Canadian J. Zoology* 76: 83-86.

Satora, L., Wegner, N.C. 2011. Reexamination of the Byczkowska-Smyk gill surface area data for European teleosts, with new measurements on the pikeperch, Sander lucioperca. Rev. Fish Biol. Fisheries April 20, 2011 on line.

Stevens, E.D. and Holeton, G.F., 1978: The partitioning of oxygen uptake from air and from waterby the large obligate air-breathing teleost pirarucu (*Arapaima gigas*). *Can.J.Zool*. 56: 974-976.

Villafane, V.E., Buma, A.G.J., Boelen, P., Helbiling, E.W., 2004: Solar UVR-induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina. *Arch. Hydrobiol*. 161:245-266.