BETTER SCIENCE, BETTER FISH, BETTER LIFE

PROCEEDINGS OF THE NINTH INTERNATIONAL SYMPOSIUM ON TILAPIA IN AQUACULTURE

Editors

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Dedication:

These proceedings are dedicated in honor
Of our dear friend

Yang Yi

It was Dr. Yang Yi who first suggested having this ISTA at Shanghai Ocean University to celebrate SHOU's move to the new Lingang Campus. It was through his hard work and constant attention with his many friends and colleagues that the entire 9AFAF and ISTA9 came together, despite the terrible illness that eventually took his life at such a young age.

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POSTERS

WHY TILAPIA IS BECOMING THE MOST IMPORTANT FOOD FISH ON THE PLANET

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ABSTRACT

Tilapia has become the shining star of aquaculture with farms starting and expanding across the globe while consumption races ahead of even the most ambitious farm building plans. 2010 saw farmed tilapia exceed 3.2 million metric tons per annum, surging further ahead of the salmon and catfish industries. We are also seeing an explosion of product forms in the grocery stores that is only matched by the variety of preparations we see in the restaurant trade. The global adoption of tilapia as a substitute for all kinds of wild-caught fish has driven demand higher every year, even through the global recession of recent years. The description of tilapia as an "aquatic chicken" becomes more accurate every day. It's wide acceptance across all cultural, religious, and economic groups is similar to chicken. A variety of breeds and strains have been developed and by most measures, tilapia is now the most highly domesticated of farmed fishes. Unique amongst the major farmed fishes, tilapia maintains a key role in rural aquaculture improving the welfare of the poorest farmers while at the same time, it is reared in the most high tech production systems and is sold into international markets for up-scale markets. Tilapia is still the darling of the environmental community and the industry continues to polish its "green" credentials.

Three or four closely related species of tilapias readily hybridize in captivity and produce fecund F1 progeny. This has provided a huge genetic base for the geneticists to perform basic selective breeding. The domestication of tilapias has been a great driver of productivity during the 1990's and 2000's. There is also a concerted effort to describe the tilapia genome. When these genetic maps are distributed we can expect a second wave of genetic research that should further improve productivity. All of this will have been accomplished without the need of transgenics or genetically modified organisms. The basic biology of the fish along with the skill of traditional breeders has provided all of the progress to this point and much more in the near future.

Tilapia continues its march towards eventually overtaking carp as the most important farmed fish crop. With a much wider distribution of production and consumption and a huge base of value added product forms, it is almost certain that tilapia production will someday eclipse that of carp. As tilapia production and consumption grows globally, it is likely to become the foundation product for all farmed fishes, just as chicken is the base for the poultry industry. So someday soon instead of referring to tilapia as the aquatic chicken we may be referring to chicken as the "terrestrial tilapia".

INTRODUCTION

Tilapia holds a somewhat unique position amongst the major aquaculture fishes as a key product in international trade produced in large vertically integrated farming operations, while at the same still being produced in large amounts as a subsistence crop by some of the world's poorest farmers. The tilapias, with their unique mouth-brooding form of reproduction and extreme hardiness, allow farmers with the most meager resources an opportunity to rear the fish. Some farmers have even been known to rear tilapia in cisterns or 200 liter barrels. To an even greater extent than carps, farmers do not need access to hatcheries, or specialized information to rear tilapia in captivity. And similar to the oft used comparison to chickens, small farmers who spawn their own tilapia, will frequently end up with problems of in-breeding and

reduced yields. But for a subsistence farmer, this may be a minor problem compared to keeping a family fed.

At the same time, cooperatives of small-scale fish farmers in Asia and Latin America have collaborated with live haulers and processing plants to produce large amounts of fish for domestic and international markets. Cage culture has proven to be a key technique for people with limited resources and experience to get into aquaculture and generate significant quantities of fish for household and ex-household consumption. Cages can be constructed of locally available materials with minimal investment and placed in small ponds or in public waters. Many countries will provide access to reservoirs, irrigation systems and public waters to farmers and fishers with limited resources or who partner with government sanctioned processors.

Tilapia aquaculture has also attracted multi-national firms who grow fish in multiple countries vertically integrating feedmills, hatcheries, production, processing, packaging, transportation, and marketing. These firms employ thousands of farmers, feeedmillers, processing plant staff, drivers, office staff and sales forces. In many cases these employees are the prime recipients of the foreign exchange generated by these operations (Fitzsimmons and Watanabe, 2010).

Finally, as mentioned above, much of the global tilapia aquaculture has been integrated into irrigation systems. By rearing fish in reservoirs, canals and farm ponds, the effluents from tilapia farming are contributing to the fertilizer value in the water delivered to irrigated crops. This contributes to tilapias "green" reputation with the environmental community while saving on chemical fertilizer costs for resource poor farmers. So tilapia truly are a key contributor to global food security on several levels.

GENETICS

One of the key reasons for tilapia's continued expansion of production in future years is based on the genetic diversity available from which to build. The farmed tilapias are derived from several species in the genus *Oreochromis*. The fact that several of the species easily hybridize and produce large numbers of fecund young has allowed fish breeders to cross several species and develop strains that incorporate various traits from each of the parent species. This further supports the contention that the tilapia have been selectively bred and domesticated to an even greater extent than the edible carps. In fact they may be even more domesticated and differentiated than koi are from wild carps.

Size and body shape – Some of the primary morphological characteristics that breeders wanted to improve were the average size of the tilapia and the body shape, especially reducing the proportion of head to fillet. In both cases the ultimate goal is to have more edible fillet product. Most of the intensive breeding programs have focused on *O. niloticus* (Nile tilapia).

The Nile tilapia strains that have been developed in recent years include:

1. The Genetically Improved Farmed Tilapia (GIFT), originally developed in the Philippines from eight farmed and wild strains collected from around the world. The breeding program continues under the auspices of the WorldFish Centre at Jitra, Malaysia.

2. The Genomar strain was developed by a partnership of biologists from Brazil and Norway. It also included a large hatchery project in China, the Trapia project in Malaysia www.trapia.com.my/ and a hatchery in the Philippines. www.genomar.com

3. The Chitralada strain was developed in Thailand, and actually was started from the stocks of tilapia given to the King of Thailand who kept them in ponds at the Chitralada Palace. Breeders in Thailand continued to work with this strain and eventually developed the line that still bears the Chitralada name. It has also been used as an important line in some of the other breeding programs.

4. The TabTim line was developed in Thailand by the CP Group as their branded tilapia strain. The line is derived from several salt tolerant red tilapia lines, including some from Thailand, the Bahamas and the University of Arizona. Tab Tim has been successfully branded as a premium tilapia which receives an increased price and now is produced and marketed in Indonesia and Malaysia as well as Thailand.

5. The GIFT Excell line is derived from some of the GIFT tilapia that were left behind in the Philippines, when the GIFT program proper was moved to Malaysia. Some of the original GIFT biologists have worked in the original location and have partnered with various hatcheries to improve the strain.

6. The GIFT Bangladesh strain is another derivation from the GIFT tilapia. In this case, Bangladeshi scientists continued a selective breeding program with the GIFT fish sent to Bangladesh. These fish have been bred to thrive under the climatic and cultural conditions found at the local farms.

YY Supermale – This novel program was envisioned by biologists at University of Wales Swansea and then put into practical operation at the Central Luzon State University in the Philippines (Mair et al, 1997). The commercial entity arising from the project is called FishGen. http://www.fishgen.com The technique produces all male progeny for stocking on farm by manipulating the reproductive morphology of the grandparent fish. By treating the juvenile grandparent fish with estrogen, breeders can produce fish with a genetically female "father". This results in 25% YY fish in the F1 which can be crossed to normal females to produce virtually all XY (normal) male progeny in the F2 generation. In 2008 and 2009 groups in Egypt and Indonesia, respectively have reported that they have developed their own YY stocks.

Color morphs – There have been several strains of red tilapia developed. These include populations from Florida, Hawaii, Taiwan and Israel. Several have arisen from random mutations in *O. mossambicus* and another one in *O. niloticus*. Diligent breeding managed to "fix" these traits and develop marketable strains. In certain Asian communities the fish fetch a premium as it is the color of "good luck". In other communities, red tilapia resemblance to red snapper or red sea bream gains a premium price.

Salinity resistance – There are several populations of *O. mossambicus* that are recognized for their tolerance for extreme levels of salinity. These populations, especially from Lakes Bardawil and Manzala in Egypt, have been used as broodlines with Red strains and other species crosses to impart the salinity tolerance. This is another major advantage that the tilapias have over several other farmed species including the carps. Salinity tolerance opens up so many more options for farming opportunities in marine and brackish coastal water, inland brackish waters, agricultural and industrial waste water, and even hydroponic solutions used for lettuce and other vegetable production (Watanabe et al. 2006).

Genome project – An international group of geneticists is rapidly working through the *O. niloticus* genome (Kocher et al., 1998; Lee et al., 2005). http://www.broadinstitute.org/ftp/pub/assemblies/fish/tilapia/Orenil1/ The project has benefited from several allied groups sequencing parts of the genetic make-up. Recently large parts have been cataloged and are now being compared to previously described portions from other cichlids and the zebrafish (*Danio rerio*). A grass carp genome project in 2010 provided the first linkage map,

many years behind the work done with tilapia (Xia et al., 2010). Again this further definition and available information will likely benefit the genetic knowledge for the tilapia sooner, and to a more full extent that that available for the various carps.

NUTRITION

Omnivores – Herbivores – One of the qualities that continues to make tilapia popular with the "green movement" is the fact that they feed primarily on a very low trophic level. In nature, the tilapias feed upon algae, fresh and decaying plant material and periphyton. In domesticated settings the various tilapias still are fed a formulated diet that consists of grains and agricultural by-products that serve to keep tilapia diets below the average for most other farmed fishes. While many of the carps have similar feeding and nutritional patterns, the fact that tilapia in general are smaller and have smaller teeth and mouths, they tend to be even more efficient at scraping off the finest biofilms and periphyton colonies.

BioFlocs - The ability of tilapia to thrive in biofloc systems is yet another benefit that tilapia have over many of the other common aquaculture species. Avnimelech (2009; and this volume) describes how tilapia are uniquely adapted to thrive under biofloc conditions that would stress most other fish. This relatively low cost system for producing healthy fish and reducing formulated feed costs could be an additional benefit that should keep tilapia prices competitive with other wild and farmed species.

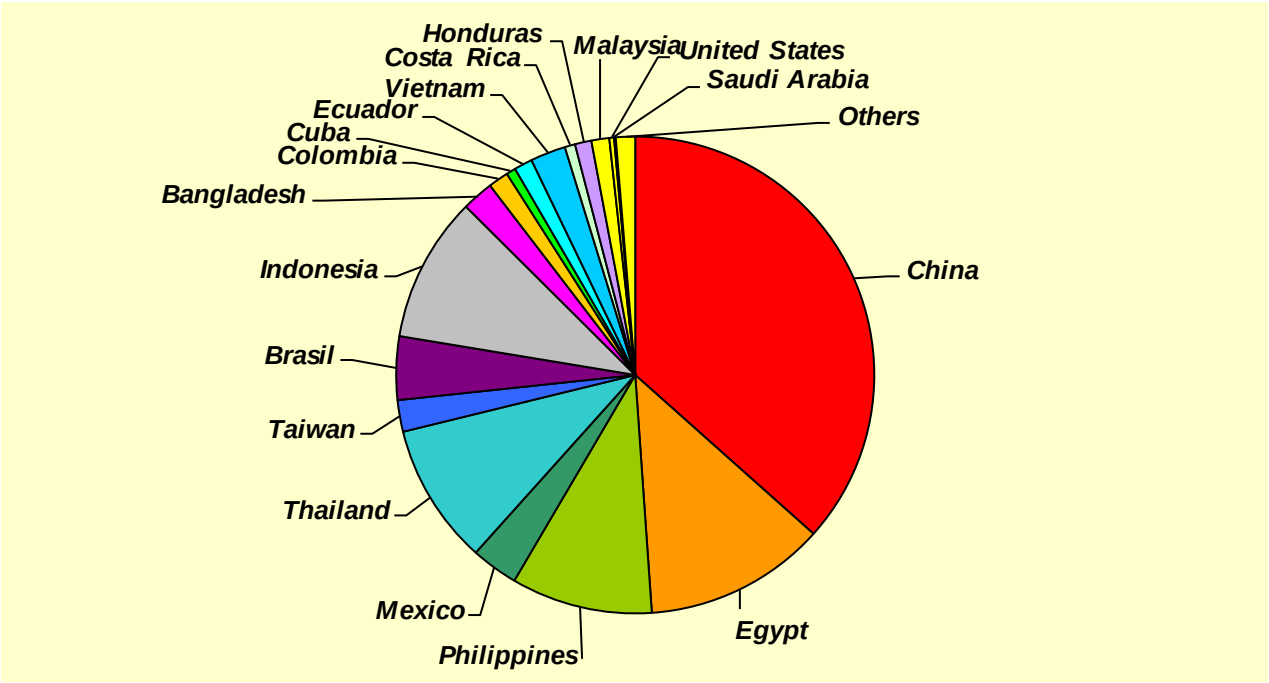
Agricultural plant wastes – Tilapia have proven to be one of the most important fishes used in alternative ingredient studies. The most common goal is to replace fish meal and fish oils. While these tend to be very minor ingredients in tilapia diets, the farmers and researcher still want to further reduce fish products in the diet and utilize locally available ingredients (Zerai et al. 2008). There are many studies available, including several more in this volume.

PRODUCTION SYSTEMS and LOCATIONS

Variety of production modes – Tilapia are unique in the array systems used to rear them in captivity. Commercial operations include: ponds, cages, raceways, tanks, net pens, lake ranching, seawater, brackish water, freshwater, aquaponics, plastic drums and computer controlled intensive recirculation systems. This variety of production exceeds that of any other farmed fish. Tilapia's usage with recirculating systems has allowed their production in urban areas, high latitude locations and even on the international space station (Fitzsimmons 2005; 2000).

Geographic distribution – FAO reports tilapia production from over 100 nations. This vast base of production and interest in the fish vastly exceeds any other farmed fish. The consumer demand is equally widespread. There are not any reports of cultural or religious restrictions on consuming tilapia. The major producing countries produced just over 3,200,000 metric tons of tilapia in 2010 (Fig. 1).

Figure 1. World Tilapia Production of 3,200,000 mt in 2010



Low cost production costs - Tilapia with their grain and vegetable based diets and ability to gather significant nutrition from grazing on algae and biofilms, have some of the lowest feed costs of any farmed fishes. With the high densities achieved on many farms, the infrastructure costs are therefore spread across a larger volume of fish. Finally, hatchery technology is relatively simple, allowing for fewer hatchery workers.

Hatchery technology – The high level of parental care provided by the female mouth brooders, simplify the activities of the tilapia hatchery manager. If the fry are left with the mother, the primary activity is to collect fry as they leave the female and start foraging. If the eggs are flushed from the mother's mouth and reared in a hatchery settling, the technology is only slightly more sophisticated; requiring hatching jars (or recycled plastic bottles) or open trays. With the hatching jars, the sick and dead eggs flow out, while trays do require maintenance to remove infected or unfertile eggs.

POLYCULTURE

An additional area in which tilapia production is rapidly increasing is polyculture. Many carp farmers in China, Vietnam and Indonesia have now incorporated tilapia into their traditional carp ponds and cages. In many cases this is for the better market price that tilapia sometimes gets and in others they appreciate the different niches (feeding and physical) that the tilapia occupy compared to their carps. Integration of tilapia and shrimp has been found to be beneficial for shrimp health and for economic return (Yuan et al 2010; Cruz et al. 2008). Across most shrimp farming regions, tilapia are increasingly being produced in cages or hapas inside shrimp ponds, or are produced in supply channels or head ponds. The increasing interest in integrated multi-trophic aquaculture systems for tropical production is certain to further contribute to overall tilapia production as most systems consider tilapia to be a key component to the systems.

Strong domestic markets – In many of the biggest producing countries, domestic demand is so strong, there are virtually no exports from countries including the Philippines, Mexico, Brazil, and

Bangladesh. Even China, with the world's biggest production, consumes more than half of all its production. In fact, Ecuador, Costa Rica and Honduras are probably the only countries which export a majority of the tilapia produced. This is a testament to the strong demand across all socio-economic groups for tilapia products.

Stronger international markets – The United States continues to be the single largest market for tilapia products. Increasing demand for all forms of tilapia products and more market share in restaurants, food-service, club stores, hypermarkets and groceries is encouraging live and on-ice tilapia sales from US farms and a flood of imports from Asia and Latin America.

Table 1. US imports of tilapia products in 2009 and 2010 (values in US$)

Product	Country	2009 Kilos	2009 Value	2010 Kilos	2010 Value
TILAPIA FILLET FRESH	BELIZE	9,304	76,620	0	0
TILAPIA FILLET FRESH	BRAZIL	264,232	1,892,361	332,471	2,445,064
TILAPIA FILLET FRESH	CHILE	643	3,589	3,218	29,250
TILAPIA FILLET FRESH	CHINA	20,769	109,200	0	0
TILAPIA FILLET FRESH	CHINA - TAIPEI	207,949	1,348,949	220,166	1,250,038
TILAPIA FILLET FRESH	COLOMBIA	1,627,884	12,655,428	1,796,060	13,549,639
TILAPIA FILLET FRESH	COSTA RICA	5,720,984	41,979,201	5,825,430	39,803,789
TILAPIA FILLET FRESH	ECUADOR	9,059,973	57,594,646	7,852,974	49,715,847
TILAPIA FILLET FRESH	EL SALVADOR	480,827	3,720,300	332,289	2,447,784
TILAPIA FILLET FRESH	FAROE IS.	0	0	3,283	25,384
TILAPIA FILLET FRESH	GUATEMALA	0	0	1,361	9,000
TILAPIA FILLET FRESH	HONDURAS	6,511,715	51,607,530	7,245,304	56,201,338
TILAPIA FILLET FRESH	NICARAGUA	430,635	3,424,958	46,428	342,391
TILAPIA FILLET FRESH	PANAMA	1,362	10,117	3,808	28,268
TILAPIA FILLET FRESH	PERU	4,009	31,199	55,044	431,899
TILAPIA FILLET FRESH	THAILAND	17,654	84,472	0	0
Subtotal		**24,357,940**	**174,538,570**	**23,717,836**	**166,279,691**
TILAPIA FILLET FROZEN	CHINA	100,691,098	363,266,149	135,522,960	517,771,039
TILAPIA FILLET FROZEN	CHINA - HONG KONG	0	0	73,935	228,090
TILAPIA FILLET FROZEN	CHINA - TAIPEI	2,332,494	12,483,161	2,248,666	10,093,980
TILAPIA FILLET FROZEN	COLOMBIA	0	0	3,832	12,128
TILAPIA FILLET FROZEN	COSTA RICA	95,838	662,839	152,776	936,587
TILAPIA FILLET FROZEN	ECUADOR	1,118,103	7,391,980	638,368	4,181,009
TILAPIA FILLET FROZEN	FIJI	0	0	16,393	63,880
TILAPIA FILLET FROZEN	HONDURAS	604,502	4,345,036	108,289	673,853
TILAPIA FILLET FROZEN	INDONESIA	8,757,932	56,464,317	10,201,574	68,590,604
TILAPIA FILLET FROZEN	MALAYSIA	0	0	319,912	1,434,481
TILAPIA FILLET FROZEN	NEW ZEALAND	51,710	579,039	0	0
TILAPIA FILLET FROZEN	NORWAY	726	4,247	0	0
TILAPIA FILLET FROZEN	PANAMA	273,499	1,250,091	193,789	871,642
TILAPIA FILLET FROZEN	PHILIPPINES	1,701	10,500	9,232	21,887
TILAPIA FILLET FROZEN	THAILAND	678,831	3,792,956	1,055,543	5,488,994
TILAPIA FILLET FROZEN	VIET NAM	156,028	555,401	224,847	705,939
Subtotal		**114,762,462**	**450,805,716**	**150,770,116**	**611,074,113**

TILAPIA FROZEN	BANGLADESH	490	2,537	1,207	2,230
TILAPIA FROZEN	CAMEROON	19,958	24,080	0	0
TILAPIA FROZEN	CANADA	2,268	10,000	0	0
TILAPIA FROZEN	CHINA	29,671,564	44,185,702	22,938,041	37,337,832
TILAPIA FROZEN	CHINA - TAIPEI	13,179,606	23,915,366	16,296,367	25,434,922
TILAPIA FROZEN	COLOMBIA	97,202	277,719	44,712	132,462
TILAPIA FROZEN	ECUADOR	5	5,162	2,000	4,551
TILAPIA FROZEN	INDIA	0	0	2,790	2,715
TILAPIA FROZEN	INDONESIA	11,026	14,431	22,401	44,939
TILAPIA FROZEN	MALAYSIA	18,144	27,550	0	0
TILAPIA FROZEN	NICARAGUA	6,037	16,395	5,527	14,520
TILAPIA FROZEN	PANAMA	65,136	121,933	158,159	242,112
TILAPIA FROZEN	PERU	42,203	78,650	0	0
TILAPIA FROZEN	PHILIPPINES	23,871	55,079	114,430	212,596
TILAPIA FROZEN	THAILAND	904,663	1,676,321	1,185,152	1,782,752
TILAPIA FROZEN	UNITED ARAB EMIRATES	0	0	7,000	11,700
TILAPIA FROZEN	VIET NAM	132,266	330,770	112,068	288,871
Subtotal		**44,174,439**	**70,741,695**	**40,889,854**	**65,512,202**
Grand Total		**183,294,841**	**696,085,981**	**215,377,806**	**842,866,006**

PROCESSING and VALUE ADDING

One of the primary constraints on the tilapia industry has been the problem off-flavor. Most often the off-flavor is caused by cyanobacteria (blue-green algae) blooming in production ponds. The industry has made a concerted effort to train farmers, custom harvesters and processing plant operators to recognize the presence of both the algae and the off-flavor odors coming from the geosmin and methyl-isoborneol imparted to the fish by the cyanobacteria. Many farms and processors have developed depuration systems and procedures to ensure that any off-flavor fish are treated before processing. More sophisticated testing and testing labs are also available to assist farmers and processors to ensure that off-flavor products do not reach consumers (Fitzsimmons 2006). It should be noted that sometimes these fish are processed and sold as lower grade product to lower price markets, especially in Russia and sub-Saharan Africa.

A second constraint is the relatively low percent recovery for tilapia fillets compared to other fishes with a more beneficial body form. This has become even more of an issue as processors implement additional trims and deeper skinning at the request of some customers. Breeders are attempting to overcome this constraint by selecting fish with a better body conformation to increase fillet yield. A second aspect is the development of co-products from the processing industry. One of the co-products has been the increase in a variety of leather goods derived from tilapia skins. This technique first appeared in Brazil, which still has the most diverse selection of products. But we are seeing additional products including tilapia skin swimwear from Thailand.

Figure 2. Tilapia skin leather goods

Sophisticated equipment and low labor costs – processing companies are continuing to utilize a mix of high technology and low skill labor to prepare the variety of tilapia goods in the market. High capital investment equipment including freezers, scalers, packaging, and computer aided weight checkers is mixed with hand fillet lines and manual packing of boxes. The low cost labor countries with tropical growing conditions will continue to be the industry leaders.

Explosion of product forms – More than any other factor, the plethora of tilapia products hitting the market is encouraging demand and will be the ultimate reason that tilapia will eventually surpass carps to become the most popular farm raised food fish. Breaded fillets, tilapia loins, stuffed fillet, ready to bake or microwave tilapia with sauces and side-dishes are flooding the markets in the US, Europe and the East Asia countries. As young women in Asia continue to join the work force, the idea of purchasing a whole fish (especially carp) and preparing it for smaller families is declining. Women shopping after a work day in the office want the convenience of a packaged fillet product that will be easy to prepare with minimal waste for disposal. Smoked and sashimi forms are also becoming more popular.

The consumption of tilapia in the US market continues to increase and Europe and East Asia are likely to follow the trend of more value added tilapia forms making up an increasing share of the market demand. Tilapia may become the fourth most popular seafood in the US by 2012.

Table 2. United States per capita consumption of seafood products in pounds per person.

2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Tuna 3.5	Shrimp 3.4	Shrimp 3.7	Shrimp 4.0	Shrimp 4.2	Shrimp 4.1	Shrimp 4.4	Shrimp 4.1	Shrimp 4.1	**Shrimp 4.1**
Shrimp 3.2	Tuna 2.9	Tuna 3.1	Tuna 3.4	Tuna 3.4	Tuna 3.1	Tuna 2.9	Tuna 2.7	Tuna 2.8	**Tuna 2.5**
Pollock 16	Salmon 2.0	Salmon 2.0	Salmon 2.2	Salmon 2.2	Salmon 2.4	Salmon 2.0	Salmon 2.4	Salmon 1.8	**Salmon 2.0**
Salmon 15	Pollock 12	Pollock 11	Pollock 17	Pollock 17	Pollock 15	Pollock 16	Pollock 17	Pollock 1.34	**Pollock 1.45**
Catfish 11	Catfish 11	Catfish 11	Catfish 11	Catfish 11	Catfish 10	**Tilapia 1.0**	**Tilapia 1.14**	**Tilapia 1.19**	**Tilapia 1.21**
Cod 0.8	Cod 0.6	Cod 0.7	Cod 0.6	Tilapia 0.7	Tilapia 0.8	Catfish 0.97	Catfish 0.90	Catfish 0.92	**Catfish 0.85**
Clams 0.5	Clams 0.5	Crabs 0.6	Crabs 0.6	Cod 0.6	Crabs 0.6	Crabs 0.7	Crabs 0.68	Crabs 0.61	**Crabs 0.59**
Crabs 0.4	Crabs 0.4	Clams 0.5	Tilapia 0.5	Crabs 0.6	Cod 0.6	Cod 0.5	Cod 0.47	Cod 0.44	**Cod 0.42**
Flatfish 0.4	Flatfish 0.4	Tilapia 0.4	Clams 0.5	Clams 0.5	Clams 0.4	Clams 0.4	Clams 0.45	Flatfish 0.43	**Clams 0.41**
Scallops 0.3	Tilapia 0.4	Flatfish 0.3	Scallops 0.3	Scallops 0.3	Scallops 0.3	Scallops 0.3	Flatfish 0.32	Clams 0.42	**Pangasius 0.35**
Tilapia 0.3									

VERTICAL INTEGRATION

Another huge reason for the rapid expansion of tilapia products and consumption has been the vertical integration in the industry that has been especially beneficial for production in developing countries and market expansion in the US and European Union. RainForest, Regal Springs,

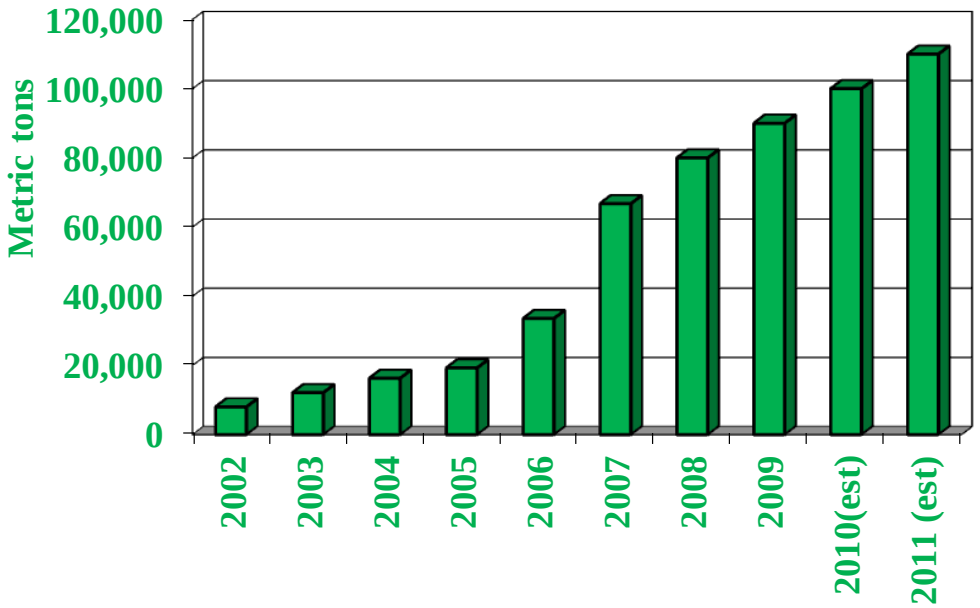
Tropical Tilapia and HQ Sustainable Maritime are multi-national firms. Some source from multiple farms in several countries and each sells to multiple countries. The application of technology across borders and multi-national, multi-lingual employees have provided these companies the ability to rapidly adjust techniques and feed formulations, genetics, processing and marketing. Brand recognition and specialized packaging have further improved the tilapia markets.

Figure 3. Packaging and brand development

CONCLUSIONS

Global farmed tilapia production has already surpassed that of the salmon and the various catfishes. New producing countries continue to enter the markets producing and consuming large volumes of tilapia. For example, Bangladesh has increased tilapia production from virtually zero in 2000 to 100,000 mt tons in 2010.

Figure 4. Production of tilapia in Bangladesh (2002 – 2011 est.)



On a global basis, while tilapia production is still far behind the carps, the convergence of stronger potential for increased production and the much wider base of consumption leads to the logical conclusion that tilapia will continue to increase production until it surpasses the carps as the most important farmed fish on the planet.

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SECTION I
HEALTH AND DISEASE

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Live Attenuated Bacterial Vaccines in Aquaculture

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水产养殖中的活性弱毒疫苗

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目前,在世界范围内,水产养殖业已经成为农业经济中的一个重要的组成部分。阻碍水产养殖业增长的一个最重要的因素就是传染性疾病,它给水产养殖业带来了巨大的经济损失。目前,已报道的致病菌种类已经多达20多个属。细菌性传染病爆发的几率是相当高的,特别是在高密度和循环水养殖系统中。预防这类疾病发生的最有效的方法是接种疫苗。这是保证养殖经济鱼类健康的一种不可或缺的重要措施。活性弱毒疫苗已经非常成功的广泛应用于预防动物与人类的多种疾病。活性弱毒疫苗能天然的模仿宿主与病原菌之间的相互作用,这就可以有效的激活宿主相应的免疫系统。因此最有效的预防鱼类细菌性传染疾病爆发的措施就是接种活性弱毒疫苗。此外,这种疫苗还具有生产成本低,运输简捷以及保护性长久等优点。下面将会讨论这类疫苗的生物安全性,保护性的强弱,经济价值,生产和运输方法,以及一些潜在的活性弱毒疫苗。

Biosecurity

Aquaculture is emerging as an important economical agribusiness, worldwide. Disease outbreaks cause severe economic losses in aquaculture production and trade. Currently, it is not possible to properly quantify the dollars losses, but disease loss estimates in Asian countries amount to more than $3 billion annually[1]. Furthermore, disease is being recognized as a primary constraint on the economic development of some countries. In addition to mortality and morbidity, disease causes reduced slaughter value, growth performance and feed conversion in fish. Other costs associated with disease are money spent to purchase chemicals and drugs to combat diseases.

A variety of pathogens are responsible for infectious diseases including viruses, parasites, fungi and bacteria. Among them, bacteria pathogens account for majority of diseases in warm water aquaculture. Bacterial species of more than 20 genera have been reported as causes of diseases. Prominent Gram negative pathogens include species of *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Francisella*, *Pasteurella*, *Piscirickettsia, Pseudomonas*, *Vibrio* and *Yersinia*. Species of *Lactococcus*, *Reinbacterium*, *Streptococcus* are examples of Gram positive pathogens while *Mycobacterium* is a Gram resistant pathogen. Diseases caused by pathogen genus, species, and available U.S. licensed vaccines are summarized in Table 1.

Table 1. Gram-negative, positive and resistant bacterial pathogen of fish

Gram-negative	**Disease**	**US licensed vaccines & type as of 10/10/2009**
Aeromonas hydrophila	Motile aeromonas septicemia (MAS)	
Aeromonas salmonicida	Furunculosis	Killed
Edwardsiella ictaluri	Enteric septicemia of catfish	Attenuated
Edwardsiella tarda	Edwardsiellosis or Putrefactive disease	
Flavobacterium columnare	Columnaris	Attenuated, Killed
Flavobacterium psychrophilum	Coldwater disease	
Francisella sp	Francisellosis	
Moritella viscosa	Water ulcers	
Pasteurella damsella pisicicida	Pseudotuberculosis	
Piscirickettsia salmonis	Piscircickettoiss	
Pseudomonas fluorescens	Generalized septemia	
Vibrio anguillarum, ordalii, parahaemolyticus, vulnificus	Vibrosis	Killed
Yersinia ruckeri	Enteric redmouth disease	Killed
Gram-positive		
Lactococcus garvieae	Lactococcosis	
Renibacterium salmoninarum	Bacterial kidney disease	
Streptococcus agalactiae	Streptococcosis	
Streptococcus iniae	Streptococcosis	
Gram-resistant		
Mycobacterium sp	Mycobacterosis	

A major threat to worldwide tilapia aquaculture is *Streptococcus* infection. Tilapia infections have occurred or are occurring in at least 26 countries of Americas, Asia, Australia, Middle East and Southern Europe. *Streptococcus iniae* and *S. agalactiae* are the two principal causes of streptococcal infections. Therefore, effective vaccines against streptococcal infections are urgently needed. In addition to these two streptococcal species, effective vaccines against *Lactococcus garvieae*, *A. hydrophila*, *E. tarda*, *F. columnare, Francisella* sp., and *Vibrio* sp. are needed.

Currently, there are only 14 licensed fish vaccines in U.S., including 11 killed bacterial, 1 killed viral, and 2 live attenuated bacterial prophylactics (http://www.aphis.usda.gov/animal_health/vet_biologics). Vaccines are licensed for given fish species and pathogen(s). They are not broadly licensed for use in other fish species that may be affected by the same pathogen as the target species. For example, currently the attenuated

vaccine, AQUAVAC-COL® (Intervet/Schering-Plough, formerly USDA, ARS, attenuated *Flavobacterium columnare* strain) is only licensed against *F. columnare* in catfish species.

Seventeen vaccines against 12 bacterial pathogens are available, worldwide[27]. The majority of these vaccines are for use in salmonids. Fewer vaccines are available for tilapia, seabass/bream, Japanese flounder, yellowtail, turbot, catfish and other species. Fish producers have considerably fewer vaccines available than producers in poultry and livestock industries with a ratio of about 1 to 10. There is a recognized need to develop new vaccines as well as to improve efficacy of existing vaccines.

A hallmark of bacterial disease is its rapid spread within a farm, between farms in a locality, nationally and internationally by a variety of means. Treatment of diseased fish is often done by the use of antibiotics or chemicals. Some drugs and chemicals may be harmful to the consumer and the environment. The use of drugs and chemicals in aquaculture is a major issue in international trade and many countries have limited detectable quantities of these substances in fish products.

Vaccine disease prevention

The best disease prevention method is vaccination. Vaccines are an integral tool in any health management strategy applicable to economically reared fish. Vaccines greatly reduce the need for drugs and chemicals. Live attenuated vaccines contain weakened or less virulent form of the pathogen that causes the disease. The concept behind such vaccines is that the pathogens are efficacious to stimulate immunity, but too weak to cause diseases. Live attenuated vaccines have been extensively and very successfully used against a number of animal and human diseases, over an extensive span of years, whereas killed vaccines were often poorly efficacious. Therefore, we believe that the most valid preventative strategy to combat infectious disease of fish is through the use of live attenuated vaccines. Aspects of biosafety, efficacy, economic benefits, methods of production, delivery of two available licensed vaccines, and some novel live attenuated bacterial vaccines will be discussed.

Vaccines types

Six types of bacterial vaccines include killed, recombinant, DNA, subunit, vector and live attenuated are currently available. The killed vaccine that is composed of killed whole bacterial cells. The most commercially available prophylactic is killed vaccine. Recombinant, DNA, subunit and vector type vaccines are developed on the basis of expression of a protein or peptide antigen that is presumed to be the protective antigen. However, only a limited number of bacterial proteins have been identified as protective. An alterative concept is that protection is confirmed by multiple antigens that are composed of lipopolysaccharides, lipoproteins, complex polysaccharides as well as proteins. Live attenuated vaccines express these multiple antigens that are needed to provide the most efficacious immunity[11,25], therefore they will be emphasized in this paper. The desirable properties of live attenuated bacterial vaccines are summarized in Table 2. These desirable properties will be discussed throughout this article.

Table 2. Desirable Properties of Attenuated Bacterial Vaccines

Desired Property	Examples
loss of virulence	Gene deletion/point mutations on more than one gene
Full antigenic complement	Protein, peptide, carbohydrate, lipopolysaccharide, lipoprotein antigens

T-cell target	T-cell presentation and activation of innate and acquired immune system
In vivo capacity for the mutant to replicate	Replication within host for more than about 72 h
Bacteriological detectable marker	Antibiotic resistance
Long duration of protection	One year or longer
Cross protection	Effective majority field strains
Easy and mass delivery	Immersion or orally
Low production cost	Fermentation
Long shelf life	Lyophilized or frozen product

Biosafety

The development and use of live attenuated bacterial vaccines is becoming more attractive due to their many advantages, despite concerns raised by some about their biosafety. The principal reason behind the concern that attenuated vaccines are considered more risky than killed vaccines is their possible reversion from the weakened state to virulent state. However, this risk appears to be unfounded as no reversion has been documented for the AQUAVAC-ESC® attenuated vaccine (formerly USDA, ARS, RE-33) which has been used in U.S. catfish industry for more than 10 years.

Development and biosafety evaluation of vaccines is regulated by the USDA, Animal and Plant Inspection Service, Center for Veterinary Biologics in the U.S. Acceptance of vaccines requires comprehensive assessments of all criteria of vaccine development. This assessment includes biological safety to both aquatic animals and environment. The assessment also includes purity and efficacy of the vaccine. U.S. protocols for studies of host animal immunogenicity/efficacy, safety, backpassage, shed/spread, immunological interference, and other areas can be found at http://www.aphis.usda.gov/animal_health/vet_biologics. U.S. environmental release- risk assessment comply with the regulations of the National Environmental Protection Act (NEPA), applies to products not exempted by categorical exclusion by 7 CFR 372.5(c). This requirement applies to conventionally derived modified live vaccine products and those derived by recombinant DNA technology.

In general, attenuated vaccines should be both safe and efficacious to achieve the desirable features of a live vaccine. Phenotypic and genotypic stability, pathogenicity to other organisms, and the potential effects to non-target organisms are major biosafety-related properties to be examined for any attenuated vaccine and its wild type parent. A risk/benefit analysis on the use of live attenuated bacterial vaccines may reveal the potential preventive benefits that outweigh the decision to preclude these vaccines from the market. Favre and Viret[8] have provided a framework on European regulations for biosafety assessment of human oral attenuated vaccines that provides valuable insight to the assessment of veterinary attenuated vaccines. Incorporation of specific genetic markers such synthetic antibiotic resistance into the attenuated vaccine strain will allow the wild type parent strain to be distinguished from the attenuated vaccine strain by appropriate bacteriological methods. This is important for monitoring the biosafety of the attenuated vaccine in the field.

Licensed attenuated vaccines

Currently two licensed attenuated bacterial vaccines are available for use in U.S. catfish. These commercially available vaccines include *E. ictulari* (AQUAVAC-ESC®) and *F. columnare* AQUAVAC-COL®[9,26]. These two vaccines were successfully developed by the USDA, ARS Aquatic Animal Health Research Laboratory and licensed from the USDA to Intervet/Schering-Plough for manufacture and distribution. The two attenuated vaccines mentioned above were developed by serial passages of the virulence wild type parent bacteria on increasing concentrations of the synthetic antibiotic, rifampicin. These mutants were demonstrated to be both safe and effective vaccines[9,26,14,32,33]. The loss of virulence was associated with alterations in their LPS[2,9,26,34].

Delivery

Attenuated bacterial vaccines, such as *E. ictaluri* vaccine, are deliverable to large numbers of fish by immersion with minimum stress[10,15]. The vaccine was licensed to be used in 7-10 post-hatched or older catfish by immersion[9]. Further, attenuated vaccines such as *F. columnare* are efficacious when delivered in 10 d post-hatched fry by immersion[26]. The relative percent survival (RPS) was 57 to 94% between 10 to 48 d post-hatch. Eyed catfish eggs were also successfully vaccinated using the *E. ictaluri* RE-33 attenuated vaccine. The RPS was 57.9% when the immunized fingerlings were challenge at 60 d post-immunization[22,24]. However, when the immunized fry were booster by immersion at 7 d post-hatch the RPS declined to 27.3%. These results indicated that the additional antigen load from the booster immunization compromised the immune protection of the attenuated vaccine. Attenuated vaccines that stimulate a strong cellular immune response have been reported to lead to immunosuppression[17]. It was believed that the persistence of the attenuated vaccine strain in the egg and fry resulted in the successful immunization of eyed eggs and their fry. The results also showed that eyed catfish eggs were safely immunized with no adverse mortality.

Eyed catfish eggs were immunized by immersion with either monovalent *F. columnare* attenuated vaccine or bivalent attenuated *F. columnare* and *E. ictaluri* vaccines[23,24]. The RPS of monovalent attenuated vaccine was 76.8% at 137 d post-immunization following *F. columare* challenge. The RPS of divalent attenuated vaccine challenged with *F. columnare* was 56.7% at 109 d post-immunization and when challenged with *E. ictaluri*, the RPS was 66.7% at 116 d post-immunization. Attenuated vaccines may be very effective at protection of fish following immunization at the nursery or fingerling stages. Application of this strategy would best protect fish throughout their production cycle. Little research has been done on feeding attenuated vaccine to fish. It seems to be a promising alterative strategy to immersion immunization[22].

Efficacy

Live attenuated vaccines activate immune responses that closely mimic a natural infection because the majority of its antigens are expressed *in vivo*. Attenuated vaccines activate both innate and acquired immune systems. While stimulating antibody and cellular responses, live attenuated vaccines induce both local and systemic immune responses[6]. Live attenuated vaccines are generally more potent than killed vaccines in activating cellular immunity. Live attenuated vaccines activate strong and long-memory T-cells[18], stimulate the production of cytokines, and produce cytotoxic T-lymphocytes[19,20]. The pathway is initiated by first interactions between naïve CD_4 T -cell and antigen presenting cell that leads to cytokine production. This is followed by pathways that activate and differentiate T -helper cell subsets, which release different types of cytokines that finally stimulate specific immunity against a pathogen.

Generally, attenuated bacterial vaccines are protective against many wild-type strains encountered in the field[9]. This is an advantage over a killed bacterial vaccine that is usually limited in its capacity to provide cross-protection against different strains. Killed vaccines are able to stimulate specific antibody responses[21]. Formalin killed *E. ictaluri* vaccine has not been efficacious[16]. This may be due to the mode of action of formalin that may result in alterations of surface antigens[3or] or the loss of the ability to enter the host fish[16]. Furthermore, killed vaccines stimulate short-lived immunity, whereas attenuated vaccines produce long term immunity. It is believed that this longer duration of immunity is the result of replication of the attenuated bacteria within the tissues of the fish[15]. It is believed that the longer the attenuated strain persists in the host, the more protection is achieved[17]. This might be due to the strength of the immune response induced by the attenuated live vaccine and/or the functions of the antigens expressed by the live vaccines. Duration of immunity following bath immersion exposure to live bacteria has been reported to last more than 4 months[9,25]. Since live attenuated bacterial vaccines are more effective in eliciting stronger cellular immune responses, they are more potent against intracellular Gram-negative pathogens such as *E. ictaluri* .

Economic benefits

Vaccines should not only reduce fish mortality and morbidity, but also provide additional economic benefits in the form of promoting faster growth rate and improved feed conversion. Economically profits of $3000 to 4000 per ha were experienced for AQUAVAC-ESC® vaccinated over non-vaccinated catfish in field trials[33]. The use of this attenuated vaccine has also been found to improve survival and to increase profits for catfish held longer in nursery ponds before being released in fingerling ponds[5]. The added economic benefits to the producer using this or similar attenuated vaccine is obvious.

The decision whether to vaccinate against a certain pathogen or not is the producers' willingness to take the risks of a disease outbreak[29]. Vaccination is a form of insurance policy against a disease outbreak occurring and its economic impact to the producer. The risks of a major economic loss occurring becomes greater in relation to the production time. A disease outbreak with high mortality and morbidity at the food size stage will be considerably greater than at fingerling stage. The loss of 50 or greater percent of fish at or about the food size stage may lead to farm closure. Vaccination with attenuated vaccines would reduce the disease risk and provide additional profits that may offset the cost of vaccination. If a particular disease occurs with some frequency on a farm, a decision against vaccination is the highest form of risk taking. A good vaccination strategy that is applied on a regular basis will result in a reduction of disease outbreaks overtime and consequently result in greater profits. Treating disease outbreak with drugs generally do not achieve this beneficial outcome. In a study of vaccine usage in the Chilean salmon industry during the period of 1999-2003, it was reported that usage by immersion increased from 97 million to 200 million doses and by injection from 2 thousand to 16.5 thousand doses[4]. The Chilean salmon industry accounts for a fish harvest at 585 thousand tons and netting an income of $1.721 million in 2005 (http:salmonchile.cl). More than 20 different vaccines are used or have been used in the Chilean salmon industry[4].

Licensed and other attenuated vaccines

Attenuated mutants have been produced using auxotrophy, transposon insertion and by chemical/drug mutagenesis (Table 3). Auxotropic mutants were produced by inactivation of the aroA gene by the insertion of a DNA fragment containing an antibiotic resistant gene. After allelic exchange using a suicide vector, aroA mutants were selected for their loss of survival in fish due to their need for aromatic metabolites[30]. The aroA attenuated vaccine was shown to have 5 $\log_{10}$ loss of virulence over the wild type. However, no viable mutant cells were detected

in catfish following immersion immunization at 48-72 h. Furthermore, the aroA attenuated vaccine was found to be not highly efficacious (RPS, 54.1- 63.8) against ESC[30].

A purA mutant of *E. ictaluri* was produced and evaluated for its attenuation, persistence and efficacy in catfish[12]. The attenuation resulted in 5 $\log_{10}$ loss of virulence compare to the wild type. The purA mutant was detected following immersion immunization for 48 h. The RPS for catfish challenged with wild type *E. ictaluri* was 63.3%.

Transposon mutagenesis was also used to produce *E. ictaluri* mutants that were deficient in lipopolysaccharide O side chain (O LPS)[13]. The O LPS attenuated mutant was shown to be highly attenuated[13]. The attenuated mutant was detectable for 14 d in catfish following immunization by immersion exposure[14]. Only i.p. injection produced protection (RPS, 90%) whereas immersion exposure resulted in a RPS of 0%. Table 3 summarized the RPS provided by the O LPS mutant compared to the RPS provided by the attenuated RE-33 mutant[14]. The RE-33 attenuated vaccine by immersion exposure had a RPS of 100% whereas the RPS of the O LPS mutant vaccine was 0% (Table 3).

Table 3. Examples of some bacterial attenuated vaccines

Pathogen	Fish	Attenuation Method	Delivery	Fish age or size	RPS (Weeks Post Vaccination)
E. ictaluri [9,14,31,32]	Catfish	Rifampicin-resistant	Immersion	7-10 d	60-100 (4)
E. ictaluri [30]	Catfish	aroA-deletion	Immersion	8 m	54.1-63.8 (4)
E. ictaluri [12]	Catfish	purA-deletion	Immersion	5 g	67 (3)
E. ictaluri [13,14]	Catfish	LPS deletion	Injection Immersion	6 m	94 (4) 0 (4)
E. tarda [28]	Japanese flounder	Rifampicin-resistant	Injection Oral plus Immersion	9.1 g	51.4 (10) 69.4 (10)
F. columnare [26]	Catfish	Rifampicin-resistant	Immersion	10 d	57-94 (5)
	Largemouth bass		Immersion	10d	74-97 (5)
F. psychrophilium [11]	Rainbow trout	Rifampicin-resistant	Injection Immersion	2.4 g	45(8) 45 (0)

The rifampicin strategy was used to produce an attenuated vaccine against *F. psychrophilium,* the cause of coldwater disease in salmonids[11]. The rifampicin resistant mutant was demonstrated to be highly attenuated and efficacious in rainbow trout. The same strategy was used to produce an attenuated vaccine against *E. tarda,* an important pathogen of marine and freshwater fish[28]. The rifampicin-resistant mutant was produced by multiple passages on growth medium containing the antibiotic rifampicin. This attenuated vaccine was shown to be safe and efficacious in Japanese flounder by injection, immersion and oral delivery[28]. Feeding plus immersion booster was shown to produce the highest RPS levels of 80.6 and 69.4% at 5 and 8 weeks, respectively. The mutant was demonstrated to survive in intestine, liver and spleen of fish for 1-10 d post vaccine feeding[28]. When administrated by immersion, the spleen, liver, kidney and blood were positive for the live attenuated vaccine at 1-14 d post immunization[28].

Conclusion

The development and use of attenuated bacterial vaccines in the U.S. has provided very promising benefits and advantages over other types of vaccines in the last 10 years in the catfish industry. The use of attenuated vaccines in world aquaculture will grow to a stage as much as they are used in livestock, poultry and humans to prevent diseases. The further development and use of attenuated vaccine in disease endemic areas is expected in the future, especially where no efficacious vaccines are available.

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ISOLATION AND CHARACTERIZATION OF *Streptococcus agalactiae* FROM RED TILAPIA CULUTURED IN THE MEKONG DELTA OF VIETNAM

Dang Thi Hoang Oanh and Nguyen Thanh Phuong

ABSTRACT

Bacterial diseases have been one of significant problem in red tilapia (*Oreochromis sp*) cage culture in the Mekong delta of Vietnam. Usual clinical signs of Streptocococcis include opacified eyes, popeye, small skin haemorrhage or petechias and skin ulcers. These clinical signs are easy to spot for the layman. However, the observation of the clinical signs from a macroscopic perspective is insufficient to determine the species of bacteria responsible for the disease as well as the biotype. These pieces of information are crucial to be collected in order to anticipate the utilization of a treatment or vaccine program. Therefore, diseased specimens were collected at a farm practicing intensive cage culture of red tilapia in Tien Giang province in 2010. Microscopic observation of fresh smear of blood, liver, kidney and spleen from these specimens revealed small cocci, gram positive bacterial cells. Bacteria isolates from brain and head kidney were recovered on brain heart agar and were analyzed as Gram positive, non-motile and oxidase negative and they were identified as *Streptococus agalactiae biotype 2* using a combination of conventional biochemical test, API 20 strep system. Histopathological examination of diseased specimens showed a typical sign of bacterial necrosis in kidney, spleen and liver. Challenge experiments using injection method showed that they can cause the observed disease signs with the LD_{50} value of about 4.89×10^4 CFU/m.

It is the first report of *Streptococcus agalactiae* biotype 2 outbreak in tilapia in Vietnam.

Keywords:Red tilapia, Streptococcus agalactiae, histopathology, pathogenicity

ECO-PHYSIOLOGICAL IMPACT OF COMMERCIAL PETROLEUM FUELS ON NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L.)

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Abstract

The pollution of commercial petroleum fuels (CPF) is one of the environmental constrains that produces aqua-toxicological effects, which are deleterious to aquatic life. Therefore, this study was conducted to explore the effect of some CPF; kerosene, gasoline or diesel, on the performance of Nile tilapia, *Oreochromis niloticus* (L.). Healthy fish (49.5±1.3 g) were distributed into glass aquaria and 12 ml of kerosene, gasoline, or diesel were separately added to 6 120-L aquaria. Fish were stocked into the aquaria containing kerosene, diesel, or gasoline for 5 minutes. Signs of poisoning in fish exposed to each fuel type included air gulping, increased opercular movement, and dyspnea. Fish lost their balance, meanwhile no poisoning signs were observed in control fish. At 0, 1, 2, 3, and 4 weeks of the recovery, blood samples were taken to measure the different physiological variables. At the end of this experiment, fish were collected, counted and weighed. Fish in control group grew up gradually to the end of the experiment, meanwhile fish exposed to kerosene, diesel, or gasoline lost their weights for 2 weeks and started to grow again. Moreover, weight gain and SGR of fish exposed to diesel and gasoline were less than that exposed to kerosene. Feed intake, FCR, and survival rate of the exposed fish were poor. RBC count and Hb in fish exposed to kerosene, diesel, or gasoline increased by time and the maximum count was obtained at the 1st week; their values decreased up gradually to the 4th week. Glucose level was maximized after the exposure to kerosene, diesel, or gasoline and decreased up gradually to the end of the experiment. Plasma lipids increased significantly by time at the treated fish groups. Plasma protein in fish increased suddenly after the exposure to kerosene, diesel, or gasoline and it decreased by time to be close to that of control group. AST and ALT activities in fish increased gradually after their exposure to CPF and the maximum values were obtained after 3-4 weeks. The lowest cortisol value was obtained at control, which was insignificantly changed throughout the experimental period. This study has demonstrated that the acute exposure to CPF had a highly significant effect on reducing the growth performance of Nile tilapia and affected their physiological status.

INTRODUCTION

The majority of studies examining the toxicity of petroleum hydrocarbons have focused on marine species, thus the toxic effects of petroleum hydrocarbons on freshwater species are relatively unknown. The main source of freshwater environments contamination by commercial petroleum fuel (CPF) is runoff from urban, industrial, and agricultural industries. The mining of oil shale reserves may also pose a risk to freshwater ecosystems. Currently, leakage of oil transport pipelines, storage tanks, and accidents involving petroleum transport vehicles are contributors to hydrocarbon pollution in the freshwater ecosystem.

Oil pollution is one of the environmental constrains that produces aqua-toxicological effects, which are deleterious to aquatic life (Kori-Siakpere 2000; Agbogidi et al. 2005). A variety

of pollutants including crude oil and its products are known to induce stress conditions, which impair the health of fish (FEPA 1991). Ekweozor (1989) reported that frequent spillages of crude oil and its products in creeks and rivers may have resulted in a marked reduction in the number of both freshwater and marine creatures. Earlier reports have also shown that oil pollution impact negatively on fishery resources (Kilnhold 1980; Afolabi et al. 1985). Ajoa et al. (1981) and Azad (2005) observed that eggs and young stages (fingerlings) of fishes are especially vulnerable to the toxic effects of crude oil and its refined products. The eco-physiological effects of crude oil on *Machaerium lunatus* had also been reported by Bamidele and Agbogidi (2006).

Nile tilapia, *Oreochromis niloticus* (L.) are native to Egypt and are worldwide distributed (El-Sayed 2006). This species has been used previously in laboratory studies and has been shown to be a suitable organism for monitoring the effects of xenobiotics. This study used Nile tilapia as a model to measure the potential toxic effects of cCPF on fish performance and to test the ability of this fish species to recover from the exposure effect. Therefore, the present study has been undertaken to evaluate the physiological alterations of Nile tilapia following the acute exposure by kerosene, gasoline, and diesel.

MATERIALS AND METHODS

Fish culture regime

The experiment design was factorial, including CPF and time intervals. Healthy Nile tilapias, O. niloticus (L.), were obtained from the nursery ponds, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish (49.5±1.3 g) were acclimated in indoor tanks for 2 weeks by feeding a commercial diet containing 20% crude protein (CP). After that they were distributed into eight120-L glass aquaria at a rate of 15 fish per aquarium, which was supplied with compressed air from air pumps via air-stones.

Kerosene, gasoline, and diesel were brought from a commercial gas station, and their specific gravities were 740, 700, and 820 g/L, respectively. Twelve mls of kerosene, gasoline, or diesel were separately added to 120-L aquarium and they were vigorously shaken with the aquaria water for 5 minutes. After that, fish were stocked into the aquaria containing kerosene, diesel, or gasoline for 5 minutes. Then, fish were transferred into other 6 120-L aquaria containing dechlorinated tap water over 4 weeks for recovery where each treatment was represented by 2 replicates. In control group, fish were not exposed to any fuels. The blood samples were taken; from 3 fish per each aquarium, within one hour of the end of the exposure to represent zero time sample. At 1, 2, 3, and 4 weeks of the recovery period, blood samples from 3 fish per each aquarium were taken to measure the different physiological variables.

During the recovery trial, fish were fed on 25% CP up to satiation twice daily at 9:00 and 14:00 h for 6 days a week. The amount of the given feed for each aquarium, calculated as a summation of given diets during the experimental period, was subsequently taken to represent feed intake. Fish in each aquarium were weekly group-weighed and dead fish were removed and recorded daily. A three quarter of each aquarium's water with fish excreta was siphoned every day and replaced by well-aerated water provided from a storage fiberglass tank.

Water quality measurements

Water samples were collected weekly at 15 cm depth from each aquarium. Dissolved oxygen (DO) and water temperature were measured *in situ* with an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, OH, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO, USA) and pH with a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). In all treatments, DO concentrations ranged from 4.1 to 4.6 mg/L, water temperature average was 26.5±0.8 ^{O}C. Unionized ammonia ranged from 1.2 to 1.6 mg/L, and pH value ranged from 7.2 to 7.6. All the water quality parameters were within the acceptable ranges for fish growth (Boyd 1984).

Fish performance

At the end of this experiment, fish were collected, counted and weighed. Growth performance was determined and feed utilization was calculated as following:
Specific growth rate (SGR; %/day) = 100 (ln W_2 – ln W_1) / T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period;
Feed conversion ratio (FCR) = feed intake (g) / weight gain (g).

Physiological measurements

At sampling date fish were not fed during the 24 h immediately prior to blood sampling. Three fish from each aquarium were anaesthetized with buffered tricaine methane sulfonate (20 mg/L) and blood was collected from the caudal vasculature. The extracted blood was divided in two sets of Eppendorf tubes. One set contained 500 U sodium heparinate/mL, used as an anticoagulant, for hematology (hemoglobin and red blood cell counting). The second set, without anticoagulant, was left to clot at 4 ^{o}C and centrifuged at 5000 rpm for 5 min. at room temperature. The collected serum was stored at –20 ^{o}C for further assays. Red blood cells (RBCs) were counted under the light microscope using a Neubauer haemocytometer after blood dilution with phosphate-buffered saline (pH 7.2). Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethaemoglobin according to Van Kampen and Zijlstra (1961). Glucose was determined colorimetrically according to Trinder (1969). Total protein and total lipid contents in plasma were determined colorimetrically according to Henry (1964) and Joseph et al. (1972), respectively. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma were determined colorimetrically according to Reitman and Frankel (1957). Plasma cortisol levels were measured by radioimmunoassay as previously validated by Chiu et al. (2003).

Statistical analysis

Data were analyzed using a two-way ANOVA with fuel sources and time intervals as factors. Statistical significance was set at the 5% probability level and means were separated using Duncan's(?) new multiple range test. The software SPSS, version 15 (SPSS, Richmond, USA) was used as described by Dytham (1999).

RESULTS

Fish subjected to the kerosene, gasoline, or diesel polluted waters were removed after 5 minutes. During the exposure period to any of the fuels, the air gulping and the increased opercular movement were observed with apparent respiration difficulties. Fish lost their balance, meanwhile no poisoning signs were observed in control fish.

Table 1. Growth performance and feed utilization of Nile tilapia exposed to commercial fuels and recovered for 4 weeks.

TRT	Control	Kerosene	Diesel	Gasoline
Initial weight (g)	49.6±1.39	49.2±1.44	49.5±0.59	49.0±1.30
Final weight (g)	73.1^{a} ±2.14	54.8^{b} ±1.56	50.1^{b} ±0.73	50.9^{b} ±1.47
Weight gain (g)	23.5^{a} ±0.75	5.6^{b} ±0.12	0.6^{d} ±0.23	1.9^{c} ±0.19
SGR (%/day)	1.62^{a} ±0.005	0.45^{b} ±0.003	0.05^{d} ±0.016	0.16^{c} ±0.010
Feed intake (g feed/fish)	39.2^{a} ±0.58	17.8^{b} ±0.55	17.6^{b} ±0.55	17.7^{b} ±0.52
FCR	1.67^{d} ±0.07	3.2^{c} ±0.03	29.3^{a} ±2.04	9.4^{b} ±0.81
Survival (%)	97.6^{a} ±2.22	84.4^{b} ±2.22	82.2^{b} ±5.87	82.2^{b} ±2.22

Means having the same letter in the same row are not significantly differed at $P < 0.05$.

The growth performance of Nile tilapia subjected to acute exposure of any of the tested pollutants was significantly affected (Table 1). Fish in control group grew up gradually to the end of the experiment, meanwhile fish exposed to kerosene, diesel, or gasoline lost their weights for 2 weeks and started to grow again (Fig 1). Moreover, weight gain and SGR of fish exposed to diesel and gasoline were less than that exposed to kerosene; the maximum fish performance was obtained in control fish ($P < 0.05$; Table 1). Feed intake, FCR, and survival rate of the exposed fish were poor.

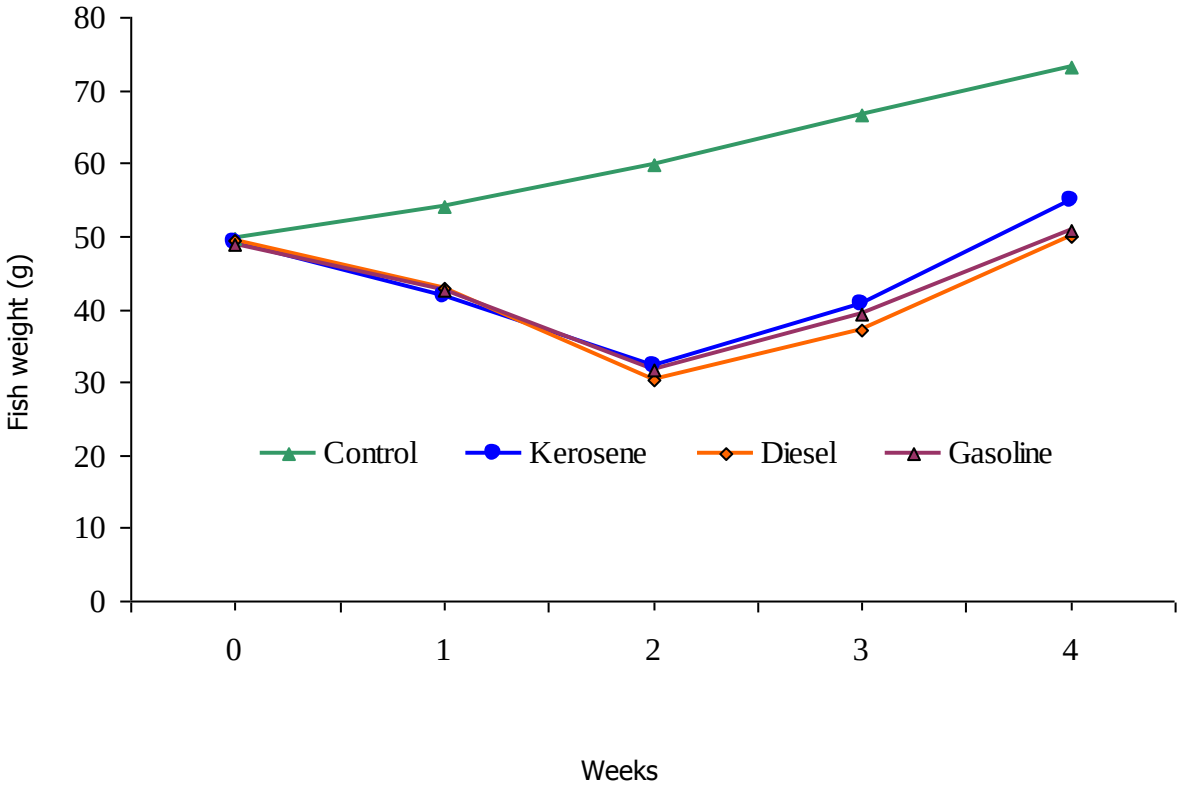
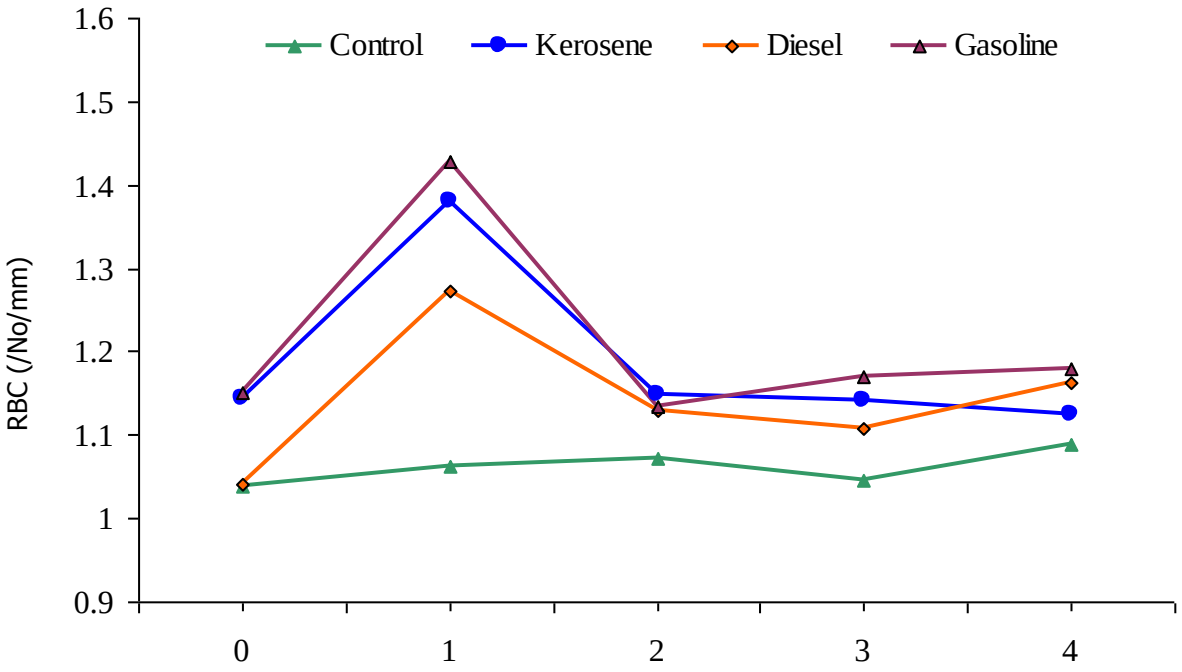


Figure 1. The mean values of live body weight (g) of Nile tilapia after short-term exposure to commercial petroleum fuels.

Red blood cells count in fish exposed to kerosene, diesel, or gasoline increased by time and the maximum count was obtained at the 1st week; their counts decreased up gradually to the 4th week (Fig 2). Hemoglobin in Nile tilapia was suddenly increased after their exposure to CPF and decreased by time up to the 4th week; they were close to that of control fish (Fig 2). Glucose level was maximized after the exposure to kerosene, diesel, or gasoline and decreased up gradually to the end of the experiment (Fig 3). Glucose levels of treated fish were higher than that of control. Plasma lipids increased significantly by time at the treated fish groups and their values were significantly higher than that of control group (Fig 3). Plasma protein in fish increased suddenly after the exposure to kerosene, diesel, or gasoline and it decreased by time to be close to that of control group (Fig 3).



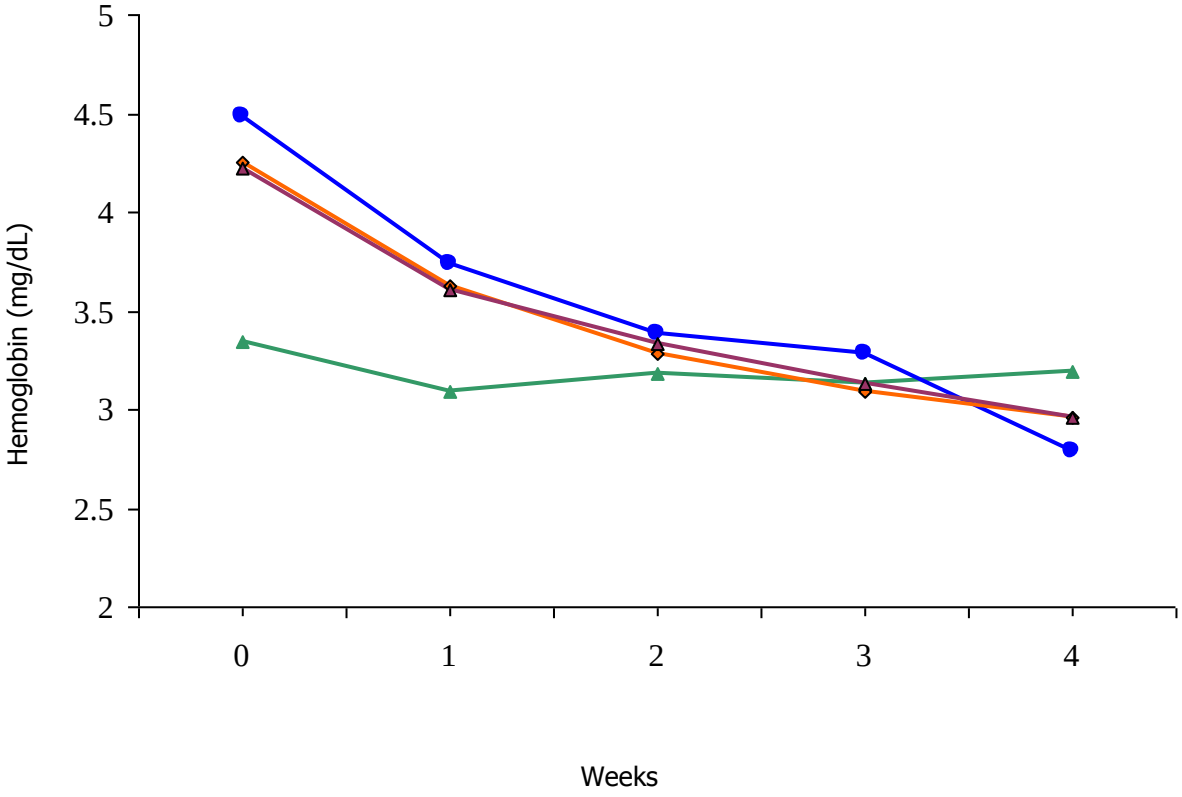
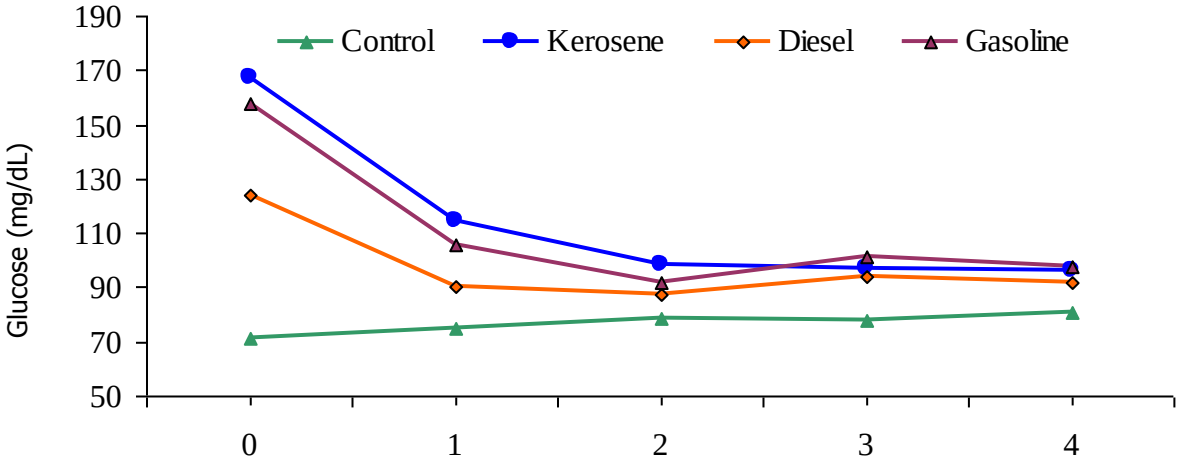


Figure 2. The mean values of RBCs count (No/mm) and hemoglobin (mg/dL) in Nile tilapia after short-term exposure to commercial petroleum fuels and recovered for 4 weeks.



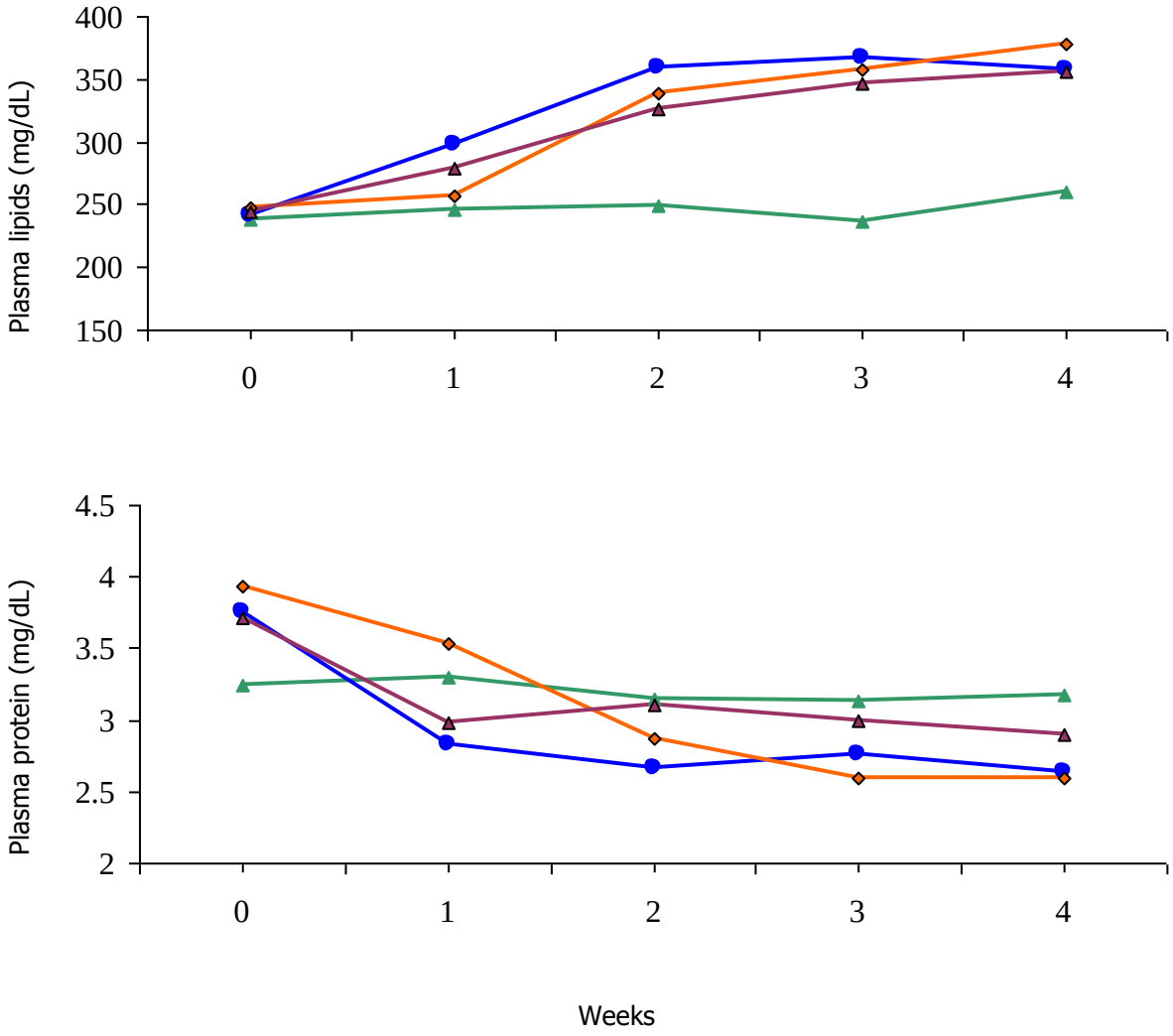


Figure 3. The mean values of glucose, lipids, and protein (mg/L) in Nile tilapia after short-term exposure to commercial petroleum fuels.

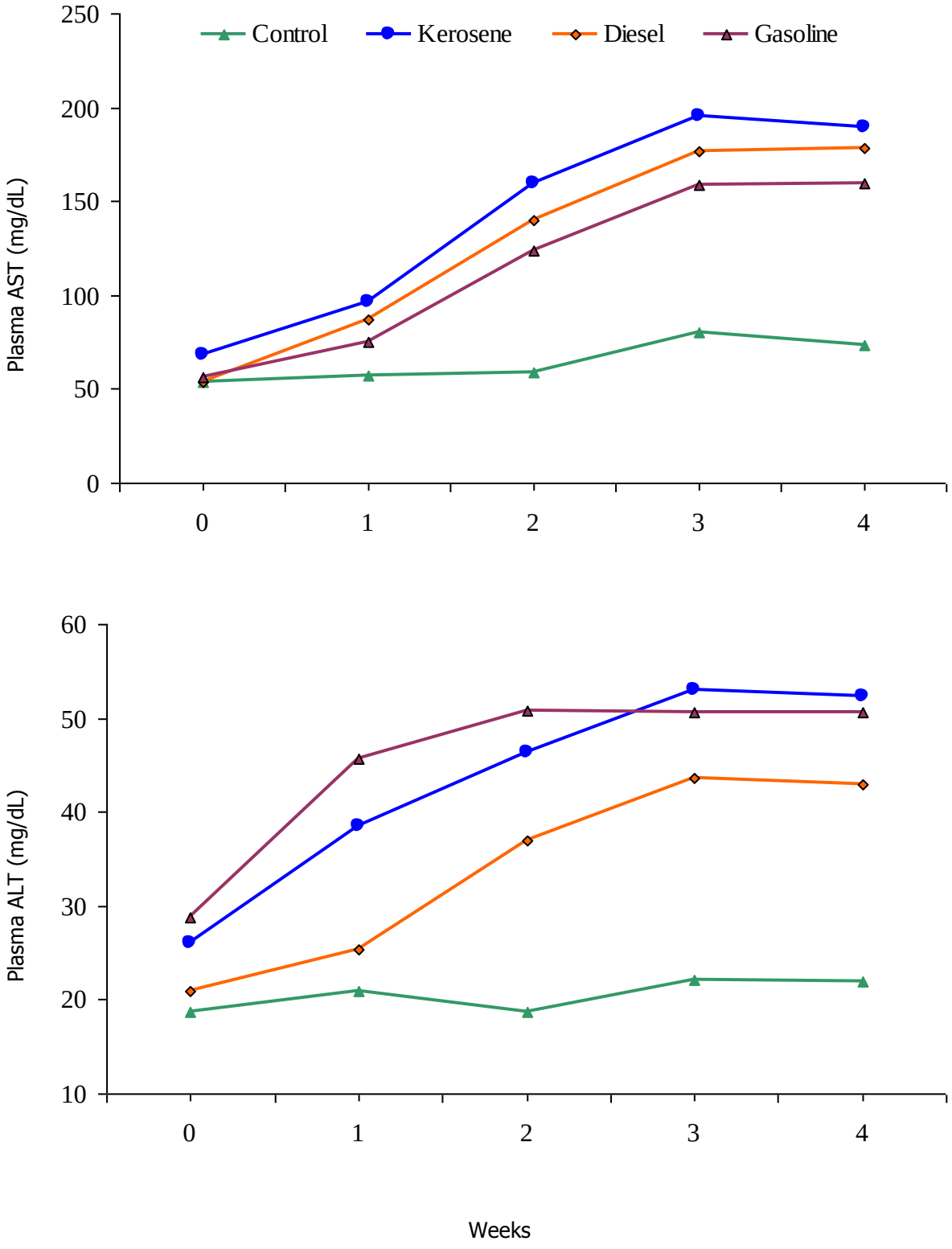


Figure 4. The mean values of AST and ALT activities (mg/dL) in Nile tilapia after short-term exposure to commercial petroleum fuels.

Activities AST and ALT increased gradually after their exposure to kerosene, diesel, or gasoline and the maximum values were obtained after 3-4 weeks (Fig 4). The lowest values of AST and ALT were obtained at control group, which did not significantly differ throughout the experimental period.

Plasma cortisol in fish was suddenly increased after their exposure to kerosene, diesel, or gasoline (Fig 5); cortisol value was significantly differed by fuel source (kerosene > gasoline > diesel). The lowest cortisol value was obtained at control which was insignificantly changed throughout the experimental period.

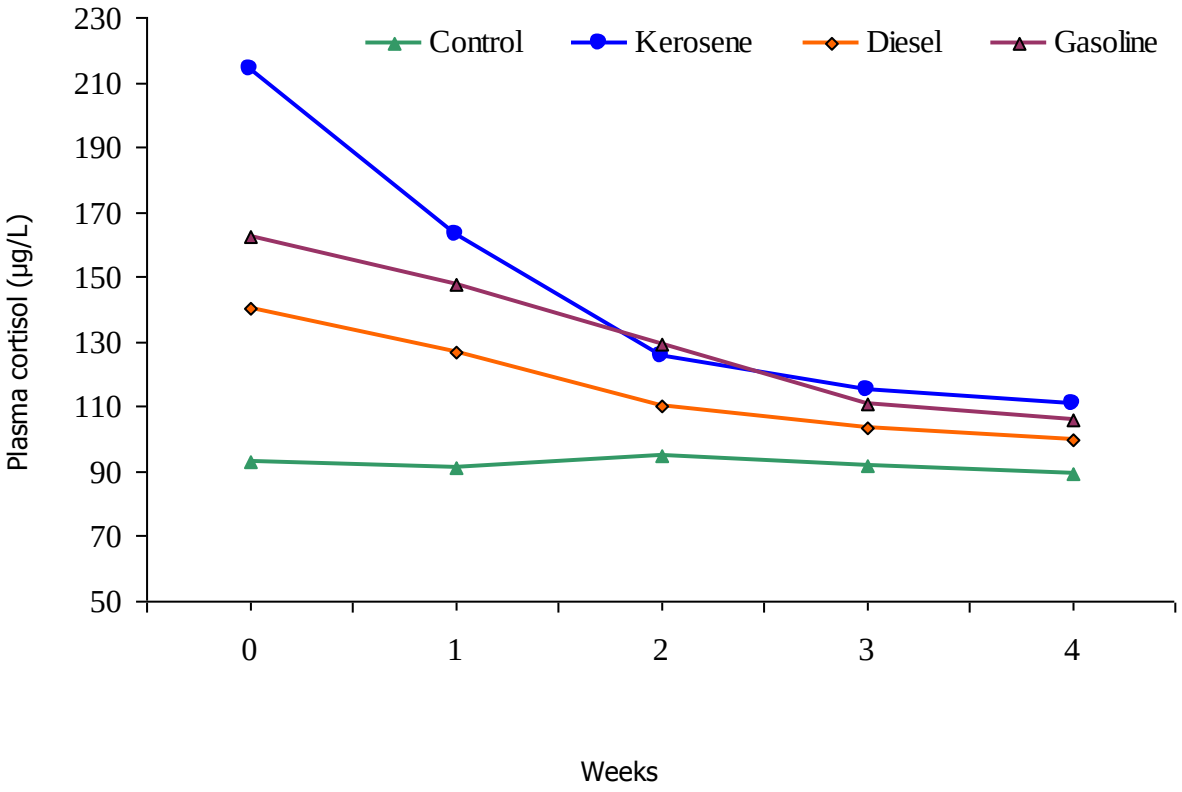


Figure 5. The mean values of plasma cortisol (µg/L) in Nile tilapia after short-term exposure to commercial petroleum fuels.

DISCUSSION

The results obtained herein indicated that CPF had negative impacts on the growth performance and survival of Nile tilapia. However, the exposure of fish to these pollutants resulted in reduced feed intake and thus lowered body weight. These results indicate that the exposure to CPF may lead to a reduction in fish appetite or complete fish fasting resulting in lower retention rate of nutrients into fish body and so growth was reduced. The findings of this study agreed with Kicheniuk and Khan (1981) and Kori-Siakpere (2000) who noted that exposure of fish to water soluble fractions (WSF) of crude oil can result in reduced feeding and lower body weight. Dede and Kaglo (2001) reported that the survival of Nile tilapia decreased by increasing concentration of diesel fuel. Ofojekwu and Onah (2002) stated that fish are known to increase their metabolic rates to metabolize and excrete aromatic hydrocarbons and consequently allocate more energy to homeostatic maintenance than storage exhibiting growth retardation. Additionally, delayed growth and reduced survival of pink salmon (*Onchorhynchys gorbuscha*)

embryos has been observed following exposure to crude oil (Heintz et al. 2000). In juvenile turbot study, fish exposed to higher concentrations of the fuel exhibited reduced growth and feed consumption (Saborido-Rey et al. 2007).

Fish hypoxia and the increased respiration of fish were observed within few minutes after their exposure to CPF. This result may be because these pollutants have been reported to cause structural damage to the respiratory lamellae of the gills (Poirier et al. 1986; Correa and Garcia 1990; Prasad 1991), as well as to have narcotic actions (Correa and Garcia 1990). Such effects would be predicted to impede gas exchange, and result in hypoxaemia (Perry et al. 1989; Ristori and Laurent 1989; Randall and Perry 1992). Other studies using flounder (*Platichtys flesus*) found that exposure to the WSF of crude oil caused declines in the plasma oxygen content, suggesting fish were experiencing respiratory problems (Alkindi et al. 1996). In addition, fusion of secondary lamellae, gill hyperplasia, and oedema have been reported in fish exposed to petroleum hydrocarbons (Correa and Garcia 1990; Prasad 1991; Dede and Kaglo 2001).

The increase of RBC, Hb, glucose, and cortisol were observed in fish following their exposure to CPF. In this regard, the study of Alkindi et al. (1996) observed that after 3 h exposure of flounder to 50% WSF of crude oil, RBC and Hb increased significantly. Kita and Itazawa (1990), Pearson et al. (1992), and Alkindi et al. (1996) reported that the exposure of fish to petroleum hydrocarbons stimulates the release of catecholamines, which could have a number of potentially beneficial effects, including stimulation of splenic release of erythrocytes to aid O2 carrying capacity, stimulation of Na+/H+ exchange in erythrocytes, and resultant increases in haemoglobin-oxygen affinity.

The rise in plasma glucose concentrations indicates a stress-induced mobilization of energy reserves. Some studies suggest that fish exposed to petroleum hydrocarbons have elevated concentrations of plasma cortisol indicating a corticosteroid stress response. In this regard, Alkindi et al. (1996) found that the exposure of flounders to a 50% dilution of the WSF of Omani crude oil, a mix of aromatic hydrocarbons (benzenes, toluene, and xylenes and lower amounts of naphthalenes), resulted in a progressive increase in plasma cortisol concentrations continuing over the 48-h exposure period. Moreover, cortisol has a direct effect on carbohydrate metabolism, stimulating glycogenolysis and gluconeogenesis, but that it also interacts with catecholamines which may exert dominant effects in the immediate stages of stress (Wright et al. 1989; Vijayan and Moon 1994; Vijayan et al. 1994).

The fluctuation in plasma lipids, protein, AST, and ALT may be due the disturbance of metabolic pathways. In addition, the increase in AST and ALT activities are indicatives to liver damage, which might have occurred due to the exposure to CPF and hence leading to the leakage of these enzymes into the blood. In this regard, Martin-Skilton et al. (2008) demonstrated that acute exposure of juvenile turbot, *Scophthalmus maximus* to the Prestige fuel oil elicits alterations in some hepatic biotransformation enzymes with different sensitivities, and leads to decreased levels of testosterone in plasma of juvenile turbot which might threaten reproductive capability of exposed individuals.

It is noticed that all variables were declined to be close to those of control group after 2 weeks, except lipids and AST, and ALT need time over 4 weeks to be near those of control group. These results may be because CPF not bioaccumulate in exposed fish, but they are rapidly metabolized to form epoxy- and hydroxyl-derivatives during phase I metabolism and subsequently converted into highly water-soluble conjugates (e.g., glucuronides or sulfates) that are excreted through the bile (Varanasi et al. 1985). Pollino and Holdway (2003) reported that the short-term exposures of petroleum hydrocarbons to rainbowfish at realistic concentrations potentially alter metabolic and detoxification enzymes, with metabolic enzymes recovering after depuration (17 days).

Conclusively, this study has demonstrated that the acute exposure to CPF significantly reduced the growth performance of Nile tilapia. The study has also showed that Nile tilapia can

serve as a bio-indicator of CPF toxicity. Particular attention should also be given to CPF process aimed at minimizing their toxicity to the aquatic ecosystem.

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ACUTE TOXICITY OF WATER-BORN ZINC IN NILE TILAPIA, *Oreochromis niloticus* (L.) FINGERLINGS

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Abstract

Zinc (Zn) is an essential trace element for most organisms including fish, but above certain limit Zn will be toxic. The present study was conducted to evaluate the toxic effect of water-born Zn on Nile tilapia, *Oreochromis niloticus* (L.) via estimating the acute 96-h median lethal concentration (LC50) value and behavioral changes. A total 140 of Nile tilapia fingerlings was subjected to 14 20-L aquaria. Fish were exposed to 0.0, 10, 40, 70, 100,130, or 160 mg Zn/L for 4 days. Each Zn dose was represented by two aquaria. Fish was daily observed and dead fish were removed immediately. The data obtained were statistically evaluated using Finney's Probit Analysis Method and Behrens–Karber's Method. The 96 h LC50 value for Nile tilapia was found to be 63.984 mg/L with 95% confidence limits of 48.029 – 78.372 mg/L. This value was calculated to be 70.0 mg/L with Behrens–Karber's Method. The behavioral changes of Nile tilapia were primarily observed as nervous and respiratory manifestations. It could be concluded that Nile tilapia is a species slightly sensitive to Zn and the two methods were relatively comparable.

INTRODUCTION

Pollution of the aquatic environment with heavy metals has become a serious health concern in recent years. These metals are introduced into the aquatic ecosystem through various routes such as industrial effluents and wastes, agricultural pesticide runoff, domestic garbage dumps and mining activities (Merian, 1991). Increased discharge of heavy metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals (van Dyk et al., 2007). Among aquatic organisms, fish cannot escape from the detrimental effects of these pollutants, and are therefore generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems (van der Oost et al., 2003).

It has been reported that heavy metals had a negative impact on all relevant parameters and caused histo-pathological changes in fish. Some heavy metals are essential elements, while others are non-essential. Zinc (Zn) is one of the most important trace metals in the body, and participates in the biological function of several proteins and enzymes (Maity et al., 2008). Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations (Ho, 2004). Since the range-finding acute test is conducted to pinpoint exposure concentrations; the definitive acute test is firstly conducted to estimate LC50 of the chemical to which organisms are exposed (Rand, 2008). Nile tilapia, *Oreochromis niloticus* (L.) is an important commercial fish in Egypt and worldwide (El-Sayed, 2006) and it could be used as test organism for evaluation the impact of heavy metals. Consequently, the objective of this study is to assess the responsiveness of Nile tilapia to Zn through determination of acute 96-h LC50 value and behavioral responses induced from exposure to different Zn concentrations.

MATERIALS AND METHODS

Fish management:

Apparently healthy Nile tilapia, *O. niloticus* (L.) (4.6 ± 0.2 g) were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Prior to the experiment, fish were acclimatized for 2 weeks in 14 40-L glass aquaria under laboratory conditions (natural photoperiod 11.58–12.38 h); 10 fish per each aquarium. The continuous aeration was maintained in each aquarium using an electric air pumping compressors. Fish were fed daily on commercial fish diet containing 25% crude protein provided for satiation twice daily at 9:00 and 14:00 h.

Analysis of the water physico-chemical variables:

Water samples were collected from each aquarium prior to Zn exposure. Dissolved oxygen and temperature were measured on site with an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). pH value was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, USA). Total alkalinity and total hardness were measured according to Boyd (1984). The mean values for test water variables were as follows: dissolved oxygen 5.84±0.72 mg/L, pH 7.5±0.1, water temperature 25.5± 0.1 ^{o}C, total alkalinity 153.7±4.8 mg/L as CaCO3, and total hardness 222.5±2.9 mg/L as CaCO3.

Experimental procedures:

The heavy metal Zn in the form of zinc sulfate anhydrous (Analar grade, Merck, Readington Township, New Jersey, USA) was used in the present study. The acute toxicity test was performed for 4 days in which two replicates of seven different Zn concentrations (0, 10, 40, 70, 100, 130, and 160 mg/L) were used (10 fish for each aquarium). At 24, 48, 72, and 96 h, fish dead were counted in the different Zn concentrations along with the control group. In this study, the acute toxic effects of Zn on Nile tilapia were determined by the use of Finney's Probit Analysis LC50 Determination Method (Finney, 1971). The computer model (Probit Program Version 1.5 software) was developed by Environmental Protection Agency (EPA, 1999). It was designed for the analysis of mortality data from acute toxicity tests with fish and other aquatic life, performed with reference toxicants by regulatory agencies and permittees under the National Pollutant Discharge Elimination System (NPDES). In addition, the data were also assessed according to Behrens–Karber's method using the following formula (Klassen, 1991):

LC50 = LC100 ∑A x B / N as mg/L;

where LC50 and LC100 indicate the lethal doses for 50% and 100% of the tested fish. Value "A" gives the differences between the two consecutive doses, "B" the arithmetic mean of the mortality caused by two consecutive doses and "N" the number of tested fish in each group. The dead fish were removed immediately. Behavioral changes, clinical toxic signs and postmortem lesions of tested fish were closely followed up and recorded daily.

RESULTS

The data obtained from the acute toxicity test of water-born Zn for Nile tilapia revealed that the Zn toxicity increased with increasing concentration and/or exposure time. The number of dead fish in relation to the Zn concentrations (40, 70, 100, 130 and160 mg/L) were assessed and counted during the exposure time at 24, 48, 72 and 96 h then they were removed immediately. No mortality was observed during the 96 h at control (0.0 mg Zn/L) and 100% mortality rate was achieved only at 130 and 160 mg Zn/L (Table 1).

Table 1. The cumulative mortalities and acute 96 h LC50 of water-born Zn in Nile tilapia fingerlings according to Behrens-Karber's method (Klassen, 1991).

Zn dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h	A	B	AB
		D1	D2	D3	D4				
0	10	0	0	0	0	0	0	0	0
10	10	0	0	0	0	0	10	0	0
40	10	0	1	2	2	2	30	1.0	30
70	10	2	3	4	4	4	30	3.0	90
100	10	8	9	9	9	9	30	6.5	195
130	10	8	10	10	10	10	30	9.5	285
									∑AB =600

Where A = differences between the two consecutive doses and B = arithmetic mean of the mortality caused by two consecutive doses.
96 h LC_{50} = LC_{100} - ∑ (A x B)/N = 130 – 600/10 = 70.0 ppm.

The relationship between the Zn concentrations and the mortality rate of Nile tilapia calculated by Finney's Probit Analysis (Table 2). The mean 96-h LC50 value with 95% confidence limits for Nile tilapia fingerlings was found to be 63.984 mg/L. This value was estimated to be 70.0 mg/L with the Behrens–Karber's method (Table 1). The two methods are in good accordance and this value indicating that the water-born Zn is definitely a slight toxic heavy metal to Nile tilapia.

Table 2. The acute 96-h LC50 values of Zn and their confidence limits in Nile tilapia fingerlings according to Finney's Probit Analysis (EPA, 1999).

Point	Zn concentration (mg/L)	95% Confidence Limits		Slope	Intercept
		Lower	Upper		
LC/EC 1.00	24.848	7.968	36.878	5.66±1.47	-5.23±2.74
LC/EC 5.00	32.781	13.835	44.830		
LC/EC 10.00	37.999	18.501	49.925		
LC/EC 15.00	41.982	22.456	53.816		
LC/EC 50.00	**63.984**	**48.029**	**78.372**		
LC/EC 85.00	97.516	79.486	147.503		
LC/EC 90.00	107.738	86.559	177.222		
LC/EC 95.00	124.888	97.285	234.828		
LC/EC 99.00	164.756	119.253	404.328		

Note: Control group (theoretical spontaneous response rate) = 0.0.
Bold value indicated the acute 96 h LC_{50} of Zn and its confidence limits in Nile tilapia fingerlings.

Figure 1 shows that large increases in fish mortality are associated with the increases in exposure concentrations (r^2 = 0.9202). Moreover, the LC50 values and the empirical probit values of the mortality rate were plotted against the water-born Zn concentrations in Fig. 2, which indicates that Zn does not have cumulative response to test concentrations.

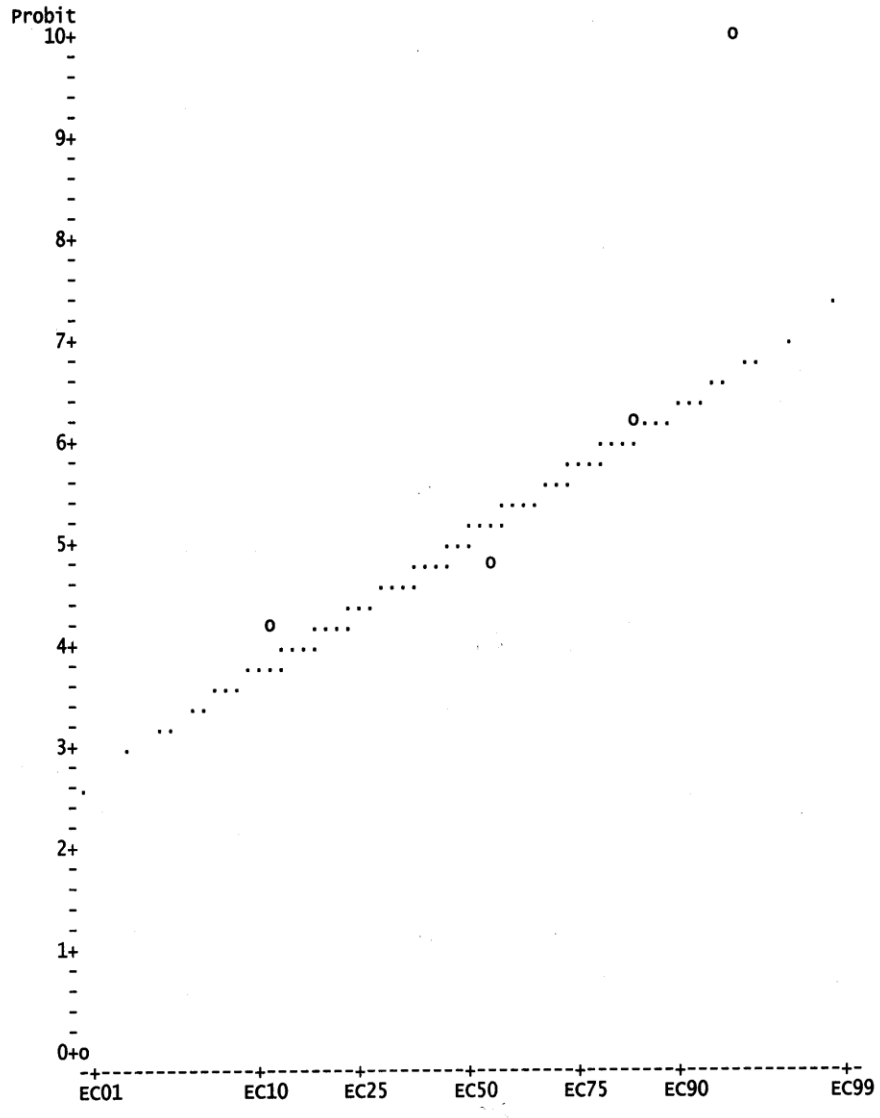

Fig 2. Plot of adjusted probits and predicted regression line.

It was observed that Nile tilapia individuals exhibited a variety of behavioral changes when subjected to different Zn concentrations. The behavioral and swimming patterns in the control group were normal and there were no deaths during the experimental period. The behavioral changes and clinical toxic symptoms in Nile tilapia subjected to different Zn concentrations are the following: sluggish movement, loss of equilibrium, and rapid operculum movement as respiratory manifestations. Variable degrees of fin erosions were seen. Fish died during the experiment were immediately removed from the aquaria and subjected to a necropsy. The necropsy revealed that there were general congestion of the kidneys and gills, and spots of congestion on the periphery of the liver at macroscopic scale.

DISCUSSION

In the present study, Zn toxicity was indicated by fish mortality. Shetty Akhila et al. (2007) reported that the determination of acute toxicity is usually an initial screening step in the

assessment and evaluation of the toxic characteristics of all compounds. Likewise, De Schamphelaere and Janssen (2004) reported that fish mortality might be a more sensitive endpoint for assessing effect of Zn exposure. The acute 96-h LC50 value of water-born Zn for Nile tilapia having an average weight 4.6 g was calculated as 63.984 mg/L by using Finney's Probit Analysis and 70.0 mg/L by the use of Behrens–Karber's method. The 96-h LC50 values obtained for both methods were found to be relatively comparable. Similar results were obtained El-Sayed et al. (2009) who used both methods to evaluate the acute toxicity of ochratoxin-A in sea bass (*Dicentrarchus labrax* L.).

Bengeri and Patil (1986) found that the 96-h LC50 of Zn for *Labeo rohita* was 65.0 mg/L. Hilmy et al. (1987) found that the 96-h LC50 of Zn for *Tilapia zillii* and *Clarias lazera* at summer (25.0 ^{o}C) was 13.0 and 26.0 mg/L, respectively. Senthil Murugan et al. (2008) found that the 96-h LC50 concentration of Zn for snakehead, *Channa punctatus* was 48.68 mg/L. The variation in LC50 values among the different studies may be due to the variations in kinetic variables that may play a role in explaining these differences. Moreover, the alkaline and hard water in the present study could be responsible for being the LC50 herein higher than the other studies. In this regard, Weatherley et al. (1980) and Wood (2001) stated that Zn bioavailability and toxicity to aquatic organisms are affected by pH, alkalinity, dissolved oxygen, and temperatures. Alabaster and Lloyd (1982) and Everall et al. (1989) stated that Zn toxicity to fish can be greatly influenced by both water hardness and pH. Hilmy et al. (1987) found that 96-h LC50 for both fishes increased with the decrease in water temperature. Eisler (1993) reported that the acute 96-h LC50 values for fish were between 66 and 40,900 µg Zn/L depending on many factors including pH, alkalinity, dissolved oxygen, and temperatures.

Previous studies have shown that Zn accumulation in fathead minnow, *Piinphales promelus*, and common carp, *Cyprinus carpio*, was reduced in hard water compared with soft waters (Everall et al., 1989). However, Bradley and Sprague (1985) found that in hard water, Zn accumulation in the gills of rainbow trout, *Salmo gairdneri*, was reduced and suggested that water hardness may protect fish by altering the dynamics of Zn exchange mechanisms. Moreover, the process of metal uptake may be dependent upon the metal exposure level, its local availability at the sites of uptake and the duration of exposure. Immediate levels of Zn exposure have been shown to affect the pattern and rate of metal uptake (Everall, 1987). It is possible that previous metal acclimation may also affect the pattern and rates of Zn uptake, dependent upon prior tissue loading and depuration (Bradley et al., 1985).

The loss of positive rheotaxis is a good indication of any toxic response, but in the case of Zn it takes place when poisoning is already irreversible. Signs of poisoning before loss of positive rheotaxis are not the same at high and lower concentrations; the air gulping and the increased opercular movement observed at high concentrations contrast with the general apathy and ataxia, but without apparent respiration difficulties observed at low concentrations. A comparable behaviour was reported by Matthiessen (1974) for *Sarotherodon mossambicus* and Hilmy et al. (1987) for *Tilapia zillii* and *Clariaz lazera*. An interpretation of the toxicity data is that two poisoning mechanism may take place, one occurring at high concentrations and provoking a rapid suffocation by destruction of the gill epithelium, the other prevailing at low concentrations and consisting of an inhibition of the main metabolic pathways.

In conclusion, Nile tilapia is a slightly susceptible species to water-born Zn and the two methods were relatively comparable and useful. The useful experimental models could be widely used to assess the aquatic toxicology of heavy metals.

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FIVE STAR CERTIFICATION PROGRAM AGAINST OFF-FLAVOR IN TILAPIA FILLETS

Tomi Hong

ACUTE TOXICITY OF AQUEOUS Morinda lucida LEAF EXTRACTS TO NILE TILAPIA, Oreochromis niloticus (LINNAEUS 1857)

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ABSTRACT

Acute toxicity tests (range finding and definitive) using aqueous extracts of *Morinda lucida* on *Oreochromis niloticus* fingerlings (mean wt., 6.2g ± 1.2g) were conducted in a static bioassay inside plastic tanks. In the range-finding test, the concentration range tested was 20, 40, 60, 80 and100g *M. lucida*/L of water while 70, 72, 74, 76 and 78 *M. lucida*/L of water was used in the definitive test. The LC_{50} at 24 hours was 1.869g *M. lucida* g/L of water. There were five concentrations with control and each treatment was replicated twice. For each test, 15 *O. niloticus* fingerlings were used in each plastic tank. The responses exhibited by *O. niloticus* fingerlings subjected to the toxicant include erratic swimming, loss of reflex, colour change, weakened motion and vertical swimming. These were enhanced by the increase in concentration of the toxicant and the duration of exposure. During the 24 hours of range finding test, no mortality occurred at concentrations 20, 40, 60, 80 and 100 g *M. lucida*/L. No mortality was recorded in the 96 hours at concentration 20.0g *M.lucida*/L. Histological changes occurred in the gills and liver of the fish in the definitive test as gill alterations (hydropic degeneration of the gill rays, degeneration of the gill lamellae and necrosis) which were usually related to gills function disorders. Liver shows hepato-cellular architecture, hydropic degeneration, vacuolation of the liver cells and spaces within the cell protoplasm filled with fluid. This effect intensified with increasing *M. lucida* concentration.

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus,* is an important cultured fish species in Nigeria. The main advantage of tilapia is relatively low cost of production, mainly for fry and seed, and the quality of its flesh. The attributes that make Nile tilapia so suitable for fish farming, are its resistance against harsh conditions, ease of breeding, rapid growth rate, ability of efficiently convert organic and domestic wastes into high quality protein, and good taste (de Graaf *et al.*, 1999). Other advantages are its herbivorous nature and its mouth brooding habits, tolerance of poor water quality and fast growth at warm temperature.

Morinda lucida is a species yielding dyes, timber, fuel and traditional medicines. The leaves are use as the remedies against different type of fever. *M. lucida* known as 'Oruwo' in south-western Nigeria, it is a medium-sized tree at maturity. The stem bark infusion is used as an anti-malarial and antidiabetic (Burkill, 1997), anti-malarial activity (Tona et al., 1999; Agomo et al., 1992; Asuzu and Chineme, 1990); Makinde and Obih, 1985; Koumaglo et al., 1992), anti-Salmonella typhi activity (Akinyemi et al.,2005),effect on contractivity of isolated uterine smooth muscle of pregnant and non pregnant mice(Elias et al.,2007),toxicity and mutagenic studies (Sowemimo *et al.,* 2007; Akinboro and Bakare, 2007; Koumaglo *et al.,* 1992; Raji *et al.,* 2005); and anti-diabetic property(Olajide *et al*., 1999) of *Morinda lucida* extracts have all been reported

M. lucida is a multipurpose species yielding dyes, timber, fuel and traditional medicines (Abbiw, 1990). The useful parts of *M. lucida* are mostly collected from wild plants. Only

occasionally are plants grown in home gardens. Propagation is possible by seed and cuttings. The genus *Morinda* comprises about 80 species and occurs throughout the tropics. In Africa five species are found. The comparatively small flowering and fruiting heads on long slender peduncles are distinctive characteristics of *M. lucida*. The growth and development of *M. lucida* is from February to May, fruiting from April to June. *M. lucida* grows in grassland, exposed hillsides, thickest, forests, often on termite mounds, sometimes in areas which are regularly flooded, from sea-level up to 1300m altitude. In West Africa, *M. lucida* is an important plant in traditional medicine. *Morinda lucida* is an important plant used in traditional medicine. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever. *M. lucida* is one of the four most used traditional medicines against fever. *M. lucida* grows in grassland, exposed hill sides, thickest forest often on the mite mounds. The useful parts of *M. lucida* are mostly collected from wild plants only occasionally are they grown in home gardens (Adesida and Adesogan, 1972). In West Africa, the roots of *M. lucida* are sold in load shops and markets, both as dyestuff and medicine. Leaves and twigs are sold in markets as a medicinal tonic for young children in Africa.

Aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals or toxicants which exhibit changes in the aquatic environment. Studies have revealed that organisms exposed to chemical or toxicants usually exhibit changes in opercula rate and may cause physical damages to fish particularly on the gill surfaces (Davis, 1973). Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homeostasis of the fish can control, it results in death or cause damages in the fish opercula and may also cause physical damages to fish particularly on the skin, liver and gill surface.

The objectives of this study are to determine the lethal concentration (LC50) value of *O. niloticus* fingerlings exposed to varying concentrations of *M. lucida* leaf extract (dry) and determine the effects of acute and sub-lethal concentrations of *M. lucida* extract on histopathology of gills and liver tissues of *O. niloticus* fingerlings.

MATERIALS AND METHODS

O. niloticus fingerlings, (mean weight 6.2g ± 1.2g) were purchased and were acclimated for 48 hours prior to toxicity tests inside plastic tanks (30 L capacity). Each tank was filled with 15 litres of water obtained from the borehole. Fish were fed to satiation daily with a commercial 35% crude protein pelleted feed. Feeding was discontinued 24 hours prior to the commencement of the tests. Individual weights of the fingerlings were measured with a top-loading mettler balance and distributed randomly in duplicate treatments at 15 fingerlings/tank. Leaves of *M. lucida* were collected around the Federal University of Technology, Akure and sun-dried at ambient temperature, and milled into powder.

A range finding test served as a preliminary test which was followed by definitive test. In the range finding test, five triplicate treatments using five test concentrations of 20.0, 40.0, 60.0, 80.0, 100.0g *M. lucida* /L of spring water were used. *O. niloticus* fingerlings were stocked into each tank for 24 hours prior to the introduction of *M. lucida* leaf extract (dry) to the water. The range finding test lasted for 96 hours and was checked initially at four hours intervals followed by 12 hours intervals. Mortality of the fingerlings was routinely monitored and recorded. The failure to respond to external stimuli was used as an index of death. LC50 is the concentration of *M. lucida* extract estimated to the lethal to 50 of the test organism after 96 hours of exposure using

probit analysis and by graphical method. Five concentrations of *M. lucida* used in the definitive test were 70.0 72.0, 74.0, 76.0g and 78.0g *M. lucida*/L. The concentrations were prepared arithmetically and followed the results obtained from the range-finding test. The test lasted for 96 hours and *O. niloticus* fingerlings mortality was monitored at 3 hours followed by 12 hours intervals. The dissolved oxygen concentration, pH and temperature were determined every 24 hours. The behaviour and mortality of the fish were observed and recorded after 24 hours.

The 96 hour LC50 was estimated by probit analysis as described by Wardlaw (1985) and by graphical method. At the end of the definitive test, *O. niloticus* fingerlings were dissected to remove the gills and liver. The organs were fixed in 10% formalin for 72 hours, dehydrated in graded level, alcohol (50, 70, 90, 100%) after which they were cleared in 50/50 mixture of alcohol and xylene for 3 hours, then 100% xylene for three hours and impregnated in molten wax, oven-dried for 6 hours after which they were embedded in Petri dishes with wax. The specimens were mounted and sectioned 8µ thickness prior to staining in haematocylin and eosin. Photomicrographs were taken with Leitz (Ortholux) microscope fitted with camera.

RESULTS

During the range finding test, *O. niloticus* exhibited various reactions which included, erratic movement, vertical swimming position, colour change to dark brown, weakened swimming motions, sudden jerky swimming movement, and changes in opercula rate. All fish in the control treatment survived throughout the 96 hours duration of the experiment. Fish mortality in the varying concentrations (Table 1) increased with increasing concentration of the *M. lucida* used. In the definitive test, fish swam weakly, settling at the bottom of the tank. They showed less movement with increase in duration of the exposure, and showed increased weakness, remaining motionless most of the time. The LC50 aws determined as 1.87 mg/L. The summary of histological observations in the gills and liver of *O. niloticus* exposed to varying concentrations of *M. lucida* are presented in Tables 1 and 2, respectively.

Table 1: Histological changes in gills of *O. niloticus* fingerlings.

Concentration (g/L)	Histological observation
0	No visible alteration on the gill ray
70	normal gill architecture
72	inflammation of the gill lamella
74	degeneration of the gill architecture
76	hydropic degeneration in the gill lamellae and disintegration of the gill architecture
78	erosion of the gill filaments and gill rakers

Table 2: Histological changes in liver of *O. niloticus* fingerlings.

Concentration (g/L)	Histological Observation
0	Normal hepato-cellular architecture
70	normal liver architecture.
72	Normal heptocellular architecture of the liver cells.
74	Hydropic degeneration in the hepatic parenchyma of the liver cells
76	Severe alteration in the liver architecture
78	vacuolation within the cell protoplasm field with fluid

DISCUSSION

Different behaviour shown demonstrated to be sensitive indicator of the physiological stress in fish subjected to sub lethal concentrations of pollutant (Davis, 1973) when the pollutant was introduced into the water containing the test fish, the fish at first displayed attempt to jump out. After being stressed, they resorted to settling at the bottom of the plastic tank and became motionless with slow opercula movement. The abnormal behaviour displayed by the fish subjected to *M. lucida* increased with increasing concentration of the pollutant used. The observation of fish response in this experiment agreed with Akinbulumo (2005) who reported that fish showed toxic reactions to *Derris elliptica* root powder by surfacing jaws and becoming stupefied. Also, according to Pascual *et al*., (1994) fish staying at the bottom of the plastic tank is a sign of stress or weakness.

The fish in the *M. lucida* solution showed erratic swimming, loss of reflex, vertical swimming position, colour changes (discolouration) and weakened swimming motion (Table1and 2). Fish response observed in the study agreed with similar observation made by White (1980) in Atlantic herring, *Clupea harengus*, exposed to dinoflagallate toxins. The abnormal swimming and changes in colour are indicative of stress which might lead to mortality (Chan, 1982). The test fish showed abnormal swimming and subsequently erratic movement and they finally settled to the bottom, showing signs of exhaustion, stress after which they move along the tank bottom and finally die off. This was in accordance with Liong *et al.,* 1988, moreover according to Pascual *et al.* (1994) suspected that fish staying at the bottom of the rubber tank is a sign of weakness.

O. niloticus fingerlings survived in low concentration of *Morinda lucida* and died at higher concentration (table 1and 2). In this study, the replicates gave different fish mortality values. This agrees with the observation of Chen and Lei (1990) who observed that juveniles of penaeus monodon showed differences in the tolerance to ammonia and nitrate solutions.

There were significant difference in the treated water in range finding test and also there were significant difference between the central medium and treated water in definitive test in terms of physico-chemical measurement (Table 4).

The results of physico-chemical parameters of the experimental water at the end of the experiment, 96 hours of introduction of toxicant are given in (table 4).Water temperature in the experimental tanks was affected by the concentration of *Morinda lucida*, also the dissolve 0_2 content of the samples were within the range desirable (>3ppm) for the optimum growth of *Orechromis niloticus* (Alex Bocek *et al*, 1991).

Water temperature was within the range desirable (24-28^{0}C) for the optimum growth of *O.niloticus* (Alex Bocek *et al.,* 1991). The pH of the test media obtained from the experiment indicated that the addition of *M.lucida* increased the pH.

Histological changes

Histological examinations of *O. niloticus* gave significant indication of toxicity of *M. lucida* (Table 5 and 6). The effects include gill alterations such as: hydropic degeneration in the gill lamellae, inflammation of the gill, and disintegration of the gill architecture and fusion which denotes gill functional disorders which may affect the fish physiology or cause death of the fish. In this study, observations showed that the damage of the liver cells increased with increasing concentration and duration of exposure to the toxicant. Liver alteration such as, normal hepato-cellular of the liver cells, hydropic degeneration in the hepatic parenchyma of the liver cells, which are usually related to liver functional disorder, which may affect the physiology and caused death and spaces within the cell protoplasm filled with fluid.

The histopathological changes detected seem to have been caused by the toxicant *M. lucida*, while the mortality recorded could be due to the malfunctioning of the gills and the disorder of the liver. The results showed that *M. lucida* is toxic to *O. niloticus* fingerlings. The results of this study showed that the survival of *O. niloticus* was directly related to the concentration of *M. lucida* in solution. The water quality parameters increased concentration of *M. lucida* with time. There was a visible effect of *M. lucida* concentration on histopathological alterations/changes in the gills of the *O. niloticus* fingerlings these include; degeneration of gill architecture, disintegration of the gill and erosion of the gill filaments and gill rakes. Histopathological evidence of the gill damaged caused by *M. lucida* toxicity was evident resulting from malfunctioning of the gill.

The liver is an important centre of metabolism of various substances and supporting the stability of intra-circumstances of organism, therefore the changes that occur in the liver would interfere with the normal metabolic function of the liver cells. Mortality may be as a result of the disorder of the liver. Changes that occur in the liver include: degeneration in hepatic parenchyma, hydropic degeneration and hepato-cellular of the liver cells and necrosis. The 96 hours LC_{50} of *M. lucida* dry extract to *O. niloticus* was at 1.869g *M. lucida*/L of water at 24 hours.

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HAEMATOLOGICAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*) JUVENILES EXPOSED TO TOBACCO (*Nicotiana tobaccum*) LEAF DUST

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ABSTRACT

Tobacco (*Nicotiana tobaccum*) leaf dust has piscicidal properties thus there is a need to study its haematological effects on Nile tilapia juveniles. A 96hour bioassay was conducted on *Oreochromis niloticus* juveniles (mean wt., 30g) to determine the median lethal concentrations (LC_{50}) .The fish were exposed to various concentrations of tobacco leaf dust (0.5g-2.5g/l).Water quality parameters and physiological parameters were monitored/determined according to standard procedures. Water quality parameters were monitored after 96hours. The LC_{50} at the end of 96hours was 1.35g/l. The monitored water quality parameters such as temperature, pH and dissolved oxygen were significantly decreased while total alkalinity and conductivity increased significantly in the exposed media, compared to the control test. The fish showed hypeventilation, erratic swimming, loss of reflex during the period of exposure and this increased with increase concentrations of tobacco leaf dust. Haematological analysis of the blood revealed significant haematological changes, the intensity of haematology damages increased with increasing concentrations and exposure to tobacco leaf dust. The reduction in blood parameters could be as a result of destruction of erythrocyte or haemodilution. However, the monitored water quality parameters revealed that the plant dust has effects on the blood parameters of the test fish and consequently the biodiversity of the organisms. The result provided baseline information and established safe limits of using tobacco leaf dust in fish ponds, hence 1.0g/l concentration of tobacco leaf dust is recommended for the use on *O. niloticus juveniles*

INTRODUCTION

Aquaculture is increasingly becoming one of the fastest growing aspect of the agricultural industry worldwide (FAO, 2004). Fish farmers often use tobacco leaf in controlling these unwanted organisms/pests (Konar, 1970; Tobor, 1990). According to Aleem (1987), the use of tobacco leaf dust is due to its inexpensiveness, local availability and easier degradability. Despite, the effective use of this plant material, eco-toxicologists are interested in the ecotoxic properties of plant origin pesticides/piscicides, such that plant origin pesticides/piscicides cannot be used directly in freshwater bodies unless their toxicity and sub-lethal long term effect have been studied on non-target animals, sharing the habitat with the target animals.

The active ingredient of the plant used, is the nicotine (Hassal, 1982), It is soluble in water, alcohol, chloroform, ether, kerosene and some fixed oils (Vogue, 1984). Tobacco leaf dust has been used in Nigeria as an effective insecticides and treatment of predators/pest in water (pond) since it is completely biodegradable (Aleem, 1987; Nile tilapia is of the commercially important species of fish for rapid aquaculture expansion in Nigeria. The choice of the test fish is attributed to the report of Rand *et al.,* (1995) that in order to extrapolate meaningful, relevant and ecological significant results from aquatic toxicity tests, not only appropriate test but also appropriate organism should be used, whenever possible, species should be studied or representative of the ecosystem that may be impacted; thus the choice of the *Oreochromis niloticus* which is of economic importance in Nigeria as an abundant cultural fish species in Nigeria and is very popular with fish farmers and consumers. The knowledge of sub-lethal effects of tobacco is very important to delineate the health of fish status and to provide a future

understanding of ecological impacts (Radhaiah *et al.,* 1987). The aim of this research is to ascertain the assumption whether tobacco leaf dust (*Nicotina tobaccum*) in a sub-lethal concentration and in a medium exposure time can influence changes in the blood of *O. niloticus* after the 96 hours exposure period.

MATERIALS AND METHODS

Juvenile *O. niloticus* of the same brood stock (30.01±0.34g) were obtained from the Federal University of Technology, Akure fish farm. They were acclimatized in a glass tank for 24 hours. The mortality and later transferred to the experimental plastic aquaria 10 fish/48L aquaria). The leaves of tobacco were sun-dried for 10 days and milled into powder, sieved and stored in a sealed plastic container until required. The concentrations of tobacco used were calculated as 50% 96h LC_{50} (96h LC_{50} of tobacco leaf dust on *O. niloticus* obtained from preliminary investigation). Thus 100 mg of tobacco leaf dust were measured and mixed in 1 litre of water to give 100 mg/L concentration of the tobacco leaf dust. These concentrations were introduced into 12 sets of aquaria with one replication.

Forty (48) liters capacity aquaria were maintained throughout the exposure period. Ten (10) juveniles each were placed in the 48L plastic aquarium. Bore-hole water was used during the acclimatization and exposure period. In order to monitor the toxicant strength, level of dissolved oxygen, the effects of evaporation; ammonia concentration and reduce stress during experimentation, the test media were replaced by 50% prepared – concentrations of the same quantity after removing its equivalent along with defaecation every 6 hours to maintain the requisite level and potency of the concentration. The exposure period lasted for 96hours during which some water quality parameters were monitored daily using APHA (1998) methods. After 96 hours, 60 fishes were sacrificed and analyzed for the haematological examination. Blood was obtained from randomly selected fish from the control and the exposed test after the 96hours, using 2.0ml plastic syringe, as described by Kori-Siakpere (1998). The blood was transferred into a lithium heparin anticoagulant tube at room temperature for 30-40 minutes (Mahoba, 1987) and stored at refrigerator until analyses.

Fish mortality data were analyzed using complete randomized design with equal replication (one-way ANOVA test) at 5% level probability. All data were presented as means ± standard error, the data from the 96hours tobacco leaf dust exposure was first analyzed using a one-way ANOVA test, after which individual means were compared, using Bonferoni multi-sample correction/test.

RESULTS AND DISCUSSION

Mean values of water quality parameters of the different sub-lethal concentrations of tobacco leaf dust and control media to which the test fish *O. niloticus* were exposed over the 96hours exposure period is as presented in Table1. The value of temperature, pH and dissolved oxygen were found to significantly ($p<0.05$) and ($p<0.01$) decreased as the concentrations of tobacco leaf dust increased. However, the values of total alkalinity and conductivity in the exposed media were significantly ($p<0.01$) increased as the concentrations of tobacco leaf dust increased, compared to the control test. Exposure of *O. niloticus* juveniles to tobacco leaf dust solution clearly disrupted haematological parameters. Haematocrit, haemoglobin values, erythrocyte and leucocyte counts, total protein and albumin of the fish exposed to different concentrations of tobacco leaf dust revealed significant haematological alteration and changes (Table 2). Erythrocyte reduces from mean value of 1.67 – 1.0mm^3 with increase in concentration of tobacco leaf dust, the decrease in these values were also observed to be both a factor of time and concentration of tobacco.

Table 1. Water quality parameters of the sub-lethal concentrations of tobacco leaf dust after 96 hours

Concentration (g/l)	Temperature (^{0}C)	Dissolved oxygen (mg/l)	pH	Conductivity
0.0	24.20 ±0.00	6.20±0.10	6.80±0.05	117.9±0.40
0.5	24.30 ±0.00	5.90±0.20	6.50±0.00	134.3±1.10
1.0	24.30 ±0.00	4.10±0.10	6.40±0.00	
1.5	24.30 ±0.00	3.80±0.10	6.40±0.05	141.0±1.20
2.0	24.30 ±0.00	3.30±0.00	6.30±0.05	145.8±2.05
2.5	24.30 ±0.00	3.10±0.10	6.20±0.00	150.1±1.05

*mean ± Standard Error (SE).

Table 2: Blood parameters of *O. niloticus* exposed to tobacco leaf dust concentrations for 96 hours.

Concentrations (mg/l)	Haematological Parameters					
	PCV (%)	RBC (mm^3)	WBC (mm^3)	HB (g/dl)	Total protein (mg/dl)	Albumin (mg/dl)
0.0	15.00 (1.00)	1.67 (0.27)	3.88 (0.10)	6.93 (2.57)	5.13 (0.12)	3.43 (0.21)
0.5	14.00 (1.00)	1.73 (0.06)	3.82 (0.10)	4.67 (0.31)	4.80 (0.20)	2.93 (0.12)
1.0	11.7 (0.58)	1.21 (0.04)	5.12 (0.10)	4.00 (0.00)	3.43 (0.21)	2.73 (0.12)
1.5	10.7 (0.58)	1.21 (0.01)	5.00 (0.10)	2.20 (0.35)	2.80 (0.20)	2.37 (0.32)
2.0	9.33 (1.16)	1.10 (0.03)	5.12 (0.08)	1.33 (0.35)	2.20 (0.20)	2.00 (0.20)
2.5	9.00 (1.00)	1.00 (0.03)	5.27 (0.76)	0.70 (0.10)	1.97 (0.15)	1.87 (0.23)

*Mean ± Standard Error (SE).

Haemoglobin values reduced from 6.93-0.70g/dl with increase in concentration from 0.0g /l control - 2.5g/ l (Table2). The erythrocyte and leucocyte counts showed intra- concentration variations, the number of the leucocytes increased as the concentration of the toxicants increases while erythrocytes deceased with increasing concentrations of tobacco leaf dust , In different concentrations of tobacco leaf dust (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5g/l), PCV values varied from 15.0 ,14.00, 11.67 ,10.67, 9.33 and 9.0%, respectively, at concentrations 0.5 and 2.5g/l, and the PCV values reduced with increase in concentration of test dye and this is traceable to different fishes having different blood parameters unlike human blood that is constant (Baker and Silverton, 1982). Total protein (g/L) varied with different concentrations of tobacco leaf dust, the mean value for total protein varies from 1.97-4.80mg/dl and 1.87-3.43mg/dl in albumin, this shows that total protein decreases with increase in concentration of tobacco leaf dust when compared with the control (0.0) with mean value of 5.13mg/dl (Table2). The 96-h LC_{50} was 1.35 g/L tobacco leaf extract, compared to other synthetic pesticides used in fish farming, such as carbamates and organophosphates, tobacco based products are certainly less toxic to fish (Wan et al., 1996). Results indicated that tilapia is more sensitive to tobacco leaf water extract than other fishes (Mamdouh et al., 2008). Changes in the water quality parameters showed that the concentrations affected the water quality, but the values were within tolerance range (Table 1).

Houston et al. (1971) reported changes in the blood parameters of fresh water fish exposed to various handling procedure before experiment and the effect of stress on the fish. The disrupted haematological parameters observed in this experiment also agreed with Akinbulumo (2005) who reported that fish showed toxic reaction to *Derris elliptica* root powder by surfacing jaws and becoming stupefied. Reduction in oxygen level in this study is in line with Lloyd (1961) who reported that toxicity of several poison on rainbow trout increased inversely to the oxygen concentrations of water .A number of poisons become more toxic at low oxygen concentrations because of an increasing respiratory rate, increasing the amount of poison to which the animal is exposed.

Haematological examination revealed adverse effect of tobacco leaf dust on the blood of *O. niloticus*, and this is similar to Mason et al. (1994) who had earlier reported similar observations when subjected *O. niloticus* to sub-lethal concentration of formalin. The result from statistical analysis shows that there is reduction in some of the blood parameters, this is an indication of anemia which is a condition characterized by a deficiency of haemoglobin, packed cell volume and erythrocytes.

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COMPARATIVE ASSESSMENT OF PARASITE INFESTATION OF TILAPIA IN NATURAL AND CULTURED ENVIRONMENTS

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ABSTRACT

The study identified and compared the prevalence and intensity of parasites of *Tilapia zillii* and *Oreochromis niloticus* from natural and culture environments. 145 samples were collected from both environments and gross observations were carried out to check for physical abnormalities and presence and identification of parasites. The samples were dissected and the skin, gills, stomach and intestine were examined for parasites presence, prevalence and intensity. Four classes of parasites comprising 410 parasites were recovered namely 106 protozoans, 148 nematodes, 7 crustaceans, 132 trematodes and 17 parasites cysts. Cultured tilapia had higher parasitic infections than the wild tilapia and the parasite intensity and prevalence; and the parasite were significantly different in the tissues and organs.

INTRODUCTION

Tilapia is now one of the most widely distributed exotic fish in the world, second only to common carp, as their introduced range now stretches to nearly every continent and include 90 different countries. Tilapias are widespread in the tropics and sub-tropics (Intervet, 2006). They are highly adaptable and easily cultured. The fish are reared in ponds, cages, or pens and they grow well in fresh water and brackish waters. The high fecundity of the fish; its few disease problems; and the availability of its fry have resulted in intensification of production (Seafood Watch, 2006). Under the original extensive or semi-intensive culture systems, Tilapias were more resistant to disease than many other fish species (Roberts and Sommerville, 1982). However the intensification of culture systems and resultant deterioration in the environment has been associated with an increase in parasitic and infections disease problems. Infections diseases are caused by parasites, but host and environmental factors also play a role in their occurrence (Thrusfield, 1997).

A parasite could be harmless, harmful or beneficial to the host. The number of parasites necessary to cause harm to a host varies considerably with species and size of the host and its health status (Carpenter et. al., 2001) Parasite infections in fish causes production and economic losses through direct fish mortality; reduction in fish growth; reproduction and energy loss; increase in the susceptibility of fish to disease and predation; and through the high cost of treatment (Cowx, 1992). Information about the mode of transmission and potential intermediate hosts is often crucial to select the most appropriate management action to reduce or eliminate the problem (Aken'ova, 2000). The aim of this study is to identify the parasites in *T. zillii* and *O. niloticus*, and compare the prevalence and intensity of parasites from the wild and cultured environments in south-western Nigeria.

METHODOLOGY

A total of 145 live fish samples comprising of two species of wild and cultured tilapia: *T. zillii* and *O. niloticus* were collected from fresh water rivers: Owena reservoir, Ogbese river, and two fish farms in Akure, South Western Nigeria. Gross physical examination of the external features of the samples were done for abnormalities (if any); and the fish were transported in a 25 liters plastic containers to the laboratory. The specimens were kept in glass tanks (72 x 30 x

35 cm) filled with freshwater. The samples were separated into species, total length (cm) measured using a measuring board; and weighed (g).

The outer layer of the skin were then scraped from the right and left sides of the back and posterior of the fish body, transferred to a slide, diluted with a drop of sterilized water, cover-slipped and examined under a microscope (Olympus CX40). Large parasites were expressed by their absolute numbers, while the microscopic parasites were expressed by decanting serially and determining their minimum, maximum and average numbers in each field of view of microscope at a definite magnification. The intestines of samples were removed and separated into stomach and intestine sections. Parasite cysts located on their surfaces were located and examined microscopically. The parasites wre transferred by a dissection needle to a slide containing sterilized water after getting rid of their slime and examined at high magnification (400 X). Smears were made from the samples of the skin, gills, stomach and intestine. These specimens were dissolved in petri dishes with few drops of 9% saline solution which kept the parasites alive. Smear was place on sterilized slide and viewed under low and high power (400 X) magnification.

Descriptive analysis was used to evaluate the data obtained. Level of significance of the mean difference on prevalence and intensity of parasites on *T. zillii* and *O. niloticus* were carried out using T-test (SPSS version 15.0).

RESULTS AND DISCUSSION

The prevalent parasite identified in *O. niloticus* and *T. zillii* samples examined from the natural and induced environments wee *Camallanus sp. Tricodina acuta, Dactylogyrus sp, Gyrodactylus sp, Echthyophithrins mutifilies,* leech and parasite cysts. Table 1 represent the prevalence parasites in the intestine of the wild and cultured Tilapia fish samples. *Camallanus sp* was highest (74.00±33.94) while leech was the least (3.50±0.95). Infection rate and intensity of the parasites were higher in culture than in the wild tilapia. 27% of males were infected while 80% of females were infected, and it agrees with the report of Ibiwoye *et al.* (2004) who reported more infection in female fish; and that they are more liable to infection with nematodes and acanthocephalan which were among the group reported in these studies. 35% of *T. zillii* were paratized while 48% of *O.* niloticus were infected, resulting that *O. niloticus* were highly infected than *T. zillii*

Among the wild species of *O. niloticus* and *T. zillii* collected (96 fish samples), 40 (42%) were infested, while 33 (67%) were infested from a total of 49 fishes from the different cultured sites. This is in line with Martin, *et. al.* 2009, which reported that higher infections levels in cultured tilapia than in wild tilapia are attributed to higher fish densities in the cultured systems and lack of adequate management techniques. And that high stocking densities favour increased parasite populations (Karvonen et al. 2006). Parasite located in the stomach organ indicated that 83% of the fish samples had parasites infestation in the stomach; while 34% is from the wild and 49% from the cultured samples. About half of the samples (51%) from the cultured habitat harbored parasites in the intestine, while 29% from the wild were infested in the intestine. Parasites infestation on gills examination indicated 45% from the cultured and 13% from the wild samples and 14% of the female *O. niloticus* from the cultured samples were paratized on the skin with leeches. *Camallanus sp* has the highest prevalence (80%). Parasitic prevalence was found highest in the following order: stomach (83%), intestine (80%), gills (58%), and skin (14%).

Fish sample with weights ranging between 20.5 – 30.5g and 110.3 -130.4g recorded the highest percentage of infection (44%) while fish with weights range of 40.7g – 60.8g and 130.5g and 190.9g recorded very low level of infection (4%). Fish samples with between 11.6cm – 18.1cm recorded the highest percentage of infection (23%) while fish with length ranging from

18.2cm – 24.7cm recorded a low percentage infection (10%). This is in-view with Goselle *et al.* (2008) who reported low level of infection in larger sizes of fishes in Lamingo reservoir, Jos, Nigeria. Total parasitic load of the fish samples (from the wild) decreased from the first sampling during March (early rainy season) to the eight sampling during June (peak rainy season). This can be due to high rain influx during the rainy season and low rain influx during the dry season. It is also supported according to Morenikeji and Adepeju (2009) and Ibiwoye *et al.* (2004) who reported that fishes are susceptible to heavy infestation with parasites mainly in the early rain when fishes are weakened by hibernation (a state of exhaustion). Table 2 shows that cultured tilapia are more infested than wild tilapia, with the cultured tilapia having highest parasitic means of 131±29.70; Table 3 indicated the difference in the infection rate between species. The result of the analysis shows that there is a significant difference at $P<0.05$ in the tilapia (Table 4). Hence, since it has been observed that parasite infection of fish affects a good number of fishes especially in the cultured ponds, outbreak of disease can be prevented by proper management techniques.

Table 1. Prevalence and intensity of parasites

Parasite	Wild Tilapia	Cultured Tilapia	Mean Parasite intensity
Tricodina acuta	20	33	26.50±9.19
Ichthyophithrins mutifilies	12	41	26.50±20.51
Dactylogyrus sp	29	37	33.00±5.66
Gyrodactylus sp	32	34	33.00±1.41
Camallanus sp	50	98	74.00±33.94
Leech	0	7	3.50±0.95

Table 2. Parasite burden in the different locations

Locations	Parasite load
Farm A	85.00±24.04
Farm B	46.00±5.66
Owena Reservoir	29.00±8.48
Ogbese River	45.00±7.07

Table 3. Relationship between infection rate of parasites and the species of cichlids

Species	Mean Number of Parasites
O. niloticus	59.25±29.36
T. zillii	43.25±18.57

Table 4. Prevalence of parasites on wild and cultured tilapias.

Environments	T	Df	Sig	Mean frequency
Wild	6.727	1	0.094	74.00±15.56
Cultured	6.238	1	0.101	131.00±29.70

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OXYTETRACYCLINE MARKING STUDIES OF TILAPIA;

Oreochromis niloticus

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Abstract

Oxytetracycline (OTC) was intraperitoneally injected into tilapia, *Oreochromis niloticus* to validate aging of this fish, to determine suitable marking dosages of OTC, to assess OTC-induced mortality and to determine the rate of OTC incorporation into 4 bony structures including scales, opercula, otoliths and pelvic fin rays' sections. The OTC was used in 3 doses; 12.5, 25 and 50 mg/kg of fish body weight. A total of 1200 fish with an average body weight of 60 grams were used for the experiment (300 fish per OTC dose and another 300 fish were injected with sterile saline solution as a control group). The fish of the 4 groups were released in the water of 4 raceways at the World Fish Centre, Abbassa, Egypt and were kept for an entire year. The results revealed that 1 annulus was laid down after the OTC mark on all bony structures. OTC was incorporated into all examined bony structures. The clearest OTC marks with minimal mortality were induced by the 25 mg OTC dosage. No significant mortalities were noticed among the injected fish with OTC.

Key words: Oxytetracycline, tilapia, annulus, scale, operculum, otolith, fin rays.

Introduction

Until the 1970s, tetracycline had been used extensively in the field of aquaculture as an antibacterial drug (Schnick, Meyer & Walsh 1986). OTC was found markedly effective in vitro and in vivo against *Aeromonas hydrophila* micro-organism and the highest concentrations of the drug were found in liver and bone tissues of the treated Oreochromis niloticus (Soliman 1994). Since it is incorporated into newly forming calcified tissue and is visible under ultraviolet light as a fluorescent band (Bavelander & Goss 1962), it had been used periodically by fisheries researchers as a marking tool (Emery & Wydoski 1987). Since that time, tetracycline has been used widely as a means of marking fish (Babaluk & Craig 1990). Applied at a known date, tetracycline has been used to validate methods of fish age determination (Casselman 1974; Babaluk & Campbell 1987; McFarlane & Beamish 1987a). Since tetracycline is incorporated relatively quickly into calcified structures (Campana & Neilson 1982; Nagiec, Dabrowski, Nagiec & Murawska 1988), it can been used to verify the existence of daily growth increments (Campana & Neilson 1982; Laurs, Nishimioto & Wetherall 1985). High dosages of tetracycline will cause mortality so it is necessary to determine a dosage that produces a suitable mark on calcified structures of a fish species

while causing minimal mortality (Kobayashi, Yuki, Furui & Kosugiyama 1964; McFarlane & Beamish 1987b).

In this study, tetracycline was injected into tilapia, Oreochromis niloticus to validate 4 age determination methods (scales, opercula, otoliths' and pelvic fin rays' sections) to assess tetracycline-induced mortality, to determine how quickly it was incorporated into calcifying tissues in fresh water and to determine the suitable marking dosage of tetracycline for this species.

Materials and methods

Fish:

A number of 1200 fingerlings of monosex *Oreochromis niloticus* were used for the study. The fish were acclimatized first and then were distributed into 4 raceways (300 per each raceway). After that the fish were kept for 4 months before launching the experiment to reach bigger sizes. The fish were fed on artificial ration of 25% protein in the rate of 3% of total fish biomass. Partial and periodical change of pond water was carried out at a monthly basis. The average body weight at the time of arrival of the fish on May 1998 was 38 gram and the average total length was 13 cm. While, the average body weight at the time of injection of the OTC on September 1st, 1998 was 60 gram and the average total length was 15 cm.

Raceways: Four raceways were used for rearing the fish for an entire year, which was the period of the experiment. The dimensions of each raceway were 50 meters long, 4 meters width and 1 meter of water depth. The experiment was carried out in the facilities of the WorldFish Centre (The Regional Centre for Africa & west Asia) at Abbassa, Abou-Hammad, Sharkia, Egypt) during the period from September 1998 until September 1999.

Anaesthesia: The fish were anaesthetized using MS222 in the rate of 75 mg/liter of water and accompanied with sodium bicarbonate in the rate of 150 mg/l to avoid the expected hyperacidity induced by MS222.

Selection of Oxytetracycline dosages: The fish in the 1st group were injected with OTC in a dose of 12.5 mg/kg fish body weight and then released into the first raceway. The fish in the 2nd group were injected with OTC in a dose of 25 mg/kg fish body weight and then released into the second raceway. While, the fish in the 3rd group were injected with OTC in a dose of 50 mg/kg fish body weight and then released into the third raceway. On the other hand, the fish of the 4th group were injected with sterile distilled water and then released to the 4th raceway to be kept as a control group. The fish were injected intraperitoneally using an automatic syringe similar to that used in mass injection of chickens in poultry farms.

Preparation of the OTC injection solution: A commercial pharmaceutical preparation known as Panterramycin (Pfizer) in a package of 50 cc was used as a stock solution of OTC. Each 50 cc bottle contained Oxytetracycline in a concentration of 30 mg/cc. Two bottles (100 cc) were used for the experiment. This stock solution of OTC (100 cc) was diluted 10 folds in a sterile distilled water (900 cc) to reach one liter of final injection solution of OTC. Thus, each cc contained 3 mg of OTC. Each experimented dose was multiplied by the average fish body weight (60 gram). The 50 mg/kg fish body weight x 60 gram = 3000 divided by 1000 to convert kg to gram = 3 mg OTC (i.e. 1 cc of the injection solution) per fish. Thus, the 50 mg/kg b.wt. was achieved by injecting each fish 1 cc of the injection solution. Consequently, the 25 mg/kg b.wt. was obtained by injecting half of the preceding dose, which was 0.5 cc/fish and finally 0.25 cc/fish for the 12.5 mg/kg b.wt. dosage. On the other hand, the fish in the control group were divided into 3 groups (100 fish per each). The first group was injected with 1 cc; the 2nd group was injected with 0.5 cc and the 3rd one with 0.25 cc of sterile distilled water.

Injection of fish: All fish were injected in the early morning; at 7 a.m, to shun the hot weather in the afternoon for minimal stress on the examined fish. The fish of the control group were injected first with sterile distilled water using the automatic syringe before being intermingled with OTC solution to avoid any undesirable entrance of OTC into the control fish. Twenty fish were injected with distilled water and kept in a hapa for sampling. Then the fish were injected with 50 mg OTC /kg b.wt. in the rate of 1 cc per fish and 50 injected fish were kept in a hapa also for sampling directly after injection. After that, injection of the fish with OTC in the rates of 25 and 12.5 mg/kg b.wt was carried out respectively. The injected fish were then released into their respective raceways, where they were kept for 1 year.

Sampling regime: One fish from the fish kept in the control hapa and 5 from the fish injected with 50 mg/kg.b.wt. were taken periodically after 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks and 4 weeks respectively. Periodical sampling of 5 fish from the 4 treatments in a monthly basis was carried out by partial fishing after half emptying of water in the 4 raceways. Those 5 sampled fish from each treatment were sacrificed. Each fish was numbered, weighed and the total body length was measured. The fish were then dissected. Different bony structures were taken from each fish, which are scales, opercula, otoliths and pelvic fins. Each bony structure was then put in a small envelope and finally all structures were kept in larger envelopes containing a full data about the fish.

Preparation of different bony structures for examination: **The scales** were soaked for 5 minutes in enumerated ice cube trays containing water plus 2 drops of liquid soap to facilitate the removal of mucus and tissue debris. The scale was then handled by a forceps, rinsed with water and rubbed by a paper tissue. After that, 3 dry scales were mounted between 2 slides and compressed by means of an adhesive tape (El-Bouhy et al., 2002). **The opercula** were

handled by a forceps and put into boiling water several times then cleaned using a paper tissue to remove the skin and tissue debris. **The otoliths** were removed from the skull of the fish after beheading. Two longitudinal sections were made along the skull passing the proximal rim of the 2 eye balls. Then the roof of the skull was reflected and the brain was removed. The 2 otoliths were then found embedded in their grooves, where they were removed by a forceps and put on a paper tissue. Finally, they were dried gently between fingers & paper tissue and kept in a small envelope. **The pelvic fin rays** were first trimmed and then placed in ice cube trays lined with Para film. After that they were embedded in epoxy resin plus hardener in a ratio of 2:1 respectively. Finally the blocks were left for 12 hours for hardening. The sections were carried out either manually using the jeweler's hand saw or automatically using an isomet electric saw. **Examination of different bony structures:** **The opercula** were examined whole on a black background with a stereoscopic microscope with a hand-held reflective ultraviolet light source. The viewing room was dark. **Scales, otolith sections, and fin ray sections** were examined under reflected ultraviolet light using a fluorescence microscope.

Results

Age validation: There was quite a bit of growth on all structures after the OTC mark. It also appears that the fish were at large long enough so that an "annulus" or "check" had formed after the OTC mark. This was especially evident on the pelvic fin rays and opercula. Thus, all examined bony structures were found to be reliable and valid for aging of *Oreochromis niloticus* in Egypt. Time of annulus formation was on May depending on scales' back calculations (Figure. 1). On the other hand, its time may be on April according to opercula's back-calculations (Figure. 2). Different results were stated by Gladys et al., 2007 who found 2 annuli on the otoliths of Nile tilapia in 2 lakes in Uganda.

Figure. 1 Back-calculations using scales radii. R = Average total scale radius in mm (sampled from 5 fish). N.B. Notice the stunted growth during winter months (In January, February& March).

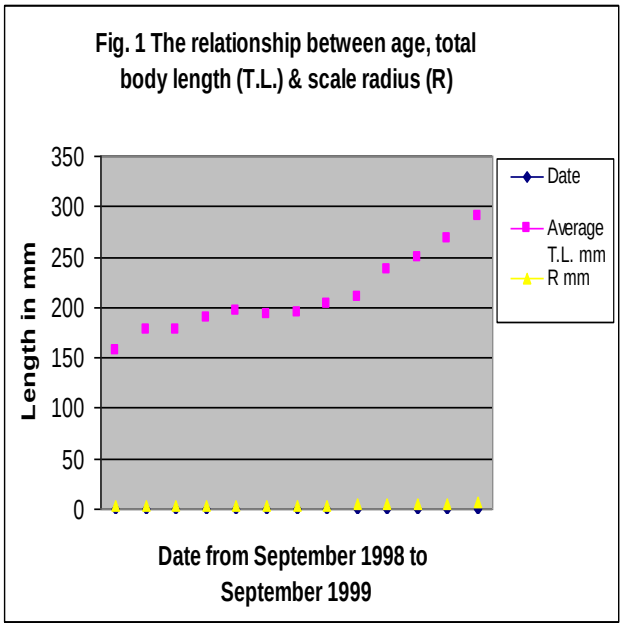
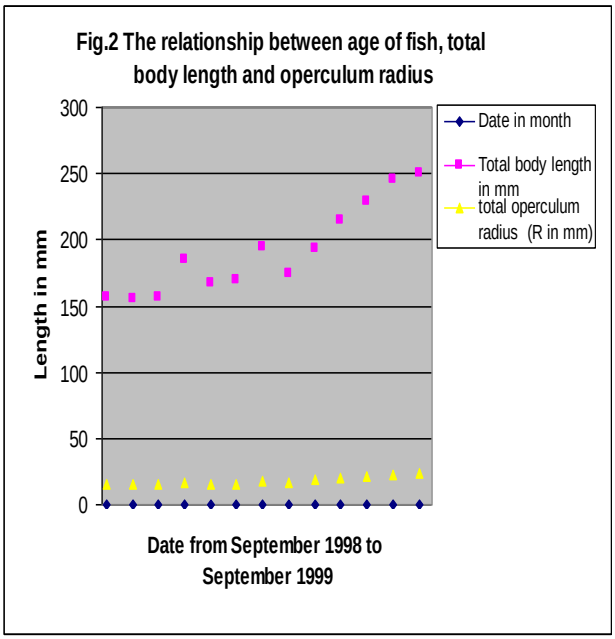


Figure. 2 Back-calculations using opercula radii. R = Average total operculum radius in mm (from 5 fish).



Oxytetracycline-induced mortality: The OTC-induced mortality was insignificant among the experimented fish. No immediate mortality recorded in the day of OTC injection. However, the fish injected with 25 mg/kg b.wt. of OTC showed the least mortality percentage (0.3 %) followed

by the 50 mg/kg b.wt. group (1.3 %). While, the fish in the 12.5 mg/kg b.wt. group and the control one revealed relatively the highest mortality percentage (2.7 %). Thus, the OTC dose of 25 mg/kg b.wt. was proved to be the best dose, which induced the most powerful OTC mark with minimal mortality. **Oxytetracycline uptake:** All the three dosages were sufficient to mark the various bony structures. OTC marks were clearly evident on all structures from OTC-injected fish that were at large for one month. In all of these cases, there was visible growth on the bony structure after the OTC mark.

- **All control** structures had no auto-fluorescence.

- **Opercula:** On the opercula from fish that had been injected and then sacrificed relatively early, a yellow fluorescence was usually evident only on the edges or tips of spiny parts. As time went on, the fluorescence tended to cover the whole surface of the opercula. Of the fish that were at large for one month, a distinct "OTC line" was visible with subsequent growth on the opercula also evident. As dense bone was being deposited in all planes, the fluorescence that would have been deposited on the operculum was covered and became less evident.

Scales: All scales taken from relatively recent injected fish showed a yellow fluorescent glow (quite dramatic when compared to the controls). Scales from fish that had been at large for one month had a distinct yellow fluorescent line as well as the glow. The glow would probably dissipate as the fish and scales grew.

Otoliths: It was more difficult to discern the fluorescence on otolith sections from the recently injected fish. The OTC appears first near the sulcus of the otolith (Figure. 3). Since the otolith is not vascularized (the other structures are), it theoretically, may take a longer time to be deposited. These results agreed with those reported by Massou et al., 2004 who got a clear OTC mark on the otoliths of Nile tilapia injected with 50 mg kg^{-1} live mass. However, the results disagreed with those recorded by Wright et al., 2002 who stated that difficulties in estimating age or backcalculating fish size from otoliths, however, have been encountered in various fish species because of the appearance of checks resulting from disruptions in otolith incremental deposition. These checks interrupt the regularity of the primary increments.

Pelvic fin rays: OTC marks were relatively easy to discern although they were easier to see on the machine-sectioned fins than hand-sawed sections.

The fact that the fish had been at large for a lengthy period of time (nine months) made it easier the discern of the OTC marks.

The optimum Oxytetracycline dosage: The 50 mg/kg dosage produced the "strongest" mark on all structures although on the scales a relatively faint line was observed. The scales had a florescent "glow" inside the OTC mark. The 25 mg/kg dosage produced "good" marks on all structures (Figure. 3) although the marks were relatively faint on the opercula and scales. The

12.5 mg/kg dosage produced faint marks on most of the structures. All that was discernable on the opercula was a florescent glow (perhaps) on the inner area of the bone.

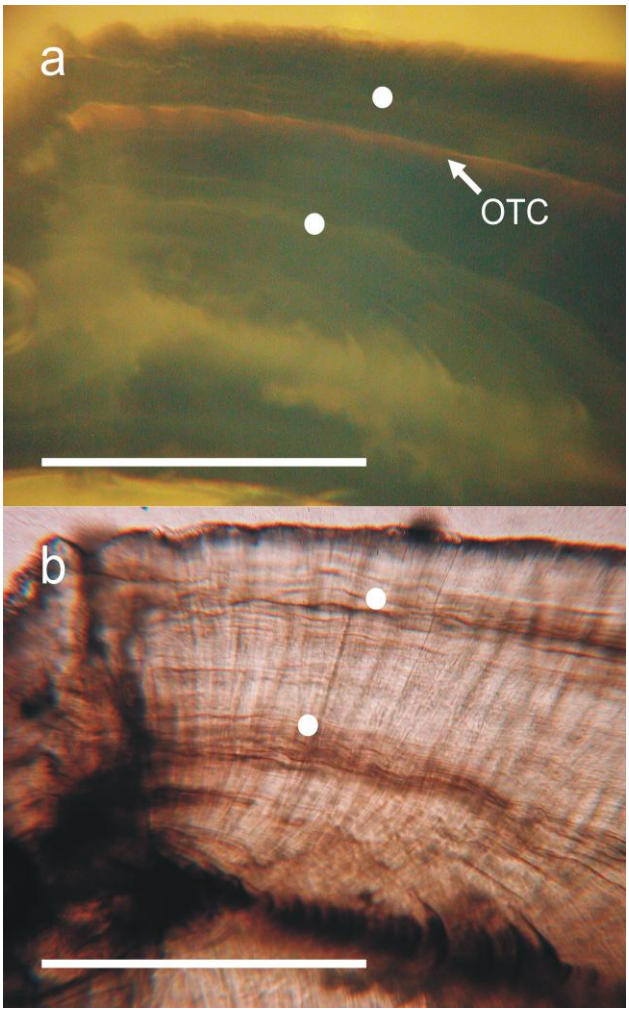


Figure. 3 Transverse section of an otolith from a tilapia injected with 25 mg oxytetracycline/kg body weight on 1 September 1998 and recaptured on 2 September 1999 under (a) reflected ultraviolet light and b) transmitted white light. Annuli are indicated by dots. Oxytetracycline mark (OTC) is also indicated. Bar = 0.5 mm.

Conclusion:

It could be concluded that tilapia fish aging is reliable in Egypt as a subtropical country and can be used as a basis for stock assessment and other fisheries management applications. The dose of 25 mg OTC/kg body weight for marking of tilapia is recommended in subsequent work & future studies.

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SECTION II
ACCELERATING AQUACULTURE DEVELOPMENT IN POORER COUNTRIES

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Oregon State University, USA

Intensity of Freshwater Use for Aquaculture in Different Countries

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Abstract

The intensity with which 172 countries use freshwater for aquaculture was estimated by dividing annual, freshwater aquaculture production (tonne/yr) by annual total natural renewable freshwater (km^3/yr). The freshwater aquaculture production:renewable freshwater ratio (AFR) varied among countries from 0 to 15,000 tonne/km^3. Country-level AFRs were assigned to AFR classes as follows: no freshwater aquaculture, 0 tonne/km^3; low, < 100 tonne/km^3; medium, 100-1,000 tonne/km^3; high, > 1,000 tonne/km^3. The number of countries in each AFR class follows: no freshwater aquaculture, 35; low, 80; medium, 45; high, 12. There seems to be adequate renewable freshwater to allow considerable expansion of freshwater aquaculture – especially outside of Asia.

Introduction

Statistics provided by the Fisheries Department of the Food and Agriculture Organization of the United Nations (FAO) (www.fao.org), reveal that total aquaculture production was 55.1 million tonne/yr in 2009, and freshwater aquaculture accounted for 35.0 million tonne/yr of this production (63.5%).

The current world population of 6.91 billion consumes about 118 tonne/yr of fisheries products, and a population of 9.15 billion that is predicted by 2050 would need about 156 million tonne/yr (an additional 34 million tonne/yr). Because capture fisheries are not projected to increase, aquaculture must supply the entire future increase in demand for fisheries products. Aquaculture production will need to be around 93 million tonne/yr by 2050 to allow the population to continue to consume fisheries products at the current rate. Assuming that freshwater and marine aquaculture grow at the same rate, freshwater aquaculture needs to increase to around 54 million tonne/yr by 2050.

The purpose of the present study was to determine the extent to which different countries use their freshwater for aquaculture.

Materials and Methods

Estimates of total natural renewable freshwater – the sum of surface runoff within a country, all surface water flowing into the country from neighboring countries, and the country's renewable groundwater – were obtained from Gleick (2009) for 172 of the world's 224 countries. Freshwater aquaculture production data were obtained for these countries from FAO fisheries statistics (www.fao.org/fishery/statistics/global-aquaculture-production/query/en). An indicator of the intensity of water use for freshwater aquaculture – referred to here as the freshwater aquaculture production to renewable freshwater ratio – was estimated for each country using the following equation:

$$\mathrm{AFR} = \frac{\mathrm{AP}}{\mathrm{RF}} \times 100$$

where AFR = freshwater aquaculture production to renewable freshwater ratio (tonne/km^3); AP = freshwater aquaculture production (tonne/yr); RF = total renewable freshwater (km^3/yr).

Results and Discussion

Of the 172 countries for which renewable freshwater data were available, 35 had no freshwater aquaculture production, or if they did, it was not reported. For the 137 countries reporting freshwater aquaculture production, AFR ranged from < 1 tonne/km^3 in several countries to 7,344 tonne/km^3 in China, 11,324 tonne/m^3 in Israel, and 15,000 tonne/km^3 in Kuwait (Table 1). Notice that the two highest AFR values were for small, water-restricted countries.

The AFR values were initially placed in five classes as follows: countries with AFR = 0 (no freshwater aquaculture); 80 countries with AFR < 100 tonne/km^3; 45 countries with AFR > 100 tonne/km^3 but < 800 tonne/km^3, ten countries with AFR > 1,000 tonne/km^3 but < 10,000 tonne/km^3; two countries with AFR > 10,000 tonne/km^3. However, it did not seem appropriate to assign the two countries – Israel and Kuwait – with AFR > 10,000 tonne/km^3 to a separate class, because they represent an insignificant proportion of world freshwater aquaculture production (19,546 tonne/yr or 0.062%). Thus, countries were placed into four AFR classes: no reported aquaculture (AFR = 0 tonne/km^3); low (AFR < 100 tonne/km^3); medium (AFR = 101-1,000 tonne/km^3); high (AFR > 1,000 tonne/km^3).

Many of the countries in the no aquaculture and low AFR classes (Table 1) need additional protein that could be obtained by increasing the amount of aquaculture. There also are countries in the medium and high AFR classes that need more protein. An example of the effect of increasing freshwater aquaculture on AFR in a country with a low FCR will be provided. Guatemala has a rapidly growing population that is expected to increase from 14,362,000 in 2010 to 22,995,000 in 2050. Suppose that Guatemala increased its freshwater aquaculture production from 3,000 tonne/yr at present to 10,000 tonne in 2050. The renewable freshwater in Guatemala is estimated at 111.3 km^3/yr (Gleick 2009); thus, AFR would rise from 27.0 tonne/km^3 to 89.8 tonne/km^3 – the country would still have a low AFR. In many Asian countries, and especially in China, increasing the amount of aquaculture will result in higher AFRs in a region where values are already much greater than in the rest of the world. Nevertheless, the data provided in Table 1 suggest that many countries could greatly increase aquaculture production without increasing AFR values to the levels found in Asia.

The main negative issues related to a large amount of freshwater aquaculture production (high AFR) at the country level are competition with other water uses and water pollution resulting from aquaculture (Pillay 2004; Boyd et al. 2007; Tucker and Hargreaves 2008). However, there are no studies revealing the extent to which aquaculture interferes with other water uses or contributes to water pollution at the country level. Based on regional studies of individual aquaculture industries such as channel catfish, (*Ictalurus punctatus*), in the southeastern United States (Boyd et al. 2000; Tucker and Hargreaves 2008), and *Pangasius* catfish in Vietnam (Bosma et al. 2009), aquaculture does not appear to be of as much concern related to water use conflicts and water pollution as many other activities. Thus, there should be opportunity to greatly increase aquaculture production in many countries, and especially those outside Asia, without resulting in major water use competition or causing serious water pollution. Nevertheless, aquaculture producers should strive to increase production per unit of water use – Verdegem and Bosma (2009) suggested that productivity could be tripled without increasing current freshwater use. Moreover, governments should pay more attention to the effects of aquaculture on the environment and require producers to comply with either discharge standards, best management practices, or both.

Table 1. Country-level estimates of annual total natural renewable freshwater (TNRF) (Gleick 2008), annual freshwater aquaculture production (AP), (www.FAO.org) and the freshwater aquaculture production:renewable freshwater ratio (AFR).

Region and country		TNRF (km^3/yr)	AP (tonne/yr)		AFR (tonne/ km^3)
AFRICA	Algeria	14.3	2215		154.00
	Angola	184	190		1.03
	Benin	25.8	180		6.98
	Botswana	14.7	0		0.00
	Burkina Faso	17.5	405		23.14
	Burundi	3.6	200		55.56
	Cameroon	285.5	340		1.19
	Cape Verde	0.3	0		0.00
	Central African Republic	144.4	0		0.00
	Chad	43	0		0.00
	Comoros	1.2	0		0.00
	Congo	832	65		0.08
	Congo, Democratic Republic (Zaire)	1283	2970		2.31
	Cote D'Ivoire	81	1150		14.20
	Djibouti	0.3	0		0.00
	Egypt	86.8	98833		1138.00
	Equatorial Guinea	26	0		0.00
	Eritrea	6.3	0		0.00
	Ethiopia	110	0		0.00
	Gabon	164	124		0.76
	Gambia	8	0		0.00
	Ghana	53.2	5594		105.15
	Guinea	226	0		0.00
	Guinea-Bisseau	31	0		0.00
	Kenya	30.2	4452		147.42
	Lesotho	5.2	91		17.50
	Liberia	232	0		0.00
	Libya	0.6	10		16.67
	Madagascar	337	2830		8.40
	Malawi	17.3	1700		98.27
	Mali	100	821		8.21
	Mauritania	11.4	0		0.00
	Mauritius	2.2	61		27.73
	Morocco	29	1185		40.86

Mozambique	216	90		0.42
Namibia	45.5	15		0.33
Niger	33.7	40		1.19
Nigeria	286.2	143207		500.37
Reunion	5	0		0.00
Rwanda	5.2	388		74.62
Senegal	39.4	160		4.06
Sierra Leone	160	0		0.00
Somalia	15.7	0		0.00
South Africa	50	1202		24.04
Sudan	154	2000		12.99
Swaziland	4.5	0		0.00
Tanzania	91	12		0.13
Togo	14.7	126		8.57
Tunisia	4.6	1117		242.83
Uganda	66	52250		791.67
Zambia	105.2	5640		53.61
Zimbabwe	20	2450		122.50

N & C AMERICA

Antigua and Barbuda	0.1	0		0.00
Bahamas	nd	0		0.00
Barbados	0.1	0		0.00
Belize	18.6	1865		100.27
Canada	3300	9314		2.82
Costa Rica	112.4	21768		193.67
Cuba	38.1	27771		728.90
Dominica	nd	0		0.00
Dominican Republic	21	140		6.67
El Salvador	25.2	3606		143.10
Guatemala	111.3	3000		26.95
Haiti	14	0		0.00
Honduras	95.2	20494		215.27
Jamaica	9.4	5812		618.30
Mexico	457.2	10618		23.22
Nicaragua	196.7	1388		7.06
Panama	148	462		3.12
St. Kits and Nevis	0.02	0		0.00
Trinidad and Tobago	3.8	0		0.00
United States of America	3069	323905		105.54

SOUTH AMERICA

Argentina	814	2465		3.03
Bolivia	622.5	631		1.01
Brazil	8233	211766		25.72
Chile	922	8717		9.45
Colombia	2132	46100		21.62
Ecuador	432	22120		51.20
Guyana	241	211		0.88
Paraguay	336	2100		6.25
Peru	1913	14987		7.83
Suriname	22	10		0.45
Uruguay	139	36		0.26
Venezuela	1233.2	2625		2.13

ASIA

Afghanistan	65	0		0.00
Bahrain	0.1	0		0.00
Bangladesh	1210.6	894205		738.65
Bhutan	95	0		0.00
Brunei	8.5	4		0.47
Cambodia	476.1	38359		80.57
China	2829.6	20781065		7344.17
India	1907.8	3342039		1751.78
Indonesia	2838	908693		320.19
Iran	137.5	150607		1095.32
Iraq	96.4	19246		199.65
Israel	1.7	19250		11323.53
Japan	430	39874		92.73
Jordan	0.9	330		366.67
Korea DPR	77.1	3700		47.99
Korea Republic	69.7	19150		274.75
Kuwait	0.02	300		15000.00
Laos	333.6	78000		233.81
Lebanon	4.8	803		167.29
Malaysia	580	95843		165.25
Maldives	0.03			0.00
Mongolia	34.8			0.00
Myanmar	1045.6	617859		590.91
Nepal	210.2	27250		129.64
Oman	1	86		86.00

Pakistan	233.8	135000		577.42
Philippines	479	311059		649.39
Qatar	0.1	36		360.00
Saudi Arabia	2.4	3753		1563.75
Singapore	0.6	283		471.67
Sri Lanka	50	5172		103.44
Syria	46.1	8595		186.44
Taiwan	67	161027		2403.39
Thailand	409.9	516405		1259.83
Turkey	234	66557		284.43
United Arab Emirates	0.2			0.00
Vietnam	891.2	1771000		1987.21
Yemen	4.1			

EUROPE

Albania	41.7	558		13.38
Austria	84	2087		24.85
Belgium	20.8	126		6.06
Bosnia and Herzegovina	37.5	7360		196.27
Bulgaria	19.4	4562		235.15
Croatia	105.5	4458		42.26
Cyprus	0.4	57		142.50
Czech Republic	16	20395		1274.69
Denmark	6.1	22661		3714.92
Estonia	21.1	813		38.53
Finland	110	2159		19.63
France	189	41340		218.73
Germany	188	36973		196.66
Greece	72	3991		55.43
Hungary	120	15687		130.73
Iceland	170	381		2.24
Ireland	46.8	850		18.16
Italy	175	39916		228.09
Luxembourg	1.6			0.00
Macedonia	6.4	1331		207.97
Malta	0.07			0.00
Netherlands	89.7	8575		95.60
Norway	381.4	90		0.24
Poland	63.1	36813		583.41
Portugal	73.6	941		12.79
Romania	211.9	12532		59.14

Slovakia	80.3	1071		13.34
Slovenia	32.1	1041		32.43
Spain	111.1	22281		200.55
Sweden	179	4016		22.44
Switzerland	53.3	1214		22.78
United Kingdom	160.6	10563		65.77
Serbia-Montenegro (Yugoslavia)	208.5	0		0.00
Russia	4498	115234		25.62
Armenia	10.5	2001		190.57
Azerbaijan	30.3	89		2.94
Belarus	58	4150		71.55
Estonia	12.8	813		63.52
Georgia	63.3	180		2.84
Kazakhstan	109.6	321		2.93
Kyrgyzstan	46.5	92		1.98
Latvia	49.9	584		11.70
Lithuania	24.5	3008		122.78
Moldova	11.7	4700		401.71
Tajikistan	99.7	26		0.26
Turkmenistan	60.9	16		0.26
Ukraine	139.5	15027		107.72
Uzbekistan	72.2	3418		47.34

OCEANIA

Australia	398	1127		2.83
Fiji	28.6	217		7.59
New Zealand	397	0		0.00
Papua New Guinea	801	80		0.10
Solomon Islands	44.7			0.00

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IMPACTS OF THE INTRODUCTION OF ALIEN TILAPIAS (*Oreochromis* spp.) ON THE FISHERIES AND BIODIVERSITY OF INDIGENOUS SPECIES IN TRI AN RESERVOIR, VIETNAM

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Abstract

This study was conducted at Tri An Reservoir of Vietnam from November 2007 to June 2009 to determine the impact of tilapias (*Oreochromis* spp.) on the fisheries and biodiversity of indigenous species in the reservoir. Historical and currently data on fish caught and fish species composition was collected. There are currently 19 different types of fishing gears in use at the reservoir, of which 14 fishing gears caught tilapias. Of the five fishing gears with highest total catches, only two caught tilapias. There were only 4.62% and 5.09% of tilapias in fishermen harvest and landing point records, repectively. However, tilapias (*Oreochromis* spp.) were 6th of 40 fish species caught from fishermen data, indicating the rather low productivity of most other fish species in the reservoir. Among the six species with highest biomass, the only economically valuable species recorded were the silver barb (*Barbonymus gonionotus*) and tilapias. The species with little or no economic value that are abundant in the reservoir (glass fish *Parambassis siamensis*, river sprat *Corica soborna*, repassan *Cyclocheilichthys repasson* and wrestling halfbeak *Dermogenys pusillus)*, accounted for 64% of estimated total fish harvest (3823 tons) in the reservoir in 2008. The high production of low value species is also evidenced by their abundance at landing points, with glass fish and river sprat accounting for 355.91 and 243.68 of the total of 1661 tons landed in 2008. These indicated that the abundance of low economic value fishes may affect fisheries and fish biodiversity much more than the impact of alien tilapias species.

By using gill nets instead of seining, fish species composition was composed of more species with high economic value. Estimated tilapia catches and landing records show that tilapia species are abundant (84.62 of the total 1661 tons at landing points), second most only to silver barb (147.59 of 1661 total tons). This pattern holds despite the fact that tilapia haven't been stocked regularly as silver barb and other cultured fish species, indicating a favorable development of tilapia species in the reservoir. During the peak catches of tilapias in August in 2008, the other top five most commonly caught fishes are not at their peak catches, indicating a likely impact of tilapias on other economically important fish species such as silver barb, common carp (*Cyprinus carpio*), repassan and *Labiobarbus spilopleura*.

Key words: Alien tilapias, biodiversity, fisheries, Tri An Reservoir

INTRODUCTION

Tilapias (*Oreochromis* spp.) support an enormous market throughout Asia. Additionally, tilapias have been promoted as a food supply for poor farmers, as they provide food security. Tilapias were introduced into Vietnam several times from 1951 to 1997, and have been widely cultured in various systems, including ponds, cages, and rice-fields (Tu 2003). There were 700 tilapia hatcheries in Ho Chi Minh City in 2003, resulting in a seed production of 400 MT per year (Tu, 2003). According to MOFI (2006), the production of tilapia in Vietnam was 54,000 MT in 2005, with total culture area of 2,148 ha in 2004. Such rapid development of tilapia culture resulted in fish escapes to natural environments, a serious concern that deserves research a great caution. The rapid expansion of tilapia populations in Vietnam's natural waters indicates that the ecosystems are able to support the invasion. Restocking tilapia in reservoirs was generally aimed at increased fish catch production (FAO-SEAFDEC, 1985).

Escaped tilapias from aquaculture have established populations in reservoirs (Tu 2003 2006). For example, tilapias accounted for about 4% and 20% of the total catch in Tri An and Thac Mo Reservoirs, respectively (Tu 2003, 2006). Some regard tilapia as beneficial to local fisheries (and sometimes for control of mosquitoes or aquatic plants); some consider them pests with stunted populations that compete with indigenous fish species; and some consider their presence to be both, with benefits and negative effects, depending on geographical area (Lowe-McConnell 2000).

The purpose of this study is to investigate the impacts of tilapias on fisheries and biodiversity of indigenous fish species in the Tri An reservoirs of Vietnam. Information on the impacts of the introduced alien species on fisheries and biodiversity of indigenous fish species will allow governmental agencies to establish policies, plans and mechanisms for the management of the introduction of alien species.

MATERIALS AND METHODS

This study was conducted at Tri An Reservoir from November 2007 to June 2009. Tri An Reservoir was constructed in 1984 for the main purpose of providing hydroelectric power to southern Vietnam, which it has been doing since 1988. It was formed as a result of a dam constructed on the upper Dongnai River. The Tri An Reservoir is the largest reservoir in Vietnam, with a surface area of 324 km^2 and 15.05 km^3 of water storage capacity. It has an electric capacity of 420 MW, generating an average of 1,700 GW $hour^{-1}$ $year^{-1}$.

Tri An Reservoir is located at 10° through 12°20'N and 107° through 108°30'E. The watershed of Tri An Reservoir is around 15,400 km^2, with a mean reservoir length of 43.5 km, mean reservoir width of 7.5 km, maximum depth of 28 m, total volume of 2.76 km^3, and mean area of 323.4 km^2.

The primary data on fish catch and fish species composition was collected during a one-year study period through interviews and field sampling.

(1) Collecting data during the annual harvest by the fisheries management companies;

(2) Collecting data at fish landing points such as Ap 1, Phu Cuong and La Nga;

(3) Investigation of fishermen for their fish catch and species composition;

(4) Field sampling to investigate seasonal fish species composition.

The secondary data of historical fish catch and fish species composition in Tri An Reservoir was collected from relevant reservoir management agencies such as the Dong Nai Fisheries Company, Dong Nai Department of Agriculture and Rural Development, Dong Nai Bureau of Fisheries Resources Protection and Management, and Dong Nai Fisheries Extension Center.

Interviews with fisherman for catch and species composition

Interviews with fishermen consisted of two main themes: the type of fishing gear used and the fish catches for each fishing gear. There were 151 fishermen interviewed at upstream, midstream and downstream of the reservoir, accounting around 15% of total fishermen in the reservoir. At the upstream area, 49 of the interviewed fishermen belonged to La Nga and Thanh Son Communes. At the midstream area, 57 of the interviewed fishermen belonged to Phu Cuong and Gia Tan Communes. At the downstream area, 45 of the interviewed fishermen belonged to Ma Da and Vinh An Communes.

The total number of each type of fishing gear was recorded from Dong Nai Fisheries Company. Detailed information that was not available at Dong Nai Fisheries Company such as catch per unit effort (CPUE), fishing times and duration, and fish species composition was investigated directly from fishermen. CPUE was defined as the daily average catch (kg/day) for each type of fishing gear.

Field sampling to investigate seasonal fish species composition

Seasonal fish species composition in Tri An Reservoir was estimated by seining fish at 4, 5 and 4 locations at upstream, midstream and downstream of the reservoir four times per year. Field sampling was also carried out using gillnets with mesh size of 40-60 mm, the net length and width were 1,000m and 5m, respectively. The gillnet was fixed by floats for whole day per location. Seasonal sampling times were November 2007, February 2008, May 2008 and August 2008 representing the dry season (November and February) and rainy season (May and August).

Data analyses

The secondary and primary data from Tri An Reservoir were calculated in the percentage changes of fish catch and fish species composition over time using Microsoft Excel software. The linear relationship was also calculated for fish catch and effort.

RESULTS

The state of fish catches over time in Tri An Reservoir

Yearly fingerling stocking, fish catch and number of fishermen are presented in Table 1. The linear relationship between fish catch and effort found an R2 of 35, so only 35% of the variation is explained by fishing effort. The fishermen population reached highs in 1998 and 2000 and then has essentially leveled off. If CPUE was defined as dividing of catch by number of fisherman, the linear relationship between CPUE and year show an increase across all years in CPUE and explains R2= 55% of the variation.

Table 1. Stocking and catch over time in Tri An Reservoir

Year	Fingerling stocking (no. of fish)	Annual fish catch (MT/year)	No. of fishermen
1993	0	800	300
1994	0	833	400
1995	1,300,000	1126	550
1996	1,900,000	1475	748
1997	5,006,000	1825	800
1998	0	1840	1234
1999	1,317,000	2269	1136
2000	1,200,000	2301	1470
2001	1,501,000	2786	1237
2002	1,170,000	3118	892
2003	868,000	3080	978
2004	0	2835	884
2005	0	2589	872
2006	500	2600	721
2007	1,000,000	2837	747
2008*	0	3823	1115

Source: Dong Nai Fisheries Company (1993-2007), * 2008 data were collected by this study.

Fishing gears and species composition in Tri An Reservoir

There were 19 main types of fishing gears used in Tri An Reservoir, with mean daily catch (CPUE) for each fishing gear ranging from 3.4 to 71.4 kg/day (Table 2). CPUEs of each fishing gear changed by seasons. The most productive gears in terms of CPUE were seine nets, magine scoop nets, lift nets with a light and lift nets without a light. In terms of quantity of fishing gears operation, gill nets, magine scoop nets, lift nets with a light and long lines were the most popular (Table 3). Seasonal fish catch of each fishing gear was also presented at Table 3 in order to combine a yearly fish catch. The five fishing gears with highest yearly catches were magine scoop nets (1 lights), lift net with a light, gillnets (mesh size 40-60 mm), seine nets and magine scoop nets (18 lights). The fish catch of top five fishing gears make up 81.7% total catch of the reservoir, with total of 3,124 tons/year.

Table 2. CPUE of fishing gears during dry and rainy seasons

No.	Fishing gears	CPUE in dry season (kg/day)	CPUE in rainy season (kg/day)
1	Seine net (2 boats)	70.7 ± 4.14	45.3 ± 3.83
2	Machine scoop net (18 light)	70.42 ± 7.65	59.28 ± 6.46
3	Machine scoop net (1 lights)	56.96 ± 5.33	38.64 ± 4.68
4	Lift net (no lights)	53.52 ± 10.9	22.48 ± 3.73
5	Mobile cast net	49.84 ± 4.92	0
6	Seine net (1 boat)	48.8 ± 4.49	40.3 ± 3.0
7	Encircle surrounding net	42 ± 8.0	0
8	Viet trawl net	31.2 ± 6.03	12.64 ± 2.16
9	Mussel trawl net	15.24 ± 1.16	0
10	Gillnet (mesh size 40-60 mm)	14.84 ± 1.2	11.82 ± 0.94
11	Cast net	14 ± 0.98	0
12	Gillnet (mesh size 70-140 mm)	10.1 ± 1.45	8.51 ± 0.73
13	Surface gillnet station	9.68 ± 1.69	7.2 ± 0.44
14	Horizontal cylinder basket trap for marble goby	8.48 ± 1.18	7.04 ± 0.84
15	Horizontal cylinder basket trap for shrimp	6.28 ± 0.58	3.4 ± 0.46
16	Long line	5.76 ± 0.44	4.32 ± 0.5
17	Lift net with light	0	71.44 ± 7.56
18	Horizontal cylinder basket trap for tilapia	0	8.08 ± 1.36
19	Trammel net	0	6.8 ± 1.16

Table 3. Fishing gears and total catches in Tri An Reservoir

No.	Fishing gears	No. fishing gears		Fish catch (MT)		Total catch (MT)
		Dry season	Rainy season	Dry season	Rainy season	
1	Magine scoop net (1 light)	104	63	889	219	1108
2	Lift net with a light	0	80	0	686	686
3	Gillnet (mesh size 40-60 mm)	228	178	406	189	595
4	Seine net (2 boat)	42	30	356	61	417
5	Magine scoop net (18 light)	25	20	211	107	318
6	Lift net	18	22	101	52	153
7	Mussel trawl net	70	0	128	0	128
8	Seine net (1 boat)	10	20	73	29	102
9	Viet trawl net	22	31	62	35	97
10	Long line	52	108	9	56	65
11	Horizontal cylinder basket trap for marble goby	25	29	25	18	44
12	Horizontal cylinder basket trap for shrimp	39	48	22	20	42
13	Mobile cast net	11	0	33	0	33
14	Surface gillnet station	6	8	5	5	10
15	Gillnet (mesh size 70-140 mm)	2	11	1	6	7
16	Horizontal cylinder basker trap for tilapia	0	12	0	6	6
17	Encircle surrounding net	2	0	5	0	5
18	Trammel net	0	6	0	4	4
19	Cast net	2	0	3	0	3
	Total	**658**	**666**	**2329**	**1493**	**3823**

Proportions of tilapias catch in various fishing gears were presented in Table 4. Gill net with various mesh size (40-140 mm) was the main fishing gear for tilapia catching. Other fishing gears with high rate of tilapias catch were horizontal cylinder basket traps and cash net. There was 14 fishing gears (73.7%) having tilapias within their catches, in which 8 fishing gears (42.1%) having tilapias catch for whole year round.

Table 4. Proportion of tilapias in various fishing gears

No.	Fishing gears	% tilapias in dry season	% tilapias in rainy season
1	Gillnet (mesh size 70-140 mm)	21.34 ± 8.01	26.63 ± 12.16
2	Gillnet (mesh size 40-60 mm)	18.81 ± 5.24	7.82 ± 2.88
3	Horizontal cylinder basket trap for marble goby	7.67 ± 3.27	25.68 ± 5.13
4	Long line	1.99 ± 0.91	15.57 ± 6.45
5	Lift net	8.07 ± 2.19	8.9 ± 3,99
6	Seine net (1 boat)	5.23 ± 1.57	6.88 ± 1.32
7	Seine net (2 boats)	5.96 ± 1.59	3.12 ± 3,22
8	Surface gillnet station	1.62 ± 1.62	3.47 ± 2.95
9	Horizontal cylinder basker trap for tilapia	0	37.46 ± 8.17
10	Trammel net	0	19.0 ± 4.4
11	Cast net	26.07 ± 2.13	0
12	Encircle surrounding net	15.95 ± 2.27	0
13	Mobile cast net	4.01 ± 2.63	0
14	Magine scoop net (1 light)	2.62 ± 1.27	0
15	Viet trawl net	0	0
16	Mussel trawl net	0	0
17	Magine scoop net (18 lights)	0	0
18	Horizontal cylinder basker trap for shrimp	0	0
19	Lift net with light	0	0

Table 5 presents the most common 40 fish species caught by the 19 fishing gears. Most abundant species such as glass fish *Parambassis siamensis*, river sprat *Corica soborna*, repassan *Cyclocheilichthys repasson* and wrestling halfbeak *Dermogenys pusillus* accounted for 64% of estimated total fish harvest (3823 tons) in the reservoir in 2008.

Table 5. Fish species composition from 19 fishing gears in Tri An Reservoir

No.	Fish species	Fish catch (ton)	%
1	*Parambassis siamensis*	727.1	19.02
2	*Corica sorbona*	666.2	17.43
3	*Cyclocheilichthys repasson*	566.1	14.81
4	*Dermogenys pusillus*	448.4	11.73
5	*Barbonymus gonionotus*	278.8	7.29
6	***Oreochromis* spp.**	**176.5**	**4.62**
7	*Kryptopterus cryptopterus*	167.7	4.39
8	*Labiobarbus spilopleura*	155.2	4.06
9	*Mystus* spp.	147.3	3.85
10	*Glossogobius giuris*	119.0	3.11
11	*Cyprinus carpio*	92.1	2.41
12	*Oxyeleotris marmoratus*	69.4	1.81
13	*Hemibagrus wyckii*	47.1	1.23
14	*Hypostomus plecostomus*	32.9	0.86
15	*Cichla ocellaris*	20.1	0.52
16	*Wallago attu*	16.9	0.44
17	*Mystus nemurus*	14.8	0.39
18	*Cirrhinus jullieni*	13.1	0.34
19	*Henicorhynchus siamensis*	12.5	0.33
20	*Mystus wyckii*	10.7	0.28
21	*Micronema bleekeri*	6.2	0.16
22	*Osteochilus hasseltii*	5.0	0.13
23	*Clarias batrachus*	4.9	0.13
24	*Macrognathus taeniagaster*	4.9	0.13
25	*Hampala macrolepidota*	4.0	0.11
26	*Labeo chrysophekadion*	3.6	0.09
27	*Ompok bimaculatus*	3.0	0.08
28	*Channa striatus*	1.9	0.05

29	*Hypophthalmichthys molitrix*	1.7	0.05
30	*Notopterus notopterus*	1.7	0.05
31	*Mastacembelus armatus*	1.5	0.04
32	*Ctenopharyngodon idellus*	0.6	0.02
33	*Labeo rohita*	0.4	0.01
34	*Clarias macrocephalus*	0.4	0.01
35	*Anguilla marmorata*	0.2	0.01
36	*Macrognathus siamensis*	0.2	0.01
37	*Macrobrachium rosenbergii*	0.2	0.01
38	*Pangasius hypophthalmus*	0.2	0.01
39	*Hypophthalmichthys nobilis*	0.06	0.002
40	*Paralaubuca barroni*	0.01	0.0002

Fluctuations of fishing seasons and locations

Fish species composition by season in seine surveys was presented in Table 6. Tilapias (*Oreochromis* spp.) were also abundant, ranking second in catch after silver barb (*Barbonymus gonionotus*). Both tilapias and silver barb catches have high fluctuations by season, with peak catches in August and February, respectively. The other fish species at high catch rate were common carp (*Cyprinus carpio*) and repassan (*Cyclocheilichthys repasson*), accounting for 16.9 and 13.6%, respectively. Within the top five fish species with highest fish catches, the peak catch season of tilapias (in August) was different with others.

Table 6. Fish species composition by season in seine surveys

No.	Species	Proportion of catch by weight (%)				
		Nov 07	Feb 08	May 08	Aug 08	All year
1	*Barbonymus gonionotus*	25.5	36.5	33.2	27.2	30.6
2	*Oreochromis* spp.	12.3	12.5	17.6	36.6	19.7
3	*Cyprinus carpio*	16.9	18.9	19.5	12.4	16.9
4	*Cyclocheilichthys repasson*	22.9	16.7	4.8	9.9	13.6
5	*Labiobarbus spilopleura*	8.6	8.5	5.8	1.1	6.0
6	*Mystus* spp.	1.9	0.8	0.8	6.8	2.6
7	*Cichla ocellaris*	0	1.5	4.3	2.3	2.0
8	*Hypostomus plecostomus*	4.1	0.4	1.7	0	1.6
9	*Mystus nemurus*	0	1.8	3.3	0	1.3
10	*Anguilla marmorata*	3.4	0	1.6	0	1.3
11	*Hypophthalmichthys nobilis*	0	0.7	3.5	0	1.0
12	*Oxyeleotris marmoratus*	2.7	0.3	0	0.7	0.9
13	*Hampala macrolepidota*	1.4	1.4	0.2	0	0.8
14	*Kryptopterus cryptopterus*	0	0	0	2.0	0.5
15	*Ompok bimaculatus*	0.3	0	0.7	0.3	0.3
16	*Hemibagrus wyckii*	0	0	1.1	0	0.3
17	*Wallago attu*	0	0	1.1	0	0.3
18	*Labeo chrysophekadion*	0	0	0.5	0.4	0.2
19	*Notopterus notopterus*	0	0	0.4	0.1	0.1
20	*Channa striatus*	0	0	0	0.2	0.1

Fish species composition by locations in seine surveys was presented in Table 7. Most of fish species have fluctuations in fish catch between upstream, midstream and downstream. At the upstream area, tilapias were the most abundant catch species (26.3% in catch weight), but it was less abundant at the downstream sites. Silver barb (*Barbonymus gonionotus*) occupied abundant at the midle and downstream of the reservoir.

Table 7. Fish species composition by locations in seine surveys

No.	Species	Percent in weight (%)			
		Upstream	Midstream	Downstream	Reservoir
1	*Barbonymus gonionotus*	25.2	33.7	32.8	30.6
2	*Oreochromis* spp.	26.3	19.1	13.9	19.7
3	*Cyprinus carpio*	14.4	16.5	19.9	16.9
4	*Cyclocheilichthys repasson*	17.9	10.3	12.5	13.6
5	*Labiobarbus spilopleura*	5.8	6.0	6.1	6.0
6	*Mystus* spp.	0	4.4	3.3	2.6
7	*Cichla ocellaris*	1.7	1.7	2.7	2.0
8	*Hypostomus plecostomus*	1.7	0.5	2.5	1.6
9	*Mystus nemurus*	0	1.2	2.7	1.3
10	*Anguilla marmorata*	1.8	0	2.0	1.3
11	*Hypophthalmichthys nobilis*	1.4	1.2	0.5	1.0
12	*Oxyeleotris marmoratus*	1.2	1.5	0	0.9
13	*Hampala macrolepidota*	0	1.1	1.2	0.8
14	*Kryptopterus cryptopterus*	0	1.5	0	0.5
15	*Ompok bimaculatus*	0.8	0.3	0	0.3
16	*Hemibagrus wyckii*	0.4	0.4	0	0.3
17	*Wallago attu*	0.8	0	0	0.3
18	*Labeo chrysophekadion*	0	0.7	0	0.2
19	*Notopterus notopterus*	0.4	0	0	0.1
20	*Channa striatus*	0.2	0	0	0.1

DISCUSSIONS

Recently in Tri An Reservoir, fingerling stocking has not been continuous, especially for tilapias, which have not been stocked for the last 10 years. Thus, current tilapia populations in the reservoir exist due to natural reproduction, while other traditional herbivorous fish are stocked continuously such as silver carp, bighead carp, common carp, etc. The fluctuations in annual fish catch showed unstable management practices at the reservoir, based mostly on fluctuations of fishermen and fishing gears. Yearly fluctuations of fish catch and CPUE indicated that fisheries resources of Tri An Reservoir are affected by multiple factors such as environment, time and seasons, etc…

As CPUEs of each fishing gear changed by seasons, recording and understanding such changes were necessary for estimating the yearly average CPUE of each fishing gear. However, most of the previous historical fish catch data from Tri An Reservoir did not record this information, making it difficult to estimate yearly total catch exactly.

The data of seasonal fish catch and total catch for each fishing gear were not contributed directly to assess of tilapias impacts, but it provides a whole picture of fishing gear diversity and activities at the reservoir. Among the top fishing gears with highest catches, magine scoop nets, lift net and seine net were the one to catch all size of fish, indicating an uncontrol fishing situation at the reservoir.

Within the top five fishing gears with highest total catch, lift nets with a light and magine scoop nets (18 lights) were not used to catch tilapia, and magine scoop nets (1 light) tilapias didn't focus on tilapia. That was probably why tilapia only accounts for 4.62% of fish species composition caught in the reservoir. However, 14 of the 19 fishing gears operating in the reservoir caught tilapia. This finding suggests a wide distribution and production of tilapias across the reservoir. When tilapias were catched mainly by gill net with its mesh size lesser than 60 mm, it indicates an overfishing situation of tilapias in the reservoir.

According to Tung and Trong (2005), there were 109 fish species in Tri An Reservoir. Although this study's effort was not similar to Tung and Trong (2005), the low number of species caught in the present study suggest a decline in biodiversity since the Tung and Trong report. However, there was no data to prove that such a decrease was caused by the impacts of fishing activity or alien species. Sy (2008) implies some negative impacts of alien carnivorous species in the reservoir, such as *Cichla ocellaris*, but not tilapias.

Among the top six species with the highest catches, only two economically valuable species were recorded: silver barb and tilapias. The fish of low economic value abundant in the reservoir were as *Parambassis siamensis*, *Corica sorbona*, *Cyclocheilichthys repasson* and *Dermogenys pusillus*. These fish represented 64% of total catch by biomass in the reservoir. This indicates over-fishing for economically valuable species in the reservoir (Li and Xu, 1995), a situation that has strongly affected fisheries and fish biodiversity much more than the impact of alien species.

By using gill nets (mesh size 40-60 mm) to seine fish at 4, 5 and 4 locations upstream, midstream and downstream of the reservoir four times per year, the data showed that fish species composition was concentrated more economically species. Tilapias were also abundant, ranking second in catch after silver barb, indicating a favorable development of such species in the reservoir as well as their strongly reproduction during a year cycle. In August, when tilapia was in their highest total catch, the other top five fish catch species does not fall into that peak catch, indicating the interaction of tilapia and other main economically fish species such as silver barb (*Barbonymus gonionotus*), common carp (*Cyprinus carpio*), repassan (*Cyclocheilichthys repasson*) and *Labiobarbus spilopleura*.

Most of fish species have fluctuations in fish catch between upstream, midstream and downstream indicating the habitat and environmental factors play an important role for fish distribution in the reservoir. The upstream area has more favorable conditions for fish growth and reproduction because of more food source and nutrients from Dong Nai and La Nga Rivers, indicating that tilapias were among dominant economically valuable species successfully occupying this area. Luong et al. (2002) indicated rather low primary and secondary productions at Tri An Reservoir (phytoplankton and zooplankton of 0.36 - 0.82 g DW/m^2), resulting less abundant of tilapias at the downstream area. On the other hand, the limitted plankton food source had also been competed by many herbivorous fish. To manage the fish species composition at Tri An Reservoir, Luong et al. (2005) suggested to stock indigenous mable goby (*Oxyeleotris marmorata*) to feed on the prawns (*Macrobrachium* spp.) and other low economic small fish species (glass fish *Parambassis siamensis*, river sprat *Corica soborna*, repassan *Cyclocheilichthys repasson*, etc.). In terms of natural food web management, reducing of low

economic small fish species may allow quick development of economically herbivorous fish such as tilapias, silver carp, bighead carp, etc.

ANTICIPATED BENEFITS

Information on the impacts of the introduced alien species on fisheries and biodiversity of indigenous fish species will allow governmental agencies to establish policies, plans and mechanisms for the management of the introduction of alien species.

As reservoirs are widely distributed throughout Asian countries and tilapias have been introduced into many reservoirs either intentionally or unintentionally, these results from this study may allow to stocking continuously of tilapias without much concerning of its negative impacts.

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DURATION OF APPETITE INHIBITION PREDICTS SOCIAL DOMINANCE IN NILE TILAPIA, *Oreochromis niloticus* L.

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Abstract

This study investigated whether the result of contest for social dominance among individuals in *Oreochromis niloticus* can be predicted by assessing the duration of appetite inhibition (DAI) during the isolation period. Fifty all-male juvenile *O. niloticus* of similar size were isolated for 10 days and were used in a social pair study. The DAI of each fish was observed when fish was transferred to the isolation unit. Body weight of dominant and subordinate individuals was recorded before and after the encounter. Eye color pattern (ECP) was also observed during the social encounter. The study revealed that tilapia with shorter DAI during the isolation had a greater possibility to win the fight for social dominance. Formation of stable dominant-subordinate relationship was observed in 24 of the 25 tested pairs. A total of seventeen fishes (70.93%) out of the 24 fishes that became dominant have shorter DAI compared to that of their conspecifics (Binomial test, $P = 0.03$). This indicates that social dominance can be predicted using the DAI of the fish during isolation. Reduced growth rate of both dominant and subordinate fish and a well-described physiological end result of social stress were observed one day after the social interaction. The significantly greater weight loss ($P < 0.01$) in subordinate fish (2.88 ± 0.21 g) compared to dominant fish (2.11 ± 0.19 g) a day after the establishment of social hierarchy was mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. Death, which is the most overwhelming effect of stress, was observed in the subordinate individuals. All subordinate fish died within a week after the social interaction.

Key words: Appetite inhibition, Behavioral stress response, Growth, *Oreochromis niloticus*, Social dominance, Social interaction

INTRODUCTION

Cultured fish live in a diverse and complex environment. Social stress has a crucial role to play in the growth of the fish. A well-characterized physiological consequence of social stress is a reduced growth rate (Sloman *et al*., 2000). Stressful conditions can also affect fish health and welfare (Barton, 2000; Barton and Iwama, 1991). Environmental and husbandry stressors weaken both the innate and adaptive immune responses of the fish against pathogens (Klesius *et al*., 2001). Due to these, stress coping style or "*the coherent set of behavioral and physiological stress responses, which is consistent over time and a characteristic of a certain species*" (Koolhaas *et al*., 1999), is of fundamental importance to the quality of life of a cultured species. This study investigated whether the outcome of contests for dominance among individuals in an unselected population can be predicted from observations made before interactions on stress coping style or behavioral stress response such as the duration of appetite inhibition (DAI) after transfer for isolation to the new environment. This behavioral stress response has a potential to be used in the breeding programs of the experimental fish to select individuals that will produce offspring that can adjust their behavior to stressful conditions.

MATERIALS AND METHODS

Experimental fish

One hundred size #20 genetically male Nile tilapia *(Oreochromis niloticus),* with average weight of 0.60 g, were obtained from the Phil-Fishgen, Central Luzon State University, Science City of Muñoz, Philippines. They were maintained in a rectangular tank (2m x 1m x 1m) receiving continuous flow of water. The fish were fed three times a day at 3% of the body weight. Prior to isolation weight of each fish was determined.

Isolation and monitoring of the DAI

Fifty fish (mean weight of 26.02 ±0.98 g) were isolated at random in glass aquarium (30cm x 15cm x 30cm) for 10 days. Each isolation unit was aerated to ensure sufficient dissolved oxygen available for the fish. Three sides of the aquarium were covered to prevent the fish seeing other fish isolated in the nearby aquaria. Upon introduction of each fish in the isolation unit, it was immediately hand fed with three pieces of floating feeds placed in a feeding ring. The duration from the time of feed introduction to the time of feed consumption was regarded as the duration of appetite inhibition (DAI). The DAI and the weight of the fish served as the bases for pairing the fish for social interaction; fish with shorter DAI against those with longer DAI; with both fish having similar weight. Fish were then fed daily at 1% of the body weight except two days prior to interaction. Water exchange was done every other day to maintain good water quality.

Fish marking for identification

After establishing the competing fish for social interaction, each fish in a pair was individually marked by a small cut on the upper or lower part of the tail fin for the purpose of identifying the fish with shorter and longer DAI. The fish in a pair with longer DAI was cut on the lower portion of the caudal fin and vice versa.

Social interaction

After marking, the pair of fish was introduced into a new environment (30cm x 15cm x 30cm aquarium) to prevent the effect of place familiarity. The period from the time of introduction to the time of first agonistic attack was recorded. The number of attacks in ten minute-time from the first agonistic attack was separately recorded from the total number of attacks during the entire interaction. Change in eye color pattern (ECP) of the competing fish was monitored at the start, during and after the competitive social interaction. Eye color was quantified as darken area of both iris and sclera (Volpato *et al.*, 2003). The circular area of the eye was divided into eight equal parts using four imaginary diagonal lines (Fig. 1). Eye color pattern value ranged from zero to eight. At the end of the interaction, social rank (dominant or subordinate) was identified by the characteristics displayed by each fish such as proactive and reactive, pursuing and retreating, erected and not erected dorsal fin and as well as changes in skin color and ECP. Canon power shot A650IS image stabilizer AIAF digital camera with resolution of 12.1 megapixels was used to document the social interaction which in turn was used in checking the observations made during the interaction.

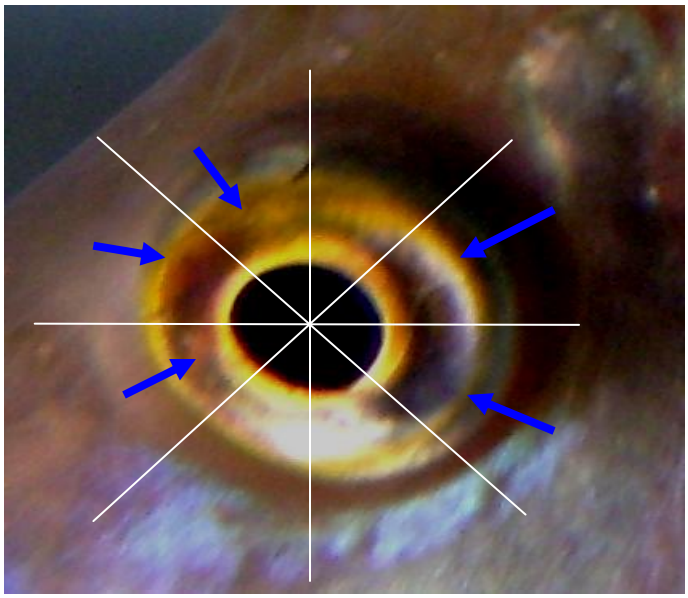

Figure 1. Eye color pattern of the fish. Picture with blue arrow shows darkening of 5 out of 8 divisions of the iris and sclera of the fish

Growth rate observation

Paired fish after the interaction were transferred to the dominant fish's aquarium to support its dominancy status. They were maintained for a week and fed once a day at 3% of their total body weight. Every aquarium was aerated and to maintain good water quality. Exchange of water was done every other day. The weight of fish was recorded a day after the fight.

Statistical analyses

Frequency difference was analyzed using Binomial test. Mean DAIs of the two groups, and mean decrease in weight a day after the social encounter between dominant and subordinate fish were compared using paired sample T-test. Linear relationship of DAI and aggression was assessed using linear regression and Pearson correlation coefficient. Statistical analyses were carried out by using the SPSS software version 16.0.

RESULTS

DAI after transfer to isolation units

The mean DAI for all isolated fish was 83.55 (±14.29) minutes. The shortest DAI was 0.31 minute and the longest was 570.76 minutes. After the matching pairs for later pairing had been established, short DAI group had a mean DAI of 33.55 (±10.15) minutes, which was significantly shorter ($P < 0.01$) than that of the long DAI group (133.54±22.86 minutes; Fig. 2).

Social interaction

During the introduction of competing individuals in the aquarium, both fish displayed pale body coloration with dark stripes. The mean duration before observance of first attack was 10.86 (±2.13) minutes. The fastest individual to adapt to the social condition and attacked the opponent took less than a minute (6.0 seconds), while the longest duration before observance of first attack was 33.66 minutes. However, at the beginning of the social encounter, it was not always the fish with shorter DAI that initiated the fight. Thirteen (52.00%) of the 25 fish with shorter DAI (compared to their respective opponents) initiated the fight while 11 social interactions were initiated by fish with longer DAI. One pair did not show any interaction.

During the social encounter, the dorsal fins of both fish were raised and both swam towards each other indicating their preparedness to fight. Then they begun aggressive interaction which involved chasing, rapid circling and biting directed against the mouth, fins and all other body parts of the opponent. During this period of intensive interaction, both fish exhibited pale body stripe coloration. However, during the later part of the interaction, challenged fish mostly rebuffed attacks and at this period, one of the fish chased and bit the flanks of the other fish that was fleeing. At this point, aggressive behaviour becomes unidirectional, and an aggressive dominant individual and a retreating subordinate fish were clearly identified. It was also observed that subordination increased the body- and eye-darkening color of the fish while dominance decreased it.

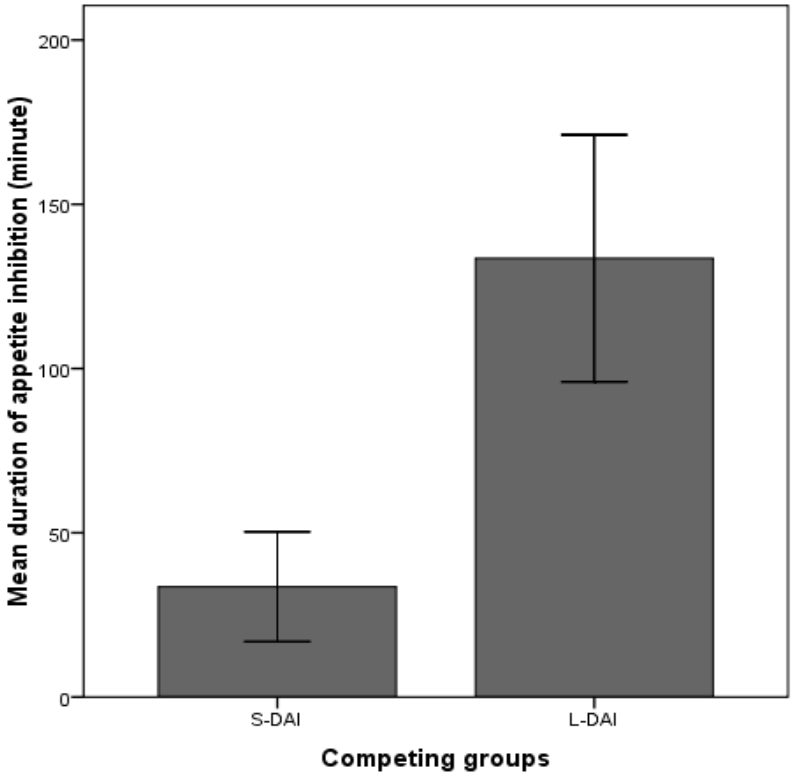

Figure 2. Mean (±S.E.) duration of appetite inhibition (minute) of the two competing groups. S-DAI: short DAI group; L-DAI: long DAI group. Mean DAI were significantly different at $P < 0.01$.

Formation of a stable dominant-subordinate relationship was observed in 24 out of the 25 tested pairs for social dominance. Seventeen dominant fish (70.83%) of the 24 had shorter

DAI during isolation compared to their opponents (Fig. 3). This frequency difference on DAI of the dominant individuals was significant (Binomial test, P = 0.03). However, as previously mentioned, social encounter was not always initiated by the earlier eaters (i.e. shorter DAI), but eleven (64.70%) of the 17 dominant earlier eaters initiated the fight and the remaining six individuals did not start up attacking the opponent yet won the fight. On the other hand, five later eaters that became dominant begun the fight, while the remain ning two did not.

Duration of appetite inhibition and level of aggression

The recorded mean number of attacks of the 24 pairs before winning the fight was 73.33 (±14.31). The most aggressive pair marked 201 attacks in 10 minute fight and had 277 attacks in the whole course of interaction. On the contrary, the least aggressive pair made no more than one attack before the establishment of dominance. The DAI difference between the competing pairs has an insignificant weak positive correlation (r = 0.28, P = 0.193) with the number of attacks.

Body weight after the fight

Reduced growth rate is a well-described physiological end result of social stress. The mean weight of subordinate fish before the interaction was 26.17 (±1.40) g and this was reduced to 23.29 (±1.36) g one day after the fight (Fig. 4). While in the dominant fish, average weight was decreased from 26.81 (±1.45) g to 24.70 (±1.36) g. The mean decrease in weight was 2.88 (±0.21) g for subordinate fish which was significantly higher (P < 0.01) compared to that of the dominant fish (2.11 ±0.19 g).

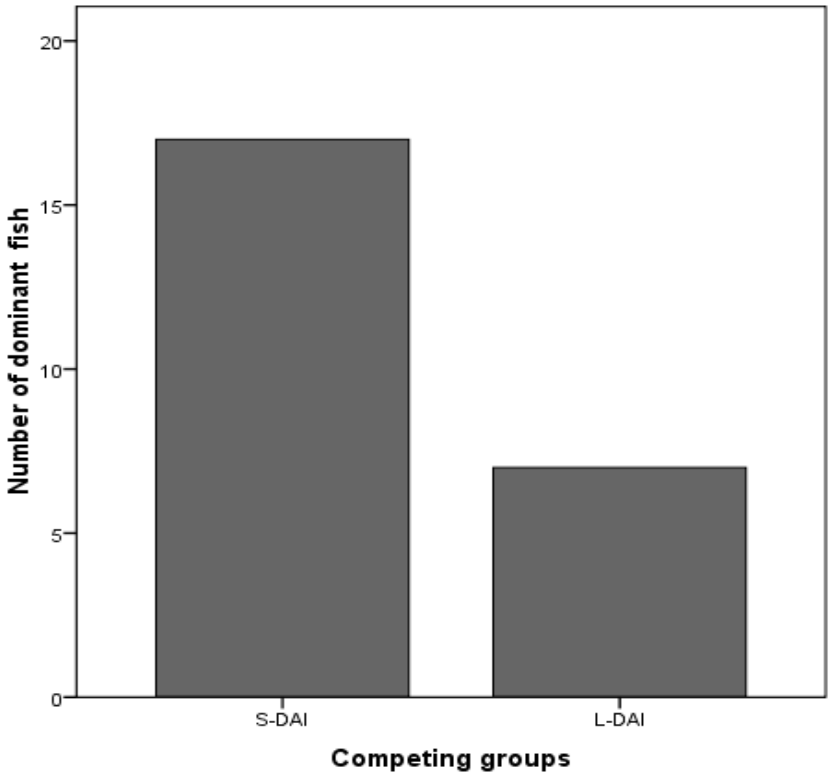

Figure 3. Number of dominant fish in the two competing groups. S-DAI: short DAI group; L-DAI: long DAI group. Frequency difference was significantly different at P < 0.05

Mortality of subordinates

Death can be the most overwhelming effect of stress. After the interaction of each pair, winner and loser individuals were easily identified by their displayed behaviors. One day after the fight, one subordinate fish immediately died followed by four on the second day, nine on the third day which was the day with the highest number of mortality. Another three died on the fourth day, five on the fifth day and one on the sixth day. The last surviving subordinate individual died on the seventh day after the interaction. It took one week from the day after the social interaction for all subordinate fish to die.

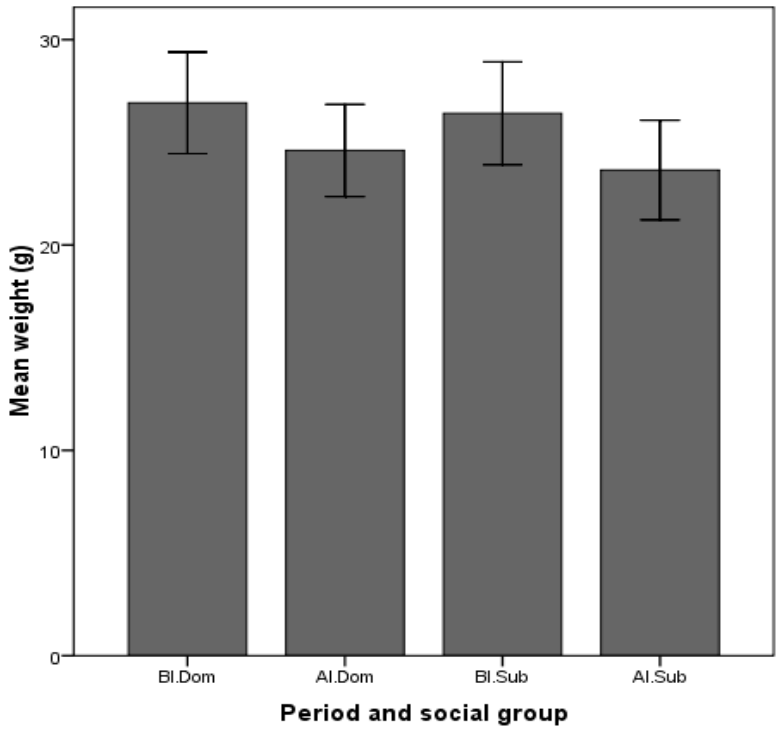

Figure 4. Mean weight (±S.E.) of dominant and subordinate fish before and after the social interaction. BI. Dom: dominant - before the interaction; AI. Dom: dominant – after the interaction; BI. Sub: Subordinate – before the interaction; AI. Sub: Subordinate - after the interaction

DISCUSSION

Behavioral stress response can be used to predict outcome of contest for social dominance. Results of the present study indicate that tilapia with shorter DAI after its transfer for isolation to a new environment before a fight is most likely or has a greater chance to become dominant. These results draw parallel with the findings of Korzan *et al*. (2006), Øverli *et al*. (2004) and Pottinger and Carrick (2001) that fish with low behavioral stress response became dominant in majority of the social pairing. The time variation of resumption of food intake (ranging from seconds to hours) of fish after being transferred to new environment most likely reflects some aspects of the physiological stress responses to confinement, which could also affect the outcome of the social interaction (Øverli *et al*., 2004). According to Bernier (2006), stress induced inhibition of food intake in fish is in part mediated by corticotrophin-releasing factor (CRF) system which plays a key role in controlling the neuroendocrine, autonomic, immune, and behavioural responses to stressors. On the other hand, the fish resumption of

feeding after they have coped-up with the stressful condition is likely to reflect a down regulation of the physiological stress response (Øverli *et al*., 1998).

The results that not all fish with shorter DAI won the fight calls for a need to refine the method of assessing the behavioral stress response in this species of fish. In a review, Øverli *et al*. (2007) described how feeding behavior can be used as indicator of stress coping style. Feeding behaviour can be assessed using point system based on the feeding behavior of the fish when fed daily for one week during isolation. This grading of fish behavior should also be tried in *O. niloticus.*

Social encounter is potentially costly and risky to the fighting opponents. The cost of fighting includes energy, time and physical injuries. The individuals engaged in social fight are integrating the costs and benefits associated with the contest and adjust their behavior accordingly (Hsu *et al*., 2006). At certain point when an individual in a pair reached its own dangerous threshold, an established dominant-subordinate relationship will be observed after one of the fish will be retreating or surrendering. In the current study, the observed changed in behavior and body and eye colors of the competing fish served as social signals to the opponents to limit aggressive interaction. When social hierarchy had been established, subordination increased the body stripes and eye-darkening patterns of the fish while dominance decreased it. These observations conform to the findings of Bero (2008), Vera Cruz and Brown (2007) and Volpato *et al*. (2003).

Social aggression is stressful for both dominant and subordinate fish (Summers and Winberg, 2006). In social interaction, defeat in many animal species is a powerful stressor that can lead to drastic alterations in physiology and behavior. Behavioral effects of defeat include appetite inhibition (Gómez-Laplaza and Morgan, 2003; Øverli *et al*., 1998; Winberg *et al*., 1993), reduce aggression (Höglund *et al*., 2001; Blanchard *et al.,* 1995), and increased submissive and defensive behaviors towards conspecifics (Blanchard et al., 1993; Siegfried *et al.,* 1984). The observed weight reduction in the current study after the interaction in both the dominant and subordinate fish supports the findings of Vera Cruz and Brown (2007). The reduced weight of subordinate fish a day after the social interaction may be more a result of appetite inhibition rather than a reflection of mobilization of stored energy for physical activity associated with social stress encountered. The subordinate fish were observed not consuming food after the social interaction and dominant fish even guarded or monopolized the food against the opponent. On the other hand, the increased physical activity of dominant fish during and after the aggressive encounters, a behaviour indicating that they have won the contest, may have contributed to the lower mean weight of the fish after the interaction. However, during the establishment of social hierarchy, the two social groups experienced similar level of physical activity. Thus, body weight differences between the two social groups during the establishment of social hierarchy were mainly attributed to physiological and behavioral differences such as appetite rather than to differences in physical activities (Fox *et al*., 1997; Øverli *et al.,* 1999). Inhibited food intake in subordinate fish may be due to social stress-induced increase in the serotonergic activity in the brain (Winberg *et al.,* 1992) and/or neuropeptide Y mRNA expression in the preoptic area (Doyon *et al.,* 2003).

The mortality of subordinates is most likely a result of exhaustion caused by social stress. This was also observed by Petrauskiené (1996) in rainbow trout reared at low densities (2 or 3 individuals). Most of the subordinate fish may have reached the exhausted state during the third day. Subordinate fish experiencing social stress when confined with a dominant fish increases the standard metabolic rate, therefore imposes metabolic disadvantage (Sloman *et al*., 2000). Lower social status, in addition, depresses hepatic insulin-like growth factor-I (IGF-I) levels while dominant status stimulates IGF-I production (Vera Cruz and Brown, 2007). Dead fish were

observed with lesions on the skin (with removed scales), on mouth part and destroyed dorsal and caudal fins.

CONCLUSION

Clear establishment of dominance hierarchy was observed in 24 of the 25 pairs. From the 24 dominants, 17 (70.83%) of them have shorter DAI during isolation compared to that of their conspecifics. This indicates that tilapia with shorter DAI during the isolation had a greater possibility to win the fight for social dominance and therefore, dominance can be predicted using the DAI of the fish during isolation.

Reduced growth rate of both dominant and subordinate fish, a well-described physiological end result of social stress, were observed one day after the social interaction. The greater weight losses in subordinate fish compared to dominant fish during and after the establishment of social hierarchy were mainly attributed to behavioral differences such as appetite rather than to differences in physical activities. Death, which is the most overwhelming effect of stress, was observed in the subordinate individuals. All subordinate fish died within a week after the social interaction.

ACKNOWLEDGMENTS

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FISHMEAL-FREE DIETS IMPROVE THE COST EFFECTIVENESS OF CULTURING NILE TILAPIA (*Oreochromis niloticus L.*) IN PONDS UNDER AN ALTERNATE DAY FEEDING STRATEGY

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ABSTRACT

Feed constitutes 60-70% of total production costs of tilapia (*Oreochromis spp.*). Reductions in quantity of feed used for fish growout and in the cost of formulated feeds are two approaches to containing feed costs. Our previous studies show that alternate day feeding at full ration produces Nile tilapia (*O. niloticus*) of comparable body size and harvest yield as those fed daily at full ration. The reduced feed consumption and 100% improved feed conversion with fish on the alternative day feeding strategy provided a significant cost savings to the semi-intensive growout of Nile tilapia in ponds in the Philippines. The cost of commercial fish feeds are rising sharply as the demand for fishmeal increases and its supply declines. We evaluated the growth performance of tilapia fed on alternate days with diets that incorporated plant ingredients widely available in the Philippines or other semi-tropical or tropical regions (cassava meal, copra meal, coconut oil, rice bran) and that contained porkmeal to replace fishmeal. Fish were grown out in ponds for 120 days with isocaloric-balanced, 0% and 6% fishmeal diets contained 31% crude protein and 6% crude fat. Fish showed similar performance on diets containing 0% and 6% fishmeal. Final body weight, total length, specific growth rate were virtually identical in fish on the two diets. Survival rates were 84% and 89% for fish on the 0% and 6% fishmeal diets, respectively. Feed consumption and feed conversion were also similar among the two groups. Total extrapolated yield at harvest was 3062 and 3080 kg fish/hectare for the 0% and 6% fishmeal groups, respectively. A marginal budget analysis showed an 8% improved return on fish fed the cheaper diet lacking fishmeal. This along with the alternative day feeding strategy previously shown to be as effective as daily feeding protocols has the potential of reducing overall feed costs for growing marketable size tilapia by > 60%. Collectively, the results show that substitution of diets containing fishmeal with cheaper and more sustainable sources of protein are effective options for reducing the costs without negatively impacting the production of tilapia.

INTRODUCTION

Feed is widely recognized as the most costly component of fish farming. A cost-farm budget analysis shows that feed constitutes 60-70% of total production costs of tilapia (*Oreochromis niloticus*) for small-scale, rural farmers in the Philippines (ADB 2005). Because of this, any reductions of feed costs can effectively increase income for Philippine farmers. Reductions in both the amount of feed used for growout of marketable fish and in the cost of formulated feeds are two approaches to containing feed costs. Our previous studies show that 1) delaying the onset of supplemental feeding to either 45-days or 75-days in fertilized ponds reduces the amount of feed consumed without any negative impact on the production of marketable tilapia, 2) feeding at a sub-satiation level of 67% did not reduce measurable production of marketable fish relative to fish fed at 100% satiation level, and 3) feeding only on alternate days saved approximately half of feed cost without a significant reduction in growth,

survival, or market yield of Nile tilapia in growout ponds (Brown et al. 2000, Bolivar et al. 2003, Bolivar et al. 2006).

The cost of commercial fish feeds are rising sharply as the market demand increases to supply growing aquaculture and the availability of fishmeal declines. About 40% of feed costs are attributable to fishmeal, which constitutes 15-20% of the feed formulation. Much of the fishmeal used for tilapia in the Philippines is imported, and costs are expected to rise in the future as global supplies become constrained by increasing demands from other aquaculture and declines in commercial bait fisheries. Because tilapia are omnivorous fish, which naturally feed on plankton, diatoms, small crustaceans, algae, higher plants and decomposing vegetable matter, they do not require fish in their diet and they are an ideal group of species to recycle food by-products into high quality food protein for humans (Brown 1983). Unlike carnivorous fishes, tilapia can digest high levels of carbohydrate in their diet (Anderson et al. 1984; National Research Council 1993), and they can effectively utilize human food by-products as feed ingredients, such as rice bran, cocoa, various flowers, soya, nut oil, milling waste, brewer's wastage, poultry by-product meal, pork meal, feather meal, cassava, and ipal-ipal leaf (Jackson et al. 1982). All of these lower-cost ingredients are readily available in the Philippines to completely replace or significantly reduce the inclusion of fishmeal in tilapia diets. Indeed, various animal protein meals (meat and bone meal, poultry by-product meal, feather meal, and blood meal) and plant proteins (soya, copra, cottonseed and others) have been shown to be either partially or completely replace fishmeal in tilapia diets (El-Sayed 1998; for reviews see Lim and Webster 2006 and El-Sayed 2006). Few studies have addressed the combinations of animal and plant protein types that might suffice in replacing or significantly reducing fishmeal in tilapia feed. Also, most investigations focus on the performance and nutritional characteristics of different protein sources rather than their ability to improve profit margins in tilapia production (see El-Sayed 2006). We examined the use of pork by-product meal as a replacement for fishmeal in diets formulated with plant and animal ingredients widely available in the Philippines on the grow out performance of Nile tilapia fed on alternate days in earthen ponds.

MATERIALS AND METHODS

In this study we evaluated the utility of using pork meal, a source of animal protein substantially lower in cost than fishmeal and widely available in the Phillipines, as a replacement of fishmeal in diets of tilapia grown in ponds. Alternate day feeding was previously shown to reduce production costs of tilapia without significantly altering final yield as almost 50% less feed could be used to grow fish than that incorporating standard daily feeding practices. Hence, this study evaluated if replacement of fishmeal with pork meal is as effective in producing tilapia under an alternate-day feed reduction strategy, as those diets containing standard levels of fishmeal.

This study was composed of two (2) treatment groups with four replicates per treatment. Groups were as follows: treatment I – formulated feeds with 6% fishmeal and treatment II – formulated feeds lacking fishmeal and containing pork meal. The constitution of the formulated tilapia grower floating feeds is shown in Table 1. The grow-out phase of this study were done in eight 500 m^2 earthen ponds at the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Table 1. Composition of caloric balanced grower test diets with 6% fishmeal and 0% fishmeal (fishmeal substituted with porkmeal). Inclusion rate of ingredients are kg ton^{-1} of feed.

RAW MATERIALS	Grower – 6% Fishmeal	Grower – 0% Fishmeal
Soybean Meal (HP) 45%	422.00	400.00
Corn Gluten	50.00	53.00
Hydrolyzed Animal Protein	30.00	30.00
Fishmeal Tuna 55%	60.00	0.00
Pork Meat Meal 55%	0.00	74.00
Copra Cake	73.00	76.00
Rice Bran	178.20	182.90
Cassava Meal	150.00	150.00
Fish Oil (Local)	5.50	5.00
Coconut Oil	5.00	5.00
Mono dicalcium phosphate	12.00	10.00
Salt	5.00	5.00
Mineral Premix	3.00	3.00
Vitamin Premix	6.30	6.10
TOTAL WEIGHT	**1000.00**	**1000.00**
DE Fish (kcal/kg)	2477.92	2484.50
Crude Protein (%)	30.99	31.07
Crude Fat (%)	6.21	6.23
Crude Fiber (%)	4.41	4.29
Starch (%)	17.36	17.40
Ash (%)	8.20	9.05
Ca (%)	0.96	0.91
Avail. Phosphorus (%)	0.67	0.66
Lysine (%)	1.51	1.50
Methionine (%)	0.54	0.50
Methionine + Cysteine (%)	0.97	0.96
Threonine (%)	1.06	1.07
Tryptophan (%)	0.33	0.42

Size 20 (weight range = 0.35- 0.37 g) sex reversed fingerlings of the GIFT strain were stocked in each pond at 4 fish m^{-2}. Fish were initially fed on alternate days with prestarter (36% crude protein) for 30 days and then starter feeds for 30 days. Following this 60-day period,

animals were fed formulated grower feeds with and without fishmeal on alternate days until the end of the experiment. The feed ration was based on the average fish biomass amd ranging from 10% down to 3% body weight per day.

Fish sampling was done every two weeks using cast net method to monitor fish growth and for feed adjustment. Estimated survival were as follow: first month – 100%, second month – 95%, third month – 90% and fourth month – 85%. Ponds were fertilized weekly with ammonium phosphate (16-20-0) and urea (46-0-0) at the rate of 28 kg N and 5.6 kg P ha^{-1} $week^{-1}$, respectively to enhance the growth of natural foods in pond water. Water quality parameters (dissolved oxygen concentration, water temperature and Secchi disc visibility) were monitored weekly between 9 to 10 o'clock in the morning. Water depth was maintained at 1 m in each pond. The total numbers of fish were counted and bulk weighed at the end of the 120 days culture period. Final mean weight, daily weight gain, gross yield and survival rates were calculated. A simple cost and return analysis were computed to compare the cost benefits between the two treatments. Data (mean ± standard deviation) were analyzed using paired t-Test.

RESULTS AND DISCUSSION

We conducted a study with an industry cooperator, Santeh Feed Corporation (Bulacan, Philippines) to evaluate porkmeal, which is widely available in the Philippines, as a substitute for fishmeal on growout of tilapia in earthen ponds in the Philippines. Cooperation with industry insured development of least cost formulated diets that incorporated locally-available ingredients including copra cake, cassava meal, local fish oils and coconut oil. We also utilized an alternate day feeding scheme that was previously shown to work as effectively as daily feeding in producing marketable fish (Bolivar et al. 2006).

Figure 1 shows growth in body weight and length of fish raised in quadruplicate in ponds for 120 days using grower diets with and without 6% fishmeal. Changes in body weight and length were virtually identical among the groups fed isocaloric diets.

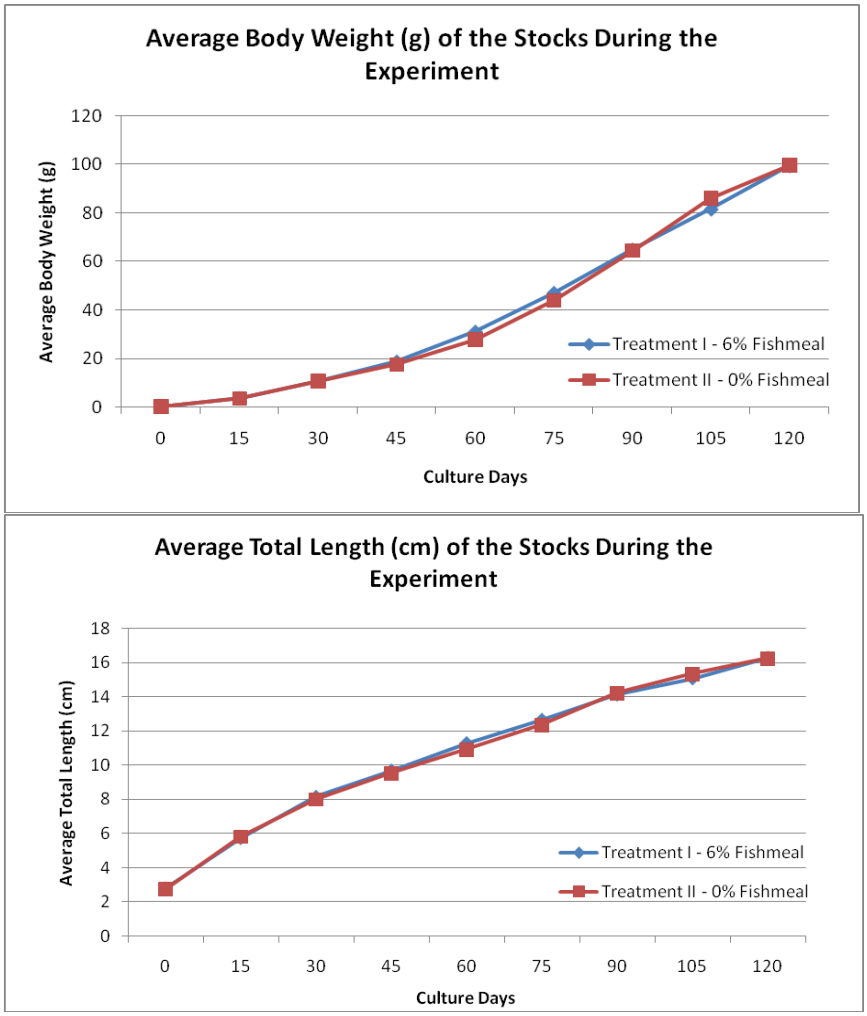

Figure 1. Mean body weight (top) and total length (bottom) of fish after a120 day culture period in earthen ponds. Fish were fed on alternate days with grower diets containing 6% fishmeal or 0% fishmeal (porkmeal substituted for fishmeal).

Table 2 summarizes the production parameters of Nile tilapia grown on the different diets including weight and length gain, feed conversion, extrapolated yield per hectare and survival rate. Overall, results show that the different production parameters did not differ among fish fed the two diets. Survival rate was high in fish fed the 0% (84.2%) and those provided the 6% fishmeal (89.3%) diet. Extrapolated yield and feed consumed per hectare was 3,080 kg/ha and 3,231.4 kg/ha, respectively, for fish on the 6% fishmeal formulated diet, and 3,062 kgs and 3,129.9 kgs per hectare, respectively, for fish on the 0% fishmeal diet. Feed conversion was slightly lower in fish fed 0% versus 6% fishmeal diets.

Table 2. Production parameters (mean ± standard deviation) of fish fed on alternate days with grower diets with 6% fishmeal or 0% fishmeal (porkmeal substituted for fishmeal). Fish were grown in ponds for 120 days.

Parameters	**Treatment I (6% Fish Meal)**	**Treatment II (0% Fish Meal)**
Initial Average Weight (g)	0.372 ± 0.049^a	0.356 ± 0.028^a
Final Average Weight (g)	99.531 ± 19.190^a	99.746 ± 14.331^a
Average Gain in Weight (g)	99.159 ± 19.175^a	99.390 ± 14.355^a
Average Daily Gain in Weight (g/day)	0.826 ± 0.160^a	0.828 ± 0.120^a
Specific Growth Rate (%)	4.652 ± 0.172^a	4.693 ± 0.176^a
Initial Average Total Length (cm)	2.8 ± 0.12^a	2.8 ± 0.06^a
Final Average Total length (cm)	16.261 ± 1.116^a	16.241 ± 0.823^a
Average Gain in Length (cm)	13.467 ± 1.107^a	13.491 ± 0.880^a
Average Daily Gain in Length (cm/day)	0.112 ± 0.009^a	0.112 ± 0.007^a
Survival Rate (%)	89.3 ± 5.3^a	84.2 ± 3.1^a
Extrapolated Feed Consumed per Hectare (kg/hectare)	3231.4 ± 711.5^a	3129.9 ± 425.7^a
Extrapolated Yield per Hectare (kg/hectare)	3080.0 ± 598.9^a	3062.0 ± 520.8^a
Feed Conversion Ratio	1.05 ± 0.05^a	1.03 ± 0.05^b

Treatment means within the same row with different superscript letters are significantly different ($P < 0.05$).

Water quality parameters including dissolved oxygen, water temperature and secchi disc visibility were similar among the two groups and fell within the range tolerable for tilapia growout. However, dissolved oxygen levels declined in ponds during the last month of growout for both groups of fish. This may have resulted in reduced feeding activity and overall growth of both groups of fish.

We conducted a simple cost and return analysis using current prices for all inputs and the value of marketable tilapia (Table 3). We found an approximate 8% higher net return for production of fish on the 0% fishmeal formulated diets (PhP 55,944.42) had than those grown on the 6% fishmeal diet (PhP 51,742.76).

Table 3. Simple cost and return analysis per hectare of production of fish grown on diets with 6% fishmeal and 0% fishmeal (porkmeal substitution of fishmeal) over a 120-day culture period. Values are shown in Philippine pesos (~44 PhP = $1 USD)

Descriptions	Treatment I – 6% Fishmeal	Treatment II – 0% Fishmeal
Gross Return	PhP 169,400.00	PhP 168,410.00
Costs (PhP, Philippines peso):		
Fingerlings	17,200.00	17,200.00
Commercial Feeds	99,043.44	93,497.46
Fertilizers		
16-20-0	526.40	658.00
46-0-0	887.40	1,110.12
Total Cost:	117,657.24	112,465.58
NET RETURN	PhP 51,742.76	PhP 55,944.42

Assumptions:

Price of Fingerling: P 0.43 piece^{-1}

Price of Commercial Feeds:

Pre-starter: P35.00 kg^{-1}

Starter: P28.25 kg^{-1}

Formulated Feeds with 6% Fishmeal: P31.00 kg^{-1}

Formulated Feeds with 0% Fishmeal: P30.00 kg^{-1}

Price of marketable Tilapia: P55.00 kg^{-1}

Price of Fertilizers:

16-20-0: P18.80 kg^{-1}

46-0-0: P17.40 kg^{-1}

CONCLUSION

It is estimated that 60-80% of total variable costs for growing tilapia is attributable to feeds.

We previously show that alternate day feeding resulted in significant cost-savings relative to daily feeding at full ration (Bolivar et al. 2006). Using this more cost-effective alternate day feeding strategy, we assessed whether elimination of fishmeal from diets and its replacement

with a cheaper animal protein (porkmeal) might provide additional cost savings to tilapia production. The diets were produced by a local feeds company and incorporated locally available Philippine ingredients where possible. We show that fish fed formulated feeds lacking fishmeal had similar daily weight gain, specific growth rate, and survivorship as fish fed fishmeal diets. Feed consumption, gross harvest yield and feed conversion were also similar among fish on the experimental diets. A cost-return analysis shows that incorporation of a diet lacking fishmeal produced an 8% or almost $100 in cost savings in feed for each hectare of tilapia farmed. This along with the alternative day feeding strategy has the potential of reduce overall feed costs for growing marketable size tilapia by 60% relative to the typical practice of applying fishmeal formulated diets on a daily basis. A future study directly comparing daily and alternative day feeding strategies with diets formulated with and without fishmeal througout the entire production cycle of tilapia is warranted to establish the actual cost savings farmers are likely to have. Collectively the results show that substitution of diets containing fishmeal with a cheaper and more sustainable source of protein, *i.e.* pork meal, is an effective option for reducing the costs without negatively impacting the production of tilapia.

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HEAT-INDUCED GERM CELL LOSS IN SUB-ADULT NILE TILAPIA *Oreochromis niloticus*

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Abstract

Reproductive failure associated with heat-stress is a well known phenomenon in higher vertebrates. To examine the effect of high temperature on ovarian development and function of fish, an experiment was conducted using immature Nile tilapia, *Oreochromis niloticus* as a model fish species. All-female Nile tilapia of 100 day after hatching (dah) were reared at 27 ^{o}C and 37 ^{o}C water temperatures for 60 days. After temperature treatment, fish were cultured in normal temperature (25-30 ^{o}C) for 3 months. The gonadal structure of fish were examined initially, and at 30, 45 and 60 days after the onset of the experiment by histological and immunohistochemical methods. Results showed the complete loss of germ cells in the gonads of fish exposed at 37 ^{o}C for 60 days, and these individuals did not recover from this condition after transfer to normal temperature for 3 months. Gonads of fish treated at both 27 ^{o}C and 37 ^{o}C temperatures for 60 days of treatment period showed strong immunopositive reactions against the major steroidogenic enzymes; P450scc, 3β-HSD, and P450arom. Plasma E_2, 11-KT and T levels were significantly lower in the 37 ^{o}C fish. The survival was significantly reduced at 37 ^{o}C (80%) than those of 27 ^{o}C (95%) during treatment period. These results suggest that exposure of Nile tilapia at 37 ^{o}C temperatures for 60 days induces complete and permanent gonadal sterility. This technique might be an easy and eco-friendly method for sex control in aquaculture.

Key words: Nile tilapia; high temperature; germ cells; gonadal sterility.

EFFECTS OF STOCKING DENSITY ON THE GROWTH, SURVIVAL AND YIELD PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*, Linn. 1858) IN AN INTEGRATED CAGE-CUM-POND CULTURE SYSTEM

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Abstract

Rural pond culture in Kenya is moving from subsistence to small-scale commercial culture of fish. Small-scale commercial farmers are utilizing improved management practices such as stocking densities, feeding regimens, and feed nutrient to enhance their economic returns (Quagrainie et al., 2009). There are several aquaculture systems in use among them being pond culture, cage culture but most recent is the integrated pond cum cage culture. This culture has been developed and practiced using combination of catfish-tilapia and tilapia-tilapia (Yang Yi *et al*., 1996). The integrated system allows the open pond water to utilize cage wastes as fertilizers, generating natural food in the pond. The integrated system is environmentally friendly because less waste nutrients are released to the public water systems.

We investigated the appropriate stocking density for rearing *O. niloticus* in cage-cum-pond fish culture that enhances optimal growth and increases fish yield in a 1300m^2 earthen pond using 9 cages each with a volume of 1 m^3. The cages had a frame made from PVC pipes covered with a half inch netting material. Hand sexed male *O. niloticus* fingerlings averaging 60g from the Mwea Aquafish Farm hatchery were stocked in the cages and the open pond water respectively. Prior to stocking, the pond was fertilized with 20kgN ha^{-1} wk^{-1} and 5kg P ha^{-1} wk^{-1} using Urea and Di-ammonium phosphate; a standard procedure. After 30 days, the rate of Urea application was lowered to 10kgN ha^{-1} wk^{-1} as a measure to correct ammonia builds up in the pond. Cages were stocked at varying densities of 50, 75 and 100 fish per m^3. Fish were fed with commercial floating feeds containing 17.60% crude protein reared for 180 days.

At the end of the trials, fish were harvested and total yield determined. A partial enterprise budget was evaluated for economic gains. Preliminary results showed that low density stocking favoured growth and that in all cages fish weight doubled within 30 days. This information will be useful to small scale fish farmers who stand to benefit from two crops in one pond.

FOOD SAFETY STUDY OF LEAFY GREENS IRRIGATED WITH TILAPIA FARM EFFLUENTS IN TAMAULIPAS.

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Epidemics of *Escherichia coli* and other related gastro-intestinal pathogens have been a common problem worldwide. Several outbreaks were traced to consumption of fresh vegetables (spinach, lettuce, green onions). For most of the cases, the vector was thought to be contamination from human or animal wastes applied through irrigation water. Very few studies have been conducted to determine any health hazards that may result from aquaponic systems. The use of UV (ultraviolet) systems is a reliable alternative to disinfect water. The determination of the efficacy of the UV during the tilapia and vegetable production in integrated systems (Aquaponics indoors and ground ponds outdoors) were evaluated.

INDOORS					
WATER SAMPLES					
TOTAL COLIFORMS			FECAL COLIFORMS		
Tank	Mean	SD	Tank	Mean	SD
1	0.207	0.3	1	0.112	0.1
2	0.609	0.4	2	0.599	0.1
3	0.157	0.1	3	0.118	0.1
4	0.748	0.3	4	0.452	0.1
5	0.192	0.2	5	0.137	0.1
6	0.854	0.4	6	0.618	0.2

OUTDOORS			
WATER SAMPLES			
TOTAL COLIFORMS			
Mean	SD	Mean	SD
4.5	4	5.5	5

The present research studied water and plant samples from both systems over 2 years period to determine the presence of total and fecal coliforms, salmonella and enterococci, and if the UV treatment makes a significant difference. A number of organisms were counted and reported. Fish and plants were grown during summer, fall and winter at different intervals. Water and plant samples were collected from indoors and outdoors systems and analyzed using standard methods NOM (Mexican official standard methods).

INDOORS									
Lettuce					Spinach				
			Mean	SD				Mean	SD
UV	Roots	TOTAL COL	0.15	0.1	UV	Roots	TOTAL COL	0.1	0
		FECAL COL	0.1	0.1			FECAL COL	0.09	0
	Leafs	TOTAL COL	0.14	0.1		Leafs	TOTAL COL	0.03	0.1
		FECAL COL	0.08	0			FECAL COL	0.12	0.1
NT	Roots	TOTAL COL	0.09	0	NT	Roots	TOTAL COL	0.12	0.1
		FECAL COL	0.11	0.1			FECAL COL	0.01	0
	Leafs	TOTAL COL	0.06	0		Leafts	TOTAL COL	0.11	0
		FECAL COL	0.06	0			FECAL COL	0.16	0.1

Water samples from indoor and outdoor systems were found contaminated with total and fecal coliforms in measurable numbers. However, tests for *Salmonella, E. coli* and Enterococci were negative. In conclusion UV treatment did significantly reduce levels compared with non-treated for fecal and total coliforms.

The absence of *Salmonella, E. coli* and Enterococci in both systems suggested that further studies using gastro intestinal pathogens should be conducted in order to determine if the low cost UV system is effective in these production conditions. And if management strategies to improve food safety for consumers of crops grown in integrated production systems are suitable to be implemented.

OUTDOORS											
Lettuce						Spinach					
				Mean	SD					Mean	SD
1	UV	Roots	TOTAL COL	0.15	0.1	1	UV	Roots	TOTAL COL	0.07	0
			FECAL COL	0.11					FECAL COL	0.08	0
		Leafs	TOTAL COL	0.15				Leafs	TOTAL COL	0.1	0.1
			FECAL COL	0.1					FECAL COL	0.09	0.1
2	NT	Roots	TOTAL COL	3.31	3.4	2	NT	Roots	TOTAL COL	4.57	4
			FECAL COL	3.58					FECAL COL	3.86	3.8
		Leafs	TOTAL COL	4.37				Leafs	TOTAL COL	3.79	3.7
			FECAL COL	3.65					FECAL COL	3.44	2.9

MASCULINIZATION OF NILE TILAPIA (*Oreochromis niloticus* L.) USING LYOPHILIZED TESTES FROM CARABAO (*Bubalus bubalis carabanesis* L.), BULL (*Bos indicus* L.) AND BOAR (*Sus domesticus* L.)

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Abstract

The study was conducted to evaluate the use of lyophilized testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) in the masculinization of Nile tilapia (*O. niloticus*) fry, specifically, their efficacy in producing phenotypic males and their influence on the growth and survival rate of Nile tilapia fry on a 28-day treatment period in outdoor tanks.

The experimental treatments evaluated were: Treatment I- lyophilized testes from carabao, Treatment II- lyophilized testes from bull, Treatment III- lyophilized testes from boar, Control I- methyltestosterone (MT)- treated diet and Control II- untreated diet. Percent phenotypic males, specific growth rate and survival rate were determined after 28 days of treatment in outdoor tanks.

Results revealed that Nile tilapia fry fed with MT-treated diet gave the highest percent phenotypic males with a mean of 96.67%. Those fry fed with lyophilized testes from bull, boar and carabao gave means 80.67, 79.33 and 72.67%, respectively. There was a significant difference ($P<0.05$) among the treatments. Based on the Chi-square test ($\alpha \leq 0.05$), the higher percentages of males produced from androgen-treated fry which are significantly different from that of untreated fry showed that lyophilized testes diets and MT-treated diet were effective in masculinizing Nile tilapia fry.

Lyophilized testes from bull, carabao and boar gave higher specific growth rate of tilapia fry with means 15.85, 15.29 and 14.82%, respectively. Tilapia fry fed with lyophilized testes from carabao and boar did not differ significantly ($P>0.05$) from MT-treated fry but differed significantly ($P<0.05$) from those untreated fry. Those fry fed with lyophilized testes fro were found to be significantly different ($P<0.05$) from the two controls. All the experi treatments gave relatively high survival rate of the tilapia fry with no significant diffe ($P>0.05$).

INTRODUCTION

Tilapia (*Oreochromis niloticus* L.) has been regarded as one of the major produced and consumed aquaculture commodities in Asia. The tilapia world production has grown rapidly at 2,515,908 metric tons in 2007 (Fitzsimmons, 2008). One of the developed management aspects considered to contribute to this growth is the production technology of monosex fingerlings through sex reversal. The production of all-male tilapia through hormone manipulation became a common methodology in the aquaculture of tilapia. Male tilapia is preferred over the female one because of its fast growth. Oral administration of sex hormones is employed to control the sexual development of this species and produce monosex fish. Various natural and synthetic hormones have been used to sex-reverse tilapia fry. At present, successful production of masculinized tilapia is done through oral administration of synthetic androgen hormone-treated

feed at 30-60mg/kg of diet for about three to four-week period (Shelton et al., 1978; Guerrero and Guerrero, 1988; Jo et al., 1988; Vera Cruz and Mair, 1994). The dosage of hormones incorporated in diets for sex reversal of tilapia varies widely from 10-70 mg of hormone/kg of diet (Abucay and Mair, 1997; Mateen and Ahmed, 2007). The use of 17α-methyltestosterone is by far the most common practice for many aquaculturists since it has been proven both effective and relatively inexpensive means of masculinizing fry of at least 95% for various tilapia species (reviewed by Macintosh and Little, 1995; Green et al., 1997; Phelps and Popma, 2000; and El-Sayed, 2006). However, some concerns have been raised on the consumption of steroid-treated tilapia in the advent of this culture practice. The use of synthetic hormones has been under increasing public criticism due to their possible health and environmental impacts. As a result, the use of methyltestosterone for sex reversal of food fish is either licensed by the U.S. Food and Drug Administration or prohibited in Europe (Penman and McAndrew, 2000). Potential disadvantage of synthetic hormone treatment is the increased risks of long term exposure of workers handling MT during food preparation and feeding which may cause adverse effects on their health (Green et al., 1997). There have been reports that hormones in the form of either active metabolites excreted by the treated fish or leachates from uneaten food can build up in a closed water system (Abucay and Mair, 1997). Hence, the waste water from the culture system with MT treatment for sex reversal can have unknown effects on the untargeted elements of the pond ecosystem.

The rapidly increasing demand for organic food in the world market has become a consideration in the aquaculture of tilapia. The demand for organic fish is rapidly increasing, while the supply is very inadequate (Aquaculture Production Technology Ltd., 2006). Most consumers want tilapia to be organically produced and with reduced or eliminated use of synthetic hormones. The idea is no antibiotics and chemicals, reduced environmental repercussions and recycled water and waste products. In Israel, organic aquaculture started at kibbutz Geva fish farm in 2000 with blue tilapia (*O. aureus*) as main species of the polyculture (Milstein and Lev, 2004). Similarly, Premier Organic Farms Group, Inc. in the US is now able to produce a superior farm-raised organic tilapia to supply the ever expanding organic market.

Among the alternatives which can be considered to mitigate the problem on using synthetic steroid for sex reversal of tilapia is the use of testes from animals which can be a potential substitute to synthetic MT. The testes from farmed animals like carabao, bull and boar which are readily available from any local market and abattoir in the country can be a good source of natural testosterone. Haylor and Pascual (1991) reported successful tilapia sex reversal using ram's testes. Phelps et al. (1996) also obtained a 65% male population using bull testes. Meyer et al. (2008) reported successful use of bull and hog testes in sex reversal of Nile tilapia fry. White (2008) also obtained high percent male of tilapia fry after sex reversal treatment using frozen bull testes. The animal testes coming from carabao, bull and boar can be potential sources of dietary testosterone. There are only few studies conducted evaluating the use of animal testes in masculinizing tilapia fry. Hence, these natural sources of testosterone can therefore be investigated for sex reversal of Nile tilapia fry.

This study was conducted at the Freshwater Aquaculture Center, Central Luzon State University, Science City Muñoz, Nueva Ecija, Philippines. The Nile tilapia fry in this study were treated with lyophilized testes for 28 days in outdoor tanks.

The general objective of this study was to evaluate the use of lyophilized testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) in the masculinization of Nile tilapia (*O. niloticus*) fry. Specifically, the study determined the efficacy of lyophilized testes from carabao, bull and boar in producing phenotypic males of *O. niloticus* fry and their influence on the growth and survival rate of *O. niloticus* fry. A simple cost and return analysis was also considered in this study.

MATERIALS AND METHODS

Fifteen net enclosures (1 x 1 x 1 m) with 1.6 mm mesh size were set in 15 outdoor tanks (3 m^3) following the Complete Randomized Design (CRD) for three treatments and two controls. The experimental treatments evaluated were: Treatment I- lyophilized testes from carabao, Treatment II- lyophilized testes from bull, Treatment III- lyophilized testes from boar, Control I- methyltestosterone (MT)- treated diet and Control II- untreated diet. These were replicated three times.

Each net enclosure was stocked with 500 fry. The net enclosures were extended at least 20 cm above the water surface to prevent the fry from escaping and were moored into the tank's bottom. The experimental units were provided with continuous flow of water, regular cleaning and water exchange. The net enclosures were washed and cleaned once a week during sampling.

A total of 7,500 tilapia fry (0.008 to 0.009 g) from the artificial incubation units of the GIFT Foundation were used in this study. These fry were of the same cohort and were taken from Generation 11 of the selected GIFT strain.

The testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) were collected at Hiyas Agro-Commodity Center in Guiguinto, Bulacan and at the Balagtas Municipal Abattoir in Balagtas, Bulacan. The age, carcass body weight and size of the testes from each animal were recorded.

The fresh testes were skinned and freed from epididymides, weighed, sliced and completely homogenized without dilution using a countertop blender. The homogenized testes were then lyophilized at the Chemistry Laboratory of De La Salle University, Philippines after freezing for a minimum of 24 hours. The testes were completely lyophilized within 72 hours using a cascade-type freeze dryer equipment. The freeze dryer can accommodate up to 6 kg of raw testes per run. Lyophilization of frozen and homogenized testes was done by placing them in a vacuum with -40^{o}C temperature to remove moisture from below zero frozen state before returning it to ambient room temperature of approximately 20^{o}C. The low processing temperature and absence of liquid water help to maintain the color, flavor and texture of the testes samples. After lyophilization, 20-25% of the weight of the raw animal testes was recovered. The resultant crumbs were pulverized and sieved before feeding to the tilapia fry for 28 days. The lyophilized testes diets were sealed in polyethylene packets and stored at room temperature.

The sex reversal of Nile tilapia fry was done through oral administration of the experimental diets for 28 days. The lyophilized testes diets and the controls were given at a rate of 20% of the fish body weight per day during the first week with gradual reduction down to 10% of fish weight until the end of treatment. The feeding frequency was five times daily during daylight, 7 days per week. Growth and survival rate were recorded every week. After the 28-day treatment period, the fish were further reared and were fed with fry mash until they reach the age of 2-month old.

Sex determination through histological examination was done following the gonadal squash method of Guerrero and Shelton (1974). After the fish reaches age of 2-month old, 50 fish which is 10% of fish population from each net enclosure were sacrificed and gonad was excised. In determining the phenotypic sex through the squash method, some criteria were used to identify male and female gonadal tissue: presence of cyst-like structures containing spermatogonia and spermatocytes and appearance of oocytes at different stages of development (Figures 1a and b).

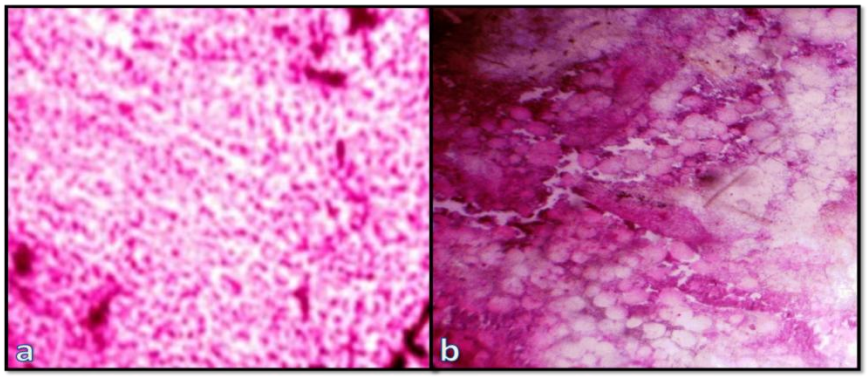

Figure 1. Tilapia gonad: (a) male; (b) female (Odin, 2009)

Water quality parameters such as temperature, dissolved oxygen and pH were monitored daily. Temperature and dissolved oxygen were measured using a YSI Model 55 DO meter while pH was measured using Fisher Model AB-15. Continuous water flow was provided to maintain desirable range of water quality parameters. Fifty percent of the total water volume of the tank was changed every other day to ensure optimum water exchange and good water quality throughout the treatment period.

The testosterone was analyzed using the Immulite 2000 analyser by a solid-phase, chemilumiscent enzyme immunometric assay. The serum collection was done after blood samples were collected from carabao, bull and boar and centrifuged at 5000 rpm for three minutes at 4^{o}C. Serum total testosterone was analyzed since it was observed to have a positive and significant correlation with the volume of Leydig cells in the testes (Costa and Paula, 2006). This means that the value of serum total testosterone is related to the capacity of the Leydig cells to secrete testosterone in the animal testes (Ewing et al., 1979).

The proximate composition of every lyophilized testes diets were also chemically analyzed to determine the crude protein, lipid, ash, fiber and moisture content of the testes diets following the standard methods of AOAC (1980). The proximate analyses of the experimental diets were done at the Nutrition Laboratory of the Philippine Carabao Center, Science City of Muñoz, Nueva Ecija.

The analyses of data were done with the statistical package of Sirichai Statistics Version 6.00. Data gathered were subjected to Analysis of Variance (ANOVA) to determine significant differences among treatments. Comparison of means was done at 5% level by Duncan's Multiple Range Test (DMRT). Sex ratio data were analyzed using the Chi-square test ($\alpha \leq 0.05$) to determine the efficacy of the treatments. Sample distributions violating assumptions were log-transformed before analysis. The data, expressed as percentages, were arc sine-transformed before analysis. Differences were regarded as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Phenotypic Males

The data on the percent phenotypic males of Nile tilapia fry after the 28-day treatment period are shown in Table 1. The results show that there was a significant difference ($P<0.05$) among the treatments at 5% probability level of DMRT.

Table 1. Summary of the results from the 28-day sex reversal treatment of Nile tilapia (*Oreochromis niloticus*) fry using lyophilized testes diets and controls

TREATMENTS	PHENOTYPIC MALES (%)	SPECIFIC GROWTH RATE (%)	SURVIVAL RATE (%)
Treatment I	72.67 ± 3.91^{b}	15.59 ± 1.26^{ab}	92.27 ± 0.02
Treatment II	80.67 ± 2.24^{b}	15.85 ± 1.24^{a}	89.67 ± 0.00
Treatment III	79.33 ± 1.66^{b}	14.82 ± 0.22^{ab}	88.07 ± 0.05
Control I	96.67 ± 1.97^{a}	14.12 ± 0.31^{bc}	92.13 ± 0.07
Control II	46.00 ± 4.17^{c}	13.20 ± 0.40^{c}	86.93 ± 0.08

*In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Tilapia fry fed with MT-treated diet (Control I) obtained the highest percent male with a mean of 96.67 ± 1.97%. Those fry fed with lyophilized carabao testes (Treatment I), lyophilized bull testes (Treatment II) and lyophilized boar testes (Treatment III) attained means 72.67 ± 3.91, 80.67 ± 2.24, and 79.33 ± 1.66% males, respectively. The treatments were not significantly different ($P>0.05$) but were significantly lower than MT-treated group and significantly higher than untreated group ($P<0.05$). Following the Chi-square test ($\alpha \leq 0.05$), it was found out that the lyophilized testes diets and the MT-treated diet have a significant effect on the masculinization of Nile tilapia fry (Figure 2). The treatment groups and the MT-treated group were significantly skewed towards males and deviated from the theoretical 50:50 sex ratio.

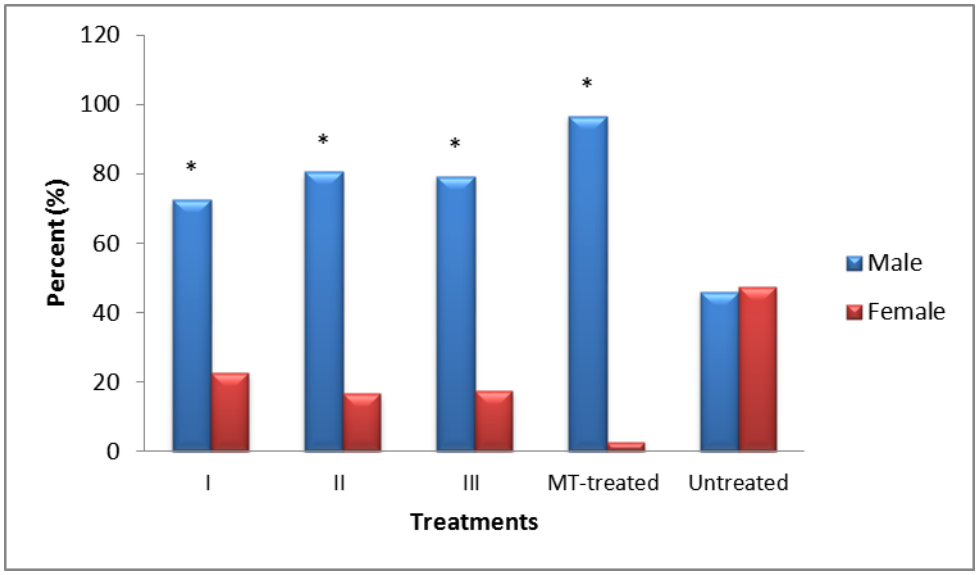

Figure 2. Percentage of Nile tilapia (*O. niloticus*) fry classified as male and female under the lyophilized testes treatments and the controls after the 28-day treatment period. Note: Asterisks indicate significant differences in proportion of males from the untreated control (from Chi-square test; $\alpha \leq 0.05$)

One of the factors that may be considered to contribute to the percent males produced from the 28-day sex reversal treatment using lyophilized testes is the presence of testosterone in the animal testes. The total testosterone from serum of each animal was analyzed using chemilumiscent enzyme immunometric assay to determine the levels of testosterone (Table 2).

Table 2. Total testosterone from serum of carabao, bull and boar

TREATMENTS	NO. OF SAMPLES	CONCENTRATION (ppb)
Treatment I (carabao)	2	2.57
Treatment II (bull)	3	9.83
Treatment III (boar)	3	13.61

As reported by Costa and Paula (2006), there is a positive and significant correlation between the serum total testosterone and the volume of Leydig cells in the testes. The values of serum total testosterone signify the capacity of the Leydig cells to secrete testosterone hormones in the animal testes (Ewing et al., 1979). Hence, the animal testes might contain concentrations of testosterone. The levels of total testosterone observed on the serum attested the presence of the androgen hormone in the testes of the animals. The testosterone in the testes is assumed to be preserved using lyophilization process which in turn promote sex reversal of tilapia fry after the 28-day lyophilized testes treatment. Furthermore, the animals from which the testes were collected were all characterized as sexually matured (Roth and Myers, 2004; Dewey and Ng, 2001; The University of Tennessee Health Science Center, 2009). Researchers have reported that mature animals have increased levels of testosterone (Becker and Snipes, 1968; Costa and Paula, 2006; Lindner, 1959; Lindner and Mann, 1960). During the age of sexual maturity of animals, testosterone level and potency is assumed to increase significantly. This idea explains the possible sex reversal of tilapia fry when treated with lyophilized testes from animals which contained concentration of potent testosterone.

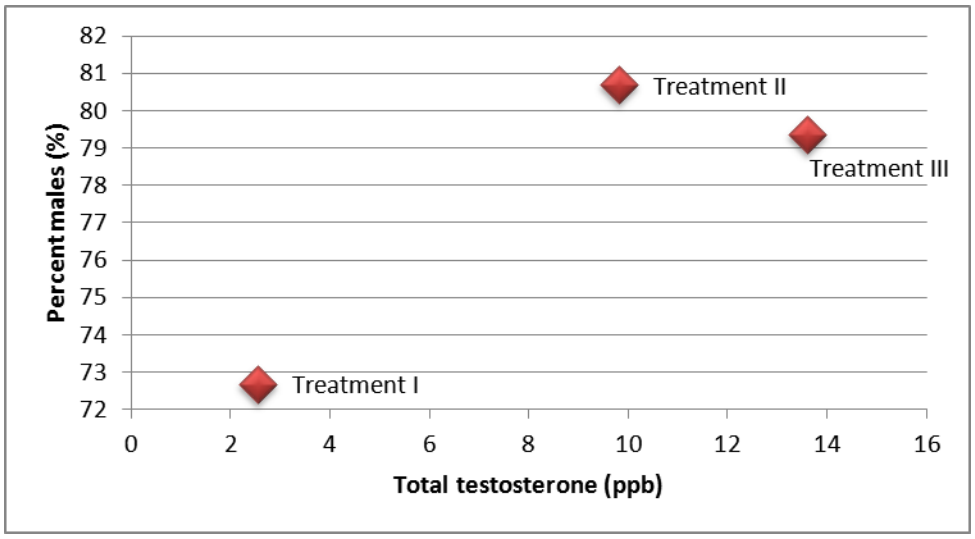

Figure 3. Total testosterone and percent males produced from 28-day sex reversal treatment using lyophilized testes

The total testosterone levels from serum of each animal and the percent males produced from lyophilized testes diets are shown in Figure 3. Treatment I with 2.57 ppb of total testosterone resulted to 72.67% males. Treatment II with 9.83 ppb of total testosterone had 80.67% males. Treatment III with 13.61 ppb of total testosterone gave 79.33% males. The highest percent males were obtained from Treatment II while the lowest percent males were found in Treatment I. Treatment III with the highest total testosterone did not obtain the highest percent male. The reason for this may be accounted to the indigestible parts of the boar testes which might have affected the digestibility of the lyophilized boar testes diet. A tough, white fibrous connective tissue capsule, the tunica albuginea, surrounds each testis and extends inward to form septa that partition the organ into lobules (Darling, 2009). It was observed that the boar testes contained the thickest and toughest tunica albuginea among the testes from other animals. During the preparation of lyophilized testes diet, the tough and fibrous septa inside the testes were not removed. These probable indigestible parts of the testes remained in the diet. In this study, it is assumed that the fry treated with lyophilized boar testes diet assimilated less amount of testosterone since some parts of the diet were indigestible in the fish body. This may explain the reason why the Treatment III with the highest total testosterone level did not obtain the highest percent males of tilapia fry after the 28-day sex reversal treatment with lyophilized testes diet from boar.

The percentages of phenotypic males produced out of the lyophilized testes from animals are relatively higher than 65% males obtained from the 28-day treatment period of lyophilized bull testes fed *ad libitum* to tilapia fry, as reported by Phelps et al. (1996). Likewise, the results are also higher than the reports of Odin et al. (2009) where 61.33, 57 and 53% males were obtained from 23-day treatment period of dehydrated hog, carabao, and cattle testes, respectively. The relatively low percent males obtained from dehydrated animal testes treatment might be affected by the diminished and suppressed testosterone level of the testes due to heat exposure (Lue et al., 2000). In this study, the high percent males obtained is assumed to be favored by the high concentration of testosterone in the testes diets which was preserved under very low temperature processing during the lyophilization process.

However, these results are lower compared to the reported 85% male population of sex-reversed tilapia fry fed with fresh ram testes for 80 days (Haylor and Pascual, 1991), to the reported 93% phenotypic males produced from *ad libitum* feeding of tilapia fry with frozen bull testes after 30-day treatment period (White, 2008) and to the reports of Meyer et al. (2008) where percent males obtained from tilapia fry fed *ad libitum* with fresh bull testes and fresh hog testes were 87 and 83%, respectively. The lower percentages of males obtained from tilapia fry fed with lyophilized testes from carabao, bull and boar might be attributed to the restricted feeding of tilapia fry with testes diets at 20% feeding rate in this study. Hence, it is assumed that fry which were not masculinized consumed fewer hormones than the required minimum amount for sex reversal during the gonadal differentiation period.

According to Phelps and Popma (2000), the age and size of the fry and the environmental factors such as temperature can impact growth and affect gonadal differentiation and in turn the treatment duration needed. In this study, the first feeding fry with less than 9 mm initial length were used. Apparently, the ample amount of high protein from lyophilized testes diets fed to the fry and the high temperature during the treatment period might have contributed to the fast growth of the tilapia fry, reaching a length greater than 18 mm on the 14^{th} day of the treatment period. This length is greater than the minimum harvestable size recommended for effective sex reversal of tilapia (Phelps and Popma, 2000; and Phelps, 2006). Hence, since growth was too fast, it may be necessary to reduce the quantity or quality of diet to reduce the growth rate and obtain effective sex reversal.

Another factor which may be considered to affect the percentages of males produced from the treatments is the flow-through system maintained throughout the experiment period. It is well established that hormones administered for sex reversal are metabolized and eliminated from the body of fish (Lone and Matty, 1981; Gomelsky et al., 1994). Abucay and Mair (1997) observed sex reversal of untreated fish reared within a system previously used with hormone treatment. He also mentioned that sex reversal treatments are more successful in closed water systems where metabolites and leachates can build up. In this study, the active metabolites of the testosterone excreted by treated fry during sex reversal and the hormones which leak from the uneaten food might have been prevented to build-up, diminished and lost from the system since continuous flow of water and regular water exchange were maintained during the treatment period. The shortened exposure of the treated fry to active metabolites of testosterone due to its loss in turn, may have affected the percentages of males produced in this study.

The percent males obtained from MT-treated group were found to be highest among the groups. This result conformed to the reports of Shelton et al. (1978), Guerrero and Guerrero (1988), Jo et al. (1988), Vera Cruz and Mair (1994) that oral administration of testosterone-treated feed (30-60mg/kg feed) to tilapia fry during a three to four-week period yields populations composed of ≥ 95% males.

The significant difference of phenotypic males in Treatments I, II, and III from that of the untreated group showed that lyophilized testes diets from bull, boar and carabao and the MT-treated diet were effective in masculinizing Nile tilapia fry. However, the higher percentage of males obtained in MT-treated group compared to those treated groups with lyophilized testes showed the greater potency of the synthetic androgen under the condition of this study. This might be due to the fact that 17α- methyltestosterone contained concentrated form of synthetic androgen. Synthetic androgens are generally more potent than natural androgens for masculinizing fish (Yamamoto, 1969).

Growth Rate

Data on the specific growth rate of Nile tilapia fry after the 28-day treatment period are shown in Table 1. The analysis of variance shows a significant difference ($P<0.05$) among the treatments at 5% probability level of DMRT (Figure 4).

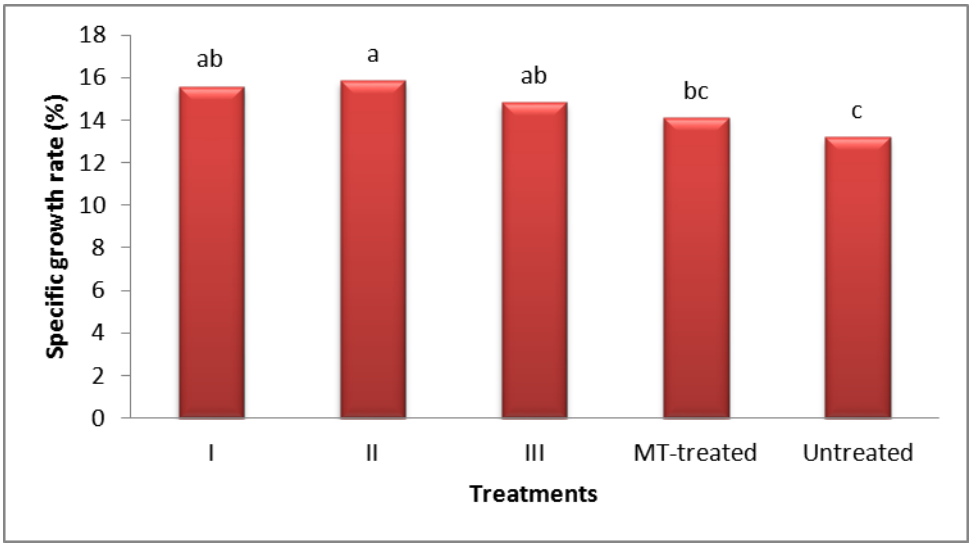

Figure 4. Specific growth rate of Nile tilapia (*Oreochromis niloticus*) fry after the 28-day treatment period. Note: Treatments with different letter superscripts indicate mean values that are significantly different at 5% level by DMRT

After the 28-day treatment period, results revealed that Nile tilapia fry fed with lyophilized carabao testes (Treatment I), lyophilized bull testes (Treatment II) and lyophilized boar testes (Treatment III) obtained the highest specific growth rate among other treatments with means 15.59 ± 1.26, 15.85 ± 1.24 and 14.82 ± 0.22%, respectively. There was no significant difference ($P>0.05$) found among the treatments using lyophilized testes. Tilapia fry fed with MT-treated diet (Control I) obtained a mean of 14.12 ± 0.31% with no significant difference from Treatments I, III and untreated fry. The untreated group (Control II) with a mean of 13.20 ± 0.40% significantly differed from the groups treated with lyophilized testes diets.

These results may be attributed to the fact that animal meal contains higher protein content which in turn results to apparent high specific growth rate of Nile tilapia fry fed with lyophilized testes diets from carabao, bull and boar. The proximate analysis evaluated on the lyophilized testes from such animals revealed their high crude protein (CP) content (Table 3). Consequently, those tilapia fry fed with lyophilized testes had the highest specific growth rate since these diets contain the high crude protein content of about 64.85 to 71.69%.

Table 3.Chemical composition of the experimental diets

TREATMENTS	CRUDE PROTEIN (%)	MOISTURE CONTENT (%)	CRUDE FAT (%)	CRUDE FIBER (%)	ASH (%)
Treatment I*	64.85	2.89	13.59	0.43	10.24
Treatment II*	71.69	1.97	13.16	0.25	10.17
Treatment III*	70.20	2.98	12.33	0.27	9.84
Control I**	33.00	12.00	6.00	5.00	12.00
Control II**	33.00	12.00	6.00	5.00	12.00

*Analyzed using AOAC (1980) method

**Guaranteed proximate analysis of TATEH Aquafeeds, SANTEH, Feeds Corp

The results on the specific growth rate of the tilapia fry fed with lyophilized testes diets conformed to the results of the study conducted by Phelps et al. (1996) who reported that growth increase of tilapia fed *ad libitum* with trout chow feed containing lyophilized bull testes may range from 0.7 to 2.0 g after 28-day treatment period in outdoor tanks. Likewise, White (2008), reported that tilapia fry fed with frozen bull testes and oven-dried bull testes for 30-day treatment period gained a mean weight of 0.79 and 0.94 g, respectively. Odin et al. (2009) also reported significant high growth rate of Nile tilapia fry fed with dehydrated testes from carabao, cattle and hog at 20% feeding rate after a 23-day treatment period in hapas in earthen ponds. Fashina-Bombata and Somotun (2008) obtained an average length of 2.9 cm for fry of 'Wesafu', a sub-group of cichlid, after 25-day feeding trial of goat testes meal with 47.33% crude protein.

In this study, the specific growth rates found among those fry treated with lyophilized testes came out to be as high as the results of similar studies. This may be due to the similar culture conditions provided during the treatment period such as the minimal stocking density of 500/unit, continuous flow of water and good water quality of outdoor tanks which ensure optimum conditions for the growth of the tilapia fry.

The trend of the growth of Nile tilapia fry during the 28-day treatment period revealed that tilapia fed with lyophilized testes consistently increases rapidly in terms of body weight (g) followed by those treated with 17α-MT diets and the control with the lowest growth increase (Figure 5). This may be due to higher protein content of the testes diets fed compared to those control treatments.

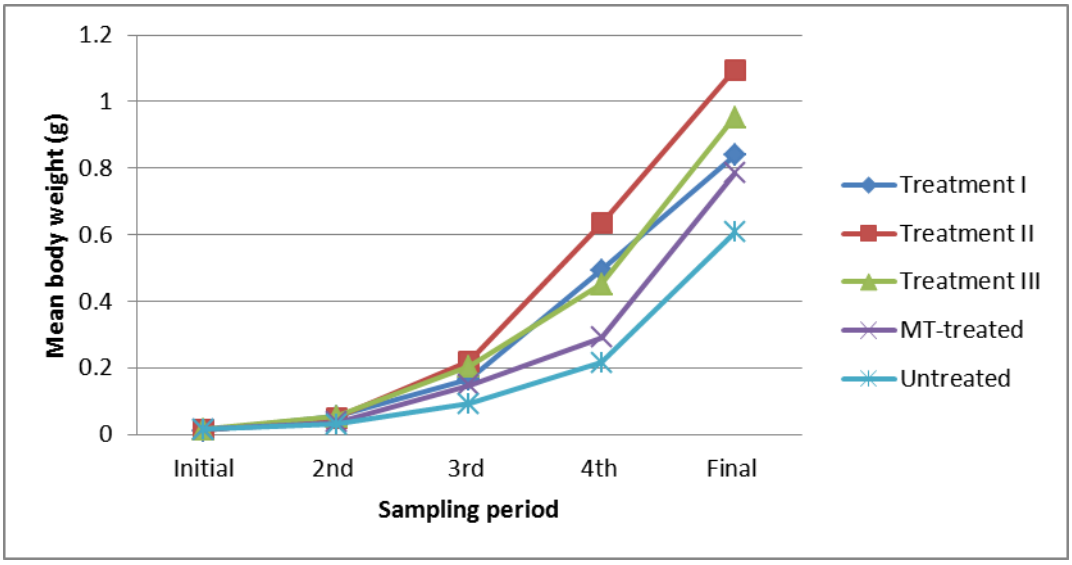

Figure 5. Growth of the Nile tilapia (*Oreochromis niloticus*) fry during the sex reversal treatment

Several dietary protein requirements of several tilapia species have been estimated to range between 20-56% (El-Sayed and Teshima, 1991). De Silva and Perera (1985) reported that the optimum dietary protein level for optimum growth of Nile tilapia fry was 30% crude protein. As reported by Ahmad et al. (2004), the growth performance of Nile tilapia fry was highest at 45% protein diets. Al-Hafedh (1999) found out that better growth of this species was obtained at high dietary protein levels (40-46%) rather than 25-35%. In this study, the lyophilized testes diets contain the highest protein level compared to the control diets which contain only about 33% crude protein. The lyophilized testes diets are also assumed to contain androgenic hormones which are beneficial for fish growth. The growth increase may be attributed to the androgenic steroids which promote release of growth hormone from pituitary somatotrops fish (Higgs et al., 1976). Hence, the presence of testosterone in the testes promotes anabolic effects which in turn lead to increased growth rate of tilapia fry after a 28-day sex reversal treatment period with lyophilized testes diets.

Tilapia fry fed with MT-treated diet did not differ significantly from those fed with lyophilized testes of carabao and boar. The MT-treated diet in this study contained a dose of 50mg/kg which is high enough to promote growth aside from sex-reversing the fry. As reported by Ahmad et al. (2004), the optimum effective dose of 17α-MT treated diet in promoting significant final weight, weight gain, and specific growth rate of Nile tilapia is 5 mg/kg. Similarly, Jo et al. (1995) found *O. niloticus* fry treated with MT at 5-25 mg/kg diet to be heavier than the control after the sex reversal period. Mateen and Ahmed (2007) also reported that different dose rates of MT significantly increased the growth of Nile tilapia fry than the control. However, in this study, it was found out that fry fed with MT-treated diet had no significant difference from untreated fry. Vera Cruz and Mair (1994) did not find significant effect of MT on the growth and survival of Nile tilapia fry during the treatment period with MT at 40 mg/kg diet.

The high growth of tilapia confirmed the findings of earlier studies regarding animal protein meal. El-Sayed (2006) stated that terrestrial animal by-products have been widely and successfully used as protein sources for tilapia due to their high protein content and essential amino acids.

Survival Rate

The data on the survival rate of Nile tilapia fry after the 28-day treatment period are shown in Table 1. Analysis of variance shows no significant difference (P>0.05) among treatments (Figure 6).

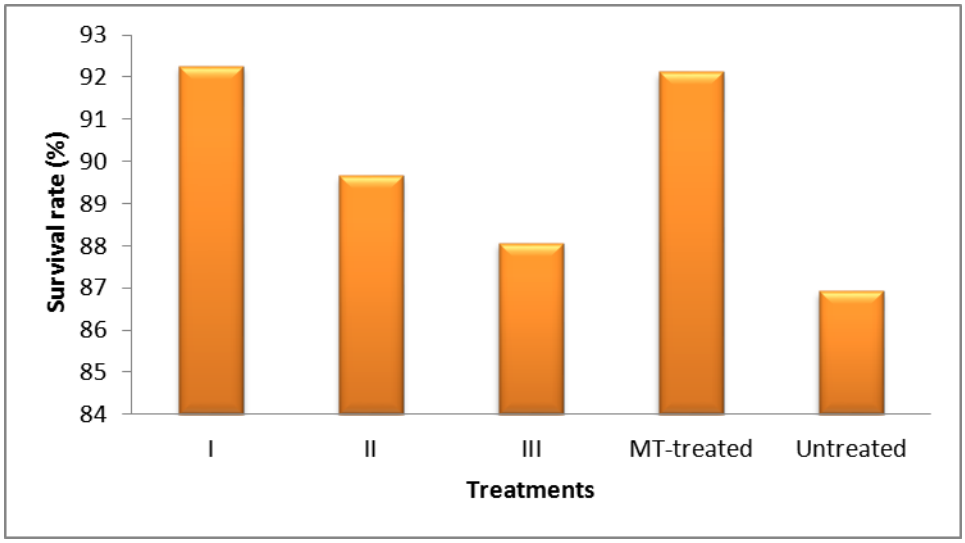

Figure 6. Survival rate of Nile tilapia (*Oreochromis niloticus*) fry after the 28-day treatment period

Results show that the survival rate among treatments did not differ significantly (P>0.05) after the 28-day treatment period. Tilapia fry fed with lyophilized testes from carabao (Treatment I) obtained the highest survival rate with a mean of 92.27 ± 0.02%. This was followed by those fry fed with MT-treated diet (Control I), lyophilized testes from bull (Treatment II), lyophilized testes from boar (Treatment III) and untreated diet (Control II) with means 92.13 ± 0.07, 89.67 ± 0.00, 88.07 ± 0.05 and 86.93 ± 0.08%, respectively.

The high survival rate of Nile tilapia fry obtained in this study confirmed the findings of White (2008) who obtained high survival rates (88-95%) of fry fed with animal testes and stocked in outdoor tanks with green water during a 30-day treatment period. The survival of fry during the sex reversal treatment are dependent on factors such as stocking density, feeding, temperature and other environmental conditions (Bocek et al., 1992). Vera Cruz (1991) stated that the sex reversal treatment unit influences the quality of fingerlings produced. In this study, the experimental units were set with continuous flow of water to ensure optimum water exchange and good water quality throughout the treatment period. Vera Cruz and Mair (1994) obtained >70% survival rate of tilapia fry utilizing outdoor tanks having at least once/day water exchange rate during the hormone treatment period. Vera Cruz and Mair (1994) also reported insignificant effect on the survival of *O. niloticus* fry during the treatment period of 40 mg/kg diet. The minimal stocking density of 500 fish per unit in outdoor tanks in this study might have favored the high number of fish surviving throughout the treatment period (Figure 7).

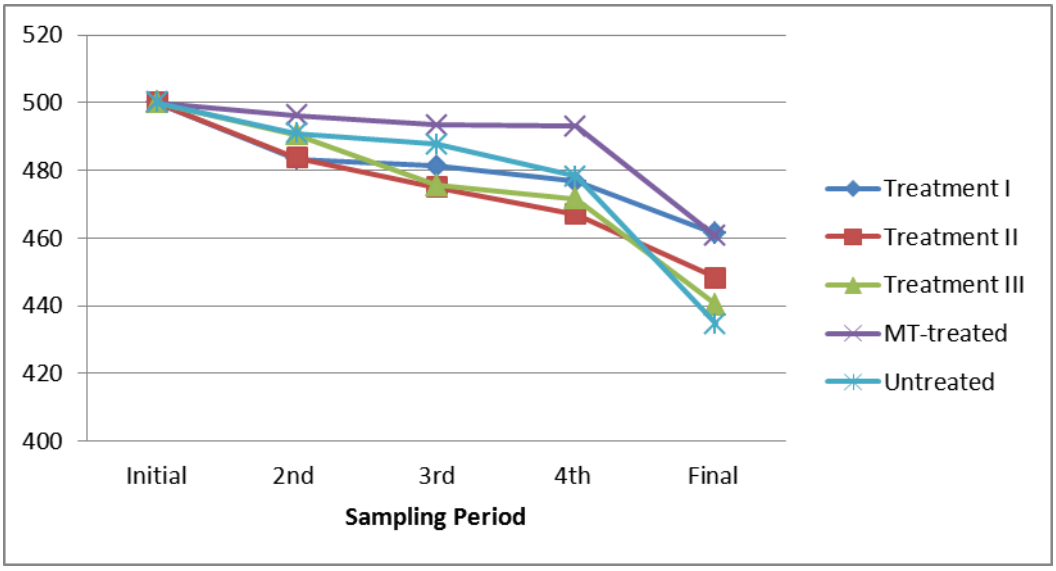

Figure 7. Number of fry surviving based on the original 500 fish per experimental unit during the 28-day treatment period

Apparently, the survival rates obtained in this experiment were higher compared to the results of earlier studies conducted on the use of animal testes in masculinizing Nile tilapia fry (Haylor and Pascual, 1991; Phelps, 1996; Meyer et al., 2008. In this study, the high survival rate of fry is assumed to be attributed to the continuous water flow and regular cleaning of the tanks and enclosure nets which in turn promoted optimum culture and environment conditions for the experimental fish during the treatment period.

Simple Cost and Return Analysis

The simple cost and return analysis of Nile tilapia fry treated with lyophilized testes on a 28-day treatment period is shown in Table 4. Treatments III and II with no significant difference ($P>0.05$) gave the highest gross income of PhP 306.58 and PhP 294.45, respectively. MT-treated diet (Control I) and Treatment I followed with a gross income of PhP 271.15 and PhP 263.17, respectively. The MT-treated group differed significantly ($P<0.05$) from Treatment III but did not differ significantly ($P>0.05$) from Treatments I and II in terms of gross income. The untreated fry (Control II) with the lowest gross income of PhP 167.42 differed significantly ($P<0.05$) from other groups.

In terms of cost, Treatment I, II and III gave the highest operating cost of Php 238.97, PhP 260.83 and PhP 197.78, respectively. The control treatments, MT-treated diet and untreated diet, obtained costs of PhP 136.02 and PhP 131.98, respectively. The cost for lyophilized testes diets significantly differed ($P<0.05$) from the control groups.

Table 4.Simple cost and return analysis of Nile tilapia (*Oreochromis niloticus*) fry fed with lyophilized testes vs controls

ITEMS	TREATMENTS				
	I	II	III	MT-treated	Untreated
Gross Income (PhP)	263.17[c]	294.45[ab]	306.58[a]	271.15[bc]	167.42[d]
Operating Cost (PhP)					
a. Fry	100	100	100	100	100
b. Lyophilized testes diet	111.37	133.23	70.18	0	0
c. MT-treated diet	0	0	0	8.42	0
d. Fry-mash	0	0	0	0	4.38
e. Labor	23	23	23	23	23
f. Electricity	4.60	4.60	4.60	4.60	4.60
Total Cost	238.97[b]	260.83[a]	197.78[c]	136.02[d]	131.98[d]
Net Returns (PhP)	23.20[b]	33.62[b]	108.80[a]	135.13[a]	35.44[b]

*In a column, means followed by a common letter are not significantly different at 5% level by DMRT

In terms of net returns, those fry fed with MT-treated diet gave the highest significant net return of PhP 135.13 and this was followed by those in Treatment III with Php 108.80 net returns. There was no significant difference (P>0.05) found between the MT-treated group and Treatment III. Treatment I and II obtained a net return of PhP 23.20 and PhP 33.62, respectively. The untreated group with PhP 35.44 net return did not differ significantly (P>0.05) from those in Treatments II and I.

The low value of net returns in Treatments I and II was assumed to be greatly affected by the high cost incurred for the production and processing of lyophilized testes diets. Treatment III, on the other hand, obtained a high net return because of the low price of raw boar testes in the market. In order to determine the market price of the sex-reversed fingerlings produced from lyophilized testes, the cost of diet preparation of the lyophilized testes must be reduced.

The result of the simple cost and return analysis revealed that it is more economical to use synthetic MT-treated diet than lyophilized testes diet in masculinizing Nile tilapia fry. The MT hormone is synthetic product and more potent in masculinizing Nile tilapia fry. This product is also a concentrated form of androgen which can be easily stored and can be easily administered to the fish. On the other hand, the testes of carabao, bull and boar are readily available from any market and local abattoirs in the country. However, the preparation and processing of these animal testes into lyophilized form to ensure prolonged shelf-life and potency for sex reversal requires a complicated, sophisticated and extensive methodology. The use of lyophilized testes can be of great relevance to the production of organic tilapia.

Water Quality

Water quality parameters such as temperature, pH and dissolved oxygen were all found to be within the desirable optimum range. Statistical analysis revealed that there were no

significant differences (P>0.05) found among the treatments in terms of the water quality parameters monitored during the 28-day treatment period (Table 5).

Table 5. Water quality parameters monitored during the 28-day treatment period

TREATMENTS	TEMPERATURE (°C)		DO (mg/L)		pH	
	AM	PM	AM	PM	AM	PM
Treatment I	27.95	29.46	5.3	8.0	8.2	8.3
Treatment II	27.97	29.38	5.4	7.7	8.1	8.3
Treatment III	27.94	29.40	5.3	8.2	8.2	8.3
MT-treated	27.96	29.39	5.3	7.8	8.1	8.3
Untreated	27.97	29.36	5.1	7.5	8.2	8.3

The readings on the water quality parameters during the experimental period demonstrated desirable levels suitable for sex reversal of Nile tilapia fry. The average temperature was recorded to be optimal at an average of 27°C in the morning. However, it was also found to be relatively high during afternoon with an average of 29°C. Phelps and Popma (2000) stated that optimum temperature suitable for sex reversal of tilapia fry falls between 26-28°C. In this study, high temperature readings which fell out of the maximum optimum range were recorded during the first to third week of the treatment period where the weather was sunny. The temperature readings started to drop to its optimum range on the last days of the treatment period where rainy weather was observed. The readings on dissolved oxygen (5.1 - 8.2 mg/l) and pH (8.1 - 8.3) recorded were all within the favorable conditions appropriate for sex reversal. Phelps and Popma (2000) suggested that dissolved oxygen concentrations should remain above 4 mg/l to ensure a strong feeding response. In terms of pH, it was mentioned that tilapia can best survive in pH of 6.0-9.0 (Popma and Masser, 1999).

CONCLUSIONS

Based on the results of this study, it can therefore be concluded that the objectives of this study were met. (1) Lyophilized testes from bull, boar and carabao were possible in masculinizing Nile tilapia fry after a 28-day treatment period in outdoor tanks but the percent phenotypic males produced is not as high as the synthetic MT. The sex reversal rates of tilapia fry using lyophilized testes were found to be significantly higher than the untreated fry. (2) Lyophilized testes from bull, carabao and boar gave higher specific growth rate of Nile tilapia fry after a 28-day treatment period in outdoor tanks. High survival rate of Nile tilapia fry fed with lyophilized testes from carabao, bull and boar were obtained after a 28-day treatment period in outdoor tanks. (3) The simple cost and return analysis revealed that it is more economical to use the synthetic MT-treated diet in masculinizing Nile tilapia fry rather than the lyophilized testes from carabao, bull and boar.

RECOMMENDATIONS

Based on the results of this study, the following recommendations are considered for future investigation: (1)Consider the influence of controlled and uncontrolled feeding in using lyophilized testes diets for sex reversal of Nile tilapia fry to come up with effective dose; (2) Consider the use of lyophilized testes diets for sex reversal of Nile tilapia fry in hapas in earthen pond; (3) Develop a procedure for the use of liquid nitrogen instead of lyophilization in the preparation method of freeze-drying the testes to reduce the cost of testes diets.

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POTENTIAL USE OF BACTERIAL DEGRADATION TO ELIMINATE METHYLTESTOSTERONE FROM INTESIVE TILAPIA MASCULINIZATION SYSTEMS

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To reduce the potential contamination of effluents with Methyltestosterone (MT) used in Tilapia hatcheries, we have proposed the use of bacteria isolated from filters that trap MT in activated charcoal. MT is commonly used in tilapia aquaculture to produce all-male populations and certain concerns have been raised regarding the potential risks to the environment and farmers. The filters we use to retain MT have a biofiltration component where microbial populations are easily formed. We hypothesized that some of the MT degradation occurs in the biofilter due to the active use of MT as carbon source. Based on this we isolated and characterized heterotrophic bacteria obtained from biofilms formed in a filtration system used for tilapia *(Oreochromis niloticus)* masculinization. We also determined the capacity of adaptation of isolated bacteria in a culture media enriched with MT as the only source of carbon. Primary isolates were obtained from biofilms collected at 7, 11, 20 and 28 days of the masculinization trial. Isolates were inoculated in nutritious agar and eosin-methylene blue agar. Identification was conducted using API WEB and dichotomic keys (Koneman *et al*.,1999). Adaptation trials were conducted in flasks containing mineral medium enriched with MT (45 mg/100 ml) as the only carbon source. Each flask was inoculated with 2ml of a bacterial suspension (0.5 in the McFarland scale) and incubated at 30 ºC with agitation at 175 rpm for 26 days. Adaptation was measured by counting bacteria daily using the plate counting method.

We isolated and characterized *Bacillus ceresus, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Serratia marcescens*. All of them were able of using MT as source of carbon and energy. *P. aeruginosa* was the species with the fastest adaptation; initial growth was perceived at 48 hours and reached the highest number of microorganisms. *B. subtilis y P. fluorescens* showed initial growth at 72 hours while *B. ceresus* y *S. marcescens* initiated growth later (96 and 198 hours, respectively (Fig. 1). *P. aeruginosa* seems to be a species capable of utilizing a large amount of organic compounds as substrate to grow. This capability allows it to colonize niches and inhospitable environments where nutrients are scarce. Our results indicate that the bacteria we isolated are potential MT biodegraders.

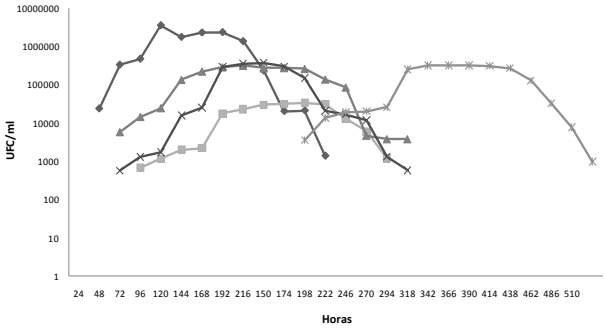

Figure 1. Growth kinetics of bacteria in mineral medium enriched with MT. *P. aeruginosa; B. ceresus; B. subtilis, P. fluorescens; S. marcescens.*

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HOW TO PRODUCE BILLIONS OF HIGH QUALITY TILAPIA FRY

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Abstract

Tilapia has now become a popular protein source to the poor, and also increasingly to middle class people. It serves as a typical model of a success story of farming outside its native area. Annual tilapia production was only 1.5 tons in 1950 which surpassed 1.5 million tons in 2002; increased by 1 million fold. Now it has surpassed even 3 million tons in 2010. Its production will still continue to grow exponentially, if high quality fry are readily available especially in countries like China where fry demand is in billions. How to produce and supply such a huge quantity of high quality tilapia fry has been a question for the countries which have potential to expand tilapia farming for domestic consumption and export markets. In Thailand, shortage of premium quality tilaia fry was realized as early as 1980s as the main constraint to the growth of commercial farming. Therefore, Asian Institute of Technology (AIT) developed a practical technique of mass-scale fry production through a series of on-station experimentation over a decade. The technology is basically to produce all-male fry by maintaining a large number of broodfish in hapas, collecting eggs, incubating them artificially in clean and controlled system and feeding with methyl-testosterone (MT) mixed with high quality feed as early as possible to ensure over 99% males in the fry population.

In addition to developing the production technology, AIT also successfully disseminated it applying all sorts of strategies involving public as well as private sector. However, a key turning occurred only after the success of a private hatchery in Thailand that triggering mushrooming of many others. There are over 100 hatcheries of such type in Thailand alone. Now the same trend can be seen in Bangladesh. The technology has now been adopted by many farmers and entrepreneurs of many countries especially in Asia and Latin America. However, in China where about half of the global tilapia is produced, most farmers use hybridization technique to produce mono-sex fry. In Thailand, three hatcheries annually produce 200 million fry each. This means, establishing about five such hatcheries could easily produce 1 billion high quality fry per year. A hatchery in Hainan island of China has been already established by a foreign company which has claimed to achieve the same level of production. However, this technology has not been widely adopted. Adoption of this technology could boost tilapia farming further increasing many folds as the demand for fish for local consumption is huge, and so the export market. Exploring potential and promoting this technology could bring a big leap in tilapia industry in China from its current level. With a view to assisting the industry, establishing functional linkages between China and Thailand and other countries that facilitate cooperation among the researchers / scholars in sharing information and organizing study visits or trainings to government officials and farm/hatchery managers could serve as solutions. This paper describes the techniques and approaches applied by AIT hoping that it provokes policy makers, extension workers, researchers and educators working especially in China, and also other countries to find various ways of collaborations.

Introduction

Background

Tilapias are natives of African continent. Nile tilapia (*Oreochromis niloticus*) was introduced to Thailand as gift to the King of Thailand from the Japanese Emperor in 1965. They were kept in Chitralada Palace. After seeing a large number of fry produced naturally in the pond, HM the King provided 10,000 fingerlings to the Department of Fisheries (DoF) requesting to produce more and distribute to the poor people perceiving the tilapia as a cheap protein source (Pullin, 1988; Bhujel and Stewart, 2007). Tilapia in Thailand is considered as precious fish because it is thought to be 'Royal Fish'. In addition to its historical background, the fish itself possess special biological characteristics i.e. caring of its eggs and young ones in the mouth by females. It also can use planktons as feed which can be produced simply by applying manures and chemical fertilizers in ponds. At the same time, agriculture byproducts such as rice / wheat bran, oil cakes and others can be used as feeds to enhance its growth thereby productivity. It can be cultured in systems ranging from backyard ponds to intensively managed tanks/ponds. It resembles with chicken in terms of farming as well as its consumption; therefore, it has been often dubbed as 'aquatic chicken' (Little, 1998). Due to its easiness of breeding and farming, it became the species of interest among the resource poor especially in rural areas. In Thailand, it became number one specie since mid-90s over-taking the hybrid catfish. More importantly, Nile tilapia has gained its popularity in China, Bangladesh, Indonesia Laos, Malaysia, Taiwan, the Philippines and Vietnam. People do not treat it as exotic fish, instead regard as a very important source of high quality animal protein and income.

Perception of the Potential and the Problem

Natural breeding without the need of any hormone injection was considered the main advantage of tilapia over other species but as the farming became more commercial, demand for large and uniform fish increased. This created high demand for good quality mono-sex fry. It was almost impossible for the traditional hatcheries to produce and supply a large quantity of fry using traditional methods. For example, production of millions of fry was almost impossible using the existing method. Therefore, the low number of eggs (approx. 1,000 per spawn) and asynchronous spawning became the constraint for mass fry production. Therefore, producing sufficiently large quantity of good quality seed was a big challenge in early 1980s (Little et al., 1997; Bhujel et al., 2000). The potential of tilapia in Thailand and lack of high quality seed was perceived as problem in advance. As a result, it provoked a research program at AIT that aimed at developing mass-scale mono-sex fry production technique. Recently, farmed tilapia has been regarded as green and good species for consumption. Demand is steadily increasing. More and more super markets and retailers are having various types of tilapia items around the globe. Some airlines in US have already started serving tilapia meal. Similarly, fast food chains such as McDonalds' and others have also tried. Indications are already there that tilapia is becoming 'Aquatic Chicken' in true sense. When it becomes everyone's meal, demand could be unimaginably higher. As the seed is one of the most components of the aquaculture development, availability of good quality seed whenever needed accelerates the industry growth. Therefore, in order to support the maximum potential growth of tilapia industry, policy makers, researchers and other involved should emphasize the seed production technology and its dissemination. Applying the method and disseminating it as a campaign is likely to solve the high demand of fry such as in PR China.

Solution to the problem

Any problem once identified, can be solved through research and development. AIT started research conducting a series of trials establishing a parental stock of Nile tilapia in 1984. Comparisons of breeding in earthen ponds, hapas-in-ponds and tanks within a recirculation system were the beginning of the research. Then trials were on developing methods of egg collection and artificial incubation systems (Little, 1989; Macintosh and Little, 1995). One of the major challenges was to explore or develop the best system or method, and container or jar for artificial incubation of eggs that could ensure high hatching rate and survival of eggs and yolk-sac larvae to swim-up fry consistently. Use of conical vessels and shaking tables (Macintosh and Little, 1995), various containers were tried such as simple coke bottles and white water bottles. However, semi-transparent fiber-glass jars (Fig. 1: left) locally made was found to be the best. They have been designed in two sizes; 4-litre and 6-litre. The larger sized incubators (Fig. 1, left) can accommodate about 0.2-0.3 million eggs. Attempts are still on-going to explore possibility of using new containers for the improvement in hatching and survival of eggs/larvae. Recently, simple plastic jars (Fig. 1: left) or jugs have been used. As they are cheap, easily available and more transparent so that the hatchery operators can see the egg movement easily and they are also lighter and easier to handle, most hatchery operators like them. However, relatively rough wall of the fiberglass jars facilitate egg hatching accelerating the process of removing egg's hulls. As tilapia eggs are heavy and remain at the bottom, they needed to be moved gently so that they would not get injured and stay at the bottom without getting adequate oxygen. For this, up- and down-welling water flows into the jars were compared and the downward water flow has been found to be better. It is commonly used by most hatchery operators.

Fig. 1 Tilapia egg incubation jar made of fiberglass (left); and a series of incubators (right) in well-designed large tilapia hatchery in Thailand.

Shallow aluminum or plastic (Fig. 2) trays are use for the rearing of yolk-sac fry after they hatch.

A large number of fry (up to 40,000 fry) can be accommodated in a tray with shallow water oxygenated by its gentle movement. Several trials studied the effects of factors such as fry density and water flow on the fry survival, showed higher densities are even better.

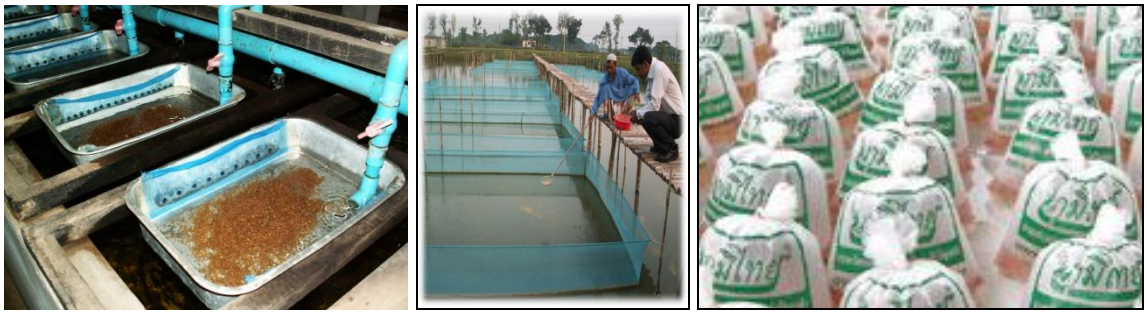

Fig. 2 Larval rearing in aluminum tray (left), feeding hormone mixed feed (middle, in Bangladesh) and fry packed in plastic bags (right) ready for transport.

Attempts were also made to maintain high survival and increase percentage of males in the fry populations. These included determining the optimum dose of methyl-testosterone in feed, frequency and length of feeding period and so on. As a result high percent of males (100% or close to) have been consistently achieved. As demand for fry is seasonal, a method of advanced nursing (Little et al., 2003) has also been developed which can be applied when they needed to keep longer period.

With a gradual improvement in each step of the whole process, a complete package of mass-scale fry production technology has been developed. Research is still on-going especially to make it more adaptable to the environmental conditions and for the manipulation of fry production and supply demand (e.g. Bhujel et al., 2001; 2007). Many research projects secured/launched, and also the student research, were either only on tilapia or in combination with other species. More than 100 student theses (M.Sc. and PhD) have been produced related to tilapia. Research areas covered varies. Initially, tilapia was used as means for waste recycling (AIT, 1994; Edwards and Pullin, 1990), its fry as feed to other species e.g. snakehead (Kaewpaitoon, 1992) and dominant species with others in polyculture e.g. carps, catfish and prawn/shrimp from semi to intensive production systems (Little, 1998). Over 150 peer reviewed journal articles have been published in tilapia alone (Bart, 2004). Significant numbers of popular articles have also been appeared in several magazines and newsletters. They served as main information outlets to the outside world and have contributed significantly to the adoption, culture tilapia technologies and overall development of aquaculture in Asia and beyond (Bhujel, 2009). For an example, Brazilian aquaculture has grown significantly as a result of Chitralada broodstock and the technology they took from Thailand.

Technology Dissemination

Any technology package after its development, it has to be disseminated. A variety of ways can be applied depending upon the local contexts keeping the benefit of end-users or farmers in mind. Some of the means applied or occurred in Thailand (Fig. 1) and around the globe are briefly discussed in this section.

Education and Training

Tilapia culture and breeding techniques were well incorporated into the post-graduate curriculum in both in theory and practicum, at AIT. In the course, all the students are assigned to

conduct tilapia breeding and grow-out trials. Many of these students are lecturers at the Universities in their home countries. They do the same when they go back to teaching. Emphasis on tilapia farming is reflected in their curricula as well as. More importantly, after acquiring knowledge and skills many graduates or alumni and staff are directly or indirectly involved in tilapia farming and its promotion. Many of them are successfully running tilapia hatcheries and farms by themselves in Thailand while few others in other countries e.g. Bangladesh, Ghana and so on.

Successful launching of aquaculture program and its activities in the region increased demand not only for formal education but also created the interest in short-term, need-based skill development training. As a result, AIT has trained over 1,000 personnel so far from about 30 countries. 'Techniques for Mass Fry Production and Grow-out' was one of the most attractive courses. Interestingly, the course on tilapia attracted more participants than by Integrated Aquaculture probably because it was completely different and about new techniques developed as compared to the traditional techniques of aquaculture dominated by carps. This training course has a significant role in promoting tilapia not only in Asia but also in Africa and Americas. In addition there were several participants for hands-on work experience in tilapia hatchery. One of the remarkable examples is that some private companies (e.g. Chareon Pokhaphand) sent their staff for training and they have established tilapia hatcheries. It served as the base for the company's tilapia business that also involves fillet export to US now. In a decade's time (1989-1999), out of 843 people trained, 26% were from Bangladesh, 22% from Vietnam and 12% Cambodia; mostly for tilapia only or in combination with other species. Many officials of the governments, research institutions have also got this training where they have established and run tilapia hatcheries in their countries, specifically e.g. Bangladesh, Thailand and Vietnam.

Demonstration and outreach

AIT has been keeping a prototype hatchery and runs as a commercial unit within the not-for-profit organization. Many visitors from abroad and students of Thai Universities, colleges visit tilapia hatchery. It has been an interesting place also for distinguished guests of AIT e.g. Thai Princess Mahachakri Sirindhorn, and the King of Sweden in 2003. When there are visits graciously paid by Royal families, the hatchery technology is additionally highlighted including live TV coverage. Many farmers also come with pick-up trucks to AIT directly to purchase fry also to farmers. In doing so, they see the system and spread the words from mouth to mouth. Interestingly, Thai fisheries stations use tilapia hatcheries as a means to generate income unlike distributing fry at free of cost in many countries. Using the funds generated further research and technology dissemination is done in the long-run. AIT engaged farmers as part of research team for field testing also called participatory research. They feel proud being a part of the scientific research. AIT continued to focus on the production of quality mixed sex tilapia in the early 1990s even when AIT was commercializing the monosex approach. This was because it was perceived that even though there was a rapidly increasing demand in the commercial sector for monosex, poor rural households needed quality tilapia to be available locally and centralized commercial monosex operations were unlikely to meet their needs in the short to medium term. Hence, AIT also focused studying on decentralized seed production resulting in large impacts in some marginal agricultural areas such as in Bangladesh. Benoy Barman's work for PhD at AIT, showed fry could be produced cheaply in rice-fish fields. There was number of research work in this aspect on-campus funded by DFID and then through various mechanisms e.g. DFID's support in Bangladesh and subsequently supports from SIDA/DANIDA in Vietnam and elsewhere in Indochina.

Aqua outreach played a considerable role in building regional institutional capacity in aquaculture and aquatic resources management and related fields through innovative approaches. It established a network of partners which included vocational colleges, research

institutes, universities and department of fisheries (provincial or national levels) under the ministries. AARM assisted to establish tilapia hatcheries under outreach activities. For example, Department of Fisheries in Udonthani Province of Thailand, a tilapia hatchery was established with a view to supplying fry to the farmers of the province. Similarly, a hatchery in an Agricultural college, which is managed by one of the AIT graduates, also serves the same purpose. More interestingly, various non-profit organizations in the same province and also in Chiang Mai established and have run tilapia hatcheries e.g. Udonpatana Foundation, as a means to serve the poor families providing an evidence for earlier the notion that tilapia is poor men's fish. Table 1 is a list of hatcheries in Thailand established with direct and indirect assistance of AIT and its partners.

Learning lessons from the promotion of tilapia culture in SE Asia, similar activities have been expanded to Nepal. A project called "Women in Aquaculture" has been launched jointly with the Institute of Agriculture and Animal Sciences (IAAS), Nepal. Tilapia culture was tested or compared with carps at the beginning. After getting positive responses, tilapia has been promoted among ethnic groups and also attempts have been made to expand further with a view to solving the problem of protein malnutrition in the rural areas (Bhujel et al., 2008).

Private-Public Partnership (P-PP)

A number of attempts were made to disseminate the technology through public / government organization with the aim of supplying large number of high quality mono-sex tilapia fry. However, the success was not up the expectation and shortage of high quality fry was still at large. It was probably due to the lack of realization on the potential of tilapia farming by those organizations and their aims were to serve as extension agents rather than doing business by themselves. However, the most obvious reason has been the lack of performance based incentives or rewards for and control over the staff in public organization. Fortunately, these problems were identified well in advance and attempts were also made to quickly shift to partnership with private sector. Unique contractual agreements were made realizing the importance of strict imposition of technological procedures or protocol was necessary in the production of high quality tilapia fry production at every step of the process that involves careful management of brood stocks, collection of eggs, artificial incubation of delicate fry and hormonal sex-reversal. A breakthrough occurred when a private company picked-up the technology in supplying large number of quality seed was possibly. Although, the technology was thought to be cumbersome but private sector adopted quickly due to its profitability and increasing demand. More importantly, after the successes in the private sector, public sector has re-focused on this technology. As a result, tilapia became number one species in Thailand in mid-90s due mainly to these reasons. Sooner or later tilapia industry may take off other countries too. Bangladesh, Malaysia and Vietnam, governments are aggressively promoting tilapia. Tilapia has been officially allowed to culture in commercial scale.

CP Food Co. Ltd., which runs five tilapia hatcheries using the AIT technology, has also played a significant role in promoting tilapia further especially red variety. The company created demand by giving "Thapthim" as a brand name which means "ruby" giving the impression to the common people that it is something special and completely different food item. The company promoted it by producing and distributing an attractive picture of a food item of red-tilapia to almost all the restaurants in Thailand in order to boost domestic demand. The company now has several tilapia growers in groups in various pocket areas under contract farming. Under the agreement, farmers get a complete package of technology, inputs such as fingerlings and feed. They also buy back the fish so that farmers would not need to worry about market. This is a very good lesson strategy to learn from CP, while promoting any new species like tilapia.

Other companies e.g. GenoMar, a Norwegian company has also made remarkable contribution hiring a consultant who was successful in establishing tilapia farming in Brazil after bringing a group of farmers to study the system and purchase broodfish in Thailand. He was initially hired by GenoMar for setting up AIT style hatcheries in the Philippines and subsequently China as well as Latin America. GenoMar also has established a hatchery in Singapore. In early 80s, David Little, who was the main researcher behind developing the technology, has worked with Regal Springs when they first started tilapia production in Java to introduce the AIT approach. More recently, many groups from Bangladesh have been supported by AIT. A large hatchery plan has also been implemented with a company in India for the transfer of tilapia farming technology in bio-secure way, where previously tilapias were not officially approved for culture. Many groups from various countries have showed interests for the establishment of AIT style hatcheries and quality control/certification systems.

Aqua Internship: on-the-job work experience

AIT and its partners in Thailand as well as in other countries offer internship programs to students from within the same country or abroad to provide on-the-job work experience at the tilapia hatchery alone so far in the past. Students, normally enrolled in their universities in their countries do not need any aquaculture background to join internship. Biology background would be sufficient as it is more in hands on work with hatchery staff and learn by doing the work as regular staff. Tilapia hatcheries have been considered as one of the best internship placements where students spend 2-3 months and understand the real field situation and also identify problems faced by the industry which can be research areas for their thesis with the aim of solving those problems faced by the industry. These hatcheries also provide opportunities to students to carry out their research afterwards and even employ in some cases. Internship program runs on cost-sharing basis. Interns or their Universities provide airfares, interns bear food costs by themselves and Asian partner institutions provide free or accommodation or with discounted fees either in their student dormitories/hostels, guest houses or arrangement to live with staff families in communities.

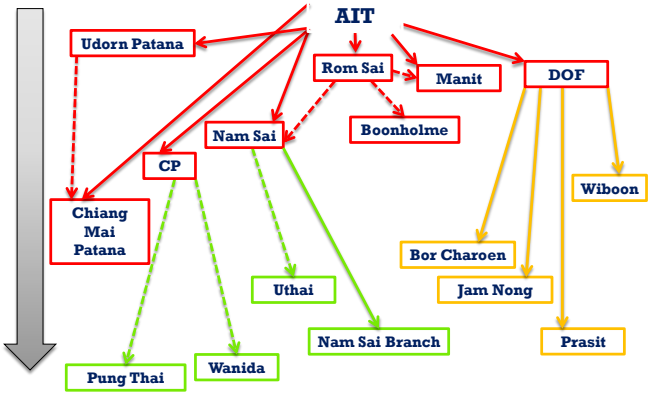

Fig 1. Technology dissemination pathways in Thailand (Belton et al., 2009)

CONCLUSIONS

Realization of the potential of tilapia as a candidate species, because of its benefits over others, identification of shortage of quality fry as the main constraint of its expansion and

continuous research carried out to find the solutions served as foundation for developing a practical technology package, for the transfer of knowledge and technology. Incorporation of the hatchery technique and its farming in post-graduate education, short-term training courses, outreach and internship programs accelerate in the promotion of tilapia culture.

Several individuals who were formally educated from, trained by and/or exposed to AIT have contributed tremendously in promoting the tilapia farming technology developed at AIT. However, the major breakthrough occurred when private sector took up the technology, successfully implemented it and success story started to spread through various means. Countries wanting to promote tilapia may need to think about establishing such hatchery either their own or encouraging and facilitating the private sector. Results have showed that five large hatcheries managed using the set of guidelines or protocol developed could easily produce over a billion high quality fry within 3-5 years period. Further promotion and expansion can be augmented through education, training, farm visits and other media means where the demand is such high e.g. PR China.

Several individuals and groups from abroad were attracted to see the operation and understand it in-depth. Many of them started by themselves and many others established their farms by making contractual arrangements. As a result, several hatcheries emerged in Thailand followed by Bangladesh where most people rely on fish for protein. Again, global expansion has occurred due mainly to the up-take of the technology by the private sector. A lot of adaptations in the technology, modifications and/or improvements have been made in different parts of the world by various groups depending upon the availability of materials and equipment. Nevertheless, ideas of several individuals have contributed in further advancing the technology. Due to which millions of people have and going to have direct or indirect benefits from tilapia research and technology transfer.

Aquaculture program of AIT has established itself in international arena because of these activities carried out over two decades. It has contributed significantly in the improvement of indigenous capacity for education, research and development in the region and beyond. Therefore, approaches/strategies used and role played by AIT in the promotion of tilapia should serve as a model for other organizations which have similar goal of contributing to food security and poverty reduction. However, there is always a room for improvement. No technology can be 100% perfect. More research is still necessary to improve the technology as well as strain itself. For examples, improvement in survival of fry, development of cold and salinity tolerance in various strains, solution to heat stress during summer and its drastic drop in egg production in some cases, various new and emerging diseases, and minimization of cost of production. One of the researchable issues has been always raised is the impacts of MT (methyl-testosterone), a steroid hormone, on the health of technicians who handle the MT on daily basis while preparing feed and feeding the fry, and the impacts on the environment.

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Improving the Supply Chain of Tilapia Industry in the Philippines

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ABSTRACT

This study was designed to evaluate and develop an efficient tilapia supply chain to foster the development of viable fast food and supermarket purchases of tilapia from small-scale producers; with the following specific objectives: Phase 1 – Evaluation: (1.) Develop tilapia supply chain maps for each market level, i.e., producer, wholesale, restaurant, supermarket, fast food stores, etc., to identify specific activities and services, key players, logistical issues, external influences, and flow of product, information and payment among market levels. (2.) Analyze tilapia supply chain performance for efficiency, flexibility and overall responsiveness. (3.) Identify areas for improvement in supply chain (i.e. behavioral, institutional and process), (4.) Provide recommendations to improve the tilapia industry, in general and specific supply chain items. Phase 2 - Development Undertaking: (1.) Design specific improvement measures based on the identified areas of improvement from Phase 1. (2.) Test the improvement measures in the market place, then assess and refine the improvement measures.(3.) Design and implement measures to ensure the sustainability of the improved supply chain of tilapia.

The country's tilapia industry supply chain is composed of the following parts, namely the hatchery and nursery farms which are responsible for the introduction of improved brood stocks to commercial or backyard fish farms which in turn responsible in providing improved quality tilapia fishes for the end-users such as consumers and institutional buyers. The institutional buyers could be further decomposed as processors, consolidators or traders, supermarkets, specialty shops, food chains, restaurants, bars and canteens, among others.

The provinces of Pampanga, Batangas and Laguna are the major tilapia sources while the cities of Metro Manila, Angeles and Baguio are the major demand centers. Dagupan City, Pangasinan being known as "bangus" or milkfish capital is a major transshipment point of tilapia and other seafood for the Northern Luzon provinces including Cagayan Valley and the Cordillera Administrative Regions. In addition to the major supply centers, Camarines Sur in Bicol Region is becoming a key source of tilapia fries. The product flow of tilapia fries from the hatchery to the nursery farms generally follows a continuous 18-day cycle while tilapia fingerlings from nursery to commercial or backyard farms follows thirty to forty five-day cycle depending on fish sizes required by the customers. Direct buying and selling, wholesaling, and retailing at central markets through agents and "consignacion" are the most common marketing operations of the tilapia industry. Consumers generally prefer whole live fish with size ranging from 250 – 300 grams per fish (or 4-5 pieces per kilogram) but the requirements of institutional buyers are more varied depending on their customers' preferences. Filleted tilapia requires about 2-3 pieces per kg or equivalent to 450 – 750 grams per fish. Grilled and barbequed tilapia are now becoming more popular recipes in the major demand centers.

The major concerns of hatcheries and nurseries are the high cost of outbound logistics, which is exacerbated by high competitive pressures of inferior quality but inexpensive stocks (e.g., non-sex reversed) and high levels of mortality due to environmental and cultural factors.

The fish farms' major concerns include; expensive but low quality feeds (at times mislabeled) and other inputs, very low fish recovery and longer culture period to reach larger fishes. Their transaction costs include the cost of waiting for buyers, delays in delivery, in-transit mortality, and toll fees or "goodwill" as well as shrinkage losses. In addition, the lack of cold storage and transport vehicles equipped with tanks and aerators or refrigeration facilities delimits them to take market opportunities. Interestingly, many farmers adapted a "circuitous" production technique to take advantage of markets preference on tilapia with darker skin.

The major concerns of processors are too few farms that could supply regularly the desired quality and volume of tilapia, the lack of capital for market expansion, and competition with cheaper imported counterparts.

The concerns of traders including "consignacion", suppliers or consolidators are the following; (a) meeting the product quality and quantity orders on schedule (b) high logistics and transaction costs of consolidating and distributing fishes from sources to destinations (c) absence of product grades and standards.

The following are some recommendations to address the various issues and concerns namely of the various chain players: (1) encourage the establishment of more nursery farms for better quality brood stocks while intensifying technology transfer to farmers for better health and management of tilapia (2) conduct market promotion activities highlighting the various niche opportunities of tilapia among growers and consumers (3) motivate the participation of small farmers in supply chains by setting up an incentive scheme through a mix of patronage refund and profit sharing (4) institutionalize an accreditation program for feed manufacturers, hatcheries, processors and the like to improve the quality assurance of products and services (5) provide capital windows to improve facilities and reduce logistics and transaction costs in the entire supply chains of tilapia.

IMPROVING SUPPLY CHAIN FOR TILAPIA IN THE PHILIPPINES (On-going)

INTRODUCTION

Tilapia (*Oreochromis niloticus)* became one of the most popular and important farmed fish commodities in the Philippines. It contributed around 12% of the gross domestic production to the aquaculture sector. It also provided a source of income and livelihood of around 1.4 million workforce and fish producers (BAS, 2010). Likewise, tilapia is an important source of food and animal protein. In 2009, the per capita consumption of tilapia was 3.81 kgs and it grew at an average annual rate of about 10% from 2005-2008. Moreover, fish accounted for about 14% of the total food expenditure of the country(Rodriguez, et.al. 2009).

Tilapia culture is widely undertaken in the country with regions III and IV as the major production areas. Due to the product attributes and factors productivity of tilapia as offshoots of research and development (R & D) efforts and programs from the mid 80's until recently, tilapia production has been a dynamic aquaculture enterprise in the country. Furthermore, markets for tilapia remains vibrant with encouraging growth potentials. All major demand areas such as cities of Metro Manila, Baguio, Angeles, among others, are now preferring other product forms and shopping venues for reasons of convenience and availability than the traditional marketing mode.

In the recent past, efforts to sustain the industry's growth momentum have been focused on the improvement of the broodstock through genetic improvement by cross-breeding strains. Likewise, improved stock management and cultural practices have been developed to decrease mortality and maintain growth vigor.

However, in the midst of the global economy, the tilapia industry remains sluggish to serve new market niches such as supermarkets, food chains and exports since it is dominated by smallhold producers that are scattered all over the country. High mortality, small marketable body size and slow growth performance are still prevalent in the industry.

Thus, this existing condition of the tilapia industry amidst pressures brought about by global competition necessitates a development framework that views the industry in a holistic manner that would bring about visible and concrete improvements in production, handling and distribution processes or activities. It is of utmost consideration that the various players of the industry are coordinated to achieve a more efficient, cost-effective, profitable and sustainable industry that thrives in an environment of increased competition due to liberalized markets.

Objectives

This study was designed to evaluate and develop an efficient tilapia supply chain to foster the development of viable fast food and supermarket purchases of tilapia from small-scale producers; with the following specific objectives:

Phase 1 – Evaluation:

1) Develop tilapia supply chain maps for each market level, i.e., producer, wholesale, restaurant, supermarket, fast food stores, etc., to identify specific activities and services, key players, logistical issues, external influences, and flow of product, information and payment among market levels.
2) Analyze tilapia supply chain performance for efficiency, flexibility and overall responsiveness.
3) Identify areas for improvement in supply chain (i.e. behavioral, institutional and process),
4) Provide recommendations to improve the tilapia industry, in general and specific supply chain items.

Phase 2 - Development Undertaking:

1) Design specific improvement measures based on the identified areas of improvement from Phase 1.
2) Test the improvement measures in the market place, then assess and refine the improvement measures.
3) Design and implement measures to ensure the sustainability of the improved supply chain of tilapia.

REVIEW OF LITERATURE

Supply Chain Management

The management of multiple relationships across the supply chain is being referred to as supply chain management (SCM). It is a network of multiple businesses and relationships and is defined as the integration of key business processes that add value to products, services and information as they move from the original suppliers to the end-users (Lambert, D. et.al., 1998). Alternatively, SCM is a process of planning, implementing and controlling the operations of supply chain as efficiently as possible, hence, the application of total systems approach to minimize the cost of flow of information, materials, and services from raw materials suppliers through factories and warehouses to the end consumer.

SCM entails the identification of the different players and how they are connected (both within and outside a particular organization) in bringing a product or in providing a service from the source to the end-users. With SCM, the exchange of information, movement of supplies and

transformation of products are facilitated through open collaboration and cooperation among these players.

One key element of managing the supply chain is to have an explicit knowledge and understanding of how the supply chain network structure is configured. In determining the network structure, it is necessary to identify who the members of the supply chain are. To integrate and manage all process links with all members across the supply chain, the key is to identify the basis for determining which members are critical to the success of the company and the supply chain, and thus should be all allocated managerial attention and resources (Lambert, D. et.al., 1998).

The value chain framework in Porter, M. (1985) is a model that helps to analyze specific activities through which firms can create value and competitive advantage. There are two sets of activities involved. The first set of activities are the primary activities (line functions) that include (a) inbound logistics - receiving, storing, inventory control, and transportation planning (b) operations - machining, packaging, assembly, equipment maintenance, testing and all other value-creating activities that transform the inputs into final product (c) outbound logistics - activities required to get the finished product at the customers: warehousing, order fulfillment, transportation, distribution management (d) marketing and sales - activities associated with getting buyers to purchase the product, including: channel selection, advertising, promotion, selling, pricing retail management, etc. and (e) service - activities that maintain and enhance the product's value. The second set of activities in a supply chain includes (a) procurement, (b) technology development to support value chain activities, (c) human resource management associated with recruiting, retention and compensation of employees and managers and (d) firm structure - general management, planning management, etc.

Three main paths are identified in the process of SCM, namely: (1) product flow that includes the movement of goods from a supplier to a customer, as well as customer returns, (2) information flow that involves transmitting orders and updating the status of delivery; and (3) financial flow that consists of credit terms, payments and payment schedules, plus consignment and title/ownership.

Supply Chain Management in the Food Sector

Food sectors such as agriculture and aquaculture involve a diverse range of distinct enterprises (producers, processors, marketers and distributors) and rely on inputs from various sources, often at distinct geographical locations (Hobbs, 1996; Williamson, 1979).

In developing countries these sectors are mostly composed of smallhold producers. These producers are information-poor and usually viewed as being the least powerful in the marketplace. On the other hand, traders are generally information-rich and are usually seen as yielding much of the power and doing so at the expense of producers. However, this is not always the case but rather there are instances that traders act as "supply chain champions". As the number of supply chains rise in developing countries, traders will have an important role in management (Ramasamy, 2007).

Smallholders operate in critical supply chains, thus, value chain becomes necessary for sustaining smallholder growth. A primary driver of the growing focus of the food sector on SCM is the changing competitive environment. Supply chain management provides one conceptual approach to build the capacity of domestic producers to match the products that exporting countries will be aiming to put into world markets. Thus, SCM in the food sector is an essential tool for integrating each step in the entire production and distribution process (from the farming of basic raw materials to delivery of final products to the consumer) so that the end product meets the value expectations of the consumers or end-users. This "biological manufacturing" is necessary to meet consumer expectations (Tveteras and Kvaloy, 2004).

For the consumers and other stakeholders, SCM focuses on improving the performance of the supply chain through the delivery of guaranteed safe, desirable, accessible and good quality food in a cost-effective manner. SCM, therefore, represents the management of the entire set of production, manufacturing/transformations, distribution and marketing activities by which a consumer is supplied with a desired product (Woods, 1999).

Benefits of Supply Chain Management

Supply chain improvement not only benefits the private sector but also creates spin-offs that stimulate social, economic, environmental and sustainable development in the country (employment generation, added value, minimization of product losses, etc.). Effective supply chain management improves data accuracy and reduces complexity in operations including supplier selection, purchasing, warehousing and distribution. Other benefits are greater productivity and lower costs, reduced inventory throughout the chain, improved forecasting precision, fewer suppliers and shorter planning cycles.

Specific gains in supply chain management include; reduction of product losses in transportation and storage, increase in sales, foster dissemination of technology and advanced techniques, provision of capital and knowledge among the chain partners, better information about the flow of products, markets and technologies, greater transparency in the supply chain, accurate tracking and tracing of product flows, better control of product safety and quality and large investments and risks are shared among partners in the chain. Efforts of targeting such potential gains were demonstrated for the Mexican tilapia (Vivanco-Aranda, et.al. 2010).

METHODOLOGY

Conceptual Framework

The framework in Figure 1 highlights the design of the study. Firstly, the inputs necessitate a comprehensive understanding of the industry and the various key players including their roles, processes, transaction flows and external influences. Secondly, an assessment of the supply chain requires the application of theories under the sphere of new institutional economics subsuming transaction cost, agency, networking, among others. Finally, the areas for improvement could be determined by evaluating the performance of the supply chain in terms of efficiency, flexibility and responsiveness.

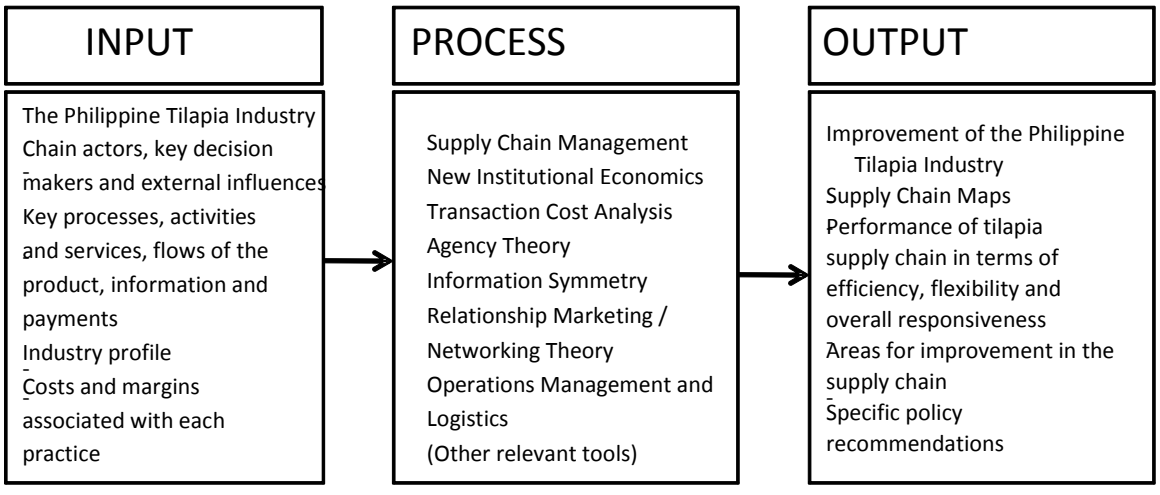

Figure 1. Research Framework for the Study

Figure 2 guided the study in the determination of the structure, business processes and management decisions in improving interdependencies of firms for customers' value (Porter, 1985 and Cooper et. al, 1997).

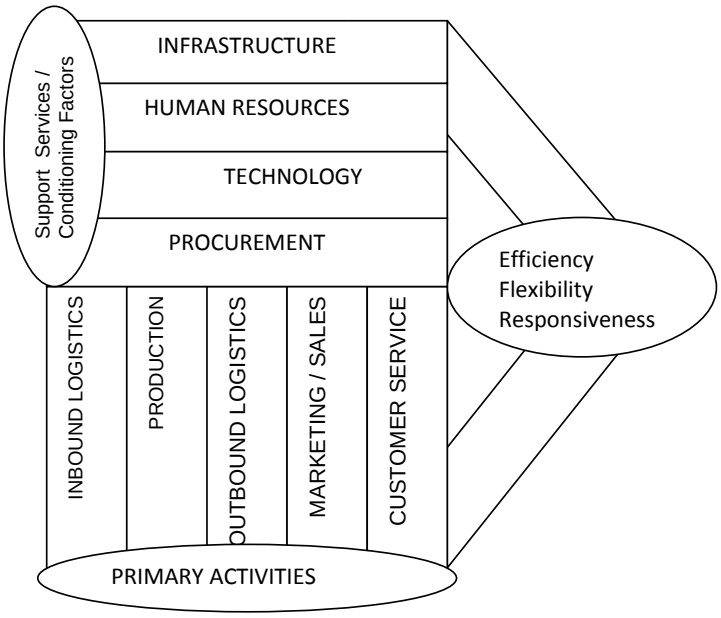

Figure 2.Value Chain, Porter (1985)

Based on the foregoing, the following activities were undertaken.

1. A synthesis of relevant studies to establish an overall picture of the industry in the study sites.

2. Identification of the members of the supply chain, key processes that required coordination (flows of the product, information and payments), activities and services conducted by supply chain members, critical logistics issues, key decision makers and external influences.

3. For each supply chain, at least one shipment from the product source to the ultimate destination was traced to: validate/verify all information in the supply chain drawn; document all practices at each stage of the chain; determine and quantify costs and margins associated with such practices; and determine and quantify the changes in product volume and quality along the chain.

After the supply chain has been validated and the impact of various practices along the chain was established, the next step was to identify areas for improvement of the supply chain. This was done through a participatory approach involving the supply chain members. Among others, identification of supply chain champions, the structure of power along the chain and the relative interest of members with regards to improvement in the chain were considered.

Study Areas and Coverage

The study covered Regions III, IV and NCR. In order to draw the major tilapia routes, at least one shipment in each of these regions, from the supply center to the ultimate end-user was traced.

Table 1 shows the total number of respondents covered, for each of the supply chains mapped. There are five chain players that perform either one process or a combination of processes depending on the degree of coordination in the chain, decomposed as follows: 5 hatchery and nursery operators, 28 farmers, 4 processors, 24 traders/consolidators/shippers and 11 institutional buyers.

Data Collection and Requirements

Primary data were obtained through survey, key informant interview and focus group discussions (FGD). FGD was also conducted to validate secondary information and to answer more specific questions related to supply chain mapping. A questionnaire was designed to answer key questions, among others: Who are the key costumers and what are their product requirements (especially quality standards)?; Who are the key players and what are their respective roles?;How do product, information and money flow through the supply chain?;What are the activities and services provided at each level in the supply chain?;What are the critical logistics issues?; and What are the external influences?

Table 1. Number of respondents covered in the tilapia supply chains

Routes of SC mapped	Supply Chain Players	Number of Respondents
Bicol-Laguna-Batangas-Manila-Baguio (Chain 1):	Hatchery and Nursery Operators	3
	Fish farmers	15
	Processors	2
	Traders/consolidators	8
	Institutional buyers	5
Pampanga-Pangasinan-Ilocos and Isabela - Baguio and Manila (Chain 2):	Hatchery and Nursery Operators	2
	Fish farmers	13
	Processors	2
	Traders/consolidators	16
	Institutional buyers	6

Secondary data series on tilapia statistics was obtained from various agencies such as the Bureau of Agricultural Statistics (BAS), Bureau of Fisheries and Aquatic Resources (BFAR) and other relevant agencies of the Department of Agriculture (DA). Previous studies on the production and marketing of tilapia also served as sources of secondary information. Likewise, Central Luzon State University and other relevant institutions served as sources of secondary information. Finally, officers and staff of appropriate government agencies and other industry personalities composed the key informants' pool.

Data Gathered

The following data were gathered: the key players and their respective roles activities and services provided at each stein the supply chain product requirements (especially quality standards)product, information and money flows critical logistics issues (including problems in production and marketing)extension services and external influences.

Data Processing and Analysis

Methods of analysis utilized for each of the stated objectives are presented in the following table:

Table 2.Objectives and methods of analysis

Objectives	Methods of Analysis
(1)To provide an overview of the tilapia industry	Synthesis of relevant studies and trends
(2)To map out the specific supply chain for tilapia	Flowchart analysis from downstream to upstream
(3) Analyze the performance of the tilapia supply chain in terms of efficiency, flexibility and overall responsiveness	Descriptive statistics, and relevant performance metrics (both qualitative and quantitative)
(4) Identify areas for improvement in the supply chain such as behavioral, institutional and process	
(5) To provide specific policy recommendations to improve the tilapia industry in general, and the specific supply chain in particular	

Areas for improvement in the supply chain were identified and specific policy recommendations were formulated with the end in view of improving the country's tilapia industry, through an improved supply chain management.

DISCUSSION OF RESULTS AND FINDINGS

The Philippine Tilapia Industry: An Overview

In 2008, the country's tilapia production was estimated at 299,813MT(BAS 2009). Out of this, regions III and IV contributed about 80% while the rest of the regions shared the remaining 20%.

The top 5 producing provinces (in descending order) include Pampanga (37.68%), Batangas (21.06%), Laguna (4.64%), Rizal (4.06%) and Bulacan (3.58%).

Tilapia production is increasing rapidly because of the following reasons: tilapias possess most of the desirable attributes of a good culture species; they reproduce readily; they are hardy; they can tolerate poor water quality (low oxygen, high ammonia); they can accommodate wide salinities; they can adapt to a variety of culture conditions (low density pond to very intensive systems; mono- to polyculture; they have versatile food habits; and they are highly marketable (Shelton,2002).

In the Philippines, tilapia is produced in freshwater fishponds, freshwater fish cages, brackishwater fishpond and freshwater fishpen with extensive to intensive culture systems. In 2002, 57% of the total tilapia production in the country came from freshwater fishponds, 38% from freshwater fish cages, 7% from brackishwater fishpond and 1% freshwater fishpen (BFAR, 2004).

There are three types of tilapia in the country: Nile, Red and Javanese (BAR digest, 2010). Nile breed of tilapia is the main species cultured in the country at freshwater ponds and cages in Central and Southern Luzon which contributed 87% to total tilapia production in 2000 (BFAR, 2001). The other important species are the Mozambique tilapia and its hybrids with O. Niloticus and O. Hornorum which are produced in brackishwater ponds.

The most popular tilapia strains adopted by the small-scale tilapia farmers in Central Luzon were the BFAR Genetically Enhanced Tilapia (GET), strain (GET Excel Tilapia) and Genomar Supreme Tilapia (GST) because of their growth performance.

Some barriers in starting tilapia farming venture include: lack of capital for financing pond construction and operating expenses; high input prices, particularly of feeds; unsuitable farm location, i.e., flood prone and no access road lack of technical expertise; unreliable water supply; low farm gate price of tilapia.

In addition, the most common problems experienced by the tilapia farmers were: high feed prices; high fertilizer prices; declining net profits; high cost of pond construction; and presence of tilapia predators, such as Channastriata (snakehead), Clarias spp. (catfish), and bullfrogs.

Important inputs to tilapia culture are the quality of seeds and broodstocks. With the efforts of Research and Development (R&D) agencies such as CLSU, BFAR, GIFT foundation among others, many hatchery farms were established across the country. However, despite the combined production outputs of the same the demand for fries and fingerlings cannot be filled up. Many producers adopt direct and indirect stocking due to high mortality rates and longer culture periods (Tan, et.al. 2009). Those who stock directly have a higher density per m^2 than their counterparts. Those who stock indirectly require larger-sized fingerlings from 17-14, hence, offering opportunities for nursery operations.

Other inputs consist of capital investments such as the land, water supply system, drainage system, pump and equipment/paraphernalia (seine net, weighing scales, tubs/buckets). It also includes the operating capital – fingerlings, fertilizer (chicken manure and ammonium phosphate), supplemental feeding (fry mash, fish starter, fish grower, fish finisher), labor, fuel/lubricant and electricity. Each item affects the operation of the tilapia industry particularly on feeds which accounts for about 73% of the operating capital (BFAR, 2010).

Marketing system of tilapia in the Philippines is as varied as the locations of the supply source. The traditional marketing of tilapia in some places was relatively simple. Traders normally picked up the harvested tilapia at the farms. Most tilapia farmers sold it to wholesalers-assemblers. Some sold the tilapia produced to retailers, consumers, and brokers. In Central Luzon, some distributors and retailers procure tilapia in "pakyaw" or (bulk and assorted) basis and these are hauled from the supply area. Moreover, there are also some traders that take charge in harvesting the produce and pay all the expenses during the activity. This practice is very common in the province of Pampanga (BFAR, 2002). Some traders, particularly wholesalers, finance small-scale farmers in order to be assured of a steady supply of fish. Under this arrangement, the farmer is mandated to sell exclusively to the trader at a pre-agreed price. Major marketing issues for tilapia include fluctuating prices, irregular supply, nonpayment of debts by traders, informal levies (particularly when transporting the product), and seasonal off-flavors that render the fish less marketable.

Moreover, the supermarket phenomenon and more liberalized trading environment have induced the emergence of market niches such as the fillet, smoked, dried and other processed forms. Links with these new markets and increased participation of small-scale producers requires a new approach to tilapia marketing.

Key Customers and Product Requirements

The tilapia chain key customers are classified into two types: the institutional buyers (hypermarkets or restaurants/specialty food shops) and the household-level/end-users or consumers.

Product Form

Generally, household customers prefer live whole tilapia with firm meat and with the size of 4-5 pieces per kg(200 - 250 g per fish).Also, regular tilapia consumers in Manila and Southern Luzon are indifferent relative to the source and skin color of tilapia. However, consumers in the Northern Luzon markets such as Pangasinan and Baguio exhibit similar product requirements except they prefer darker skinned tilapia because of more belly fats and tastier as perceived by them. The common food recipes for tilapia are charcoal grilled, fried, boiled and "paksiw". Most of these customers buy tilapia from fish vendors at local public markets or stalls. Regular customers in Laguna require daily volume of about 1,700 kgs while Manila customers require 2,500 kgs a day.

Institutional buyers such as specialty shops, hypermarkets or malls, restaurants and food chains cater to relative affluent customers with varied product requirements. Hypermarkets normally require live whole fish with size from 3-4 pieces per kg (250 – 350 g per fish).Institutional buyers of this sort are indifferent to the source of tilapia as long as suppliers meet the fish size, volume and delivery requirements as stipulated in the marketing contract. Hypermarkets in Manila and Laguna normally require 500 – 1000 kgs of tilapia per day while Pangasinan and Baguio require a daily volume of 65-70 kgs, respectively.

In the case of specialty shops and food chains such as Monterey shops and SM South Mall, Central Barbeque Plaza in Parañaque and Ineng's Barbeque shop in Global City, tilapia fillet and whole frozen fish are more preferred than whole live fish. By-products of filleting are absorbed by specialty restaurants selling fish soups, tilapia belly and deep fried tilapia skin. The specialty shops require consistent fillet size and volume. The total volume requirements of these institutional buyers is 1000 kgs daily with a fish size of 1-2 pieces per kg (450- 600 g per fish).

Volume requirement

The total volume requirements of the supply chains serving major customers in Luzon average about 5,335 kgs daily or approximately 1, 947,275 kgs (or ~ 1,947.28MT) yearly. With the per capita consumption of around 3.81 kg (or ~323,850 MT), these chains could barely meet 1% of the consumption requirement of the country.

Major Players and Their Activities

The major players and their activities are highlighted in Figure 3. The **hatchery and nursery operators** supply the fries and fingerlings as well as provide techno-guides to fish producer - customers. Both operators are closely linked and coordinated with each other. The **hatchery operator** handles about 3,000 breeders (Genomar crossed with IDRC strain from BFAR and CLSU) that produce 1.5 million sex-reversed fries every 18 days. The hatchery farm is located in Ligao City, Camarines Sur which is around 600 kms south of the nursery operator in Cabuyao, Laguna. Due to the travel distance, 33 plastic bags(i.e. imported from Taiwan) with no bottom corners each containing 400,000 fries were used to reduce stress and minimize fries mortality while being transported in 4 rented trucks each with 6-ton capacity. Oftentimes, the **hatchery operator** pays "goodwill fees" to traffic enforcers on top of the regular toll fees charged by the superhighways in Manila. The operator maintains also a nursery pond to serve other farmer-

customers requiring bigger fingerlings in nearby towns of Buhi, Baao and Bato, Camarines Sur. These customers usually require also 400 thousand fries every 18 days. Normally, the hatchery operator charges P0.05 (or 25%) higher than other competitors in the area because fries are already sex-reversed. Upon reaching Laguna, delivered fries are immediately unloaded to the conditioning pond for acclimatization. The **nursery operator** will then manage and maintain the fries until reaching its marketable sizes of 14 and 12 as ordered by regular producer-customers (chain members) in Laguna and Batangas. Normally fries will be rested for 1 month period then it takes 3-4 weeks to reach size 14 for sex-reversed fingerlings otherwise it takes 1 and 1½ months for non-sex reversed. The nursery ponds handle various tilapia strains since the regular customers would tend to try other strains in their operations. Usually, the **nursery operator** delivers fingerlings 4 times per week to its regular customers. Also, the sizes of fingerlings delivered vary with "season" i.e. smaller size (22-20) during on-season months (May, June, July, August) while 14-12 on off-season (September, October, November, December). Finally, the nursery operator prefers tilapia nilotica as a better strain compared with other tilapias.

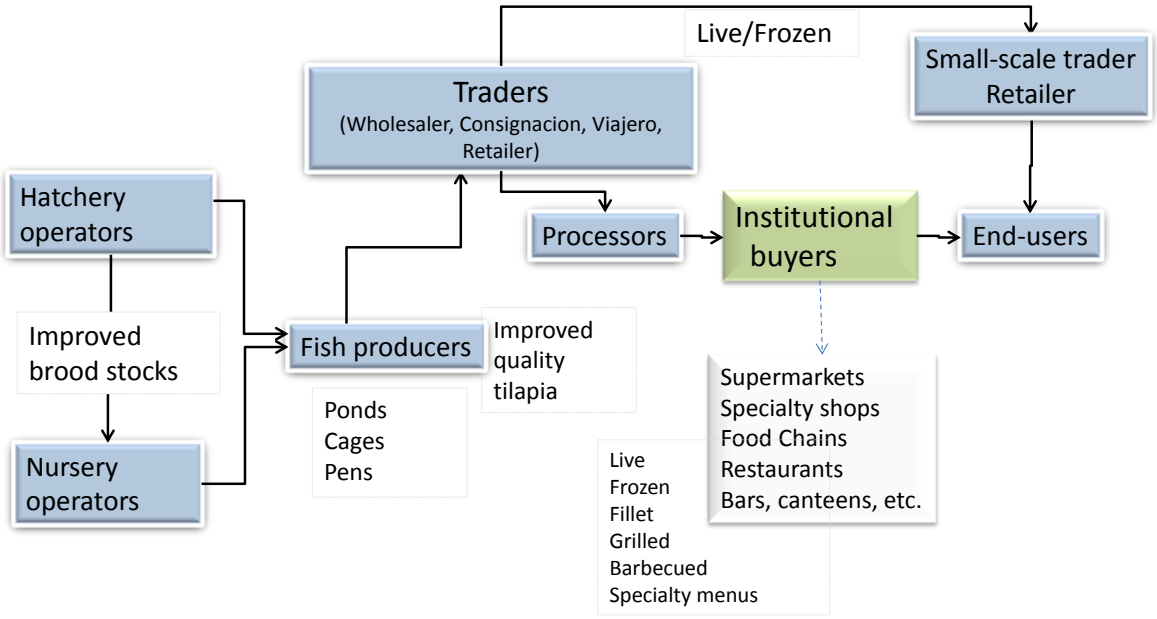

Figure 3. Major Players and their Activities

The **fish producers** are the main "production centers" of the tilapia chain whose focus is to produce marketable tilapia for distribution through traders for end-user or consumers and processors for institutional buyers. The regular producer- customers usually take 2.5 – 3 months to grow their fishes to reach marketable size of 4-5 pieces per kg and about 3.5-4.5 months to reach 2-3 pieces per kg. **Fish producers** (chain-members) are aware of the product, volume and delivery requirements of their trader-customers. To reach markets in the Northern Luzon which prefer darker colored skin tilapia, fish producers will grow the tilapia first in Laguna under semi-extensive feeding regime say 2-3 months then transfer the fish to Taal Lake in Batangas for conditioning say in 3 weeks before harvest – a kind of "circuitous" production technique. Most **fish producers** do not have delivery trucks and cold storage facility, thus, they have to wait for their customers to haul their fish harvests on farmsite. Other producers (non-chain member) in Laguna and Batangas persisted to stock non-sex reversed fingerlings since its cheaper and it perform at par with sex-reversed counterparts as they claimed. Contrary, it takes longer time to reach equivalent weights or sizes of fingerlings. Hence, they are losing more in terms of low fish recovery of about 18-20% in Laguna lake while 25-30% in Taal lake and high feed costs.

Occasionally, customers of non-chain member complained about the off-flavor taste of their tilapia. Accordingly, farmers' knowledge on preventing repeat of such incidents is fairly limited.

Traders generally subsume the terms **consolidators/wholesalers/retailers** and brokers or agents a.k.a. **consignacion** since they are all engaged in the buying, selling and distribution of tilapia from sources to various destinations depending on which market level they operate. The **consolidators** are the big traders who supply regularly supermarkets and bulk buyers in major fish terminal markets (or transshipment points). At the terminal markets, the **consignacion** facilitates the transactions between the traders (shippers or viajeros) and bulk buyers (provincial traders) for a "commission fee". Since they own a stall at the terminal market, they also act as gatekeeper of the traders and key player in the price discovery process, thus, they also perform price monitoring and occasional small-scale trading. The **wholesalers** are themselves shippers or viajeros who buys tilapia from the terminal markets in bulk and ship them to other bulkbuyers serving other geographical markets. Strategically, some wholesalers resort to backward integration by self-producing tilapia and contracting other farmers to meet market commitments and reduce supply risks. The **retailers** are the smallest traders in the market chain which finally caters to the end-users. They owned stalls at public markets and small delivery vehicles such as tricycles and owner jeeps with aerators. Their sales volume depends on the deliveries by local traders. Since they compete with many retailers, they handle about 100-150 kg of live fish (with average size of 5-6 pieces per kg) for easier disposal and minimize unsold products for each transaction day.

Processors are those who supply regularly the specific product forms such as the fillet, cubes, whole frozen and choice portions or trimmings by institutional buyers(either as supermarket, specialty food shop, food chain, bar and restaurants, canteens, among others. It had been operating and registered in 1995 as Fishda Enterprises. Now it is incorporated and named as Unavis. Its operation is accredited by Department of Food and Drugs. Product forms, volumes and deliveries depend on the arrangements with the various customers. Presently, the customers include Monterey specialty shops, SM Southmall, Metro Bank canteens, Central Barbeque Plaza in Parañaque, Ineng's Barbeque at Global city, Setton Golf Club, etc. To maintain customers, the **processor** should ensure that raw materials meet the size, volume and meat quality requirements needed for processing. The processing plant has 1.5-2 ton capacity (processing-in - demand) with 6 – ton cold storage capacity of filleted tilapia at one time. The plant maintains a "comfort" or safety stock level of 5 tons. The filleting process for a per kilogram raw tilapia (2-3 pieces per kg) yields the following: 30-35% fillet, 18% belly, 25% innards, 21% head and 1% skin. Because the cost of filleting is about P35 per pack (1 pack = 300 grams), cost recovery has to be taken from the sales of the by-products. The **processor** could not raise the price of tilapia fillet due to cheaper import alternatives like the pangasius and dowry fillets. To increase the shelf-life and maintain quality of products, quick or blast freezing is necessary. Increasing the present capacity of cold storage and blast freezers entails additional cost which is unaffordable at the moment. Finally, only few tilapia producers can meet and assure volume, size and quality of raw materials, the processor cannot expand market coverage. Many orders and inquiries from potential high-end customers including Philippine Airlines, Cathay Pacific, five star hotels and restaurants, etc. were turned down.

Major Routes of Products

Figure 4 shows the tilapia supply chain's major routes in Luzon. The cities of Malabon in Manila, Angeles in Pampanga and Dagupan are the major transshipment points of tilapia in Luzon; with cities in Metro Manila, Angeles and Baguio; La Union and Ilocos provinces, Isabela and Cagayan Valley provinces and the Cordillera Administrative Region, being the major demand centers. Pampanga, Batangas and Laguna provinces are the major production centers while Pampanga, Laguna and Camarines Sur are the major hatcheries concentration.

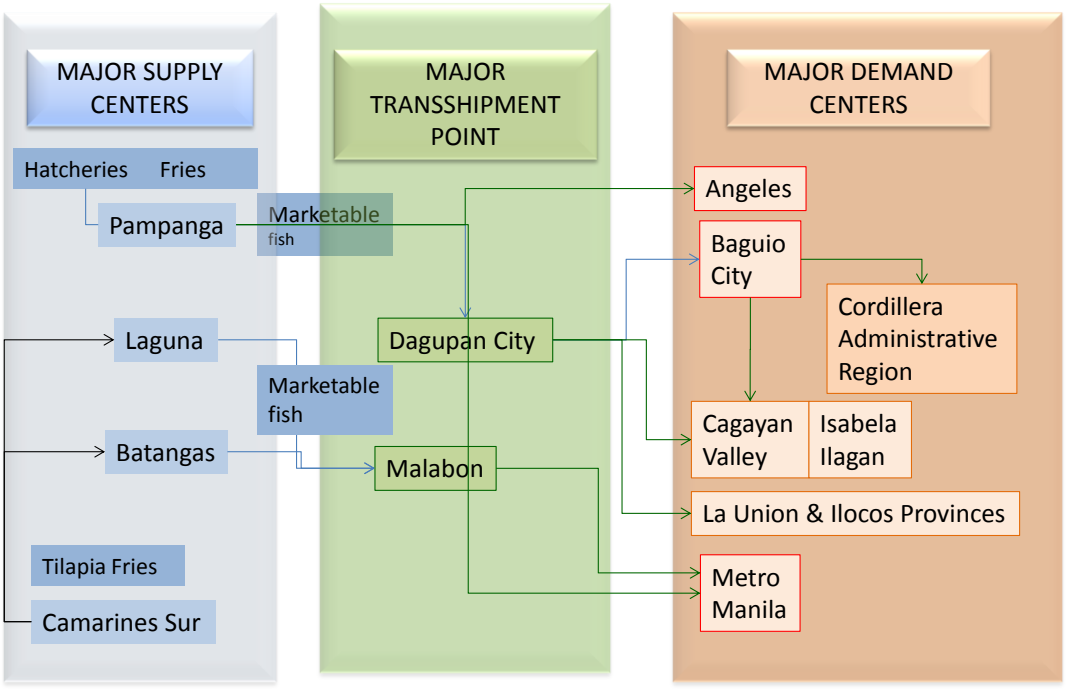

Figure 4. Major Routes of Products

The geographical locations of the major routes are depicted in Figure 5. There are two major routes traced namely: (1) Laguna/Batangas – Manila/Baguio; (2) Pampanga – Pangasinan/Baguio. These routes normally take from 1 to 3 days to distribute from source to final destination points. The time period used to assemble the required volume of tilapia with consistent size is the bottle neck for meeting the entire delivery schedule of tilapia in the supply chain.

Figure 5. Major Routes of the TilapiaSupply Chain

Figure 6 describes the product flow of route 1. Eighteen-day old tilapia fries from Camarines Sur hatchery are brought to the nursery operators in Cabuyao, Laguna, for conditioning and growth in about 45 days to reach fingerling size of 14-12. It will then be delivered to grow-out operators in Laguna and Batangas for 2-3 months before harvest. Fish sizes range from 4-5, 3-4 and 2-3 pieces per kg. Small fishes will be sold to local markets inLos Baños and Pila, Laguna. The larger fishes will be delivered to supermarkets in Calamba and Sta. Cruz, Laguna. Largest fishes will be delivered to the processors in Los Baños, Laguna and Parañaque. Fishes intended for the Northern Luzon supermarkets such as Rosales, Pangasinan and Baguio City will be transferred and conditioned in Taal Lake for a period of 3-4 weeks to ensure that fishes will have dark colored skin before marketing.

In the case of Batangas, fries with size 22-20 from Calauan, Laguna hatcheries are brought to grow-out operators in Tanauan, Talisay and other towns along the Taal lake. Large fish producers (with 500 cages) in Batangas usually stock 200/m^3. Their fish recovery in Taal lake ranges from 25%-30%. Furthermore, their FCR on the average is 1.5:1 while Laguna producers have lesser. After 6-8 months culture period, marketable tilapias are picked up by wholesalers and brought to Malabon, in Manila being the major transshipment point for seafood to the rest of the markets in Metro Manila and the rest of Luzon. Smaller traders and other provincial traders will both source their fishes from wholesalers through a consignacion in Malabon market. Fishes are then packed in ice boxes for distribution to supermarkets and far-flung markets.

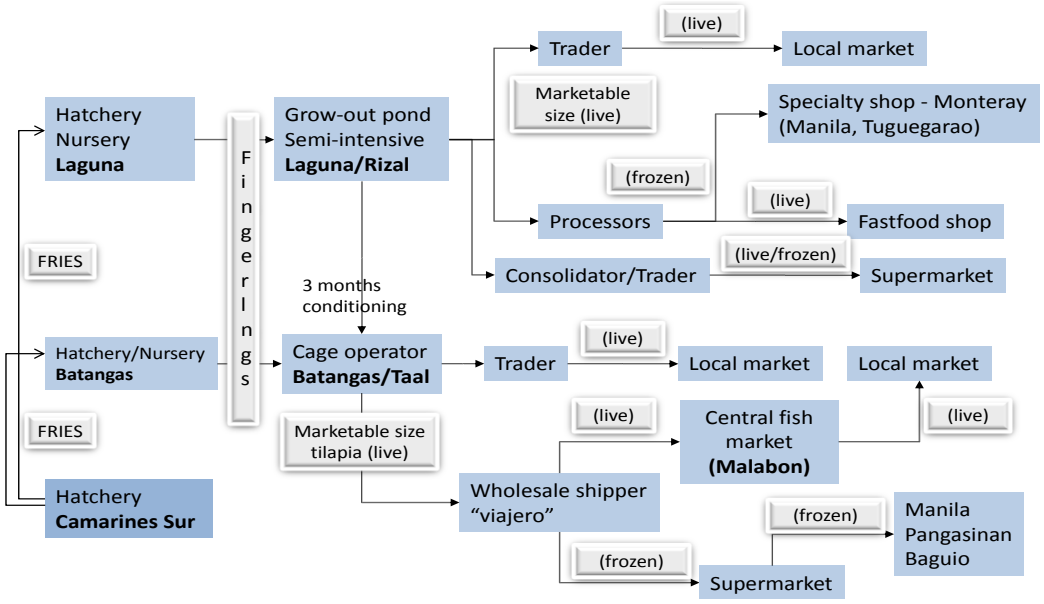

Figure 6. Product Flow (Laguna/Batangas-Manila/Baguio-Route 1)

The flow of products in route 2 is shown in Figure 7. Pampanga fish producers usually source their fries from nearby hatcheries and stock them directly in their grow-out ponds. After 6-8 months culture period, tilapia are harvested using mesh nets since ponds sizes range from 2-12 hectares on the average. Some larger farms have about 25-100 hectares surface areas. Most farmers do not have trucks equipped with tanks and aerators, thus, they have to wait for traders through an agent to pick up their harvest and bring it to the fish terminal market in Pampang, Angeles City as the major transshipment point of Pampanga.

Wholesalers and local traders including consolidators source their live tilapia at this market. Wholesalers then bring their tilapia to consignacion market in Dagupan City, Pangasinan serving as transshipment point to the rest of the Northern Luzon markets. Fishes usually reach this market alive which is preferred by most if not all customers. However, once passed on to other provincial traders whose markets are farther, fishes must be stacked with ice to retain freshness upon reaching destination points. Normally it takes around 1-2 days to reach some markets in Ilocos and Isabela provinces together with Cagayan Valley and Cordillera Administrative regions.

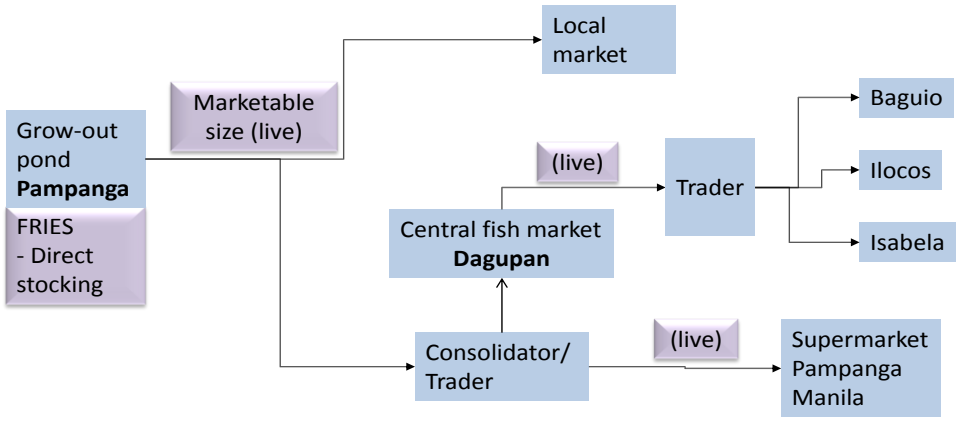

Figure 7. Product Flow (Pampanga-Pangasinan/Baguio-Route 2)

Payment Flow

In general, payments are made on spot cash and cash-on-delivery (COD) between the local consumers and retailers; wholesaler and trader/consolidators; processor and producers; small-scale traders and producers. However, bank payments through 7 day post-dated checks are made among hatchery/nursery operators and producers; supermarket and processors/traders or consolidators (Figure 8).

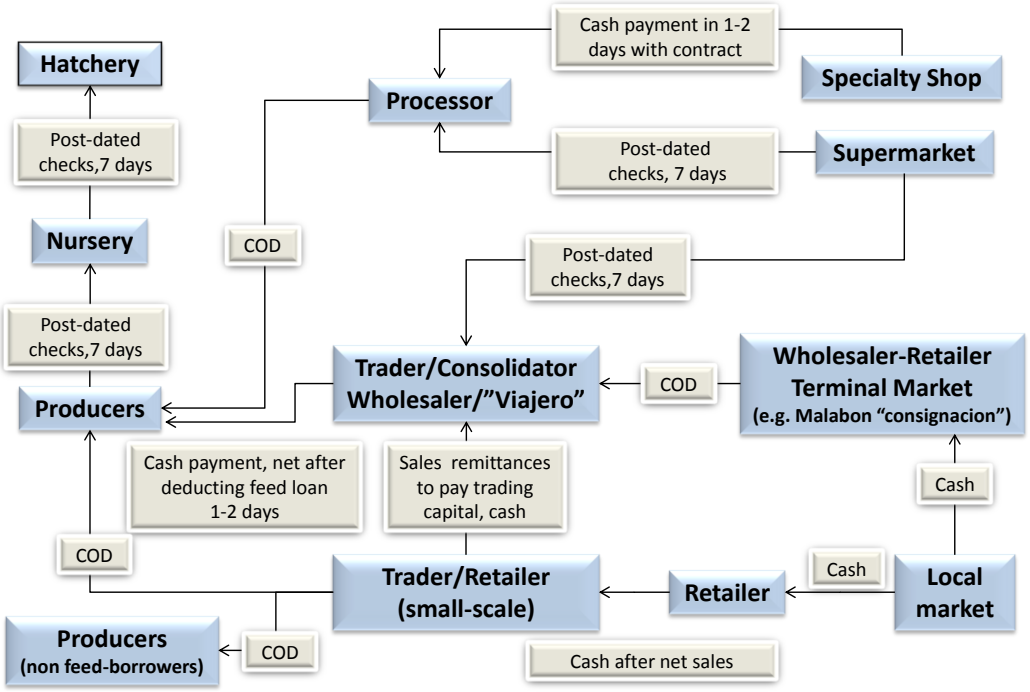

Figure 8. Payment Flow of the Supply Chains

Only the specialty shops advance payments for about 1-2 days before product delivery to the processor as stipulated in a contract. On the other, the trader/consolidator who loan out feeds to producers and trading capital to small-scale traders depict a different arrangement. The small scale-traders will check on the exact harvesting dates of the farmer-borrowers. Upon harvest, these traders will weight, transport and consign the tilapia harvest to the local retailers at an agreed price. After each transaction day, the retailers remit the net sales proceeds to the small-scale traders who will in turn remit the same plus the trading capital equivalent to the fish volume purchases from the producer-borrowers to the trader/consolidator. After deducting the cost of feeds, the trader will pay the producers the net sales value of their tilapia.

Information Flow

Figure 9 shows the flow of information among the supply chain members. Information exchange between and among the chain members and the mode of contact are done through face-to-face and mobile or telephones which are concluded in a short period of time. The price, sources, quality, availability and delivery schedules of tilapia are the major information required by the chain members. Farm gate prices are low and more unstable than those in the wholesale and retail levels. Such behavior is prevalent because institutional buyers are slow to react with price

changes. Another, the processor maintains a price level that was two years ago for fear of losing customers with or without contracts. Retail prices tend to be sticky upwards but faster to adjust downwards.

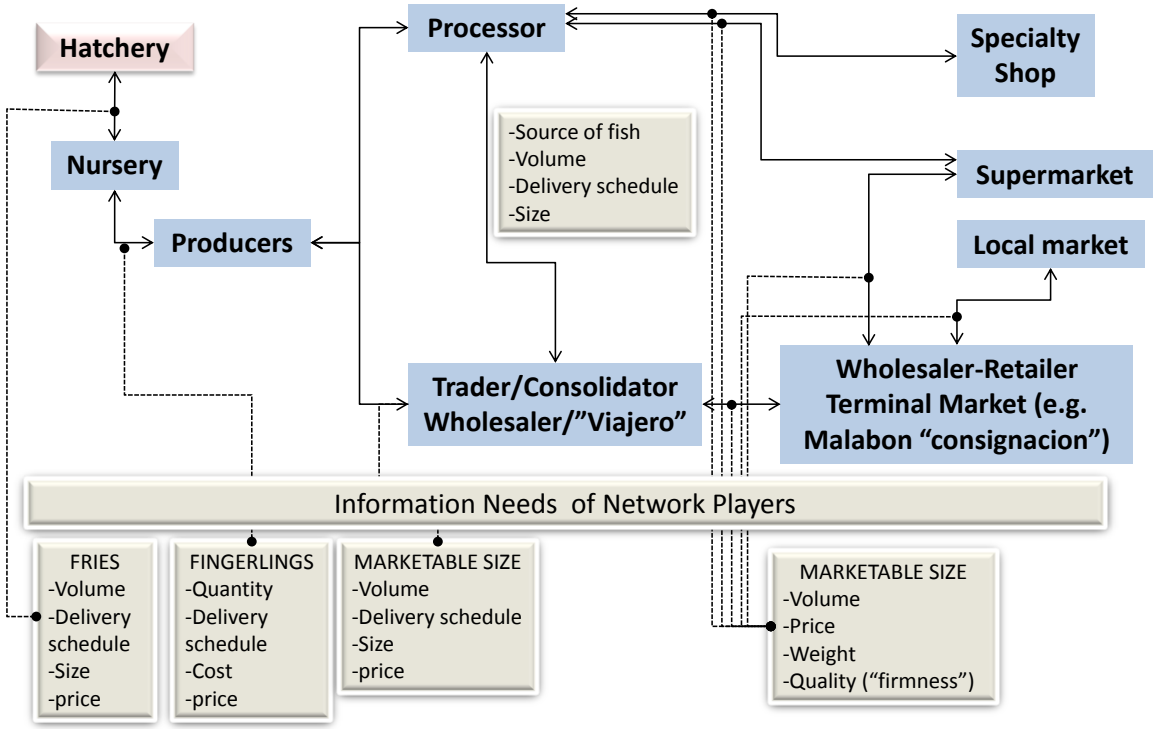

Figure 9.Information Flow of the Supply Chains

External Influences

(1) Production and market support programs of the government

Recognizing the vital importance of tilapia to address poverty alleviation and development of the country side, the government had embarked on tilapia upgrading program through genetic improvement projects espoused by CLSU, GIFT, BFAR and other international R&D agencies. This program had effected the participation and entry of many tilapia industry players. Additionally, the establishment of hatcheries and dispersal programs of BFAR had facilitated the extension of broodstock quality improvement of tilapia into the countryside. Likewise, training and capacity building activities among tilapia farmers in terms of improved technologies in management, nutrition and health aspects of tilapia growing. The continuing improvement of broodstock and dispersal program will help foster the growth of the industry. On the other, the market support program of the GOP is limited to market matching and participation in aqua fairs.

(2) Food safety through permits and accreditation

In preparation towards globalization, BFAR had instituted an accreditation protocol for quality assurance of meeting export standards. However, many found it to be very rigid hence restricts

the potential exporters to qualify. Unfortunately, fish imports continually to flood the domestic market which dampens further the competitiveness of the local industry.

(3) Presence of "rent-seeking" behavior of law enforcers

In addition to the toll fees in superhighways paid by traders or viajeros including hatchery operators, unreceipted fees are charged that serve as goodwill to rent-seeking law enforcers at check points. Such expense is usually passed on to the final consumer.

Issues and Concerns

The major concerns of hatcheries and nurseries are the high cost of outbound logistics, which is exacerbated by high competitive pressures of inferior quality but inexpensive stocks (e.g., non-sex reversed) and high levels of mortality due to environmental and cultural factors.

The fish farms' major concern, on the other hand, is the expensive but low quality feeds (at times mislabeled) and other inputs coupled by very low fish recovery of about 25% in lakes' cages or pens and about 60% in ponds system. In addition, the more pronounced variability in climate pattern had induced more variability in production volume hence, overstocking became a "recouping mechanism" among fish growers. Moreover, the grow-out period ranges from 6-8 months to reach a marketable size of 250-400 grams per fish across production systems. Their transaction costs include the cost of waiting for buyers, delays in delivery, in-transit mortality, and toll fees or "goodwill" as well as shrinkage losses. In addition, the lack of cold storage and transport vehicles equipped with tanks and aerators or refrigeration facilities delimits farmers to take market opportunities in terms of value-adding and processing activities. Interestingly, due to the high consumers' preference on "darker tilapia", many farmers adapted a "circuitous" production technique i.e. fries from the hatcheries say in Bicol (pond based) were transferred to nurseries' pond in Cabuyao, Laguna then moved and raised in a semi-intensive grow-out environments in Laguna lake then finally transferred and conditioned as "dark tilapia" within 3 weeks in another place like Taal, Batangas to take advantage of such marketing premium.

The major concerns of processors are too few farms that could supply regularly the desired quality and volume of tilapia at each process-in-demand period, likewise, the lack of blast freezers to maintain higher quality products while maintaining longer shelf-life of products and other derivatives. Moreover, due to high cost of filleting and low dressing recovery, processors' could hardly compete with the influx of cheaper imported alternative fillets like pangasius, sea bass and others, saved by the revenues derived from by-products such as heads, bellies and skin. Demand for choice portions and trimmings by high-end institutional buyers like Philippine Airlines and Cathay Pacific remained untapped. Also, other test markets that showed bright prospects are the tilapia nuggets and fingers.

The concerns of traders including "consignacion", suppliers or consolidators are the following; (a) regularly meeting the quantity and delivery schedules of their customers is undermined by their defaulting "contract tilapia farmers" (b) high logistics and transaction costs of searching, locating, assembling and distributing fishes from sources to destinations (c) lag responses in unexpected price movements and the absence of product grades and standards contribute to the difficulty of maintaining a "profitable" volume of operation.

RECOMMENDATIONS

The following are some recommendations to address the various issues and concerns namely of the various chain players: (1) encourage the establishment of more nursery farms of better quality brood stocks while intensifying technology transfer to farmers for better health and management of tilapia (2) conduct market promotion activities highlighting the various niche

opportunities of tilapia among growers and consumers (3) motivate the participation of small farmers in supply chains by setting up an incentive scheme through a mix of patronage refund and profit sharing (4) institutionalize an accreditation program for feed manufacturers, hatcheries, processors and the like to improve the quality assurance of products and services (5) provide capital windows to improve facilities and reduce logistics and transaction costs in the entire supply chains of tilapia.

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Appendix Figures

Appendix Figure 1.Majorchain players

Consignacion

Dagupan and Malabon

Traders

Wholesaler/Viajero

Retailers

Appendix Figure 2. Traders and retailers at fish terminal markets

End-users

Institutional Buyers/Processors/ Specialty Shops







Appendix Figure 3. Fillet-in-process, final products, by-products and product display

DEVELOPMENT OF SUSTAINABLE AQUACULTURE PRACTICES IN TABASCO, MEXICO USING NOVEL IAA TECHNOLOGY

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Abstract

The treatment and discharge of aquaculture effluent and resulting negative impacts on the environment remains a critical issue that is threatening the sustainable growth of the aquaculture industry. Three optimal sites has been selected to carry out IAA systems in order to deplete eutrophication, two indigenous communities, one from the highlands and other one from the wetlands were selected to produce agro and aqua products with the same amount of energy, also a demonstration system is building at UJAT. Part of our progress so far is: two workshops; the first one on integrated systems and the second one on bioflocs systems with more than 60 attendants among farmers, students and technicians. In Caridad Guerrero the highland indigenous community we have a 90% progress for the setting up phase, habanero pepper will be growth with Tilapia water effluents. In the wetland community there is a progress of 40% the group is already organize and training is given, the demonstration system at UJAT has a 30% progress, materials and instruments have been already purchased and the design was made. In overall the project suffered a delayed due to major flooding events in the region.

Introduction

The treatment and discharge of aquaculture effluent and resulting negative impacts on the environment remains a critical issue that is threatening the sustainable growth of the aquaculture industry. Even the discharge of effluent that has been treated to levels acceptable under limitation guidelines (ELGs) through the use of dilutions, may pose long-term environmental risks. The resulting negative impacts will continue to intensify in the future if sustainable practices are not developed in commercial scale.

The first phase of a two phase project focused on the development and testing of sustainable aquaculture systems—specifically, the development of technology that combined agriculture and aquaculture technology. Such systems are often referred to as aquaponic systems as the water in which the fish are living in is used as a nutrient rich water source to stimulate plant growth. The technologies in the current research program were a novel variation from existing concepts, however, as they were developed as re-circulating systems and the plants were imbedded in soil and supplied with irrigation water from surface drip systems. Subsequently, the water migrates through the soil profile, collects in sub-surface collecting drainage canals built at negative slopes, and migrates down the canals and collects in a terminal basin. From this basin, the water is pumped back into the aquaculture tanks. Alternatively, plants in many aquaponic systems are grown in styrofoam trays that float on top of a water medium. Roots from the plants are allowed to grow submerged in the water which is infused with the nutrient rich aquaculture effluent, thus eliminating the need for using soil mediums. While hydroponic and aquaponic technologies have proven to be highly productive and efficient in commercial scale, the technologies developed in phase I of this program allow for a more diverse number of crops to be generated. Moreover,

these systems are relatively simple and were developed with the goal of implementation in Tabasco, Mexico. Because more plants can be grown in soil based mediums compared to hydroponics systems, RIAA technology offers the advantage of diversifying the product for improved income as well as providing a more diverse diet to local communities.

Approach

The approach towards implementing novel agriculture technology in Tabasco is being carried out, through the training of local farmers, demonstration of concept through development and support, and longer term monitoring. The project team was formed through an existing strong partnership between the University of Arizona's College of Agriculture and Life Sciences (CALS) and the Universidad Juarez Autonoma de Tabasco (UJAT). Business management practices were also supported by Arizona's Eller College of Business Management.

Firstly, our student led team held a training program at UJAT for rural and commercial farmers in the nearby regions. This training seminar was given by Dr. Kevin Fitzsimmons, Dr. Dennis McIntosh and Dr. Rafael Martinez-Garcia, which were designed to engage nearby farmers and to describe to them the techniques and practices of RIAA systems including technical aspects, animal and plant management, and essential simplified business practices. The second component of this project was to build demonstration systems in two small indigenous communities in Tabasco. Along with the demonstrations, our student led team will provide workshops in the indigenous languages (with collaboration with the Universidad Intercultural del Estado de Tabasco; an Indigenous University) (Fig. 1) describing the benefits of the integrated farms and how best to manage the farm to increase income and reduce chemical costs.

Figure 1. Training on site of IAAS systems and hands on practical activities

Summary of Findings:

The project suffered a major delay in its development due to severe flooding events that impact the three selected sites. Fig. 2.

a)

b)

Figure 2. A) Flooded State highway Villahermosa-Teapa. B) Before and after flooding event, massive erosion and damage

All the progress in Caridad Guerrero site suffered severe impacts. Agricultural beds were destroyed with heavy sediments and water erosion, the whole project had to be rebuild. The other two sites also suffered flooding impacts for more than three months. The Villages were abandoned and people stayed in emergency shelters. All the activities programmed at these sites were delayed; at UJAT a portion of land was filled with sand to raise the land level. We have rescheduled our activities in both sides for January 2011.

Workshop 1: Integrated Agriculture-Aquaculture

From August 12 - 19, students from the P3 award team along with Drs. Dennis McIntosh, Kevin Fitzsimmons and Rafael Martinez from the Universities of Delaware and Arizona respectively, presented an overview of integrated agriculture aquaculture systems (IAAS) at the Universidad Juárez Autónoma de Tabasco (UJAT) to 34 participants. This workshop provided an opportunity to introduce project group members to other members of the University, to indigenous community farmers, extension agents, Professors and also participating members of nearby farming cooperatives attending the workshop. In conjunction with educational training, the workshop included several visits to nearby communities where development of small-scale integrated farming operations were either being planned or currently being developed.

Workshop 2: Bio-Floc

From September 6 – 10, a second workshop was held at UJAT to discuss the many aspects of maintaining bio-floc in integrated systems. Several of these more advanced themes attracted approximately 30 participants each, representing farmers, hatchery staff, professors and students. Maintaining bio-floc material in aquaculture systems is appropriate in regions like Tabasco, Mexico where high protein diets may be prohibitively expensive, but power for aeration is subsidized and reliable. Bio-flocs are characterized by a mixture of nitrifying , autotrophic and heterotrophic bacteria along with several kinds of algae, and have been shown to be a very nutritious for several kinds of filter feeding juvenile fish (such as those used in our proposed technology) as well as crustaceans including shrimp. Originally, a large component of this workshop was dedicated to visiting a number of aquaculture facilities in nearby rural regions, where on-the-ground training would occur. Unfortunately, our group was unable to make these visits due to massive flooding caused by substantial rainfall and high intensity storms.

Support visit 1: Kelly Green

From March 18 – 28, 2011, support visit was held in order to support P3 projects, Kelly Green designated from the Farmer to Farmer program to collaborate in the duties of the project. The first part of the collaboration was to visit the site project in Tacotalpa, where Kelly help with sampling activities, she stayed for over 3 days in the Tacotalpa area and could observed the situation of the population. After this she visited the second work site in a Chontal indigenous community in Nacajuca municipality, where he had a training talk and a water sampling activity. The rest of her visit she expended collaborating in other Aquafish CRSP projects.

Early Phase IAA Implementation.

The P3 project has identified several regions with indigenous communities that have connections to the University Juarez de Tabasco in collaboration with an Indigenous University (Universidad Intercultural del Estado de Tabasco). The P3 team has already begun to implement the integrated agriculture-aquaculture technology in (Caridad Guerrero) Lacandon village in Tacolapla, (Fig. 3A) where a farmer's cooperative has already formed and there is existing aquaculture infrastructure (Fig. 3B). The farmers have identified a suitable location (Fig. 4A) where they would like to produce vegetables, though currently do not have the knowledge to set-up an integrated agriculture-aquaculture system (IAAS). The intent is to produce a variety of crops both for their own use, but also for generating income, in this phase they will grow habanero pepper, which has a very high demand and price. The proposed site needed to be selectively cleared to make planting space for the IAAS; however, most of the larger trees were retained.

Figure 3. A) Lacadon village in the Mexican state of Tabasco. B) And an aquaculture tank in use by the farmer's cooperative to grow tilapia.

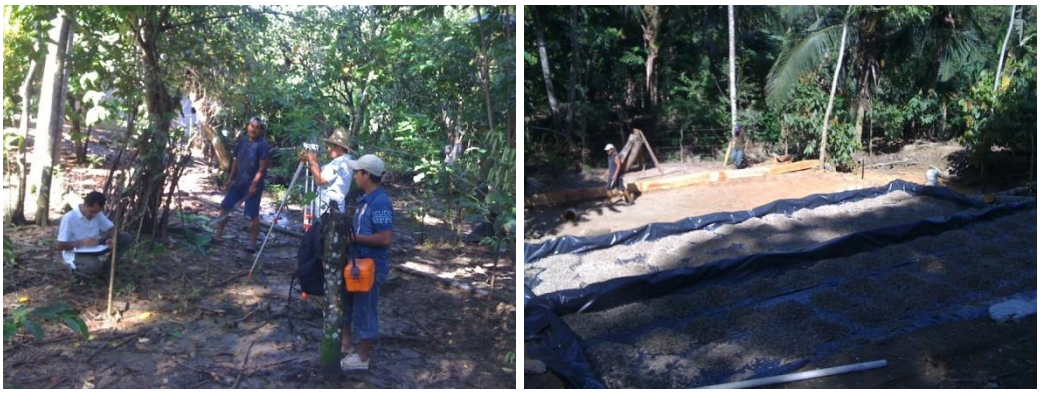

Figure 4. A) Personnel measuring levees and levels in the selected site. B) View of the three agriculture beds part of the IAAS system

Following the evaluation of the supply of water quality and quantity, aquaculture system set-up, proposed site, and listening to the farmers' expectations, our team proposed to utilize a series of flood irrigated raised growing beds (Fig. 4B). These beds are currently being developed with an expected completion by the end of 2010 (Fig. 5) There was a delay in the bed construction due to major flooding events in September, the beds were destroyed and reconstruction was needed. One of the principal construction goals is to develop a simplified surface irrigation system by which the aquaculture effluent or 'waste water' is delivered to the plants (Fig. 5) .

One challenge the team has had to overcome in this regard is managing the timing of the irrigation without automated pumps. The aquaculture tanks require frequent water replacement to avoid the accumulation of suspended solids and both organic and inorganic nutrients. These water outflows are greater than plant demands. The solution identified is to build a settling basin in which to collect the aquaculture outflows and to distribute the water to a variety of plant production beds. The excess treated aquaculture effluent in the settling tank will be discharge back into the stream.

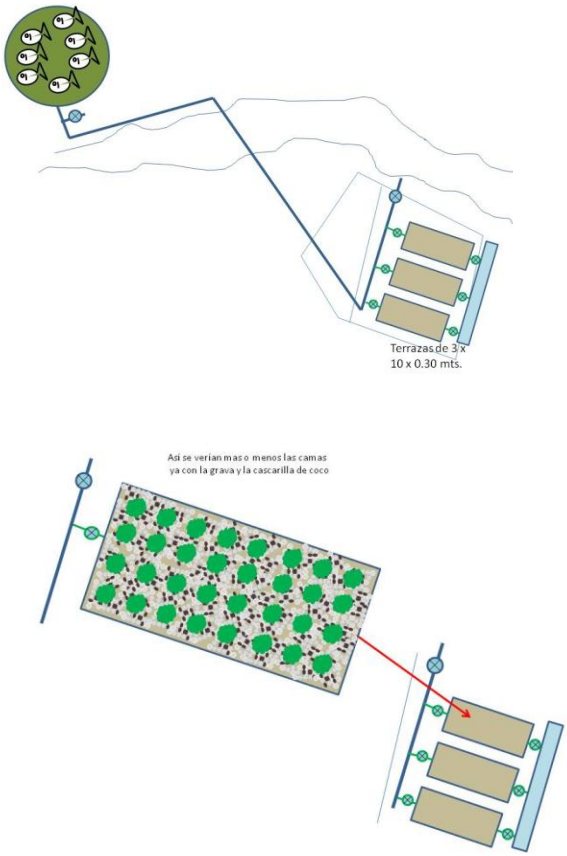

Figure 5. IAAS design in Caridad Guerrero, Tacotalpa

Continued Future Work

Development of the integrated aquaculture-agriculture technology is being developed to suit the needs of the indigenous communities and farmers cooperatives in the Lacandon village in Tacotalpa. This work is expected to be completed and operational by early 2011. Additional nearby communities have also been identified and have expressed interest to work with our P3 team to develop and implement similar technologies. These are expected to occur by May 2011. Following the implementation of the integrated agriculture-aquaculture technology, the student team will continue to work with community representatives to manage the operations using simplified business practices. For example, our team is working with the farmers to keep track of harvest quantities, market prices, and profits made. The goal of communicating these management tools, is that the communities will not only become self reliant but also use the information to make investment decisions to maximize their supplemental incomes. Academic products will result of this project, two undergraduate students are working on their thesis, the first one is evaluating the feasibility of the IAAS system (Tilapia and habanero growth) as long with agriculture parameters, the other thesis is focusing in the socioeconomic impact of the project in the community and in the surrounding communities.

Conclusions:

At this moment we are ready to transplant habanero peppers and start the IAAS system, organization and training phase on the other two sites is done, and we will begin setting up IAAS system in the rest of the sites. The P3 project, aimed at the development of Sustainable Aquaculture Practices in Tabasco, Mexico Using Novel RIAA Technology, has thus far been met with promising results. Our team has identified that success of this project is dependent upon

satisfying the economic, environmental as well as social equity benefits. The social components of this project have thus far been the most critical. Students from the team have worked with indigenous community leaders as well as farmers from local communities to better understand their needs and to modify the technologies developed in the early phases of this project to maximize the economics and environmental benefits. In less than a year from the start of the project, the team has helped educate and train more than 200 participants, technologies to enhance the livelihood of local level farmers are being developed in one site with anticipation of development in two additional sites, and critical relationships have been developed which will extend the life of the project beyond the completion of the P3 component. Utilization of the technologies will have low impact on the environment, generate sufficient revenues to cover both long and short terms costs, and the communities are being supplied with the knowledge and tools to administer farming production effectively. Thus, the farming technologies implemented through this project are expected to remain operationally sustainable beyond the life of the P3 project.

Figure 6. Habanero pepper plants ready for transplant

CONSTRAINTS AND OPPORTUNITIES IN CAGE AQUACULTURE IN GHANA

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Abstract

This study was conducted to identify why the overall contribution of the aquaculture industry to local fish production in Ghana is low (<1%) although cage aquaculture has a potential to increase production. We administered 106 questionnaires to six respondent groups (current cage fish farmers, potential adopters of cage aquaculture, farmers who have abandoned cage aquaculture, Fisheries Commission, regional and district fisheries officers, and financial institutions) to obtain insight into the constraints in cage aquaculture as well as opportunities that can be exploited to promote cage aquaculture adoption. For the purpose of this study, potential adopters are individuals who have fish-related livelihoods including fishermen, pond-based fish farmers and fish traders. We also interviewed key informants in relevant government institutions. Preliminary results indicate that lack of funds and lack of government extension services are the main constraints in cage aquaculture in Ghana. Lack of funds manifests in farmers' inability to afford quality floating feed and could explain low production levels of current cage farmers, although most (95%) suggested they could market their fish if they increased production. Lack of funds also accounted for the inability of potential adopters and farmers who have abandoned cage aquaculture to start or continue cage aquaculture respectively. Major opportunities identified include 1) a high interest among potential adopters (97%) to start cage aquaculture and farmers who have abandoned cage aquaculture (100%) to resume if constraints are removed, 2) development of a feed production plant in Ghana by a private enterprise, 3) willingness of some financial institutions to provide loans for cage farmers, and 4) a number of government initiatives to promote cage aquaculture. Our preliminary recommendations are that the Fisheries Commission should work with the financial institutions to help determine farmers' ability to repay loans and guarantee loans made by the financial institutions. Also, there is a need for a more specialized aquaculture extension service accessible to farmers to help with technical issues built on the model of agricultural extension services in Ghana.

Key words: Tilapia; Cage aquaculture; Adoption constraints; finance; extension; Ghana

Introduction

Aquaculture in Ghana has been predominantly land-based since its inception in the 1950's. There are currently about 4,500 ponds operated by more than 2,800 fish farmers in Ashanti, Brong Ahafo, Central and Western Regions of Ghana (Lionel Awity, unpublished data). Despite these numbers the contribution of aquaculture to local fish production is still insignificant. Available data suggests that the output from aquaculture in 2006 was estimated to be less than 1% of local fish production (Abban *et al*. 2006). Increasing aquaculture production will be a major step towards food security in Ghana and a further step in achieving 20% of local

production, similar to the global mean, which the government seeks (Abban *et al*. 2006). In order to achieve this goal in addition to meeting the estimated annual deficit of 400, 000 mt (Asmah 2008), cage aquaculture must be given serious consideration since land-based aquaculture in Ghana is mostly extensive and the land is finite.

The country offers considerable opportunity for small-holder and commercial-scale development of freshwater cage aquaculture, especially in the Volta Lake. Utilizing only 1% of the area of Volta Lake (approximately 8502 km^2) (ILEC 1999) corresponds to about 8500 hectares of water. This quantity of water is more than 10 times the area used for land-based aquaculture, about 468 hectares, estimated with 1,300 farms with mean size of 0.36 hectares (Asmah 2008). The culture of other desirable species such as the catfishes can also be expanded through cage aquaculture in addition to Nile tilapia (*Oreochromis niloticus*) which is currently the only species cultured in cages in Ghana (Blow and Leonard 2007). There is no doubt that cage aquaculture has the potential to make significant contribution to total fish production and food security in Ghana. China is a good example of a country where cage aquaculture has played an important role in inland fish yields. During1978 to1993, production from cage aquaculture accounted for 67.5% of total fish production of inland water bodies (Baotong and Yeping 1997). Even in Ghana, a single commercial cage farm contributed about 21% (200 tons out of 950 tons) to total aquaculture production in 2004 (Awity 2005). It has been suggested that if cage farmers in Ghana can produce yields of 50-150 kg/m^3/9 months as done elsewhere in Africa, less than 100 hectares of fish cages can produce yields matching the current capture fisheries production of 90,000 mt (Ofori *et al*. 2010).

Evidently cage aquaculture is not without negative environmental impacts. However, most impacts can be avoided if appropriate policies are implemented to limit the area of water allocated for cage aquaculture, which is currently being considered (Lionel Awity, pers. comm.). Existing irrigation reservoirs also have the potential to be used for cage aquaculture since they are less likely to raise major concerns.

Obviously, having significant national water resources for cage aquaculture is an important first step, but national development policy for cage aquaculture should be cognizant of other complex and interacting constraints to cage aquaculture development as have already been documented elsewhere (Hambrey 2006). Cage aquaculture has been developing in Ghana consistently in the last decade but there have been no significant reflection in the overall aquaculture production figures. Major constraints to aquaculture development suggested for Sub-Saharan Africa are feed and seed quality and availability, cost of cage design and construction, and financing (Ridler and Hishamunda 2001; Halwart and Moehl 2006; Moehl *et al*. 2006; Blow and Leonard 2007; Asmah 2008). Other constraints identified include lack of technical know-how (Ridler and Hishamunda 2001; Halwart and Moehl 2006; Blow and Leonard 2007; Asmah 2008), lack of market (Hambrey 2006; Moehl *et al*. 2006), lack of processing (Blow and Leonard 2007), lack of access to information and support (Ridler and Hishamunda 2001; Moehl *et al*. 2006; Asmah 2008), conflict over water use (Halwart and Moehl 2006) among others.

Many of the constraint suggested have been attributed to aquaculture in general and are likely to be constraints facing cage farmers but because they are mostly described for the entire sub-Saharan Africa, it becomes difficult to develop policy strategies and solutions targeting specific constraints. It is imperative that each country identifies its specific set of constraints and prioritize development interventions accordingly.

Our goal was to identify why the overall contribution of the aquaculture industry to local fish production in Ghana is low although cage aquaculture has a potential to increase production, and make necessary recommendation to the Fisheries Commission aimed at developing interventions for expanding cage aquaculture. Our specific objective was to identify the main

constraints to cage aquaculture in Ghana. We also sought to identify any opportunities that could be exploited to increase the contribution of cage aquaculture to fish production in Ghana.

Description of the study area

The study was conducted in communities around the Volta Lake where there are present or past cage aquaculture activities. Lake Volta is currently the main inland water body used for cage aquaculture in Ghana. It presents enormous opportunities for aquaculture expansion. Communities around the lake are mainly engaged in fishing and farming employing mostly men with the women focusing on fish processing and trading. Lake Volta and its tributaries drain 70% of the entire area of Ghana (FAO 2005) covering mostly Northern, Volta, Eastern and Brong Ahafo regions. The Eastern and Volta regions were the focus of this study. We selected the respondent groups from several districts in these regions based on the recommendations from the Fisheries Commission.

Methods

Sample selection and data collection

The surveys were done with three main respondents including current cage fish farmers (Adopters), cage fish farmers who have abandoned the trade (Abandoned), and Potential Adopters represented by people with fish-related livelihoods such as pond aquaculture and trading in fish. The other respondents were the Fisheries Commission, regional and district Fisheries Officers, and representatives of financial institutions. The group consisted of people already employed in fish activities including fishermen, pond and pen-based fish farmers and fish traders. Regional and district Fisheries Officers of the Fisheries Commission function as extension officers to fish farmers in addition to their prescribed duties. Therefore we included this respondent group to learn about their perspectives of what the constraints in cage aquaculture in Ghana were.

With the exception of Potential Adopters and financial institutions, all respondents identified for this study had small populations which were easily accessed through census. We obtained a list of Adopters and Abandoned from the Fisheries Officers and contacted as many as were available. Where we could not contact farmers directly, we employed opinion leaders to help access them. We also interviewed financial institutions based on their availability and preparedness to voluntarily answer questions.

The field studies were conducted between June and August 2010. We employed both surveys and interviews in this study. We administered most of the questionnaires in person to ensure answers provided were directed to exact questions asked. A total of 106 questionnaires were administered. Questionnaires were structured to suit respondent groups but we incorporated similar questions in some questionnaires to aid comparison among groups. We interviewed 43 Adopters, 20 Abandoned (including 10 individuals who had abandoned pen fish farming), and 31 Potential Adopters. We also administered 1 questionnaire to the Fisheries Commission, 5 questionnaires to regional and district Fisheries Officers, and 5 financial institutions identified in the two regions used in this study. We further interviewed key informants in relevant government institutions.

Questionnaire design

Based on the information available in the literature about constraints in aquaculture in general and cage aquaculture in specific we developed nine items representing constraints that could be evaluated by Adopters, Abandoned and Potential Adopters. The nine items were presented and scored on a four-point interval scale ranging from "not important" to "very important" modified from Vagias (2006) level of problem type-scale. Respondents were to rank the constraints according to how important they were in their cage aquaculture operations, their decision to abandon or adopt the business. Additionally we presented the same set of constraints to the Fisheries Commission and the regional and district Fisheries Officers. We also provided an open-ended option for respondents to state other constraints that thought were very important.

Adopters, Abandoned and Potential Adopters were asked to indicate (yes/no) whether they had had specific cage aquaculture training. We followed up with an open ended question of the type of training, where and when they had the training. We used these multiple measures of training as a way of assessing the level of knowledge of respondents in cage aquaculture.

We also wanted to evaluate the market availability for products, the profitability of cage aquaculture from the respondents' perspective, and interest in the business. To do this we developed a series of binary response questions which were presented to the appropriate respondent groups. We asked Adopters to indicate (yes/no) whether they thought they would be able to sell more fish if they could expand production above their current level. Then we asked them if they would recommend cage aquaculture to potential farmers. To evaluate the level of interest, we asked Potential Adopters to indicate (yes/no) whether they were interested in starting cage aquaculture on the Volta Lake. We further asked both Abandoned and Potential Adopters to indicate (yes/no) if they were interested in resuming or starting cage aquaculture if constraints are removed, and to provide reasons for their responses.

Opportunities available for farmers to access loans from banks and financial institutions were explored through both close-ended and open-ended questions. Financial institutions were asked to indicate (yes/no) if they had given loans to fish farmers in the past. When the response was yes, they were further asked to indicate the percentages of farmers who paid the loan at the appointed time, sometime after the appointed time or never repaid the loan. Future opportunities for loans were explored by asking financial institutions to indicate (yes/no) whether they had some form of budget for fish farmers currently. For those that responded in the affirmative, we asked them to provide specific requirements that farmers needed to meet in order to access a loan.

Interviews

We used select questions from the questionnaires as an interview guide in conducting the interviews with the key informants in government research institutions. We asked interviewees their opinions about the constraints in cage aquaculture in Ghana and opportunities they knew existed which could improve the industry. We took notes in all interviews but recorded none of the interviews to avoid making interviewees uncomfortable.

In this preliminary analysis, the quantitative questions in the surveys were analyzed using descriptive statistics such as arithmetic means, percentages and proportions and the qualitative questions were either coded and analyzed using descriptive statistics or analyzed qualitatively. All interviews were transcribed and stored to await analysis with the surveys.

Results and Discussion

Constraints in cage fish farming

Overall, the three main respondents groups (Adopters, Abandoned and Potential Adopters), the Fisheries Commission, and regional and district fisheries officers ranked lack of funds high on a 4-point scale. Mean ranking of lack of funds was 3.58 for Adopters (Figure 1). Abandoned and Potential Adopters had mean rankings of 3.25 and 3.81 respectively. Due to the small sample size of the Fisheries Commission, and regional and district fisheries officers (n = 1 and 5 respectively), their means were not included in the comparisons but it is worth mentioning that the Fisheries Commission ranked lack of funds as very important (4) whiles the regional and district officers had a mean ranking of 4 for the same constraint.

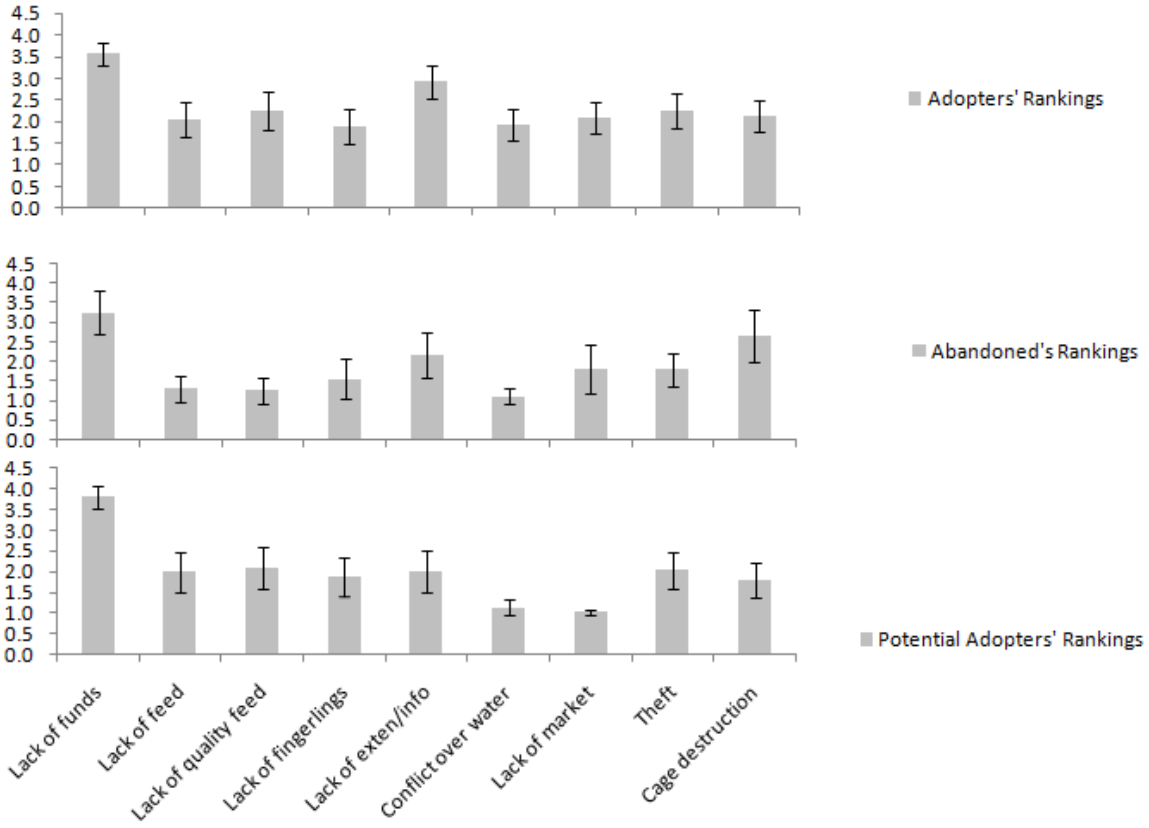

Figure 1.- Mean rankings of nine constraints for Adopters, Abandoned and Potential Adopters. The ranking is based on a 4-point scale from not-important to very-important. Total sample size (n) for Adopters, Abandoned and Potential Adopters are 43, 20 and 31 respectively. Error bars are 95% confidence intervals.

The results from the survey suggest that lack of funds is the main constraint in cage aquaculture in Ghana and not lack of feed and fingerlings as has been suggested for Sub-Saharan Africa (Halwart and Moehl 2006). Rather, the problem appears to be high input cost, specifically, feed cost due to the importance of feed in the relatively intensive system of tilapia cage aquaculture. Lack of good fingerlings may have been a constraint in the past for Ghana but with the extensive research conducted by the Aquaculture Research and Development Centre of the Water Research Institute (CSIR-WRI) to improve the genetic quality of tilapia broodstock and fingerlings in the country and the availability of many commercial hatcheries, lack of fingerlings is

probably a problem of the past in Ghana. When the respondents were asked to state other constraints they thought were important, high feed cost emerged as the most important constraint. Additionally, extra information provided by some respondents indicated that high feed cost was an important constraint not the lack of feed or lack of good quality feed (Figure 2). It is therefore reasonable to conclude that farmers lack funds to buy feed for their business because quality feed are often imported. This result corroborates the opinion of Blow and Leonard (2007) who said the availability of high-quality locally produced feeds at competitive prices in sub-Saharan Africa was a constraint in cage aquaculture. High feed cost also translated into high fish price, which some farmers felt affected their profit (Figure 3) even though lack of market was not necessarily a major constraint according to the survey results.

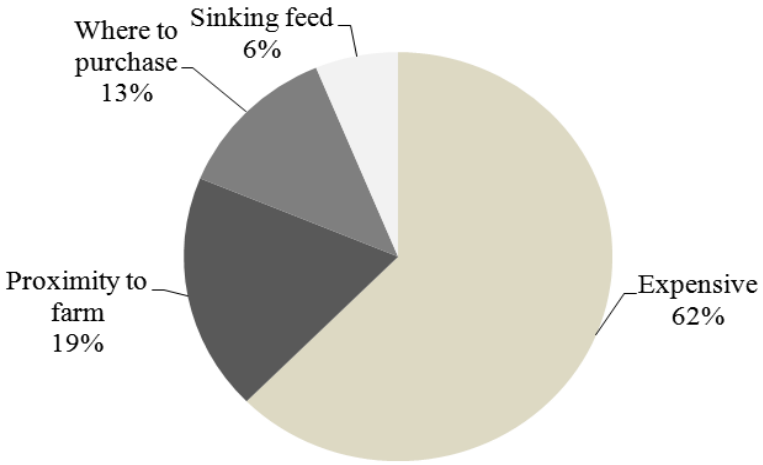

Figure 2. Proportion of respondents who provided additional information about other factors they considered constraints in relation to lack of feed and good quality feed. Sample size n = 15.

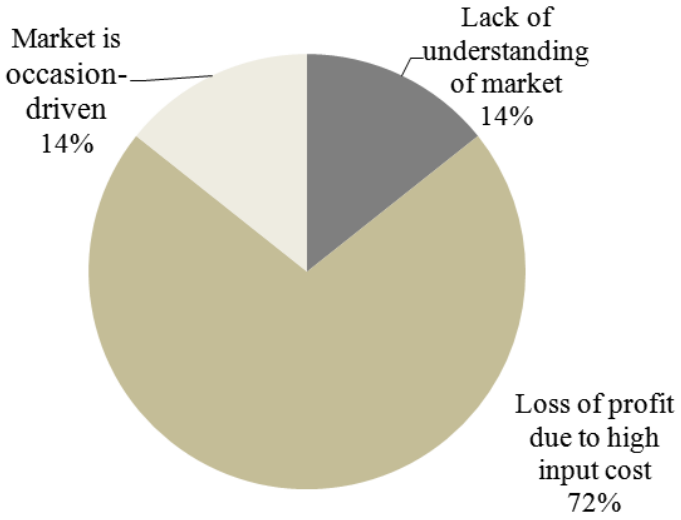

Figure 3. Proportion of respondents (n = 7).who provided additional information about other factors they considered constraints in relation to lack of market.

For the Abandoned and Potential Adopters, lack of funds could explain why they are not currently practicing cage aquaculture. When asked if they were interested in resuming the business, all 20 respondents (100%) in the Abandoned group (including 10 farmers who have abandoned pen fish farming) were interested in resuming cage aquaculture if they had capital. The pen farmers were interested in adopting cage aquaculture but not pen farming because they had received some training in cage aquaculture and found it more desirable than pen aquaculture

Lack of extension was ranked as the second most important constraint by Adopters with a mean of 2.93 (Figure 1). The Fisheries Commission ranked lack of extension as very important (4), however, both the Abandoned and Potential Adopters rated lack of extension or lack of information (for Potential Adopters) as a slightly unimportant constraint. In contrast, the regional and district fisheries officers ranked lack of extension quiet low with a mean of 1.6. This is probably because the regional and district fisheries officers felt they were doing their best doubling as extension officers in addition to their assigned duties.

Apart from lack of funds and lack of extension, respondents ranked all other constraint as slightly unimportant (mean rank of 2.3 or lower). The only exceptions are cage destruction by storms which was ranked higher by Abandoned (mean rank of 2.67) and theft which was ranked 4 and 3.4 by the Fisheries Commission, and regional and district fisheries officers respectively. Cage destruction by storms was ranked as slightly important because 50% of the cage farmers who had quit the business did so because their cages had been destroyed by storms. Theft was probably ranked high by the Fisheries Commission, and the regional and district fisheries officers because of individual reports by some farmers but it appears that once funds are available to hire security personnel on farms, the problem of theft is easily dealt with.

Interview results shared some similarities with survey result in terms of lack of extension being a major constraint in cage fish farming. Whereas all three interviewees mentioned lack of extension specifically, only one mentioned lack of funds as a constraint. Interestingly, all three interviewees stated lack of knowledge in cage aquaculture as the main constraint. However, this was not evident in the survey because when asked if they had specific training in cage aquaculture, we had yes response of 72%, 85% and 55% for Adopters, Abandoned and Potential Adopters respectively.

Opportunities that can be exploited

In response to whether they would be able to market their produce if they could expand their production above current level, 95% of Adopters responded yes, suggesting a potential to expand the aquaculture industry through cage fish farming. Farmers also appear to be making profits judging from the fact that 93% of all Adopters said they would recommend cage aquaculture to potential farmers, with 65% of them recommending cage aquaculture on the basis of its profitability.

Another opportunity that can be exploited to expand production was evident when 90% of Potential Adopters said they were interested in starting cage aquaculture on the Volta Lake. Some fisher folk in the group indicated they could hardly wait to start due to the advantage of getting fish all year round compared to the seasonality of fishing. Additionally, 97% and 100% of Potential Adopters and Abandoned respectively, responded yes when asked whether they were interested to start or resume cage farming if constraints are removed. The prospects of making profit was a strong indication why both Abandoned and Potential Adopters were interested in cage aquaculture but they also indicated that they found management of cages relatively easy.

We also learned through the interviews that a private enterprise has started producing floating feed for fish farmers in Ghana. Hopefully, this should ease the burden of high feed cost on farmers especially if local ingredients are used. We expect locally produced floating feed to be cheaper but the price and quality of locally produced floating feed will need to be verified in future studies before a definite advantage for cage aquaculture development can be ascribed.

In exploring the possibility of cage fish farmers being able to access loans from banks and other financial institution, we learned that some banks have had unpleasant experiences with fish farmers in the past and indeed were skeptical about future loans to fish farmers. Nevertheless, some institutions were willing to provide loans to fish farmers if they had guarantors, mortgage collateral, and the institution had sufficient knowledge about the entire project. There were also opportunities for groups to access micro-finance with relatively less stringent criteria. Our findings are consistent with that of another study by Hishamunda and Manning (2002) who investigated the role of banks in aquaculture development in six countries in Sub-Saharan Africa (Cote d'Ivoire, Madagascar, Malawi, Mozambique, Nigeria, and Zambia) and found that banks were skeptic about giving fish farmers loans because of past failures but there still existed opportunities for acquiring loans if farmers had a convincing proof of success.

Certain government initiatives were also identified as avenues to improve to cage fish farming in Ghana. Results from the survey revealed that some interested individuals had received training in cage fish farming organized by the government and were awaiting inputs from the government to commence business. This is probably a part of a "Youth in Agriculture" proposal by the Ministry of Agriculture which we learned about during the interviews. Finally, our study also revealed that limited government supported microfinance and small loans centers were in operation in Ghana and could be accessed by fish farmers.

Conclusions and Recommendations

Our study suggests that the main constraint in cage aquaculture in Ghana is the lack of funds to purchase input such as feed. While lack of funds prevented farmers who have abandoned cage farming from resuming, the constraint also barred potential adopters from starting cage aquaculture even though they showed a high interest in the business. In addition, farmers appeared to have knowledge in their operations but it seems the knowledge is inadequate and they could use more extension services.

In light of these results, our preliminary recommendations are that the Fisheries Commission should work with the financial institutions to help determine farmers' ability to repay loans and guarantee loans made by the financial institutions. In the long run, aquaculture could be made more attractive and competitive by subsidizing feed cost for small-holders, especially if quality floating feed is produced locally. It would seem appropriate to provide subsidies for some of the most expensive inputs for aquaculture since farmers in crop production receive similar subsidies on fertilizers and inputs. Also, there is a need for a more specialized aquaculture extension service accessible to farmers to help with technical issues built on the model of agricultural extension services in Ghana. Farmers who cannot afford private extension services would greatly benefit from such a program especially if this is a cheaper alternative.

Acknowledgement

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GEOSPATIAL MODELING OF SITE SUITABILITY FOR POND BASED TILAPIA AND CLARIAS FARMING IN UGANDA

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Abstract

The study set out to implement geospatial modeling of site suitability for Tilapia and Clarias farming for Uganda. Seven criteria of water requirement, water temperature, soil texture, terrain slope, potential farm gate sales, availability of farm inputs, and access to local and regional markets were analyzed. The crisp and fuzzy approaches of criterion classification were implemented and the results compared. The weighted linear aggregation method was used to generate the overall suitability maps. There was a statistically significant difference between suitability values generated by crisp and fuzzy approaches. For both the crisp and the fuzzy approaches, over 99 % of the land was classified as moderately suitable or as suitable. However, the distributions of the suitable and moderately suitable classifications varied between the two approaches. The differences were more dominant in the Northeastern part of the country and areas around the shores of the major Lakes. For the same location, the fuzzy method gave slightly higher suitability values at the lower extreme (unsuitable) and gave slightly lower suitability values at the upper extreme (very suitable). Overall, the crisp method classified 59,203 ha (0.34 %) as very suitable for Tilapia and Clarias farming compared to 230 ha (0 %) by the fuzzy method. Simultaneously, the crisp method gave 10,794 ha (0.06) as unsuitable compared to 7,150 ha (0.04 %) by the fuzzy method. Of the 138 fish ponds with operational pond status, the crisp method classified 71 % as suitable while 29 % as moderately suitable while the fuzzy method classified 71.7 % as suitable while 28.3 % as moderately suitable. The paper provides a nice template for duplicating this assessment in other regions of the world.

Keywords: GIS, fish farming, fuzzy logic, crisp sets, multicriterion evaluation, and pairwise comparisons

WHAT INFLUENCES THE SUCCESS OF AQUACULTURAL RESEARCH PROJECTS?

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Introduction

No research program can enjoy long-run success without a periodic assessment of how it is performing and what factors influence success and failure. While most such assessments are informal and specific to a particular study, formal evaluations eventually become important at the program level. A formal analysis the same as an informal one in the sense of comparing research outputs with the inputs or efforts expended to achieve them (a "knowledge production function"). Approaches to research assessment thus differ only in how such outputs and inputs are to be understood, measured, and compared.

Assessment methods can be either quantitative – typically statistical – or the kinds of institutional evaluation one sees in a case study. In biological disciplines, at least, most statistical analyses of the factors affecting research success employ the scientists' publication counts, citations, or intellectual property as measures of research output. Methods are parametric or non-parametric, dynamic or static. In the parametric approach, the bibliographic output measures are regressed against current and lagged research expenditures, against other inputs or conditions poorly represented by expenditures, and sometimes against a time trend.

Adams and Griliches (1996), for example, examine U.S. university research performance in eight scientific fields during the 1980s. They find at the level of a particular university or field that diminishing returns to scale prevail in academic research. That is, increases in study size bring less-than-proportionate increases in the study's publication output. At the aggregate level, however, there appears to be approximate equality between research expenditure and publication (or citation) performance, possibly because aggregate data incorporate cross-study knowledge spillovers that are not captured in more disaggregated data. These methods have more recently been applied to life-science research by Smith (1998), Xia and Buccola (2005), Groot and Garcia-Valderrama (2006), Buccola, Ervin, and Yang (2009).

The bibliometric approach is, despite its widespread use, inadequate in a number of respects. The first and probably most important difficulty is that publication, patent, and journal citation rates mask much of the detail of a study's findings and thus only grossly reflect the findings' nature, magnitude, and importance. Much published output also becomes available only

years after the study has been completed. Finally, the bibliometric approach is poorly suited to an exact matching of a study's outputs and inputs.

To help solve these difficulties, we examine here a new approach to research assessment. The new method focuses directly on the information a research study has generated, enabling a more exact and more contemporaneous matching of that information to the skills, expenditures, and capital devoted to the study. We apply the approach to the 55 past and on-going aquacultural research investigations which AquaFish CRSP is pursuing in eleven countries.

Conceptual Framework

To place this approach in its broadest context, let *K* represent the knowledge an aquacultural study has generated; *E* the study's expenditures; *M* the research management policies, such as choice between control and survey methods; *C* the human capital devoted to the study (and which can be only imperfectly captured in expenditures); *I* the institutional, cultural, and environmental conditions under which the study is pursued; and ε the unexplained variation. We then can relate the magnitude *K* of the study outcome to the inputs affecting that magnitude:

$$(1) \qquad K = f(E, M, C, I, \varepsilon)$$

and in this way individually examine the impacts of factors *E*, *M*, *C*, and *I* on knowledge output *K*. For example, we can estimate the effectiveness of research management policies while holding expenditure, such human-capital dimensions as researcher education and experience, and environmental conditions statistically constant.

Difficulties in Measuring Research Output

Relationship (1) is best characterized with disaggregated data, that is with observations on many individual studies. With disaggregate data, information about research expenditures, the composition of the research team, research management practices, and the institutional environment will be available if enough effort is made to collect it. The more difficult task, even in disaggregate settings, is to characterize and measure research output magnitude *K*. Output measurement difficulties arise from several sources.

First, an aquacultural research study typically focuses not only on a variety of research treatments but on a variety of outcomes per treatment. An examination of alternative fish feed rations, for instance, typically is interested in the survival rate, feed conversion, final body weight, and flesh quality associated with each ration. The reason is that all such outcomes affect profitability in a separate way. Yet these outcomes are incommensurable with one another unless expressed in terms of their impact on profit, an impact that varies with market conditions and hence is unknown to the investigator at the time the project is initiated. Even greater outcome incommensurability is encountered from one study to another. For example, one study in our AquaFish dataset may be seeking to boost tilapia exports, while another seeks to improve pond water quality.

Second, an aquacultural research study's contribution is information rather than something tangible like per-hectare fish yield or pond oxygen content. Because information is intangible, we cannot measure its magnitude by counting tons or hectares.

Third, research studies in our dataset frequently will be found to have started on different dates and to have progressed at different rates. Our assessment procedures must therefore account for differences in the stages at which a given study will be encountered. That is possible only if our assessment method is updatable, permitting us to evaluate progress as a study proceeds as well as when it is completed.

Fourth, some research projects fail in the sense that the hypothesized improvement – enhanced oyster management practices, say – does not materialize. Such failures do not necessarily imply that the study expenditures have been wasted, since the experimental disappointment can be valuable in pointing to more fruitful directions in subsequent research (CGIAR Science Council 2009).

The first or incommensurability problem can be solved by expressing study output units in terms of percentage changes, relative either to the originally anticipated improvement, the improvement the study manages to obtain, or the outcome levels already achieved before the study was inaugurated. Percentage changes in, say, one study's per-hectare yields are directly comparable to percentage changes in another study's water quality index. However, such proportional measurement makes no progress in solving the remaining three measurement difficulties.

Bayesian Approach to Research Output Measurement

An approach that does solve the second through fourth problem is to take advantage of Bayesian statistics, which are complementary to the classical statistics employed in most aquacultural research (Robert 2001; Schimmelpfennig and Norton 2003; Carlin and Louis 1996; Press 1989; Winkler 1972). In contrast to classical statistics' focus on estimating a population parameter like a water-quality improvement rate, Bayesian statistics concentrates on the *new* information the water-quality study provides. The old information can be either the results from earlier experiments or surveys or the aquaculturalist's subjective, pre-experiment hunches about how the experiment will turn out.

More particularly, the Bayesian method regards any new information as the difference between the study outcome probabilities (e.g. fish mortality rate or feed/gain ratio) that the researcher had surmised before the study began and the probabilities she encountered during or as a result of the study. Provided those outcomes are expressible in frequency terms, they can never generate less information than what the researcher originally had possessed, since the least the new outcomes can do is to exactly confirm the researcher's prior expectations. For example, early success of a proposed new Tilapia seed production method may boost the pond yield rates relative to those farmers presently achieve or to those the investigators had anticipated. If, on the other hand, the study fails to boost yields, its success is reflected instead in the experimental lesson learned, which in turn is expressed as the narrowing of the probability distribution of possible yield outcomes.

To summarize the Bayesian approach, let Y be the percentage improvement in a particular study outcome such as pond water quality. Let Z be the performance of the new technology the researchers are examining and which is intended to improve pond water quality. The probability the investigator initially assigns to a particular level of water-quality improvement is, in light of the study's current progress, updated as

$$p(Y \mid Z) = \frac{p(Z \mid Y) \cdot p(Y)}{p(Z)} \tag{2}$$

where $p(\cdot)$ is the probability of the first term in each parenthesis, and $|$ indicates that the following term is held constant. That is, after the researchers have observed performance Z of the new technology, the probability of a particular pond improvement level equals the probability that experimental outcome Z will occur given that the new technology improves pond quality by a stated amount, times the prior probability of pond improvement and divided by the prior probability of experimental outcome Z.

The likelihood that experimental outcome Z will occur depends in turn on study expenditures and other inputs **X** such as the investigators' human capital. We may represent this dependence as $Z = Z(\mathbf{X}, \varepsilon)$, where ε are unanticipated research findings. Furthermore, once the experiment is complete, $p(Z)$ is a known constant. Combining these two considerations gives, at the end of the experiment,

$$p[Y \mid Z(\mathbf{X}, \varepsilon)] \quad \propto \quad p[Z(\mathbf{X}, \varepsilon) \mid Y] \cdot p(Y) \tag{3}$$

in which $\propto$ signifies "is proportionate to." That is, the probability of the given pond improvement once experimental results have been obtained is proportionate to the probability that experimental outcome Z (a function of research inputs **X)** will be obtained assuming the new technology will be pond-enhancing at a stated level, times the pond enhancement originally expected. Expression $p(Y)$ in equation (2) is the investigator's prior (baseline) pond quality probability. In contrast, $p\,[\mathrm{Z}(\mathbf{X}, \varepsilon) \mid Y]$ is the posterior improvement expectation elicited at a midpoint – then at the termination – of the research process, reflecting the intervening experimental or other study results. Well-developed methods are available for eliciting the prior probabilities (Stael von Holstein 1970). They involve casting the probability questions in the context of the study's institutional and technical environment and employ the researcher's judgment about the situation at hand, a judgment that will partly be based on the previous literature.

The value to a decision maker of basing her decisions on the prior probability distribution $p(Y)$ rather than on posterior distribution $p(Y \mid Z)$, that is the value the aquacultural study has provided her, is reflected in the "loss" difference

$$L(d, Z, \Omega) \quad = \quad U(d \mid \Omega) \quad - \quad U(d \mid Z(\mathbf{X}), \Omega) \tag{4}$$

where Ω is the prior information available to the decision maker. This loss is the difference between the utility of acting in the absence of the study results and the (higher) utility of acting after knowing those results. Thus also, it is the disutility a fish farmer, buyer, supplier, or other interested party suffers if deprived of the research study. Function (4) can be specified in a variety of functional forms (Robert 2001).

For example suppose an oyster producer's decision d is that he promises, on the basis of his present management practices **X**, to deliver oysters at a particular quality grade A. If he delivers the oysters at lower than the promised grade, his quality reputation suffers; if at a higher grade, he sells at a price lower than he could have achieved had he predicted the quality more accurately. His profit, that is, rises with his accuracy in predicting quality grade. By improving our understanding of the true quality probabilities related to a particular management practice, oyster management research is precisely what improves such prediction accuracy. The negative of Loss (4) thus reflects the value of the oyster study's contribution to the improvement in prediction accuracy. A measure of the study's knowledge output K that solves all four of the research-output measurement difficulties listed above is therefore

(5) $$K = -L[d, Z(\mathbf{X}), \Omega]$$

the negative of the Bayesian Loss value (4). If we consider decision *d* to be a function of sample information Z itself, and prior information Ω to be unobserved error ε, we get

(6) $$K = -L[d(Z), Z(\mathbf{X})] = f(\mathbf{X}) = f(E, M, C, I, \varepsilon)$$

namely, knowledge production function (1).

Characterizing the Knowledge Production Relationship

In order to characterize how the magnitude of such new research knowledge responds to the type and quantity of research inputs, namely in order to depict relationship $K = f(E, M, C, I, \varepsilon)$, we treat each AquaFish research study as a production unit employing research inputs (E, M, C, I) -- such as money and personnel – to produce knowledge outputs. Most AquaFish studies are designed to examine a variety of experimental or survey dimensions, and they involve a variety of alternative treatments for a given dimension. For example, a controlled-experiment investigation focusing on feed rations can examine both the weight-gain and fish mortality effects of a given ration. A variety of rations are examined in each study – each constituting a separate research treatment – for their weight-gain and mortality effects. Considering instead a non-controlled-experiment situation, a survey investigation focusing on fish export opportunities can examine a marketing program's implications for both fresh and dried fish.

If the investigator employs *m* alternative feed-ration or marketing-program treatments, and examines the implications of each of them for such *n* outcome dimensions as mortality, weight gain, or dried exports, she will generate *mn* utilities (3), that is *mn* items of utility-enhancing new knowledge. Because *p* AquaFish investigations have been pursued over *t* years, we thus have *N* = *mnpt* observations for estimating equation (1). The result is to reveal, for each of a group of investigations, how much research output *K* has been achieved for each given set of research inputs and each scenario of institutional, management, and environmental conditions. Importantly, our method can be used to examine research performance not only at its conclusion, but at earlier project stages; that is relative not only to other studies but to its own earlier efforts.

Research Inputs We Are Examining

We examine in knowledge production function (1) the research impacts of the following study inputs and conditions:

Expenditures (E)

At U.S. Project University

In Host Country

Research Management (M)

Controlled Experiment *versus* Statistical Survey

Sample Size

Investigator and Collaborator FTE and Human Capital (C)

Scientist and Collaborator Age Distributions

Distributions of Highest Degrees

Position Rank Distributions

Institutional and Environmental Conditions (I)

On-Station *versus* Off-Station research

Transportation Modes

Road Conditions and Distances

Climate and other Environmental Conditions

Knowledge production function (1) or, equivalently, (6) can be estimated with distance-function methods (Fare and Primont 1995). Attention to unexplained variation ε in equation (1) is useful. If we consider ε to be the sum two independent random variables, $\varepsilon = u + v$, the latter may be used to represent frontier or best-research-practice performance, and the former to represent negative deviations from that frontier. Error u thus provides the basis for conducting cross-study comparisons of the efficiency with which research resources are used to achieve knowledge.

Utility Computation Example

An example from Investigation 07MNE04UM, a University of Michigan AquaFish study conducted in China, will illustrate the method we are using to compute an AquaFish study's knowledge contribution. The purpose of 07MNE04UM is to examine ways of reducing effluent and settling-pond pollution from Chinese shrimp production. Fish yield is compared in that study with water quality as measured by settling-pond chemical content. Statistical-survey rather than experimental-control methods are used. Relevant data in one of its treatments are:

Outcome	Level	Prior probability	Posterior mean	Posterior standard deviation
Fish yield (kg/ha)	Low 7000	0.10	8255.06	1438.05
	Medium 8000	0.60		
	High 9000	0.30		
Dissolved oxygen (mg/L)	Low 6	0.20	6.74	1.05
	Medium 7	0.70		
	High 8	0.10		
Total suspended solids (mg/L)	Low 20	0.10	47.12	12.33
	Medium 30	0.70		
	High 40	0.20		

One thing that is clear from this table is that an econometric model in which several dimensions of outcome – such as fish yield, dissolved oxygen, and suspended solids – are pooled, we first must normalize the outputs in a way permitting comparisons among them. Kilograms per hectare and micrograms per liter are not directly comparable. A natural way to do so is to divide each outcome level by the sum of its Low, Medium, and High levels, so that outcome levels are expressed essentially as percentage differences from their mean. Thus, for example, we can restate the three alternative fish yield levels as 7/24, 8/24, and 9/24, where 24 = 7+8+9. Loss function values are invariant to this normalization.

The prior probabilities in the table are elicited from the scientists on the basis of their best understanding, prior to conducting the research, of what the yield and chemical readings would be in response to the particular treatment or set of management practices. The posterior mean and standard deviation are, in contrast, taken from the statistical survey's ANOVA results. To demonstrate the value of this research, we compare the advantage of using these posterior rather than prior distributions of when making shrimp production management decisions.

The mean of the shrimp producer's prior distribution is

$$Mean_{PR} = P_L L + P_M M + P_H H \tag{7}$$

where *L*, *M*, and *H* are the low, medium, and high levels shown in the table, and the *P*s are the associated prior probabilities. This is what, prior to the research study, the producer expects his yields or dissolved oxygen levels to be. To derive the mean of the posterior distribution – namely what the producer expects, by virtue of the new study, the yields or dissolved oxygen to be, we first generate the normal density function corresponding to the posterior mean and posterior standard deviation shown. We then compute from that density function the low, medium, and high outcomes (L', M', H') whose cumulative probabilities would – to keep our results comparable to the prior distribution – equal to those in the prior. The result is the mean outcome as corrected by the research results, namely by the information in the posterior distribution:

$$Mean_{P0} = P_L L' + P_M M' + P_H H' \tag{8}$$

We next use these two respective means in (7) and (8) to compute two important measures of random variation in the producer's shrimp yield or pond dissolved-oxygen content. The first is the variation the producer anticipates around his naïve (pre-research) outcome expectation $Mean_{PR}$, but evaluated in terms of the outcome probabilities *estimated from the new research*. This models the risk which the new study shows the producer actually was facing when she used the pre-research outcome expectation. It is the post-study view of the producer's pre-study thinking. The relevant measure of this variation is the outcome variance expressed in terms of the outcome deviations from the pre-study mean but in which the outcome levels (L', M', H') are those the study indicates are associated with the given probability levels – that is, essentially in terms of the *posterior* probability distribution:

$$Var_{PR}^{PO} = P_L(L' - Mean_{PR})^2 + P_M(M' - Mean_{PR})^2 + P_H(H' - Mean_{PR})^2 \tag{9}$$

The appropriate variance of the posterior distribution is based on the respective distances between the posterior mean and the same three outcomes predicted. This of course is the posterior variance itself:

(10) $$Var_{PO} = P_L(L' - Mean_{PO})^2 + P_M(M' - Mean_{PO})^2 + P_H(H' - Mean_{PO})^2$$

The knowledge or utility gained from the research is the difference between this prior and posterior variance:

(11) $$K = -L(d, Z, \Omega) = U(d|Z, \Omega) - U(d|\Omega) = Var_{PO} - Var_{PR}^{PO}$$

It is easy to show that this difference is non-negative, so that the knowledge gained from properly conducted research can never be negative.

Data on AquaFish research treatments, probabilities, and inputs were provided by the host-country AquaFish investigators. Coordinators were (by US university base of respective project): Steven Amisah (Purdue U), Gertrude Atukunda (Auburn U), Remedios Bolivar (North Carolina Stat U), Wilfrido Contreras (U Arizona), Eladio Gaxiola (U of Hawaii), So Nam (U of Connecticut), and Gao Zexia (U of Michigan). Our study covers 55 aquacultural research studies conducted during AquaFish's 2007 – 2009 and 2009 – 2011 phases. Twenty-seven of these were conducted during the 2007 – 2009 phase and 28 in the 2009 – 2011 phase.

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METHODS FOR ASSESSING ECONOMIC, ENVIRONMENTAL AND SOCIAL IMPACTS OF AQUACULTURE TECHNOLOGIES: ADOPTION OF INTEGRATED AGRICULTURE-AQUACULTURE IN MALAWI

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Abstract

There is a growing demand for assessment of economic, environmental and social impacts of new food-related technologies, including the impacts of new methods for aquaculture management. This paper presents a new "minimum-data Tradeoff Analysis" (TOA-MD) model that can be applied to assess economic, environmental and social impacts in a wide array of agricultural systems that incorporate aquaculture, crops, and livestock (Antle 2011; Antle and Valdivia 2010). This model is widely applicable to assess impacts because it utilizes a generic model structure that can be parameterized with data available from a variety of sources, including farm surveys, experimental data, simulated data from bio-physical simulation models, and expert judgment. A key feature of this model is that it takes into account the fact that farmers systematically selected themselves into adopting and non-adopting groups. Analysis shows that this selection must be taken into account to obtain accurate estimates of impact.

To illustrate the use of the TOA-MD model, we use it to implement an impact assessment of integrated agriculture-aquaculture (IAA) systems in southern Malawi developed by the World Fish Center, using a WorldFish farm survey data collected in 2004, together with data from other public sources. We use the TOA-MD model to demonstrate how it is possible to use available data to move a conventional economic impact assessment "along the impact assessment pathway" to estimate adoption rates in the relevant populations, and to quantify impacts on distributional outcomes such as poverty, environmental impacts such as soil and water quality, and social and health-related outcomes such as nutrition or gender impacts. The analysis predicts an adoption rate of about 44%. In two districts, there is a substantial increase in protein consumption associated with the adoption of IAA and substantial reductions in poverty, whereas in others the effects are smaller.

Introduction

One of the great challenges in impact assessment is to "move assessment along the impact pathway" to quantify distributional, environmental impacts, and social impacts of agricultural technologies being developed and disseminated. As noted in a recent report sponsored by the Standing Panel on Impact Assessment of the CGIAR (Walker et al. 2008), a major impediment to meeting the growing demand for broader impact assessments is their cost in time and other resources, particularly when donors expect impact assessments to be carried out as part of a technology-related project. As Walker et al. (2008) observe, "In terms of both budgetary support and human capital, a disaggregated multi-dimensional impact study can be quite demanding and costly. The supply of these studies is more likely to be constrained by lack

of funding than the other types. (p. 7). Nevertheless, Walker et al. (2008) conclude, "The desirability of moving along the impact pathway is unquestioned. As donors want to see ever more comprehensive impact assessments, so ways have to be found to accommodate their wishes… even when resources for carrying out these studies are not forthcoming." (p. 14).

A recent development in impact assessment methodology is the use of a "parsimonious" approach that moves the focus from site-specific, processes based models and data, to the use of simulation models parameterized with population data (Antle 2011). This approach has been implemented in the form of a generic "minimum-data Tradeoff Analysis" (TOA-MD) model that can be applied to assess impacts in a wide array of agricultural systems that incorporate crops, livestock and aquaculture (Antle and Valdivia 2010). The TOA-MD model is a unique simulation tool that uses a statistical description of a *heterogeneous farm population* to simulate the proportion of farms that utilizes a baseline system (in this case, farms not using integrated agriculture-aquaculture, or IAA) and the proportion of farms that would adopt an alternative system (in this case, farms using IAA) within defined *strata* of the population. We apply this model using those data from the World Fish impact assessment of IAA in Malawi made public by Dey et al (2010), together with data from other public sources such as the national agricultural census. We use the TOA-MD model to demonstrate how it is possible to move a conventional economic impact assessment "along the impact assessment pathway" to estimate adoption rates in the relevant populations, and to quantify impacts on distributional outcomes such as poverty, environmental impacts such as soil and water quality, and social and health-related outcomes such as nutrition or gender impacts.

The adoption and impacts of World Fish Center research on integrated agriculture-aquaculture (IAA) systems in Malawi has been studied and publicized by Dey et al. (2006, 2010), Russell et al. (2008), Government of Malawi, (2005), NSO-GoM (2010), FAO (2008) and related sources. The impact assessment that was carried out by World Fish Center analyzed factors influencing adoption, and using an *assumed* adoption rate, evaluated aggregate economic impacts and estimated a rate of return on investment. While the study collected some data on outcomes such as nutrition, this was done for a stratified random sample of a small number of farms not using and using aquaculture. Thus, while it was possible to conclude that if farms adopted IAA they would be better off in terms of income and nutrition, it was not possible to estimate an overall adoption rate or make statements about the overall impacts of IAA in the relevant population of farms that potentially could adopt the IAA technology. Moreover, the assessment also did not provide estimates of environmental impacts (CGIAR Science Council 2007). In this report we show how the TOA-MD approach can utilize data that was collected in the original impact assessment surveys, along with other publicly available data, to carry out a disaggregated, multi-dimensional impact assessment. The TOA-MD approach also provides the basis for carrying out sensitivity analysis to parameters that cannot be estimated with the available data, thus providing guidance about the types of data that should be collected in future impact assessments.

Impact Assessment using TOA-MD

The TOA-MD model is a unique simulation tool that uses a statistical description of a *heterogeneous farm population* to simulate the proportion of farms that utilizes a baseline system (in this case, farms with small ponds and low integration) and the proportion of farms that would adopt an alternative system (in this case, farms with larger ponds, higher integration, and vegetable production) within defined *strata* of the population. Based on the predicted adoption rate of the alternative system, the TOA-MD model simulates associated economic, environmental and social *impacts* on adopters, non-adopters and the entire population. The version of the model used for the analysis presented here is a new version developed for impact assessment (Antle 2011). It is

based on the earlier version developed for ecosystem service analysis (Antle and Valdivia 2006, 2010).

One unique feature of the TOA-MD model is its capability to exploit statistical relationships between technology adoption and the environmental, economic and social outcomes associated with adoption. Economic research shows that taking these inter-relationships between adoption and outcomes is critical to obtain accurate estimates of impact. This fact also has important implications for data collection that we discuss in the conclusions of this study.

Another unique feature of the TOA-MD model is its parsimonious, generic structure, which means that it can be used to simulate virtually any farm system. One virtue of this model design is that, unlike many large, complex simulation models, it is easy to relate results to particular features of the system. TOA-MD is also well-suited to address the uncertainty in impact assessments, by using sensitivity analysis to explore how results change with different assumptions. The TOA-MD model is programmed in Excel, and is easy to learn and use.

The model utilizes the following types of data:

- population means and variances of production, output price and cost of production, by crop, aquaculture and livestock activity
- population means and variances of environmental and social outcomes associated with each system
- correlations between system returns and environmental and social outcomes
- population means and variances of farm household characteristics (farm size, pond size, household size, off-farm income).

Population and Strata

The population represented is farms in southern Malawi that could adopt aquaculture, or that have aquaculture operating at a low level of integration that could be improved. The strata are 5 districts where survey data were collected : Zomba East and West (pop. 670500), Mulanje (pop. 428322), Mwanza (pop. 138000), Thyolo (pop. 458000), and Mangochi (pop. 610000), see Figure 1.

Brooks (1992) estimated the potential areas for aquaculture in Malawi based on some physiographic factors (land formations, altitude, temperature, precipitation, run-off and soils). Brooks estimated that the areas under or that have potential for aquaculture in Malawi was about 11,650 km2 and about 7,200km2 corresponded to the southern regions of Malawi. More recently, the project "Determination of High-Potential Aquaculture Development Areas and Impact in Africa and Asia (funded by the Federal ministry of Economic Cooperation and Development of Germany and in coordination with the WorldFish and other partner institutions) developed a decision-support package that can be used to identify areas where aquaculture is feasible. One of the studies of this project was carried out in southern Malawi where they identified areas with existing and potential aquaculture adoption. They used biophysical (e.g. water availability, land conditions) and socio-economic (market, knowledge and inputs, labor and finance) criteria to evaluate the suitability of the area. The overall area suitable for Southern Malawi estimated was about 35,400km^2 (Kam et al., 2008; Kam and Teoh, 2008).

Agriculture in Southern Malawi is characterized by small farms (average 0.89 ha)[1] growing mixed crop systems of maize, beans and some vegetables. Poverty rates are very high, approximately 70% based on the Malawi poverty line of about $0.41/person/day. Some farms have non-agricultural income. Various NGOs have been involved for several years in encouraging adoption of more highly integrated agriculture-aquaculture (IAA) systems. Adopters of IAA tend to be larger farms growing irrigated vegetables and have higher incomes and lower poverty.

Integrated Aquaculture-Agriculture (IAA) Farming Systems

The IAA farming system is based on the utilization of organic wastes and by-products, such as crop residues, as feed inputs to the fish pond, and the recycling of pond mud and water containing nutrient wastes back to cropland. In Southern Malawi, maize bran is the most common pond input. (Dey et al. 2007).

The Malawi data differentiate the farms according to a) the adoption or non-adoption of IAA, and b) the level of integration of IAA. Based on this data we defined the systems for our analysis as:

System 1: Crop-based system of maize, beans and some other crops, with the addition of small ponds with a low level of integration with agriculture. The level of integration is defined by the number of bio-resource flows in the farm, where 2 or less bio-resources is considered to be the low integration case.

System 2: more highly integrated system with larger ponds and irrigated vegetables that utilize water from ponds.

Impact Indicators

Mean farm income and per-capita income

The mean farm income in the study area is about $420/yr while the per-capita income is about $160/yr. Fish culture contributes in average between 8% and 10% to the annual farm income (Dey et al., 2007, 2010)

Poverty rate

Poverty rates are high in the region, several sources indicate poverty rates ranging from 65% to 78% (NSO-GoM, 1998, IFAD, 2006). According to the survey data, using the official poverty line of 16,165 Malawi Kwacha/year and an exchange rate of 108 Kwacha per US dollar, the poverty rate of the farms in the survey is about 90 percent.

Human nutrition

A survey performed by the GoM's National Nutrition in December 2005 for the rural Malawian population, concluded that the national average number of meals per day for an adult was 2.0; 45% of adults had two meals the day before the survey, roughly one-third of adults had three meals and 19% had only one meal the day before the survey. The percentage of households reporting at least one member regularly reducing the amount of food they consumed at mealtimes was 82% and 49% of households reported that at least one member did not eat

[1] Average farm size in Dey et al. (2010) survey data was about 1.4ha because it included *dambo* areas used for IAA

during a whole day in the last month due to lack of food (GoM and UNICEF, 2005). An important component of diet is protein consumption.

Data

The TOA-MD model utilizes statistics (means, variances, correlations) estimated from the data. The model set-up with all of the data used for the analysis is available from the authors.

Farm Data

The average farm size in the population is about 1.9 ha, with an average household size of 5 people. The low integrated farms is characterized by having small ponds averaging 150m^2 (0.015 ha), while high integrated farms own larger ponds with an average of 300m^2. Non-agricultural income per farm varies across strata and ranges from $44 to about $100, with an average of $76/farm.

Economic Data

Maize is the main staple crop in this region, but farmers also grow a combination of other crops (e.g. beans, pigeon peas, cowpeas, etc.). For this analysis we use maize and beans to represent the crop activities in the farms with low integration. Farmers with high integration of IAA grow vegetables in addition to maize and beans. Data on yields as well as production costs and prices were obtained from GoM (2005), Chilongo (2005) and NoS (2010).

Nutrition

Using the survey data, the protein consumption in each household (kg/person/month) was calculated. The data showed that the average for non-IAA farms was about 1.32 kg/person/month, whereas the average for IAA farms was about 1.64.

Results

Results of the analysis are presented in Figures 2, 3 and 4, and summarized in Table 1. Figure 2 presents curves showing the simulated adoption rate of IAA as a function of the opportunity cost of changing from System 1 to System 2. The rate that would occur if farmers are behaving economically rationally and maximizing expected returns to their farms, is the point where the curves cross the horizontal axis. This rate ranges from 38 to 49 percent.

Figure 3 presents the predicted poverty rates in relation to the adoption rate of IAA. The baseline poverty rates are at the zero adoption rate, and as noted above, average about 90 percent, and range from 74 to 99 percent. At the economically-efficient rates of adoption (the rates where the adoption curves cross the horizontal axis in Figure 2), the poverty rates decline by 8 to 14 percent, when averaged over the entire population of adopting and non-adopting farms. However, when only adopting farms are considered, the poverty rates decline for adopters by 19 to 35 percent (Table 1).

Figure 4. shows the impacts on protein consumption. The baseline (the rate at zero adoption) shows that protein consumption varies substantially across the regions. Adoption of IAA has relatively small impacts on those areas where consumption is relatively high, but has substantial impacts in Mulange and Mangochi, the two districts with the lowest protein consumption. In those areas, protein consumption among adopters increases from less than 1 kg/person/month to over 2 kg/person/month.

Conclusions

This paper demonstrates the use of the TOA-MD model to carry out an integrated impact assessment of technology adoption, using the case of integrated agriculture-aquaculture in Malawi. Using TOA-MD, it is possible to implement an integrated assessment of economic, environmental and social impacts at low cost relative to methods that rely on case-specific, complex bio-economic simulation models. Cost is reduced in two ways. First, by using a generic model that can be applied to virtually any system, the time and resources needed to design a new model for each case are largely eliminated. Second, by identifying in advance the indicators that need to be quantified, any data collection activities can be focused on the relevant information, thus eliminating the cost and respondent burden caused by the "kitchen sink" approach to survey design. Moreover, the TOA-MD approach shows that correlations between economic, environmental and social data are often needed to obtain accurate estimates of impact. By recognizing this need in advance, the cost of collecting data can be reduced, and the quality of impact assessment can be enhanced.

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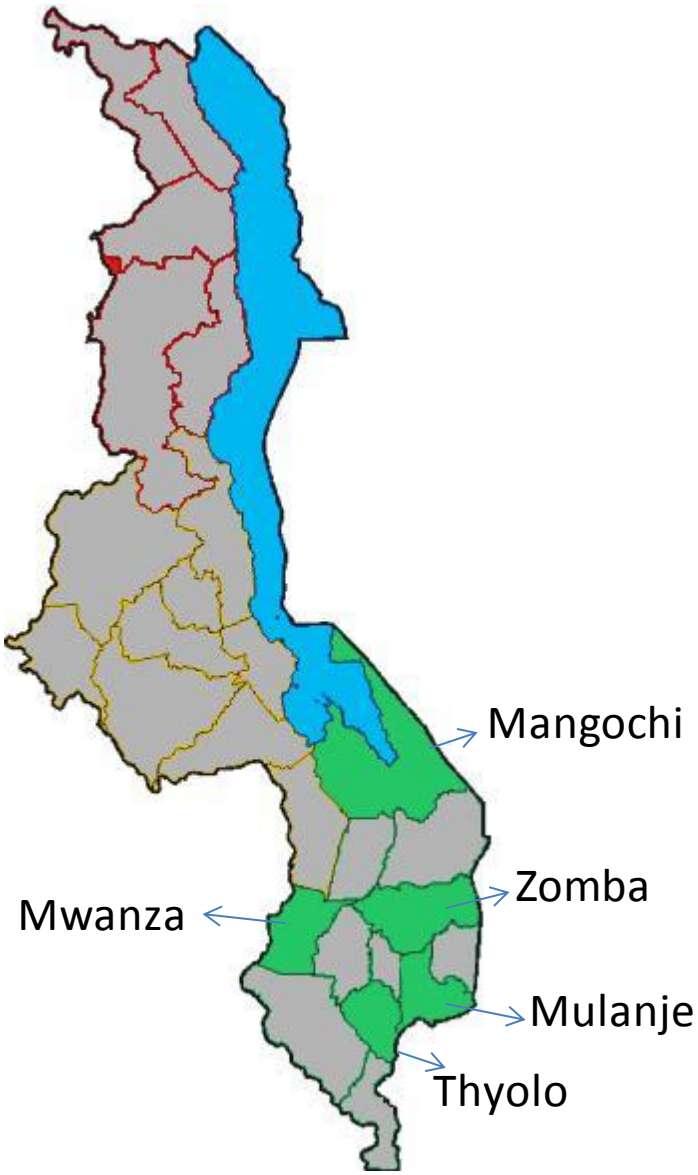

Figure 1. Study area: Map of Malawi

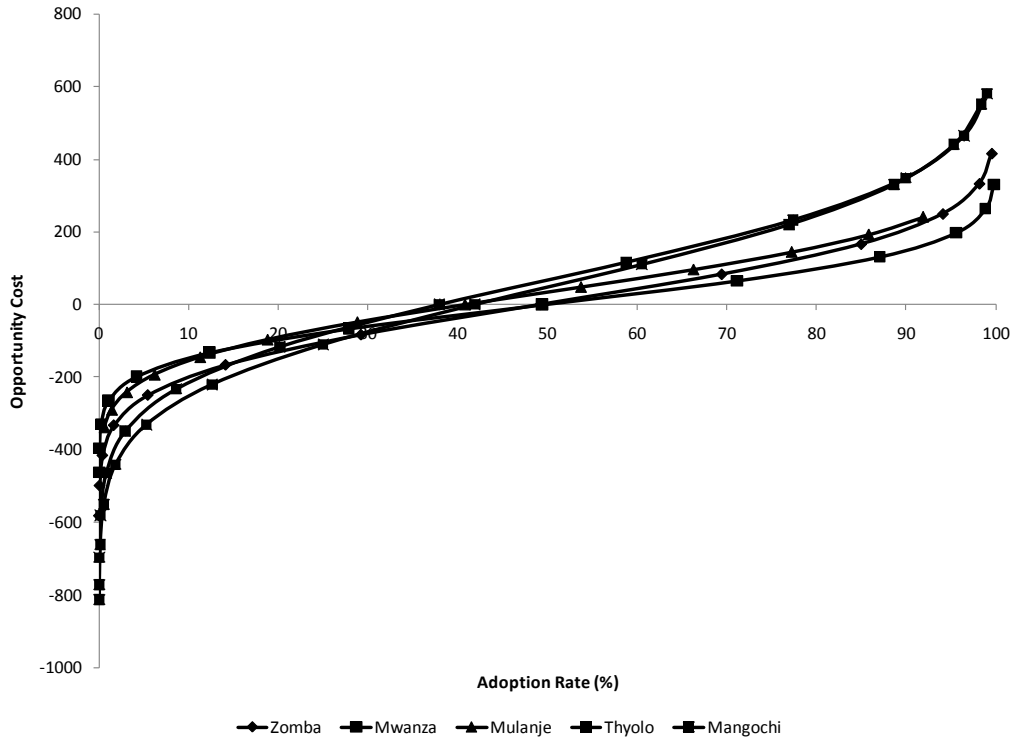

Figure 2. Adoption Rate and Opportunity Cost of Adopting IAA in Southern Malawi – Predicted Adoption Rate is Point Where Curves Cross the Horizontal Axis

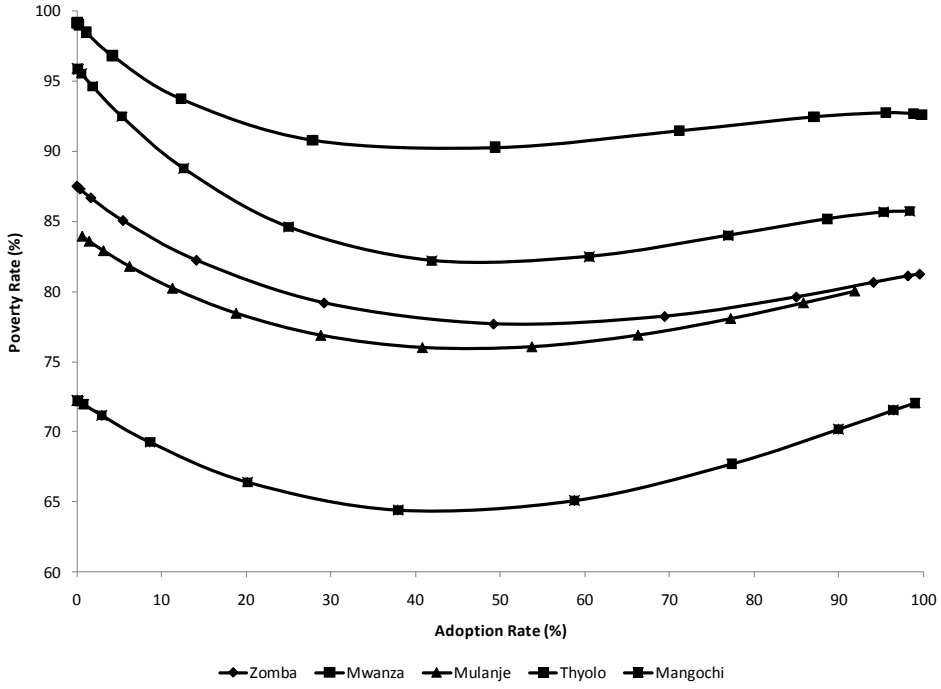

Figure 3. Poverty Rate and Adoption Rate of IAA, Southern Malawi.

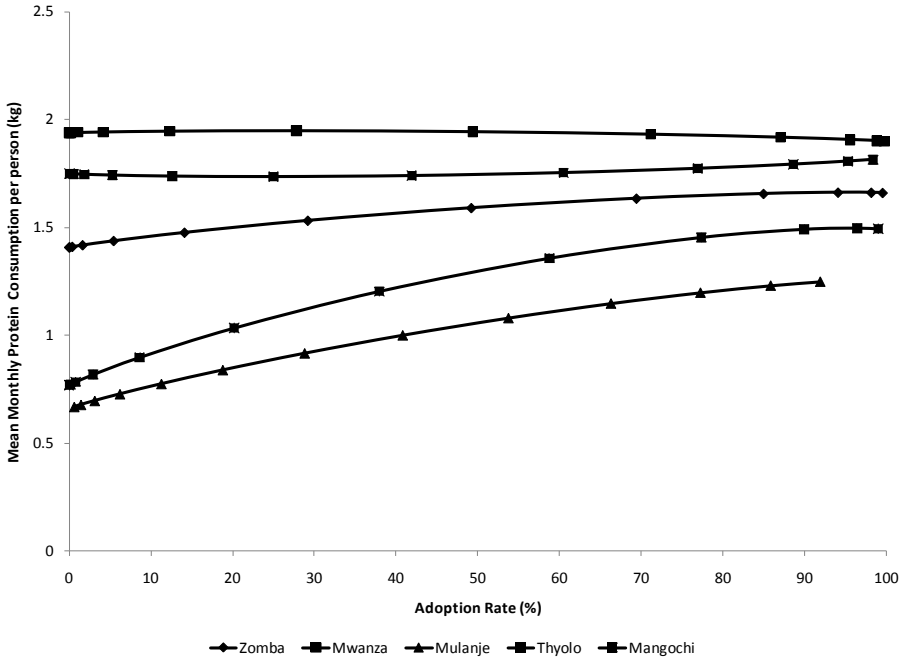

Figure 4. Mean Monthly Protein Consumption and Adoption of IAA, Southern Malawi.

Table 1. Summary results

Strata	Adoption rate (%)	Ave. farm income ($/year)			Poverty rate (%)			Mean Monthly Protein Consumption (kg/person)		
		base (no adoption)	% Change on population	% Change on adopters	base (no adoption)	% Change on population	% Change on adopters	base (no adoption)	% Change on population	% Change on adopters
ZOMBA	49.22	112.47	54.60%	135.62%	87.50	-15.81%	-42.48%	1.41	12.86%	38.95%
MWANZA	49.40	89.01	50.77%	137.61%	99.16	-16.01%	-51.88%	1.94	0.30%	10.64%
MULANJE	40.81	81.01	54.46%	179.51%	84.30	-11.38%	-44.26%	0.65	53.10%	191.35%
THYOLO	41.92	170.85	41.85%	116.92%	95.93	-16.48%	-56.11%	1.75	-0.49%	28.63%
MANGOCHI	37.95	188.62	30.77%	116.63%	72.24	-10.53%	-53.66%	0.77	56.42%	178.33%
REGION	**44.49**	123.90	45.23%	132.70%	87.11	-11.25%	-30.45%	1.29	15.32%	59.00%

VALUE CHAIN OF CULTURED SNAKEHEAD FISH IN THE MEKONG DELTA OF VIETNAM

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ABSTRACT

Snakehead fish is the fish species which is mostly preferred by consumers in the Mekong Delta of Vietnam. However, it is difficult to develop this industry due to a number of reasons. This study was carried out with the aims to describe the value chain of cultured snakeheads and to analyze the distribution of cost-benefit among the chain actors in the delta. Among 10 common market channels, two most important ones in term of total production were Channel 3 (Fish farmers – Wholesalers – Retailers – End consumers in the Mekong Delta), and Channel 9 (Fish farmers – Wholesalers – Wholesalers in Ho Chi Minh City). Profit was unbalant distributed among the chain actors, mainly for the wholesalers (87.9-93.4% of total profit of the whole chain). In order to have an appropriate development of snakehead industry, to improve profit of the whole chain and to have a better competition power, the followings should be given more consideration: (i) more proper planning of cultured area and technological supports, and marketing of fish products; (ii) To encourage the application of pelette feed in order to reduce the pressure on fresh water wild fish stocks; and (iii) To have incentive policies/regulations that help to encourage the processors to export, especially processed products for a long-term market expansion in terms of higher production, more export value, and stable price of snakehead products.

Key words: *chain actor, cost, profit, snakehead fish, value added, value chain, yield.*

INTRODUCTION

The cage culture of giant snakehead (*Channa micropeltes*) was started in Vietnam in the 1960s, while the farming of common snakehead fish (*Channa striatus*) was begun in the 1990s and spread by different farming systems in the flood-prone areas of the Mekong Delta. However, the development of snakehead fish culture was unstable and said to contribute to the depletion of wild fish resources, more water pollution, and unsustainable development. This study was carried out over a one year period beginning in September 2009. It covers four main provinces of snakehead farming in the Mekong Delta (An Giang, Dong Thap, Can Tho and Hau Giang). The study aims to describe the value chain of cultured snakeheads and the distribution of cost-benefit among the chain actors in the study area. Five main groups of actors of snakehead value chain (farmers, traders, processors, retailers and end consumers) were interviewed. In addition, two chain supporters, including market managers and government officers were also interviewed.

Reports from provinces in the Mekong Delta in 2010 revealed that estimated total production of snakehead fish in the Delta was about 40,000 tons, increasing about 1,000 tons compared to that of the previous year, of which giant snakeheads made up approximately 20% of the total production. The average stocking duration was 4-6 months/crop depending on cultured species and selling price at the harvest. Average stocking density was 204 fish/m^3 (or 114 fish/m^3) with the average survival rate of 53.2% and the average yield was 41.9 kg/m^3/crop. Production cost was VND 29,700 per kg and if the cost of self-captured trash fish was not taken into account, this cost was reduced to about VND 24,400 per kg. Most of local traders bought snakeheads directly from the grow-out farmers (54.7%) and resold the fish to bigger traders in Ho Chi Minh City (58.8%). All retailers in the local markets sold their fish to local consumers.

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For dried snakehead processors, the average amount of raw fish bought was 8.2 tons/processor/year, of which 84.4% was bought from fish traders. Approximately 60% sold their dried fish to HCMC after processing. The average purchased quantity of raw snakehead bought by fish sauce processors was 9.0 tones/processor/year, of which 39.6% was bought from grow-out farms. Today, some processors of fish sauce bought wild snakeheads from Cambodia (5.7%) due to the depletion of wild fish in the local markets. The wild snakeheads from Cambodia were mainly traded in the flood season (September to December).

There were 10 marketing channels of snakehead fish identified, of which two are most important *in terms of total production*: (1) *"Fish farmers – Wholesalers – Retailers – End consumers in the Mekong Delta"* and (2) *"Fish farmers – Wholesalers – Wholesalers in Ho Chi Minh City".* Profit was not distributed fairly among the chain actors. Traders received more profit than others (about 87.9-93.4% of total chain profit). Retailers received the highest level of profit/kg but their total profit was lower than other actors due to small amount of fish purchased.

There were five independent variables found that could affect fish yield at the same time ($p<0.05$). They were: (i) Own hatchery; (ii) Own nursery; (iii) Stocking density; (iv) Species of giant snakehead; and (v) Costs of medicines and chemicals for prevention and treatment of snakehead diseases. Most of these independent variables were positively related to fish yield, except the own hatchery (negatively relationship). If stocking density is increased to more than 150 fish/m^3, the total production costs were found to increase very much. The net income also increased if stocking density increased but the best result was to stock at a density of 120-150 fish/m^3. The costs of medicines and chemicals for fish health management can be increased compared to the mean value of that cost item in order to increase the fish yield, but VND 28,000-35,000/m^3/crop can help to provide the best benefit.

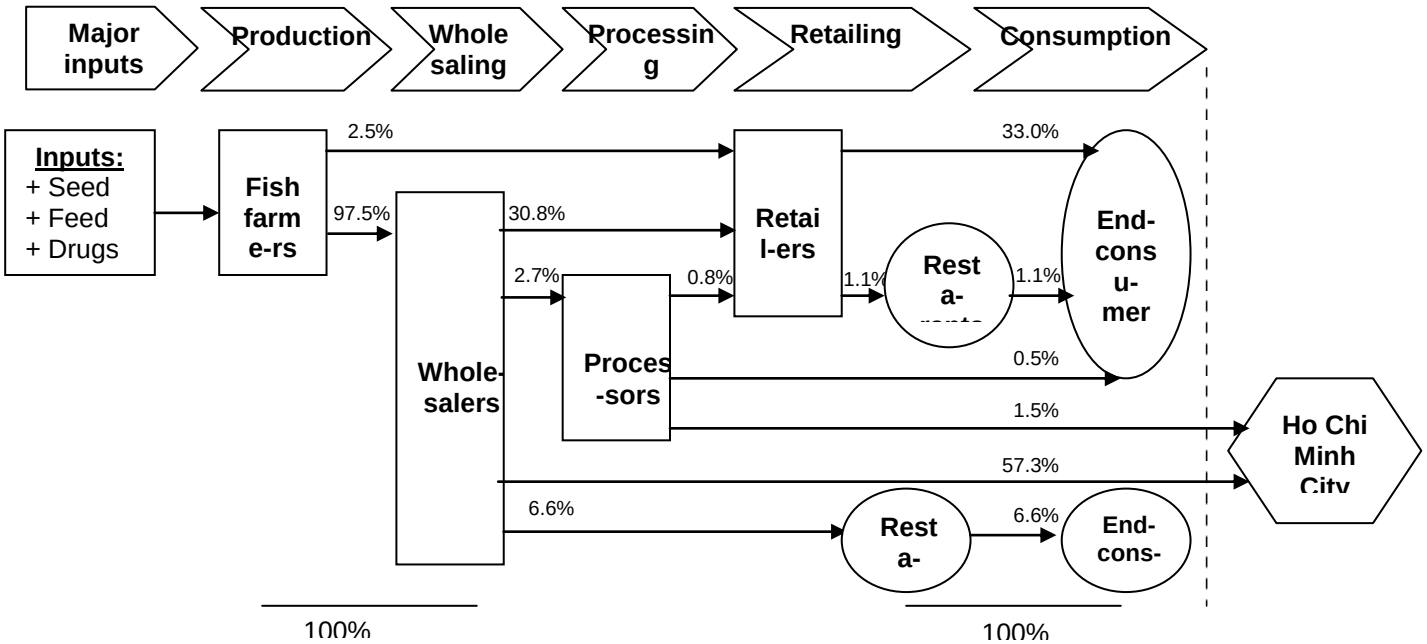

Figure 1. Marketing channels of snakehead fish in the Mekong Delta

In order to have an appropriate development of the snakehead industry, to enhance the competitive advantage, and to increase income throughout the whole value chain, in particular for farmers, the following issues should be give more concern: (i) Planning and management of snakehead industry in association with protection of

aquatic resources and capital and technical support as well as a better organization of production and marketing of snakeheads; (ii) Development of concentrated grow-out areas for snakeheads with more application of pellet feed, aiming to increase the production of snakeheads and reduce the pressure on wild freshwater fish resources; and (iii) Some policies to support the processors to process and to export snakehead products for market expansion, better and more stable price.

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USE OF GONADOTROPIN RELEASING HORMONE ANALOGS ON THE INDUCED REPRODUCTION OF CHAME *Dormitator latifrons*

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Abstract

Chame (Pacific Fat Sleeper) is considered a relevant upcoming fish species for aquaculture; particularly in Ecuador and some preliminary trials in Mexico. Nevertheless, the reported production for the last 15 to 20 years in culture has been dependant of wild-caught juveniles. Thus, we are conducting research focused on the achievement of controlled reproduction and larvae production as well as to get relevant information on the reproductive biology of the fish. At this moment we have successfully induced gamete release in both genders using the following procedures: An experiment was conducted with 16 females divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly^{10}-Ala^{6} LHRHa injected at 40 µg/kg (priming dosage) and 80 µg/kg (resolving dose), 2 injections of Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®). Spawning results showed 100% success within 24h and 48h for the Ovaplant group, and 25% for the LHRHa treatment but 0% for Ovaprim group within 48-72h. Only one natural spawn was observed. Obtained data establishes oocyte size as 300 µm and a relative fecundity of 80,000 to 100,000 cells per gram. All delivery treatments were effective to induce spermiation in volumes from 0.5 to 10 ml per male (LHRHa injected at 40 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®); however several males released sperm naturally up to 1 ml throughout the reproductive season. Obtained data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to 2X10^{9} cells per milliliter. Increased sperm motility is achieved after predilution on a 1:10-1:40 ratio in Ringer´s solution. As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity; similar data were recorded for optimal incubation salinity as no hatching was observed above 5‰ salinity. These findings are relevant due to the differences with other spawning protocols previously used, given that other trials reported the need of repeated injections of Human chorionic gonadotropin (HcG) up to 10,000 UI per fish. Another difference with previous studies was the observance of only partial spawns. We conclude that these protocols allow to successfully obtaining viable gametes for chame larvae production.

Introduction

At present, medium-scale commercial aquaculture in Ecuador, as well as initial experiences of chame culture in Mexico, are conducted with wild caught juvenile fish. There is also interest in this fish in Nicaragua, where freshwater fishes such as tilapia are currently fetching higher prices than cultured shrimp. Therefore, the goal of this work is the production of juveniles under laboratory conditions and minimize the dependency on wild fish supply. Available information indicates that in Ecuador, chame aquaculture has continuously decreased over the last eight years due to the shortage of juvenile fish since controlled propagation has not been achieved. Research in this area was largely abandoned over ten years ago. For Mexico, there is a

steadily demand on the central and the southern Pacific Coast. Also, as surveyed by the authors, there are already fish farmers interested in acquiring laboratory produced juveniles for commercial aquaculture in Oaxaca State. In addition, the species is not considered for protection under Mexican laws, and controlled juvenile production will provide a considerable benefit for the diversification of fish culture in Mexico. The main goals of this proposal are the following: 1) attempt hormonally induced reproduction by outlining the viability of the utilization of newer spawning techniques; 2) fertilization and egg incubation at different salinities to evaluate hatching success; 3) a series of trials with larvae offered live and dry food as exogenous starter diets have been conducted at a preliminary stage. This manuscripts details the first trails with hormone induced spawning and spermation in chame.

Materials and Methods

Broodstock fish were collected in a 100 km radius of Mazatlán, Sinaloa Mexico and later transported and acclimated to FACIMAR-UAS (23°12´57" N; 106°25´31" W). Fish were fed with a combination of 60% floating pellets (32% protein 8% lipids) and 40% sinking pellets (35% protein 10% lipid). Fish of both genders were tagged using PIT-Tags (Passive Integrated Transponder tag, Biomark®) and potential breeders with visible signs of gonad maturation such as swollen abdomen, significant individual weight gain and changes in coloration on males and females, both in the papilla and the abdomen (Bonifaz et al, 1985; Estuardo Campoverde, pers. comm.), were separated and monitored, however gonad biopsies were not possible due to the significantly reduced size of the pore at the papilla. An. experiment was conducted with 12 females divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly^{10}-Ala^{6} LHRHa (sigma®) injected at 40 µg/kg as priming dosage and 80 µg/kg as resolving dose, 2 injections of Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®) (Syndel®). Number of spawners 24 and 48 h after hormone treatments, number of oocytes per gram (relative fecundity) and oocyte diameter was measured in spawned fish per treatment.

For males, an experiment with twelve fish was carried out to induce spermiation with the following treatments: control group (0.5 ml/kg 0.9% saline solution), desgly10-Ala6 LHRHa injected at 40 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®). Sperm quality as motility, activation time and sperm concentration were evaluated in spermiating fish per treatment.

An alternative protocol for sperm activation and fertilization as well as hatching success in terms of water salinity was conducted as follows: sperm samples were pre-diluted in ringer´s solution at several dilution ratios (1:1-1:40) (Arias-Rodriguez L. UJAT-Tabasco, per. comm.) as sperm viscosity was too high to allow effective activation with direct dilution in activation media (10 µm filtered, UV sterilized water). Once pre-dilution was completed, again 50-100 µl of Ringer´s diluted sperm samples were activated in 900 µl of activation media (0, 5, 15, 25, 35, 45, 55 and 65 ‰) to establish best activation conditions as water salinity value.

Also, once spawns were achieved, water salinity incubation conditions were estimated by placing 1000-1500 fertilized eggs in 1 l containers with 10 µm filtered, UV sterilized water at 0, 5, 15, 25, 35, 45, 55 and 65 ‰ with three replicas per salinity. Survival (%) per salinity and total length and morphological characteristics of larvae at hatching and thereafter were observed using digital image analysis with Motic Image Plus 2.0 software (Fig. 1).

Figure 1. Computer digital analysis for measurement of day-2 larvae using Motic Image Plus 2.0 software

Result and Discussions

The use of gonadotropin releasing hormones is a useful technique for the induced reproduction of chame. For females, spawning results showed 100% success within 24h and 48h for the Ovaplant group, and 25% for the LHRHa treatment but 0% for Ovaprim group within 48-72h after injections or implantations (Fig. 2). Ovaplant females released oocytes both 24 or 48 h after implantation; as extra information in overall a total of 29 spawns were achieved on this first attempt to produce viable oocytes for larvae production of chame using implantation delivery techniques for synthetic analogs of GnRHa (Ovaplant®) at a 75 µg single implant; other inducing spawning treatments were not as effective as implants, nevertheless still LHRHa showed some interesting results to be verified in a follow-up experiment during chame next reproductive season in Mexico (Sept-Oct).

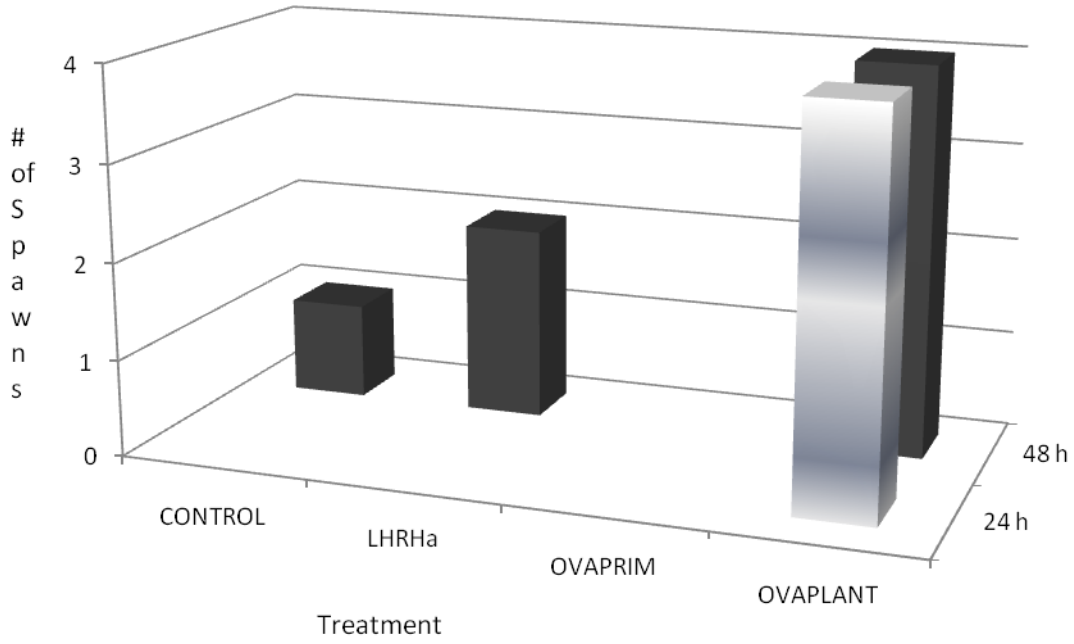

Figure 2. Spawning success of females per treatment (n=4) within 24 and 48 h after injection or implantation.

Only one natural spawn was observed for all females either within the experiment and for all collected fish. As extra information, we estimated that mean oocyte diameter is 300 µm and fish showed a relative fecundity of 80,000 to 100,000 cells per gram (Table 1) Apparently, only partial spawns were recorded for all observed spawns. Thus, we were able to validate GnRH analogs as valuable tools to achieve controlled spawning of chame, in a similar fashion to bullseye puffer *Sphoeroides annulatus* (Duncan et al., 2003) and with better results that previous trials in Ecuador (several per comm.) were large amounts of HcG had to be injected (up to 10,000 UI) to obtain viable oocytes or no spawn achieved as tested with fatsleeper *Dormitator maculatus* using HcG, LHRHa and Ovaprim (Gaude et al, 2010).

Table 1. Estimated values of spawning females for all experimental treatmens within the experiment.

Variable	Control	LHRHa	Ovaprim	Ovaplant
Weight (g)	393.3±185.1	486.15±205.2	388.9±151.6	388.6±216.1
% of spawning fish	25%	50%	0%	100%*
Relative fecundity (cell g^{-1})	83000	59000	n/a	50000±10000**
Oocyte diameter (µm)	392.6±51.8	327.7±18.5	n/a	353.8±106.6**

*n=4 **Pooled from 4 females

For males, sperm quality issues are noticeable given that in most cases, milt collected can show very low sperm motility; either after hormone injection or with testicle removal and maceration from fish (Estuardo Campoverde, pers. comm.). We were able to induce spermiation in all hormone treatments with minimal changes in estimated sperm quality variables (Table 2).

As main difference, both and Ovaprim and Ovaplant groups released as significantly higher amount of milt, with noticeable sperm fluid mixed with sperm fluid; however it did not affect motility or sperm concentration (Table 2). LHRHa was an effective spermiation inducing agent as proved with many other fish such as bullseye puffer *Sphoeroides annulatus* (Rodriguez, 2001). Several males released sperm naturally up to 1 ml throughout the reproductive season as observed in this experiment. Obtained data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to $2X10^9$ cells per milliliter (table 2). No spermatocrit values were recorded as chame sperm viscosity probed to high in undiluted sperm. Therefore, GnRHa can be used to induce sperm release in chame, result that in our knowledge is the first report of similar findings.

Predilution of sperm in Ringer´s solution on a 1:10 ratio was determined to be the most favorable ratio, both for sperm activation and fertilization. As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity; similar data were recorded for optimal incubation salinity as no hatching was observed above the same salinity value. Therefore all fertilization and incubation trials were conducted in 1 µm filtered, UV sterilized fresh water.

Table 2. Estimated values of sperm quality for all experimental treatments

Variable	Control	LHRHa	Ovaprim	Ovaplant
Weight (g)	622.7±54.7	434.7±139.4	538.75±187.4	540.6±202.1
# of spermiating fish	3	4	3	4
Mean volume ml	0.5	2.3	4.3	8.2
Motility (%)	93.3±11.5	83.3±11.5	80.0±26.4	93.3±5.77
Activation time (Min)	4:24±0:22	4:57±1:91	2:47±1:37	2:90±1:02
Concentration (cell ml^{-1})	1.96E+09± 1.29E+09	2.29E+09± 7.8E+08	1.26E+09± 2.37E+08	2.31E+09± 7.84E+08

Eggs are demersal and have an adhesive layer, transparent and spherical with a 300 µm average diameter (Fig. 3a). Hatching occurs at 14-17 hours at 26°C, larvae length is close to 1288.2±137.2 µm, yolk sack diameter is around 171.2±10.6 µm with a single lipid droplet, no eyes or mouth are visible and show a vertical floating position with no active movement (Fig. 3b). At 24 h (1 day posthatching DPH), yolk sack diameter reduces to 137.1±8.3 µm, eyes are perceptible, with no pigmentation and digestive tract is noticeable (Fig. 3c). Mouth opening occurs at 2 DPH, ayes are well pigmented and digestive tract structures become more discernable (intestine, vestigial anus); yolk sack diameters is significantly smaller 93.8±10.7 µm (Fig. 3d). At 3 DPH, yolk sack is fully consumed and oral movements are perceptible and digestive tract has an evident circumvolution and pigmentation (Fig. 3e). Anus fully opens at 4 DPH, and some other internal structures are visible (i.e. liver) and body pigmentation increases considerably.

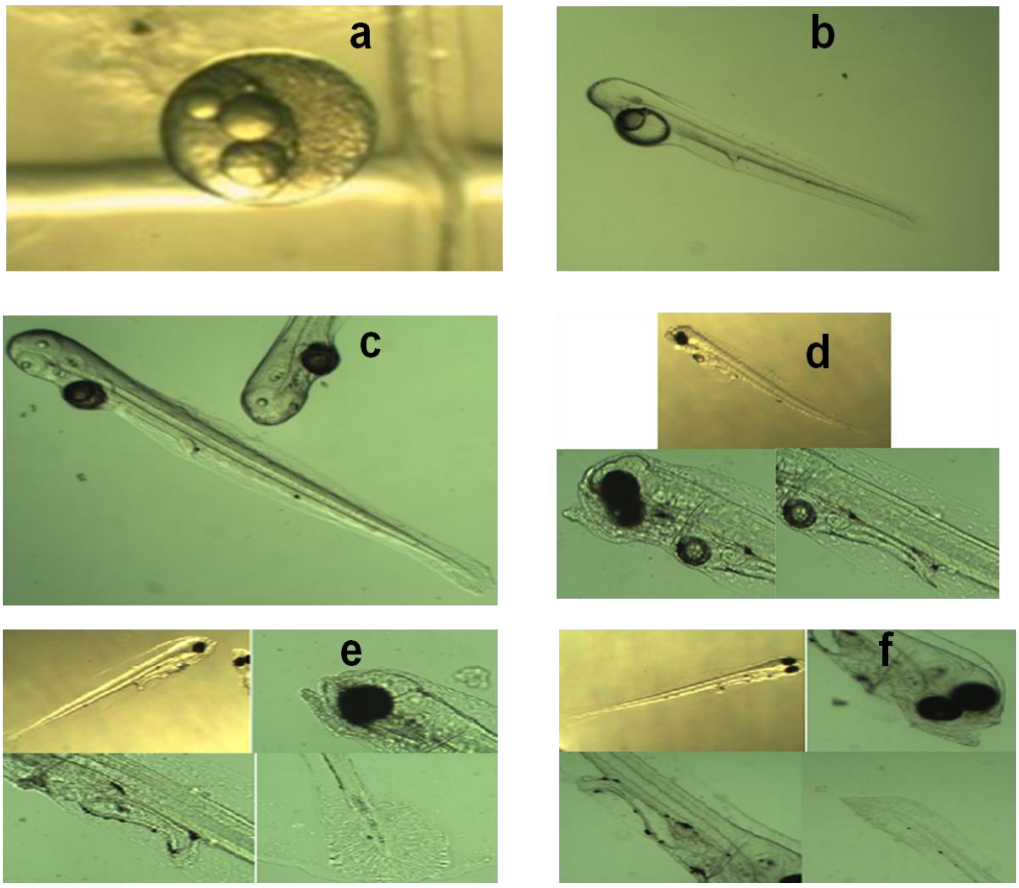

Figure 3. Early morphological development of chame larvae at 26°C.

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SECTION III
GENETICS and REPRODUCTION

Chair: Professor Gideon Hulata
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IMPROVING SALINITY TOLERANCE IN TILAPIAS: A REVIEW

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Abstract

With increasing scarcity of fresh water available for aquaculture, especially in arid regions, development of tilapias that tolerate high salinity would increase fish (and hence, animal protein) production. We review culture practices, nutrition, physiology and genetics, and propose approaches to improving salinity tolerance in tilapias. Dietary supplementation with NaCl and optimized acclimation protocols are immediate and practical ways to improve salt tolerance. Inter-specific variation in salinity tolerance may be used to select salt-tolerant species and develop salt-tolerant hybrids. Physiological studies of biochemical pathways underlying phenotypic differences in salt tolerance can lead to genetic studies of intra- and inter-specific variation. Molecular technology can lead to studies on osmoregulation-related biochemical pathways, for which the euryhaline tilapia is an attractive model. Functional genomics and proteomics are powerful tools for studying the molecular bases of environmental adaptation and metabolic connections to osmoregulatory physiology. Both provide avenues for discovering novel pathways related to osmoregulation with relevance to aquaculture. In the long term, quantitative trait loci associated with, or genes involved in saltwater tolerance may facilitate marker-assisted or gene-assisted selection for this trait in tilapia.

We recently examined the possible interrelationships between the allelic polymorphism of the tilapia Prolactin gene and growth performance in brackish water. Comparative sequencing revealed one amino-acid substitution at a highly conserved site adjacent to the receptor binding site, where the conserved leucine in *O. mossambicus* is replaced by phenylalanine in *O. niloticus*. We also studied allelic variation in a microsatellite marker at the promoter region of the Prolactin 1 gene. Two distinct alleles in *O. niloticus* as well as two additional alleles in *O. mossambicus* were detected. Parental crossing was performed between *O. mossambicus* and *O. niloticus* in search for correlations between the allelic composition and growth performance of F_2 families in brackish water. Correlation between genotypes (allelic composition) and growth performance was found in some but not all families. One allele appears to be associated with good growth in brackish water.

INTRODUCTION

With the increasing scarcity of freshwater available for aquaculture in general, and for tilapia culture in arid regions (like Israel) in particular, tilapias tolerating high salinity would increase global tilapia production (and hence, animal protein production) by expanding the range of production in many regions of the world. Cnaani and Hulata (2011) have recently reviewed the subject, aiming to show what can be learned from the past experience in areas of culture management practices and nutrition as well as physiology and genetics, and to propose the best approaches for improvement of salinity tolerance in tilapias. The present paper brings the highlights from that review, focusing on the more recent findings, and some results of additional work carried out recently in our laboratory.

PHYSIOLOGICAL STUDIES

Control of salt and water balance within a narrow limit is critical to life in all multicellular organisms, including teleost fishes. Salt tolerance is a term describing the overall fitness, or productivity, of the fish in a saline environment. It is a combination of different quantitative traits, such as metabolism, growth, osmoregulation, immunocompetence and fecundity.

Inter-specific variation in salinity tolerance may be used to select salt-tolerant species and develop salt-tolerant hybrids. Growth of *O. niloticus* at high salinity is significantly lower than that in freshwater (Fineman Kalio, 1988), whereas survival is not affected by salinity. High salinity does seem to suppress, or at least delay, onset of reproduction in *O. niloticus,* thus presenting a practical method of population control.

Chloride cells (known also as Mitochondrion Rich Cells - MRC) in the gill epithelium are important osmoregulatory sites in all fish species. Their large surface area at both sides, the apical and basolateral, is a placement of ion transporting proteins such as sodium-potassium ATPase (Na^+/K^+-ATPase) and $Na^+/K^+/2Cl^-$ co-transporter (NKCC). Studies on changes in chloride cells characteristics and function in response to salinity challenges, mostly in *O. mossambicus*, revealed a significant increase in the abundance of chloride cells and in ion transporters activity in the gills (Fiess et al., 2007). Differences in ion transporters type and membrane location on the chloride cells were also found between fresh- and salt-water challenged fish.

Hormones of the neuroendocrine system are essential players in the control of osmoregulatory mechanisms, and extensive studies on endocrine pathways involved in osmoregulation clarified the role of prolactin (PRL) and growth hormone (GH) in osmoregulation. PRL and GH are closely related and thought to be derived from the same ancestral gene. They exhibit a variety of functions in growth, development, osmoregulation and reproduction that are variously distinct, overlapping or opposing (Sakamoto and McCormick, 2006; Mancera and McCormick, 2007). Tilapias possess two PRLs that are encoded by separate genes (Specker et al., 1985). One form of PRL, PRL_{177}, binds to the GH receptor and has somatotropic actions of stimulating growth and cell proliferation (Shepherd et al., 1997). GH is produced and secreted from the anterior pituitary gland and has been shown to have an osmoregulatory role in seawater, where it promotes ion regulation by stimulating chloride cell proliferation and up-regulating ion transporters tied to extrusion pathways (Sakamoto and McCormick, 2006; Mancera and McCormick, 2007). Several isoforms of the PRL receptor exist in tilapia, with unique intracellular signaling pathways (Fiol et al., 2009). It is becoming increasingly apparent that the existence of an array of receptor subtypes has a major role in the pleiotropic nature of GH and PRL.

Osmoregulation, somatic growth, and reproduction are among the most energetically costly metabolic activities engaged by teleost fishes. Boeuf and Payan (2001) discussed four possible pathways of interaction between osmoregulation and growth: (1) difference in standard metabolic rate, (2) increase in food intake, (3) increase in digestibility, and (4) hormonal stimulation. These four pathways can interact, and none can be considered as a unique route connecting osmoregulation and growth.

Growth and development are directed by an integration of environmental, physiological and genetic factors. The high energetic cost of osmoregulation, usually estimated as 25-50% of metabolic output, means that there is a link between osmoregulatory and growth capacities. This might explain the observation that growth and osmoregulation are governed by many of the same hormones, notably PRL and GH. It has been demonstrated that genetic variation in the tilapia PRL gene is associated with differential gene expression and growth rate in saline water (Streelman and Kocher, 2002).

Functional genomics (i.e., the field of molecular biology that attempts to answer questions about the function of DNA at the levels of genes, RNA transcripts, and protein products) and proteomic (i.e. the study of the entire complement of proteins, particularly their structures and functions) approaches represent powerful tools for gaining insight into the molecular bases of environmental adaptation. Gene transcripts for ion transporters, enzymes, hormones and components of cellular stress signaling were characterized in the brain, gill, gut and kidney of Mozambique tilapia (Fiol et al., 2006) and black-chinned tilapia (D'Cotta et al., 2006; Tine et al., 2008). The transcriptional response to tilapias salinity challenge was studied for the immediate and long term response in two highly salinity tolerant species. In the Mozambique tilapia genes involved in the immediate hyperosmotic stress response were analyzed in gill epithelial cells. Most genes show an immediate response with peak levels observed between 2 and 8 h after seawater transfer. Pathway analysis of the newly identified genes revealed that more than half of the identified immediate hyperosmotic stress genes interact closely within a cellular stress response signaling network. The genes cluster together in six molecular processes that are rapidly activated in tilapia gills upon salinity transfer: (1) stress response signal transduction, (2) compatible organic osmolyte accumulation, (3) energy metabolism, (4) lipid transport and cell membrane protection, (5) actin-based cytoskeleton dynamics, and (6) protein and mRNA stability (Fiol et al., 2006). In the black-chinned tilapia genes whose transcription is induced by 45 days acclimation to either hyper-saline waters or to fresh water were analyzed in the gills. The suppression subtractive hybridization (SSH) resulted in the isolation of a wide spectrum of differentially expressed genes, classified according to functional annotations. These genes were clustered into 14 functional categories of biological processes. Cellular processes, metabolic processes, and localization were the most abundant categories in the high salinity library (D'Cotta et al., 2006; Tine et al., 2008).

APPROACHES TO IMPROVING SALT TOLERANCE IN TILAPIAS

Adding salt to feed: Saltwater survival of *O. mossambicus* improved by 84% after two weeks of feeding the salt diet, and that of the *O. aureus* x *O. niloticus* hybrids by 62%. Three weeks of feeding the salt diet were required to improve survival of *O. spilurus* by 50%. Contrary to the sudden increase in plasma osmotic concentration recorded in the fish transferred directly from freshwater to 60% sea water, feeding the high-salt diet prior to the transfer resulted in only a slight increase in the plasma osmotic concentration in sea water (Al-Amoudi, 1987). In all-male tilapia hybrids (*O. aureus* X *O. niloticus*) fed with feed that did not contain fish meal, 3% dietary salt supplementation resulted in about 20% improvement in specific growth rate and feed conversion ratio while cultured in freshwater for two months (Cnaani et al., 2010).

Acclimation: *O. mossambicus* can be acclimated in a single step (intermediate salinity directly to final full sea water salinity) and requires only one day at the intermediate salinity for sea water acclimation with no mortality, *O. aureus* requires four days and *O. niloticus* eight days for acclimation (Perschbacher, 1992). Yao et al. (2008) investigated the best conditions for transfer of Nile tilapia (*O. niloticus*) from freshwater to salt water. Fingerlings (8 to 12 g) were transferred, either directly or gradually, from freshwater to water of variable salinities, and survival was monitored after 3 weeks. Survival of fish transferred directly to saline water was high (84.3% to 96.8%) until 17 ppt, but mortalities were significant (60-70%) above that salinity. High rate of survival (78 to 81%) was, however, achieved by gradual acclimation to salinity of 30 ppt over two days.

These management practices are useful, yet genetic approaches may be more sustainable.

Variation among species and hybrids: Villegas (1990) found that *O. niloticus* was significantly less saline-tolerant than *O. mossambicus* and their reciprocal F_1 hybrids. Similarly, Kamal and Mair (2005) evaluated *O. niloticus, O. mossambicus* and *O. mossambicus* x *O. niloticus* hybrids over a series of salinities. *O. niloticus* exhibited faster growth at low salinity and *O. mossambicus* at the higher salinities; the hybrid was superior to *O. mossambicus* at all salinities and to *O. niloticus* at salinities above 10 ppt.

Several red tilapias, such as the Taiwanese (Cheong et al., 1987), Florida (e.g., Thourad et al., 1990; Watanabe et al., 1990; Ernst et al., 1991; Head et al., 1996), Philippine (Romana-Eguia and Eguia, 1999) and Thai (Yi et al., 2002) strains, originating by hybridization of either *O. niloticus* or *O. mossambicus*, are also considered saline-tolerant. Significant differences in the growth among five strains of Asian red tilapia (*O. mossambicus* or *O. mossambicus-hornorum* hybrid crossed with *O. niloticus*) were found when grown in fresh, brackish and salt water (Romana-Eguia and Eguia, 1999) using *O. mossambicus* as a reference strain. However, they also observed a significant interaction between strain and rearing condition. Ignoring the interaction effects, their results suggested that overall growth in length was more rapid in brackish water (17 ppt) than in either freshwater or salt water (34 ppt).

***Variation within species*:** To the best of our knowledge, there is not much divergence for salt tolerance within Nile tilapia (*O. niloticus*). Selection of more salt-tolerant strain(s)/population(s) of *O. niloticus* would be based on documenting the salinity tolerance of various wild stocks in their native waters, and comparing them under standard conditions. Basiao et al. (2005) evaluated three commercial strains of *O. niloticus* for growth rate in freshwater and saline water (32 ppt) relative to an 'internal reference' population and found significant strain effects on specific growth in standard length in both saline and freshwater environments.

***Hybridization*:** A saline-tolerant hybrid, produced by crossing the salt-tolerant *O. mossambicus* with a commercial ND9 line is characterized by good growth rate and high salinity tolerance. Males of this F_3 hybrid were crossed with orange-colored females of the ND5 commercial line, resulting in a mostly homogenous red tilapia with good growth rate and salinity tolerance, termed ND60. The good performance qualities were confirmed in growth trials in tanks (compared to a commercially-cultured hybrid of *O. niloticus* x *O. aureus*) and in commercial sea-cages. Consequently, this hybrid was introduced into a brackish water farm in Surinam and a marine farm in Guatemala (Lahav and Ra'anan, 1997). A synthetic strain of fast-growing tilapia with high salinity tolerance that breeds naturally in brackish water was developed in the Philippines starting in 1999 through a series of repeated backcrosses of the saline-tolerant *O. mossambicus* to the hybrids, coupled with selection for growth rate, and was named "molobicus". The first stage of the project produced a hybrid population that was 1/3 *O. niloticus*, 2/3 *O. mossambicus* with a good salinity tolerance (Mateo et al., 2004; Rosario et al., 2004). Another inter-generic hybrid of interest regarding salinity tolerance was produced by artificial propagation - the two reciprocal hybrids between the fast-growing *O. niloticus* and the highly euryhaline *S. melanotheron* (Toguyeni et al., 1997; Baroiller et al., 2000). Both hybrids were viable and fertile, and their growth rate was intermediate to that of the two parental species, but their relative salinity tolerance was not reported.

***Selective breeding*:** Four *Oreochromis* species were used in an evaluation of salinity tolerance conducted by Tayamen et al. (2002) in the Philippines. A diallel cross of the different species/strains was carried out involving *O. spilurus*, *O. aureus*, *O. mossambicus* and three genetically improved strains of *O. niloticus*, namely: sixth-generation improved GIFT strain, FAC selected line (FaST), and all-male YY tilapia. Progenies from the 27 cross combinations (5 purebreds and 22 crossbreds) were evaluated in 10 environments with different salinity levels and agro-climatic conditions using a communal rearing design. Among the different cross combinations reared across environments, *O. aureus x O. spilurus gave the highest body weight*

and O. mossambicus x O. spilurus, the highest survival rate. Tayamen et al. (2004) continued the selection program by breeding the selected fish and testing the progeny in different culture systems. Salinity tolerance in terms of growth and survival was positively influenced by having *O. spilurus* as sires, while *O. niloticus* FaST dams contributed most to increased growth rate. Different rankings in terms of growth and survival were obtained across environments. The second stage of the "molobicus" project was initiated in 2003 with selection process based on a simple within-family selective breeding scheme in a saline environment (Rosario et al., 2004). Fish of the first selected generation of "molobicus" currently are used on a small scale in the Philippines, while the selection process is going on (P. Morissens, CIRAD, France, and W. Rosario, BFAR-NIFTDC, Philippines, pers. comm.).

Armas-Rosales (2006) took a quantitative genetics approach to evaluate genetic effects influencing tilapia salinity tolerance using a diallel mating design. Six parental strains were used [*O. aureus*, *O. mossambicus*, *O. niloticus*, Stirling red *O. niloticus*, Florida red tilapia (originated from an *O. urolepis hornorum* x O. *mossambicus* male hybrid) and a commercial hybrid (originated from the Rocky Mountain White® tilapia)], resulting in 36 genetic groups. Twenty-four salinity levels were used in the growth trial. Salinity tolerance was determined for all strains and crosses, and genetic effects influencing salinity tolerance were estimated. Several lines exhibited highly significant line and maternal effects. Several crosses exhibited highly significant heterosis effects. The results suggest that improvement in salinity tolerance could be accomplished by developing a breeding program combining selection, hybridization and backcrossing among *O. aureus, O. mossambicus* and Florida red tilapia. Experiments were conducted at the Research Institute for Aquaculture No.1 (RIA1), northern Vietnam, to evaluate the growth and survival of the GIFT and Vietnamese strains of Nile tilapia in fresh and brackish water earthen ponds. The heritability estimates for harvest weight in both test environments were moderate (~0.2) for both brackish and fresh water. The genetic correlations of harvest body weight and survival were relatively low (>0.4) between the two test environments. The results suggest a substantial additive genetics variance for the traits that can be further exploited through a selective breeding program. However, in view of the strong genotype by environment interaction for harvest weight and survival traits observed, separate breeding programs should be considered for Nile tilapia in fresh and brackish water farming (Luan et al., 2008).

Genomic approaches: Genomic approaches may offer contribution to aquaculture over a longer term. With the application of modern molecular biology techniques, it may be possible to identify genes encoding specific proteins active in salt-tolerant species that are lacking or are less active in less-tolerant species, or specific proteins that are induced under salt stress. One such gene is prolactin1 (*prl1*); this gene has a central role in adaptation of marine species to freshwater by reducing Na^+/K^+-ATPase activity and consequently increasing the osmotic level of the plasma (e.g., Sakamoto et al., 1997). Streelman and Kocher (2002) reported that microsatellite polymorphism in the tilapia *prl1* promoter is associated with differences in *prl1* gene expression and growth response of salt-challenged fishes. They crossed females of the salt-tolerant *O. mossambicus* (homozygous for long alleles) with a freshwater-adapted *O. niloticus* male heterozygous for microsatellite alleles that differed by 17 repeat units (CA_{31} vs. CA_{14}). Fish homozygous for the long allele grew more slowly at 16 ppt and their weight was only half those of the other two genotypes, while in freshwater growth rate did not differ significantly among the three genotypes.

We have recently re-examined this association in nine F_2 families of *O. mossambicus* X *O. niloticus* hybrids (Velan et al., 2011). Both parental fish were heterozygous for different alleles (CA_{33} and CA_{38} in *O. mossambicus*, CA_{30} and CA_{35} in *O. niloticus*, resulting with PCR products of 253, 263, 247 and 257 bp, respectively). The association reported earlier by Streelman and Kocher (2002) was observed in only three of the nine families. In two of those three families, full-sibs were also grown in freshwater where no correlation between the genetic polymorphism and

growth was found. In these two families, fish carrying the allelic combination 247/253 grew better in saline water and worst in fresh water (Figure 1).

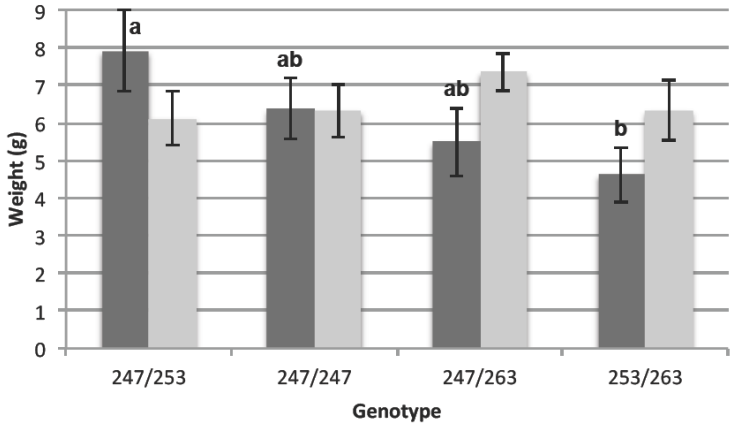

Figure 1. Weight of the four genotypes of offspring in one family, grown in salt water (dark bars) and fresh water (light bars). Groups sharing the same letter are not significantly different (α = 0.05).

We concluded that this variation is probably not a major contributor to the total genetic variation in salinity tolerance, and that there may be a large environmental influence underlying the differential growth in saline water. We have sequenced the *prl1* gene in the parental species and discovered one point mutation, within a conserved motif, that cause substitution of phenylalanine with leucine and forming two isoforms of the tilapia *prl1* (Figure 2).

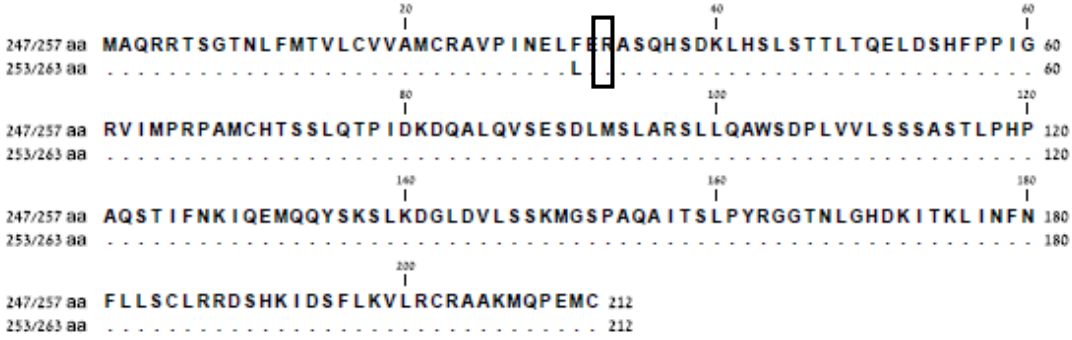

Figure 2. Comparison of *prl1* sequences between *O. niloticus* (top) and *O. mossambicus* (bottom) alleles.

The F31L mutation within a conserved motif is marked.

Rengmark et al. (2007) identified several candidate genes associated with salt tolerance in tilapia through a study that identified a number of genes differentially expressed in saltwater and freshwater – *beta haemoglobin, Ca^{2+} transporting plasma membrane ATPase, pro-opiomelanocortin* (all up-regulated in saltwater) and *beta-actin* (down-regulated). Rengmark and Lingaas (2007) investigated the role of transferrin, an iron-binding glycoprotein known to have an important role in the immune system, on salinity tolerance. They cloned and sequenced the entire transferrin gene of tilapia, and identified two microsatellites closely linked to the gene as well as many single nucleotide polymorphisms (SNPs) within it. Studies of the segregation of alleles in these two closely-linked microsatellite loci showed that they defined two haplotypes

(combinations of alleles); salt-tolerant individuals showed a strong tendency to possess haplotype 2, whereas the less salt-tolerant ones tended to possess haplotype 1. Expression levels of transferrin were compared in saltwater- and freshwater-reared tilapia using real-time PCR. Transferrin showed an 85% up-regulation in tilapia kept in saltwater compared to freshwater, suggesting that transferrin may be involved in saltwater tolerance or that closely-linked genes may be directly involved in saltwater tolerance. This gene was partially cloned and mapped to linkage group (LG) 21 of the tilapia linkage map by Cnaani et al. (2002), LG18 in the more recent Lee et al. (2005) map.

CONCLUSIONS

Studies on the molecular basis of osmoregulatory properties of the gills, kidney, gut and brain have revealed a wealth of genomic knowledge that can lead to genetic studies of intra- and inter-specific variation for salinity tolerance. Once relevant genes are identified, genetic polymorphisms can be searched for in cultured and natural populations. The emerging knowledge of quantitative trait loci (QTL) associated with, or genes directly involved in saltwater tolerance may facilitate marker-assisted or gene-assisted selection for this trait in tilapia in the future. Hence, the two routes that hold the keys for improving salinity tolerance are: (1) exploring and revealing biochemical pathways and gene networks involved in osmoregulation, thereby realizing a better understanding of both the salt tolerance phenotype and the genotypic background; and (2) screening domesticated and natural populations, searching for genetic variation in the biochemical pathways that underlie the observed phenotypic differences. Knowledge so gained can be exploited in selective breeding of tilapia stocks performing well in saline waters.

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COMPARISON BETWEEN GREEN WATER AND CLEAR WATER SYSTEMS DURING THE MASCULINIZATION PROCESS OF SILVER TILAPIA, *Oreochromis niloticus*

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Key words: *Oreochromis niloticus*, masculization, 17 alpha methyltestosterone, sex reversal, green water.

ABSTRACT

The growth and survival of Silver or Nile Tilapia (Oreochromis *niloticus*) was compared between green water systems (containing green algae) and clear water systems (no algae). Both treatments (green and clear water treatments) were replicated four times using 45.72cm x 20.32cm x 20.32cm tanks, stocked with 100 fry each. The hormone 17 alpha methyl testosterone was administered four times per day for twenty eight days, in an attempt to obtain an all male stock. Water chemistry including pH, ammonia and nitrite levels was monitored weekly and temperature was monitored daily. An algal count and species identification was done to estimate the composition and density of green algae culture. The fish length, wet mass and mortality of the fish were monitored on the 14^{th} and 29^{th} day.

It was found that compared to clear water fish raised in green water were significantly larger and heavier ($F_{1, 575}$ = 1028.28: $p<0.001$ for length and $F_{1, 575}$ = 566.48 for mass). In addition there was a significantly higher survivorship in the green water system (mean of 85% survival in green water and mean survival of 61% in clear water respectfully, χ^2 =67.18, $p< 0.001$). There were also significantly higher levels of nitrite and ammonia in the clear water tanks. Green water systems were found to be overall superior for producing sex reversed or masculinized male Silver tilapia fingerlings, than clear water systems.

OSMOREGULATORY CAPACITY OF THE NILE TILAPIA (*Oreochromis niloticus* (L.)) DURING EARLY LIFE STAGES.

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ABSTRACT

Although not considered to be amongst the most tolerant of the cultured tilapia species, the Nile tilapia still offers considerable potential for culture in low-salinity water. The ontogeny of osmoregulation in the Nile tilapia was studied from spawning to yolk-sac absorption after exposure to different experimental conditions ranging from freshwater to 25 ppt. Eggs were able to withstand elevated rearing salinities up to 20 ppt, but transfer to 25 ppt induced 100% mortality by 48 h post-fertilisation. At all stages embryos and larvae hyper-regulated at lower salinities and hypo-regulated at higher salinities. Osmoregulatory capacity increased during development and from 2 days post-hatch onwards remained constant until yolk-sac absorption. Adjustments to larval osmolality, following abrupt transfer from freshwater to experimental salinities (12.5 and 20 ppt), appeared to follow a pattern of crisis and regulation, with values for larvae stabilising at *c.* 48 h post-transfer for all treatments, regardless of age at time of transfer. Age at transfer to experimental salinities (7.5 – 25 ppt) had a significant positive effect on larval ability to osmoregulate; larvae transferred at 8 dph maintained a more constant range of whole body osmolality over the experimental salinities tested than larvae at hatch. Concomitantly, survival following transfer to experimental salinities increased with age. There was a significant effect (GLM; $p < 0.05$) of salinity of incubation and rearing media on the incidence of gross larval malformation that was seen to decline over the developmental period studied.

INTRODUCTION

In recent times, diminishing freshwater resources, due to the rapidly increasing drain of urban, industrial and agricultural activities in combination with the impact of climate change, has called for an urgent need to manage marine and brackish water environments more efficiently. Therefore the diversification of aquacultural practices, either by the introduction of new candidate species or by the adaptation of culture methods for existing species is vital at a time when innovation and adaptability of the aquaculture industry is fundamental in order to maintain its sustainability.

The Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758), which has now extended well beyond its natural range, dominates tilapia aquaculture because of its adaptability and fast growth rate. Although not considered to be amongst the most tolerant of the cultured tilapia species, the Nile tilapia still offers considerable potential for culture in low-salinity water. An increase in knowledge of the limits of salinity tolerance of this species during the sensitive early life stages and the ability to predict responses of critical life-history stages to environmental change could prove invaluable in improving larval rearing techniques and extend the scope of this globally important fish species. It is well established that measurement of osmolality provides a valid route for the evaluation of the osmoregulatory status of fishes (Alderdice, 1988). Recent reports on ontogenic changes in osmoregulatory capacity during early life stage have been mainly confined to marine teleost species in an attempt to explain species and developmental stage-specific distribution i.e. turbot (*Scophthalmus maximus*) (Brown and Tytler, 1993), chum salmon (*Oncorhynchus keta*) (Kaneko et al., 1995), sea bass (*Dicentrachus labrax*) (Varsamos et al., 2001), Japanese eel (*Anquilla japonica*) (Unuma et al., 2005; Okamoto et al., 2009),

Mozambique tilapia (*Oreochromis mossambicus*) (Yanagie et al., 2009) and the gilt-head sea bream (*Spaurus aurata*) (Bodinier et al., 2010).

In the present study, the responses and physiological effects of osmotic challenge during ontogeny in the Nile tilapia were assessed through the measurement of embryo and larval osmolality and the resulting osmoregulatory capacity. In addition, the short-term osmoregulatory responses of yolk-sac larvae to abrupt transfer to a range of salinities (7.5 – 25 ppt) in terms of osmoregulatory capacity, survival and the related incidence of deformity were investigated. This is the first study to give a complete picture of the ontogeny of osmoregulatory capacity over a range of salinities during successive early life stages in the euryhaline Nile tilapia and provides valuable insights into ontogenic variations in the capacity of this species to hyper- and hypo-regulate over a range of salinities.

MATERIALS AND METHODS

Egg supply, preparation of media and rearing systems

All eggs were obtained from Nile tilapia (*O. niloticus*) breeding populations held at the Tropical Aquarium, Institute of Aquaculture, University of Stirling. Broodstock were maintained individually in partitioned 200 L glass tanks with re-circulated, pre-conditioned freshwater (local tap water aerated and heated to 28 ºC ± 1 for 24 h prior to use) heated to 26 - 28 ºC and fed on artificial pellets (#5 trout pellet, Trouw Aquaculture Limited, Skretting, U.K.). The light régime was maintained at a 12:12 hour day: night photoperiod. Eggs were obtained from ripe females by manual stripping with the addition of sperm from two males per female. Incubation of eggs and rearing of yolk-sac larvae in freshwater was carried out in a down-welling incubation system (Rana, 1985) at 28 ºC ± 1. The experimental hyper-saline media was prepared using pre-conditioned freshwater (as above) and commercial salt (Tropic Marin, Aquarientechnic, D-36367, Germany) and salinity was measured using a salinity refractometer (Instant Ocean Hydrometer, Marineland Labs., USA) accurate to 1 ppt. Incubation of eggs and rearing of yolk-sac larvae in the experimental salinities was carried out in independent test incubation units consisted of 20 L plastic aquaria, each with an individual Eheim pump (Series 94051) and with 6 x 1 L plastic bottles with a down-welling system. (Figure 1). Temperature in the incubation units was maintained at 28 ºC ± 1 with individual 300 W thermostatically controlled heaters (Visi-therm, Aquarium-systems, Mentor, Ohio, U.S.A.). Approximately 10% of water was replaced daily to compensate for evaporation and salinity was adjusted accordingly.

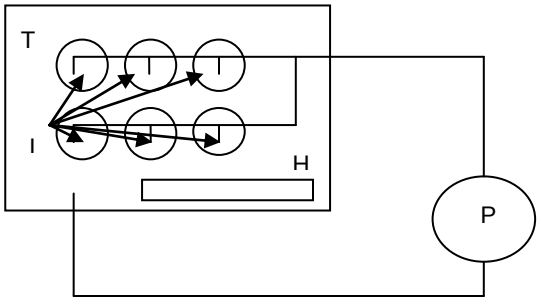

Figure 1. Schematic representation of incubation unit for experimental salinity consisting of a water pump (P), six plastic round-bottom incubators (I) and a thermostatically controlled heater (H) in a 20 Ll plastic aquarium (T)

Ontogenic profile of osmoregulatory capacity

In the first experiment, ovarian fluid and pre-fertilised eggs were initially sampled for osmolality. Eggs were then fertilised in freshwater and transferred at 3 - 4 h post-fertilisation to the experimental salinities *i.e.* 7.5, 12.5, 17.5, 20 and 25 ppt. Control eggs remained in freshwater. Sampling was initially performed at time of transfer and subsequently at developmental points during embryogenesis *i.e.* gastrula (*c.* 24 h post-fertilisation) and completion of segmentation period (*c.* 48 h post-fertilisation) and then at hatch, 2, 4 and 6 dph and finally at yolk-sac absorption. Triplicate experiments were conducted using three different batches of eggs, and each batch was divided into three replicate round-bottomed incubators within each incubation unit. A pooled sample of 30 eggs or larvae was collected at each sampling point (10 from each replicate) and immediately frozen at -70 °C. The small size of Nile tilapia embryos and yolk-sac larvae prevented efficient collection of blood or specific body fluids for osmolality measurements therefore whole-body measurements were used for osmolality measurements; the pools of whole larvae were thawed on ice, homogenised with a motorised Teflon pestle (Pellet Pestle® Motor, Kontes) and the homogenate centrifuged at 10 °C for 10 min at 14 000 g (Eppendorf centrifuge 5417R). The supernatant overlying the pellet was carefully removed into a single well of a 96-well plate and thoroughly mixed with a pipette to ensure homogeneity of each sample. Osmolality was determined using an Advanced 3MO Plus MicroOsmometer (Advanced Instruments, MA, U.S.A.) using three replicates of 20 µl aliquots of supernatant from each pool and accuracy of the machine was regularly checked against calibration standards of 50 and 850 mOsm kg^{-1}. Osmolality was expressed either as whole body osmolality (mOsmol kg^{-1}) or as osmoregulatory capacity (OC; mOsmol kg^{-1}), defined as the difference between the mean osmolality of the pooled larvae to that of the osmolality of their corresponding incubation or rearing media.

Adaptation time

In the second experiment, the acclimation time of yolk-sac larvae either at hatch, 3 and 6 days post-hatch (dph) to abrupt salinity challenge was carried out to determine the time necessary for whole-body osmolality to reach a steady-state after abrupt transfer from the rearing medium (freshwater) to two experimental salinities (12.5 and 20 ppt). Triplicate experiments were conducted using three different batches of eggs. Pooled samples, consisting of 30 whole larvae collected at 1.5, 3, 6, 12, 24, 48 and 72 hours after transfer were immediately frozen at -70 °C. Whole body osmolality (mOsmol kg^{-1}) was determined as described above.

Osmoregulation and survival following abrupt salinity challenge

In a third experiment, healthy yolk-sac larvae were transferred directly from freshwater to 7.5, 12.5, 17.5 or 25 ppt at hatch, 2, 4, 6 and 8 dph. Larvae were exposed to their experimental salinity for 48 h prior to sampling. Control larvae remained in freshwater. Triplicate experiments were conducted using three different batches of eggs. Pooled samples, consisting of 30 whole larvae (10 from each replicate), were immediately frozen at -70 °C. Osmolality was determined as described above and expressed either as whole body osmolality (mOsmol kg^{-1}) or as osmoregulatory capacity (OC; mOsmol kg^{-1}).

Incidence of larval malformation

Thirty newly-hatched larvae from each of the three batches from the first experiment were selected at random from freshwater, 12.5 and 20 ppt and examined under a dissecting microscope and type and incidence of malformations were noted. Thereafter, thirty live larvae were selected at regular time points during yolk-sac absorption *i.e.* 2, 4, 6 dph and yolk-sac absorption and malformations were assessed as before. The percentage of abnormality was calculated, based on the numbers of normal and malformed larvae as follows: percentage of malformed larvae (%) = 100 x number of malformed larvae/number of normal larvae.

Statistics

Statistical analyses were carried out with Minitab 16 using a General Linear Model (GLM) or One-way analysis of variance (ANOVA) with Tukey's post-hoc pair-wise comparisons ($p < 0.05$). Homogeneity of variance was tested using Levene's test and normality was tested using the Anderson-Darling test. Where data failed these assumptions, they were transformed using an appropriate transformation *i.e.* squareroot. All percentage data were normalised by arcsine square transformation prior to statistical analyses to homogenise the variation and data are presented as back-transformed mean and upper and lower 95% confidence limits. Significance was accepted when $p < 0.05$ and results were expressed as mean ± SE.

RESULTS

Ontogenic profile of osmoregulatory capacity

Osmolality of unfertilised eggs (358.2 ± 4.95 mOsmol kg^{-1}) was similar to that of ovarian fluid (370.7 ± 2.30 mOsmol kg^{-1}) but was seen to drop significantly (One-way ANOVA; $p < 0.05$) to 216.9 ± 8.89 mOsmol kg^{-1} after 3 - 4 hours post-fertilisation in freshwater (Figure 2). There was always a significantly higher whole body osmolality in eggs and larvae maintained in elevated salinities as compared to those in freshwater. Osmolality during embryogenesis in freshwater dropped further to a low of 174.6 ± 4.15 mOsmol kg^{-1} at completion of segmentation period at *c.* 48 h post-fertilisation, and then was seen to increase significantly (GLM with Tukey's post-hoc pair-wise comparisons; $p < 0.05$) by hatching to 230.3 ± 2.53 mOsmol kg^{-1}. Osmolality of larvae in freshwater was then seen to rise again significantly (GLM; $p < 0.05$) by 4 dph and, thereafter, maintained a relatively constant level of 319.5 ± 4.91 – 324.8 ± 7.41 mOsmol kg^{-1} until yolk-sac absorption (Figure 2.). In contrast, the osmolality of eggs transferred to elevated salinities at 3 - 4 h post-fertilisation increased with increasing salinity immediately upon transfer. Transfer to 25 ppt induced 100% mortality by 48 h post-fertilisation. In the higher salinities of 17.5 and 20 ppt, osmolality was seen, after the initial abrupt rise, to steadily increase, reaching a maximal value of 434.0 ± 2.07 mOsmol kg^{-1} and 497.8 ± 2.79 mOsmol kg^{-1} at hatch for larvae maintained in 17.5 and 20 ppt respectively, declining significantly (GLM; $p < 0.05$) at 2 dph and thereafter maintaining a relatively constant level until yolk-sac absorption (Figure 2.). For the lower salinities of 7.5 and 15 ppt, following a similar, abrupt rise at transfer, osmolality appears to drop slightly at *c.* 48 h post-fertilisation and then rise significantly (GLM; $p < 0.05$) by 4 dph, similarly maintaining a relatively constant level thereafter until yolk-sac absorption.

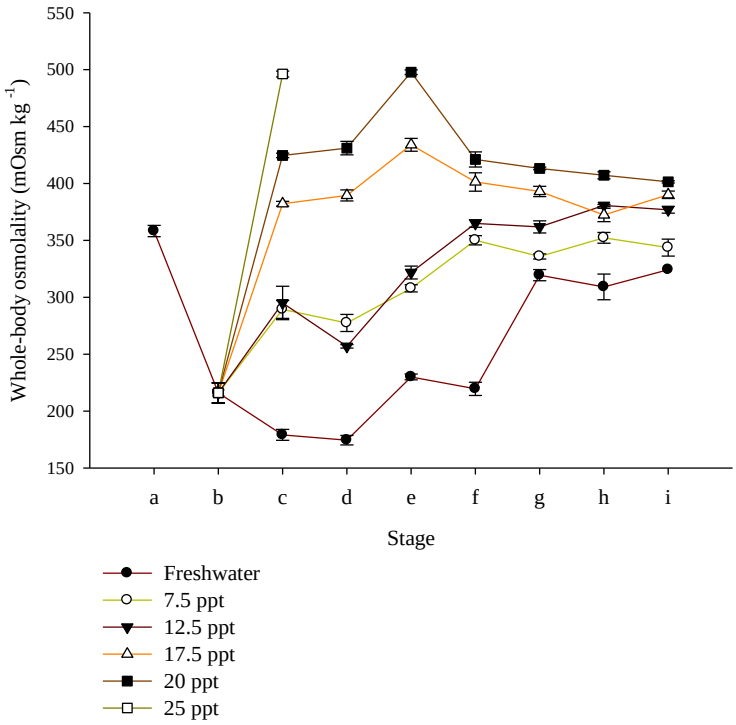

Figure 2. Ontogenic changes in whole body osmolality of Nile tilapia larvae. Mean ± S.E. *x* axis (Stage): a; pre-fertilised eggs; b: 3 – 4 h post-fertilisation; c: 24 h post-fertilisation-f; d: 48 h post-fertilisation; e: hatch; f: 2 dph; g: 4 dph; h: 6 dph; i: yolk-sac absorption. Different numerals indicate significant difference between pre-fertilised eggs and those at 3 - 4 h post-fertilisation (One-way ANOVA with Tukey's post-hoc pair-wise comparisons; $p < 0.05$).

The ability to osmoregulate increased throughout the developmental period studied, as evidenced by variations in osmoregulatory capacity (OC; defined as the difference between the mean osmolality of the pooled larvae to that of the osmolality of their corresponding incubation or rearing media) (Figure 3.).

Hyper-OC in freshwater increased progressively in absolute value from 176.1 ± 3.66 mOsmol kg^{-1} at 24 h post-fertilisation to 321.2 ± 4.99 mOsmol kg^{-1} until yolk-sac absorption; OC values during embryogenesis remained similar but rose significantly (GLM; $p< 0.05$) at hatch. Osmoregulatory capacity was again seen to increase significantly (GLM; $p< 0.05$) by 4 dph to 316.4 ± 2.92 with levels remaining constant thereafter until yolk-sac absorption. A similar pattern was observed for embryos and yolk-sac larvae adapted to 7.5 ppt, although OC levels were significantly (GLM; $p < 0.05$) lower throughout ontogeny than corresponding freshwater values Figure 3.). Whilst at the elevated salinities of 17.5 and 20 ppt, OC levels remained constant during embryogenesis with no significant change in absolute value from 24 hours post-fertilisation until yolk-sac absorption, a significant drop (GLM; $p < 0.05$) in OC was observed at hatch (Figure 3.), but which then rose again by 2 dph. In the iso-osmotic salinity of 12.5, embryos hypo-regulated until hatch, and thereafter were either iso-osmotic to the environmental salinity or slightly hyper-regulated (Figure 3.).

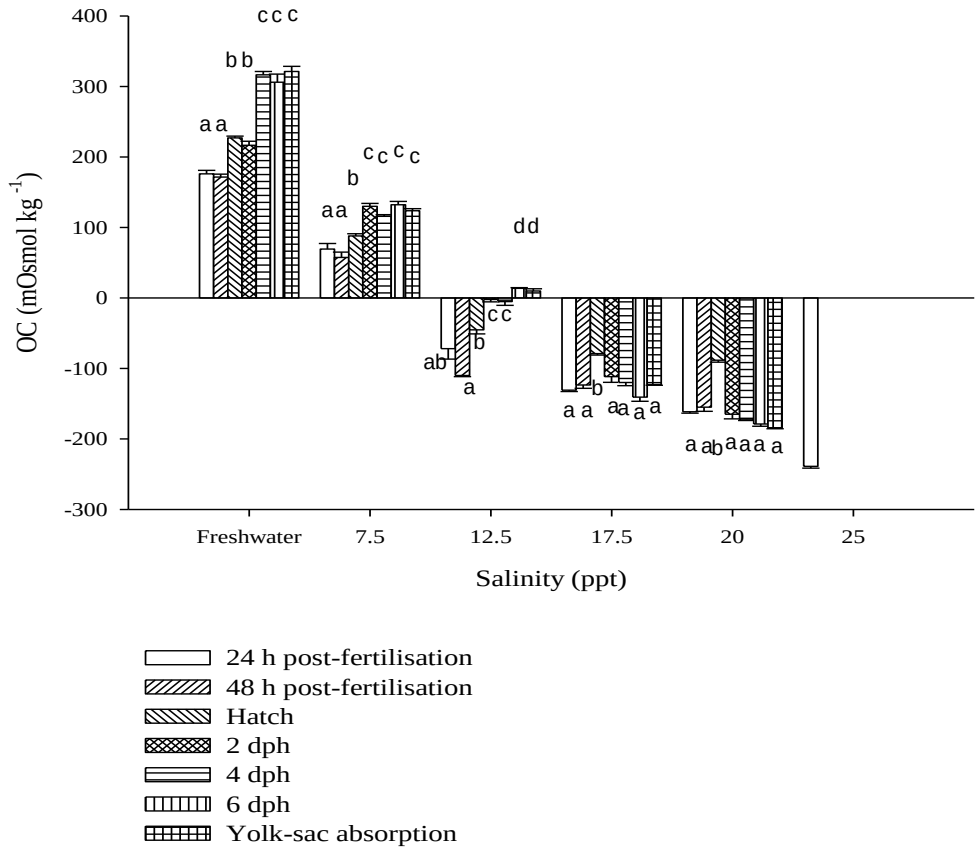

Figure 3. Variations in osmoregulatory capacity (OC) during ontogeny in relation to the osmolality of the medium. Mean ± S.E; different letters represent significant differences between sampling points (General Linear Model with Tukey's post-hoc pairwise comparisons; $p < 0.05$).

Salinity tolerance

The time required for whole-body osmolality to stabilise following an abrupt transfer to an elevated salinity did not appear to vary according to age at transfer (Figure 4.). In general, the changes in osmolality appeared to follow a pattern of crisis and regulation, with values for larvae stabilising at c. 48 h for all treatments, regardless of age at time of transfer, and subsequently remaining the same with no significant change (One-way ANOVA; $p < 0.05$) until 72 h post-transfer. According to these results, the subsequent experiments on osmolality and osmoregulatory capacity were made on larvae having reached a steady-state osmolality following 48 h exposure to experimental salinities.

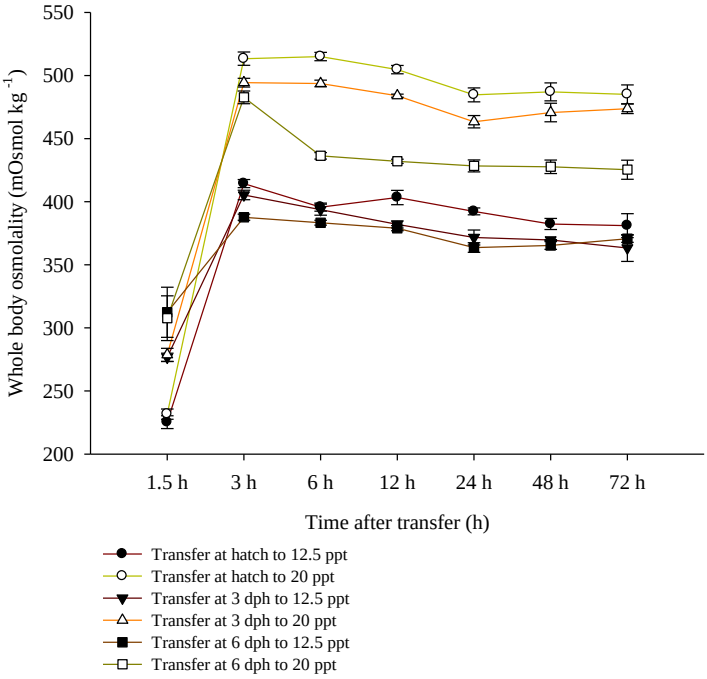

Figure 4. Time-course of whole body osmolality in Nile tilapia yolk-sac larvae following direct transfer from freshwater to 12.5 and 20 ppt at hatch, 3 dph and 6 dph. Mean ± S.E.

Ontogeny had a significant (GLM with Tukey's post-hoc pair-wise comparisons; $p < 0.05$) effect on larval ability to withstand abrupt osmotic challenge; larvae at 8 dph maintained a more constant osmolality over the experimental salinities tested (range 341.4 ± 11.06 to 427.0 ± 2.34 mOsmol kg^{-1}) than larvae transferred at hatch (360.9 ± 3.33 to 487.7 ± 4.92 mOsmol kg^{-1}) (Figure 5.). Similarly, a statistical comparison of OC values showed a clear pattern of age at transfer positively influencing osmoregulatory status. However, there was no significant (GLM; $p < 0.05$) effect of age of transfer on osmoregulatory capacity (OC) to 7.5 ppt (Figure 6.).

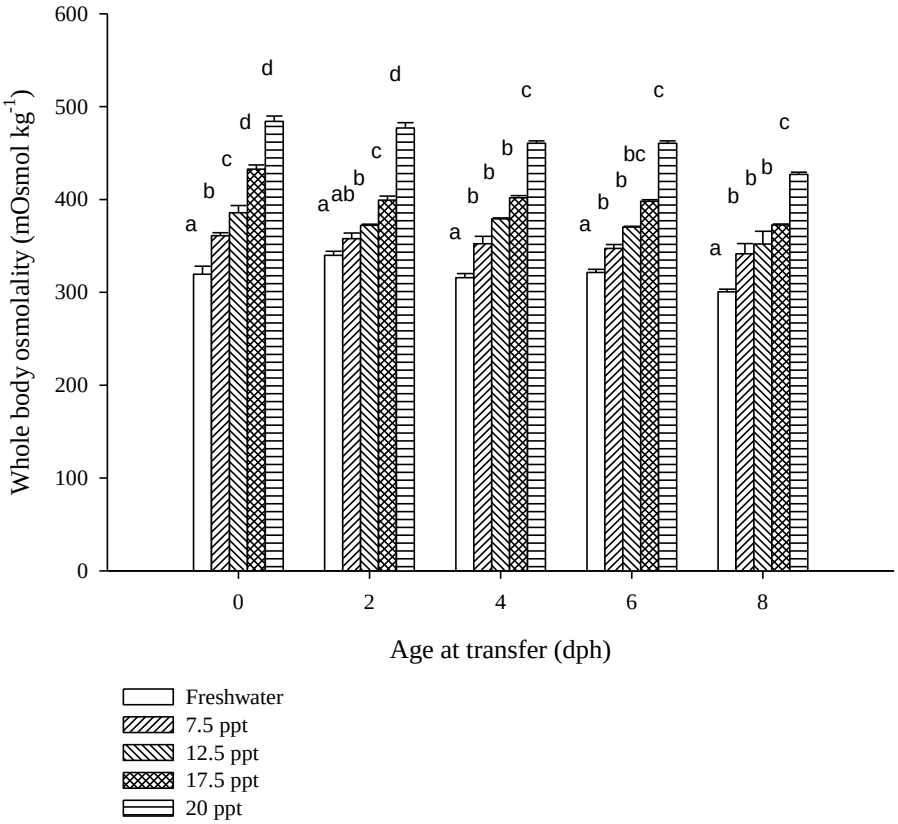

Figure 5. Whole-body osmolality following 48 h after transfer to elevated salinities. Mean ± S.E.; different letters represent significant differences between treatments (General Linear Model with Tukey's post-hoc pairwise comparisons; $p < 0.05$).

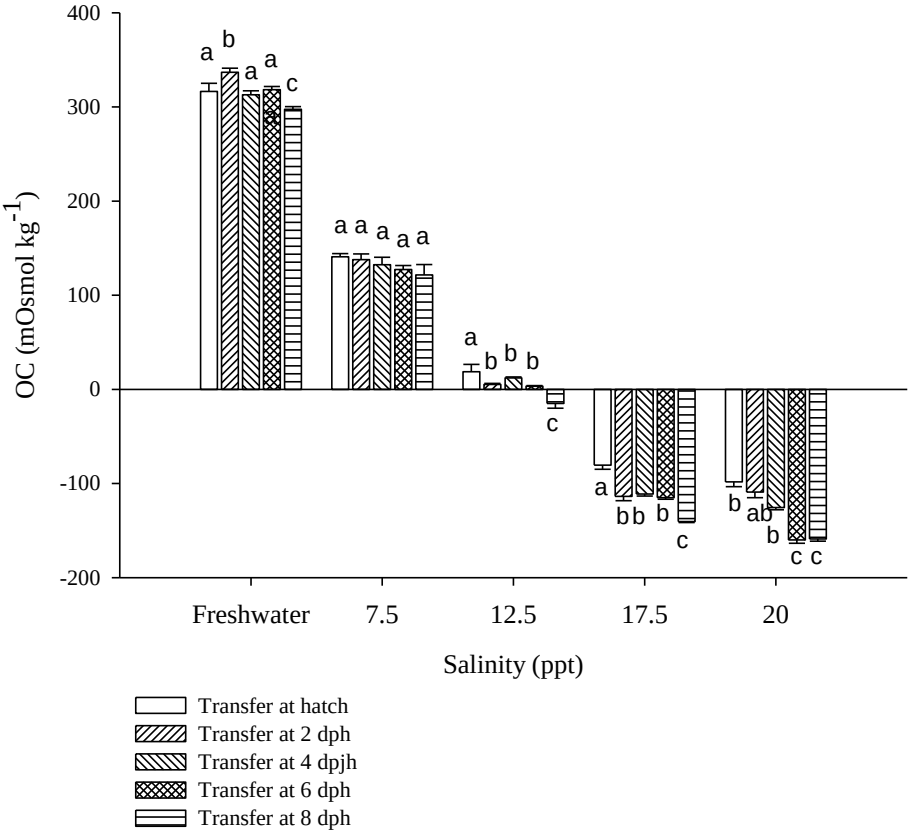

Figure 6. Variations in osmoregulatory capacity (OC) at different post-embryonic stages in relation to the osmolality of the medium following 48 h exposure to experimental salinities. Mean ± S.E.; different letters represent significant differences between time of transfer (General Linear Model with Tukey's post-hoc pairwise comparisons; $p < 0.05$).

Survival generally decreased with increasing salinity but increased with successive developmental stages (Table 1.). Survival rates of 98 % were recorded for larvae maintained in freshwater at hatch yet lower survival rates, in the range of 83 - 92 %, were recorded for those transferred, at hatch, to elevated salinities. Larvae transferred to salinities of 7.5 – 17.5 ppt at 2 and 4 dph exhibited an improved survival rate than at hatch, yet larvae transferred to 20 ppt still displayed a significantly lower survival rate (GLM; $p < 0.05$) than other salinities. From 6 dph onwards, no significant differences were observed between survival rates amongst salinities (GLM; $p < 0.05$) (Table 1.).

Table 1. Effect of various salinities on larval survival (%) at 48 h post-transfer at various developmental stages during yolk-sac period. Mean and 95% confidence limits. Different superscript letters represent significant differences between treatments; different subscript letters represent significant differences between age at transfer (General Linear Model with Tukey's post-hoc pairwise comparisons; $p < 0.05$).

Larval survival (%)					
Salinity	**Freshwater**	**7.5 ppt**	**12.5 ppt**	**17.5 ppt**	**20 ppt**
Time of transfer:					
Hatch	98 (94.4–9.9) $^{ab}_{a}$	86 (70.6–96.4) $^{b}_{a}$	92 (82.9–97.8) $^{ab}_{a}$	83 (73.7 – 90.2) $^{c}_{a}$	85 (70.7 – 95.6) $^{bc}_{a}$
2 dph	98 (95.4–99.9) $^{a}_{a}$	98 (94.5–99.9) $^{a}_{b}$	95 (79.5–99.9) $^{a}_{a}$	97 (93.5–99.6) $^{a}_{b}$	85 (69.4–95.9) $^{b}_{a}$
4 dph	99 (98.4–99.9) $^{a}_{a}$	96 (90.7–99.5) $^{a}_{b}$	92 (86.2–96.9) $^{a}_{a}$	95 (90.1–98.8) $^{a}_{b}$	77 (69.6–84.2) $^{b}_{a}$
6 dph	99 (95.1–99.8) $^{a}_{a}$	98 (94.8–99.9) $^{a}_{b}$	96 (87.1–99.9) $^{a}_{a}$	95 (85.1–99.7) $^{a}_{b}$	99 (95.5–99.3) $^{a}_{b}$
8 dph	99 (96.8–99.9) $^{a}_{a}$	99 (94.8–99.9) $^{a}_{b}$	97 (90.8–99.9) $^{a}_{a}$	99 (96.8–99.9) $^{a}_{b}$	99 (96.8–99.9) $^{a}_{b}$

Incidence of malformation

Gross larval malformation was defined as pericardial oedema, sub-epithelial oedema of the yolk-sac, non-specific haemorrhaging of blood vessels associated with the yolk-sac syncytium and body or abnormal neurocranium (Figure 7.). Incidence of malformation of yolk-sac larvae was always significantly higher in salinities than in freshwater at all stages (GLM; $p < 0.05$). Incidence of malformation was seen to decline significantly (GLM; $p < 0.05$) from hatch until yolk-sac absorption (Table 2.).

Table 2. Effect of salinity on larval malformation during yolk-sac period. Mean and 95% confidence limits were calculated on arcsine square transformed data. Different superscript letters represent significant differences between treatments; different subscript letters represent significant differences between days (General Linear Model with Tukey's post-hoc pairwise comparisons; $p < 0.05$).

Incidence of malformation (%)			
Salinity	**Freshwater**	**12.5 ppt**	**20 ppt**
Time of transfer:			
Hatch	14 (12-59.6) $^{a}_{a}$	22 (20.6-41.9) $^{b}_{a}$	23 (19.9-32) $^{b}_{a}$
2 dph	2 (0.5-17.6) $^{a}_{b}$	8 (6.2-34.8) $^{b}_{ab}$	29 (22.6-35.6) $^{c}_{a}$
4 dph	2 (0.4-4.7) $^{a}_{b}$	8 (2.23-18.1) $^{b}_{ab}$	10 (2.4-23.6) $^{b}_{b}$
6 dph	1 (0.1-15.1) $^{a}_{b}$	2 (0.1-15.1) $^{a}_{b}$	6 (1.9-13.6) $^{b}_{b}$
Yolk-sac absorption	1 (0.5-6.0) $^{a}_{b}$	7 (5.6-8.5) $^{b}_{ab}$	9 (7.5-11.7) $^{b}_{b}$

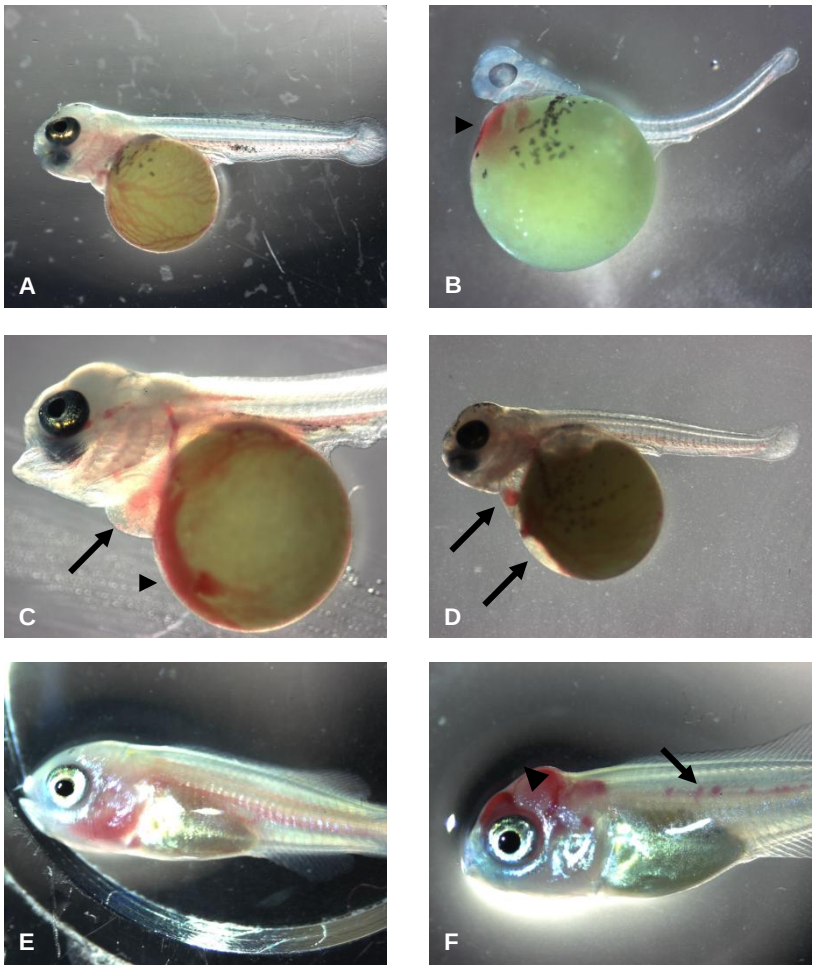

Figure 7. Malformation during yolk-sac absorption period in Nile tilapia. **A)** Normal larvae at hatch in freshwater showing network of blood vessels associated with yolk-sac syncytium, **B)** Malformed larvae at hatch maintained in 17.5 ppt showing curvature of stunted tail and pericardial haemorrhaging (arrowhead), **C)** 2 dph larvae maintained in 20 ppt showing pericardial oedema (arrow) and haemorrhaging of blood vessels associated with the yolk-sac syncytium (arrowhead), **D)** 2 dph larvae maintained in 20 ppt with pericardial oedema, enlarged heart (arrow) and sub-epithelium oedema of the yolk-sac (arrowhead), **E)** Normally developing larvae at yolk-sac absorption maintained in freshwater, **F)** 8 dph larvae maintained in 20 ppt showing distortion of neurocranium (arrowhead) and pooling of blood along spine (arrow).

DISCUSSION

Ontogenic pattern of osmoregulatory capacity and salinity tolerance

This study confirms that newly extruded Nile tilapia eggs, prior to fertilisation, have the same osmo-concentration to that of the ovarian fluid, which has been confirmed in a number of marine teleost species e.g. herring (*Clupea harengus*) (Holliday and Blaxter, 1960; Alderdice et al., 1979), plaice (*Pleuronectes platessa*) (Holliday and Jones, 1967), long rough dab (*Hippoglossoides platessoides limandoides*) (Lonning and Davenport, 1980), cod (*Gadus morhua*) (Davenport *et al.,* 1981; Mangor-Jensen, 1987), lumpsucker (*Cydopterus lumpus*) (Kjorsvik *et al.,* 1984) and Atlantic halibut (*Hippoglossus hippoglossus*) (Østby *et al*., 2000). Indeed, it has been recognised that marine teleost eggs, prior to ovulation take up a large amount of water leading to swelling of 4 - 7 times resulting in a relative water content on 90 -92 % (Craik and Harvey, 1987; Østby *et al*., 2000). Indeed, both prior to and post ovulation, the plasma membrane of eggs are relatively permeable to water and respond to changes in the ovarian fluid (Sower *et al.,* 1982) and they are therefore assumed to be iso-osmotic with maternal blood. After spawning, fertilisation and activation of the egg results in cortical alveolar exocytosis, a process that causes imbibition of water from the external environment across the chorion to form the perivitelline fluid (PVF), blocking the micropyle and therefore preventing polyspermy (Yamamoto, 1944). Lonning and Davenport, (1980) report swelling to be complete at 24 h post-fertilisation, but may have ceased between 4 – 24 h in the eggs of the long rough dab (*H. platessoides limandoides*). Similarly, Shanklin (1959) comments that the PVF of the egg, upon spawning, rapidly establishes equilibrium with the external media, and this is confirmed by Lasker and Theilacker (1962) in the developing eggs of the Pacific sardine (*Sardinops caerulea*). Similarly, a rapid increase in osmolality after spawning into sea water is reported in newly extruded eggs in the Atlantic herring (*C. harengus*) (Holliday and Jones, (1965), the cod (*G. morhua*) (Davenport *et al.,* 1981), the long rough dab (*H. platessoides limandoides*) (Lonning and Davenport, 1980) and the lumpsucker (*C. lumpus*) (Kjorsvik *et al*., 1984). This could explain the abrupt decline in osmolality of eggs at 3 - 4 h post fertilisation into hypo-osmotic freshwater that is reported in this study.

It has been demonstrated in this study that, during embryogenesis, a constant osmolality is maintained regardless of the external media until hatch. Therefore the question arises, how do embryos maintain some sort of osmoregulatory control during these early stages of embryogenesis. At spawning the yolk is enclosed by a double membrane enclosing a thin layer of cytoplasm which concentrates on the animal pole forming a blastodisc. During gastrulation the peripheral cells of the morula begin to cover the yolk sac coinciding with the appearance of cutaneous mitochondria-rich cells (MRCs) *i.e.* on the epithelium of the body surface and yolk-sac of the developing embryo, thus marking the start of the selective restriction of ions and water transfer or active ionoregulation (Guggino, 1980). The first appearance of MRCs on the yolk-sac epithelium of dechorionated freshwater maintained tilapia (*Oreochromis mossambicus*) embryos was reported at 26 h post-fertilization but no apical crypt was found until 48 h post-fertilization (Lin *et al*., 1999). Similarly, Ayson *et al.* (1994) observed MRCs on the yolk-sac epithelium of the tilapia (*O. mossambicus)* embryos at 30 h post-fertilization in both freshwater and seawater, but apical openings of MRCs were first observed at a low density at 48 h post-fertilization or half-way to hatching. The presence of functional MRCs, therefore, may offer an explanation for the ability of embryos, as demonstrated in this study, to maintain osmotic control *i.e.* to hyper-regulate in low salinity waters (*i.e.* freshwater and 7.5 ppt) and to hypo-regulate in elevated salinities (*i.e.* 12.5 – 20 ppt) at 48 h post-fertilisation following completion of epiboly. Whilst osmolality levels of embryos initially showed a rapid rise following transfer to hyper-osmotic environments, embryos still displayed some sort of regulative control, with the exception of embryos transferred to 25 ppt, who were unable to survive.

Ontogenic changes in salinity tolerance appear, in this study, to be related to developmental stage. Results suggest that abrupt osmotic challenge gave rise to different osmoregulatory responses which were dependant on the ontogenic stage of the larvae and, moreover, a gradual improvement in ability to osmoregulate occurs during ontogeny. Indeed this ability to maintain osmotic homeostasis is reflected in survival patterns of larvae following transfer; from 6 dph onwards, no significant difference is evident in survival between salinities. The study by Watanabe *et al.* (1985) on the ontogeny of salinity tolerance in various tilapiine *spp.* (*e.g. Oreochromis aureus*, *O. niloticus* and *O. mossambicus* x *O. niloticus* hybrid) spawned and reared in freshwater but transferred to elevated salinities (0 – 32 ppt) from 7 – 120 dph suggested that changes in salinity tolerance were more closely related to body size than chronological age, and was probably related to maturational events such as the functional development of the osmoregulatory system. Although the fish in that study were older than those used in the present study, it is still interesting to note that ontogenic physiological changes may confer osmoregulatory ability and salinity tolerance.

Larval malformation

In the present study, haemorrhaging and pooling of blood appears to be linked to oedematous build up during yolk-sac stages of the Nile tilapia. It is possible that oedema may compress the delicate blood capillary network on the yolk-sac syncytium, and have a damaging, systemic effect on whole larvae by impairing circulation. Hill et al. (2003) examined the negative impacts of the contaminant Polychlorinated dibenzo-*p*-dioxins (PCDDs) on the epithelium of zebrafish during early life stages and reported a build-up of oedema and ensuing organ compression that led to decreased kidney and circulatory function. They concluded that this model also predicts that many different types of stresses, within which salinity must be included, might lead to the same outcome, and this therefore offers a possible explanation to what is happening in this study.

In this study, there was a significant negative effect (GLM; $p < 0.05$) of increasing salinity on the occurrence of larval malformations during the yolk-sac period. A high incidence of larval abnormalities has been previously reported during early life stages of marine teleosts, when challenged with variations in salinity. Larvae of the navaga (*Eleginus nava*), polar cod (*Boreofadus saida*) and Arctic flounder (*Liopsetta glacialis*) exhibited a high incidence of malformation in low salinities (Doroshev and Aronovich, 1974), as did the Atlantic halibut (*H. hippoglossus*) (Bolla and Ottensen, 1998). A lower percentage of abnormalities in the newly hatched larvae of the pomfret (*Pampus punctatissimus*) was reported at 29 – 30 ppt than either at < 25 ppt or > 40 ppt (Shi *et al.,* 2008) and, similarly, the percentage of deformities was significantly lower at 36 ppt than at either lower (24 – 33 ppt) or higher (36 - 42 ppt) salinities in the Japanese eel (*A. japonica*) (Okamoto *et al*., 2009). These results would therefore seem to suggest that, once the incubation and rearing salinity moves away from that which is encountered in nature, detrimental effects become more pronounced, a trend that is apparent in the current study.

It is clear from this study that there also exists a significant effect of ontogeny on the incidence of malformation during the yolk-sac period. The development of the branchial system and an ontogenic shift in location of active MRCs from extrabranchial to branchial sites is widely accepted and has been reported in the Mozambique tilapia (*O. mossambicus*) by Li *et al*. (1995) and van der Heijden et al. (1999) and in the Nile tilapia (*O. niloticus*) (Fridman *et al*. in press) which would appear to confer an increasing osmoregulatory capacity which is apparent in the reported pattern of survival in elevated salinities following hatch. This observed reduction of pericardial and sub-epithelial oedema as yolk-sac larvae develop appears to reflect an increasing ability to maintain ionic and osmotic balance throughout the yolk-sac period. In agreement, oedema is not observed in zebrafish larvae after exposure to contaminants if exposure is delayed

during ontogeny suggesting that larvae are particularly vulnerable shortly after hatching (Belair et al., 2001).

To conclude, assessment of whole body osmolality has provided a method that has allowed an evaluation of the osmoregulatory status during the early life stages of the Nile tilapia; these measurements appear to offer valuable insight into the emerging pattern of the adaptive capacity to hypo- and hyper-regulate during ontogeny. Osmolality levels of embryos immediately post-transfer to elevated salinities appear to be proportional to and directly related to the osmolality of the external media, but then drop to a more steady state during embryogenesis and yolk-sac period, suggesting that an ontogenic regulatory control is evident which is, in turn, reflected in larval ability to withstand transfer to elevated salinities and decrease in the incidence of larval deformity.

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Tilapia Germplasm in China: Chance and Challenge

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Abstract

Tilapia aquaculture has experienced rapid development in China in recent years, which has become the largest tilapia producing country in the world. Tilapias are actually several exotic species and have been introduced repeatedly to China since 50 years ago. The major species are *Oreochromis niloticus* and *O. aurea*. The superior strain of introduced Nile tilapia is the Genetically Improved Farmed Tilapia (GIFT). Now, a further bred GIFT is widely used in practice for its high growth rate, another is the hybrid of *O. niloticus* × *O. aurea* for its high male percentage. Genetic breeding and biotechnology are extensively being applied to improve tilapia performance such as sality tolerance, resistance to lower temperature and some diseases. However, it may not be rational and healthy for rapid development of Chinese tilapia industry. Rapid development driven by profits may not be the best indicator for the tilapia industry. An environmentally and economically sustainable industry should be the goal for development in China. The largest production of tilapia in China is mainly relying on international market, local market for acceptance and consumption is less developed. Tilapia germplasms in China are mainly from international introduction, our capacity of germplasm creation and genetic breeding is still at a low level, which could constrain our development in long run. Much attention has been given to the total production but less on fish quality. Strong renovation technologies for improving limited tilapia germplasm are expected to support the largest Chinese tilapia industry.

Keywords: Tilapia, germplasm, chance, challenge

Outlines

A. The largest tilapia industry in China is totally depended on some limited introductions

Tilapias are exotic species and originated from African countries. Since 1950s, nine species (*O. mossambicus*, *O. niloticus*, *O. aurea* etc) have been introduced to China, which formed the founder of Chinese tilapia industry (Table 1) After 40 years experiment and exploration, China successfully became the biggest tilapia producer in the world.

Table 1 The introduction of tilapia in China

Species	Year	Source	Introducing institute
O. mossambicus	1956	Vietnam	Guangdong Fisheries Institute
O. niloticus	1978	Sudan	Yangtze Fisheries Institute
	1978	Thailand	Zhujiang Fisheries Institute
	1988	Egypt	Hunan Fisheries Institute
	1992	America	Freshwater Fisheries Research Center
	1994	Philippine	Shanghai Fisheries University
	1998	Egypt	Shanghai Fisheries University
	2005	Malaysia	Freshwater Fisheries Research Center
O. aurea	1981	Taiwan	Guangzhou Fisheries Institute
	1983	America	Freshwater Fisheries Research Center
	1998	Egypt	Shanghai Fisheries University
O. hornorum	2000	America	Zhujiang Fisheries Institute

S. melanotheron	2002	America	Shanghai Fisheries University

The actual introductions should be bigger than the above table. However, the most introductions were in-directed, second-handed or more, a few introduction was from the original rivers. **Thus, these germplasm now supporting Chinese tilapia industry is relatively smaller.**

Through many species and many times introductions, GIFT strain and the hybrid *O. niloticus*×*O. aurea* are two commonly cultured species in inland fisheries (Table 2). The NEW GIFT strain is famous for its high growth rate, the hybrid of *O. niloticus* × *O. aurea* for its high male percentage, other speices or strain are not extensively used due to laking of superior performance. Meantime, Red tilapia, GILI (*O. niloticus*× *S. melanotheron*) and Mohe (*O. mossambica*×*O. hornorum*) are partially applied in some seawater.

Table 2. The major strains and species cultured in China

Common name	Species/strain/variety
GIFT	Selected from a mixed population of 4 native and 4 domesticated of *O. niloticus*
NEW GIFT	Selected from GIFT strain of *O. niloticus*
Genomar GIFT	*O. niloticus*
Baolu GIFT	*O. niloticus*
Ni ao	*O. niloticus*× *O. aurea*
Gili	*O. niloticus*× *S. melanotheron* F2
Red tilapia	*O. niloticus*× *O. mossambica* variety
Mo he	*O. mossambica*×*O. hornorum*

Due to warm climate requirement, the major tilapia production area is in south China (>90%), it also be cultured in the north China using waste heat generation (Table 3).

Table 3. The major production areas in China

Area	Production percentage
Guangdong	47%
Hainan	20%
Guangxi	15%
Fujian	8%
Other	10%

Good strain, huge human labors, large amount of water area, new markets, together with the aquaculture experience promote the tilapia production quickly. Chinese tilapia production is about one-third of total tilapia production in the world (Table 4).

Table 4. Tilapia production in recent year in China

Year	Production ($\times 10^4$ tons)
2000	63
2001	67
2002	71
2003	81
2004	90
2005	98
2006	99
2007	113
2008	95
2009	115
2010	100

In those years, the export production also keeps a strong increase and export to many other countries (Table 5).

Table 5. Tilapia export production

Year	Production ($\times 10^4$ tons)
2007	21.5
2008	22.4
2009	25.9
2010	26.0

Problems on introduction

(1) Unorganized introduction

The purposes of tilapia introduction were for research or commercial use, fish introduction was never organized by the government. These tilapias are kept in some separated institutes or fish farms, their genetic variation may not be fully utilized. Introducing source, number and their characters are less considered for industry view.

(2) Small population

The population size is determined by the actual mating parent's numbers rather than the total parental numbers. Because of long-distance transplantation, only few individuals could survive at last. Therefore, the effective population is much smaller than its original amount; the genetic variation of introduced population was rather small.

(3) Loss of genetic variation

Under the circumstance of small population size, genetic drift happened and severely changed their genetic variation generation by generation. In depth, low genetic diversity is the ultimate limitation for its future genetic improving.

(4) Poor management

Inbreeding is easily happened for tilapia because they are from the common ancestor, especially from some small population (Table 6). Meantime, unjust mating scheme are another source of inbreeding during generation transition.

Table 6. The estimated inbreeding coefficient for introduced tilapia in China

Species	Survival No.	Effective size	Coefficient index
N78-1	10♀,12♂	21.8	0.00965
N78-2	30	30	0.03333
N85	9♀,1♂	3.6	0.01389
N95	24♀,29♂	52	0.00952
N98	3000	3000	0.00017

(5) Hybridization

Because of easy interspecific hybridization, more than two kinds of species kept in the same fish farms would produce interspecific hybrid, these hybrids were often mixed with the brooder stocks. Genetic introgression was found in tilapia fish farm.

In respect of the germplasm, low genetic variation, inbreeding of these introduced population further limited and constrained their long term utilization.

B Genetic improvements is still on development

Growth Rate

Growth rate is the first demand for a good variety. GIFT strain was selected from the combined base populations of four African strains and four Asian strains in Philippines. It was introduced to China in 1994, and showed some superior performances (growth, capture) than that of the extant strains in China, thus became a superior introduced variety.

New GIFT was developed from the introduced GIFT strain by Shanghai Ocean University. After eight generations of mass selection on growth rate and morphology, the NEW GIFT possessed higher growth rate than the control group (>30%), it now become the most popular cultured species in China.

Although there are many introductions for different tilapia species, the small effective population size resulted in the genetic drift or genetic bottle, the loss of genetic variation made its selection are not effective in most strain.

Male percentage

Sexual maturation ahead of the commercial size is also perplexing tilapia aquaculture. The hormone administration was easily applied at the fingerling stage to increase male percentage, however, its safety is still on debated. Another practical method for producing male-offspring is interspecific hybridization. The best hybridization combination for high male percentage is *O. niloticus*× *O. aurea*, which claimed to more than 95% male percentage. In fact, some other factors (genetic and environmental) also affected the male percentage.

YY-male tilapia

The sexual chromosome type of Nile tilapia is **XY** for male and XX female, after sexual reversal **XY(♂)**→XY (△♀), then identified the **YY(♂)** among XY(△♀)×XY(♂) progeny, In genetics, XX(♀)×YY(♂) will produce 100% male percentage. Development and application of super male tilapia natural breeding system are carried out in Guangzhou Luye Fisheries Co. Ltd

Salt-tolerance

O. niloticus grow fast but low sality tolerance (<5), *S. melanotheron* grow slowly but high sality tolerance (0-100). It is easy for interspecific hybridization among some tilapia species, far interspecific hybridization of *O niloticus* and *S. melanotheron* are less successfully probably due to their different genera. Secondly, the mouth hatching parent is female in *O. niloticus*, while mouth hatching parent is male in *S. melanotheron*. The growth and sality of *O. niloticus*× *S. melanotheron* was better than that of the reciprocal hybrid *S. melanotheron*× *O. niloticus*. The difficulty to get enough F1 greatly confined their application.

In contrast, the F2 generation could easily be obtained by the natural mating among F1, and kept sality tolerance and growth as F1. They could be largely propagated in practical. Now, they have been cultured in seawater ponds (15) or polycultured with shrimp. Meantime, the meat quality also was improved under salinity culture. Also brackish-alkaline water tolerance tilapia is expected in north China.

Disease resistance

Disease is another problem perplexing the industry. A Tilapia epidemic has broken out in

main producing area, South China since 2009, it mostly attacked tilapia at 200g, with 20-30% morbidity and 95% mortality, thus greatly decrease the total production. Recently, *Streptococcicosis agalactiae* was isolated and identified as the main pathogen in Guangdong and Hainan. A new program has been initiated to prevent and control this disease during the whole production process, also disease resistance strain is expected urgently to adjust the aquacultural environmental.

Cold tolerance

Naturally, these temperate species couldn't survive the winter in most part China. The lethal temperature for *O. niloticus* is 10℃, 8℃ for *O. aurea*. In north China, tilapia was only cultured under circulated warm water supplied by electricity power plant. In south China, they could survive the winter in the simple plastic-roof rooms, the warm climate also provide a long growth period for tilapias. Therefore, the major production area is south China.

Since 2008, the bad cold climate often intruded south China. The low temperature killed the adult fish and decreased the total production; it also killed tilapia breeders and caused the shortage of the next year seed supply.

The best resolution for safe winter is providing some warm-keeping apparatus, it is effective way to escaping from the cold, but would add some cost to producer. Improving their cold tolerance is put forward in recent years; however, it may be a long way to conquer their biological ability and climate change.

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EFFECTS OF *Aloe vera* (Liliaceae) ON THE GONAD DEVELOPMENT IN NILE TILAPIA, *Oreochromis niloticus* (Linnaeus 1758)

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ABSTRACT

There is need to control undesirable tilapia recruitment in ponds using natural reproductive inhibitory agents in plants because they are less expensive and constitutes appropriate technology in developing countries. *Aloe vera* latex (AL) was added to a basal diet (350g crude protein and 18.5MJ gross energy/kg diet) at 0, 0.5, 1.0, 1.5 or 2.0 ml/kg diets and fed to mixed-sex *Oreochromis niloticus* for 60 days to evaluate the effects on growth and feed utilization, reproduction traits, and histology of gonads. There were no significant difference ($p > 0.05$) in the growth parameters and food conversion ratio. Indices of reproduction traits decreased with increasing dietary *A. vera* latex (AL) levels. Fish fed with the control diet and 0.5ml AL/kg diet had significantly higher and better indices of reproduction traits ($P<0.05$) than the other fish fed with 1.0,1.5 and 2.0ml AL/kg diets respectively. Fish fed 0ml AL/kg diet (control) showed normal testicular and ovarian tissues architecture, typical bilateral lobes of the ovaries were evident, normal olive green colour was maintained; and no pathological lesions occurred. Fish fed 0.5ml AL/kg diet showed no visible alteration in the ovaries, testicular architecture and cystic seminiferous tubules. Fish fed 1.0ml AL/kg diet showed testicular atrophy. Fish fed 1.5ml AL/kg diet exhibited cystic seminiferous tubule and atrophy of tissues. Fish fed 2.0ml AL/kg diet showed severe tissue atrophy, sperm cells disintegration and necrosis. Also, in fish fed 2.0ml AL/kg diet, ovirectomy revealed a change in the colour of ovaries, histology showed ruptured follicle, granulomatous inflammation in the insterstitium and necrosis of the ovaries. Reproduction traits and histological observations of gonads in *O. niloticus* fed high dietary levels of AL revealed that *A. vera* latex may be effective as reproduction inhibitory agent.

INTRODUCTION

Aloe vera is a succulent, almost sessile perennial herb. Its leaves 30–50 cm long and 10cm broad at the base; colour is pea-green (when young they are spotted with white) and has bright yellow tubular flowers, 25–35 cm in length arranged in a slender loose spike. It contains a colourless mucilaginous gel called *A. vera* gel (Bruneton 1995). *A. vera* is grown commercially, especially in the Netherlands Antilles, for the latex which is used medicinally (Christman 2005). It is frequently used in herbal medicine and can be grown as an ornamental plant. Preliminary evidences have shown that *A. vera* extracts (bitter yellow *A. vera* latex) are useful in the treatment of fungi and bacterial infections in humans and as laxative (Boudreau and Beland 2006). Compounds extracted from *A. vera* have been used as an immunostimulant that aids in fighting cancers in cats and dogs (King *et al* 1995). King *et al.* (1995) and Eshun and He (2004) reported that the active compounds in *A. vera* include polysaccharides, mannans, anthraquinones, lectins, salicyclic acid, urea, nitrogen, cinnamic acid, phenol and sulfur. It also contains amino acids, lipids, sterols tannin and enzyme.

Though many scientific studies on the use of *A. vera* have been undertaken, some of them are said to be conflicting (Ernst 2000 and Vogler and Ernst 1999) and this include the fact that the bitter yellow latex from *A. vera* leaf (which active ingredients are aloe-emodin, aloin and barbaloin)can cause abdominal cramps,impair fertility or cause miscarriage in humans and

animals during overdose or misuse. Active ingredients from *A. vera* latex are used extensively in commercial laxative preparation, though its use has been banned.

Tilapias constitute one of the most productive and internationally traded food fish in the world (Modadugu and Belen 2004). They are a major protein source in many of the developing countries. The commodity is not only the second most important farmed fish globally (next to carp) but also described as the most important aquaculture species of the 21st century (Shelton 2002). (FAO 2006) reported that farmed Nile tilapia (*O. niloticus*) production reached 1,703,125mt, which is about 84% of total farmed tilapia production in 2006. However, tilapias are yet to reach their full aquaculture potential because of the problem of precocious maturity and uncontrolled reproduction, which often results in the overpopulation of production ponds with young (stunted) fish. Population control in farmed tilapias has been reviewed (Guerrero, 1982; Mair and Little, 1991); such control methods include monosex culture, sex reversal by androgenic hormones, cage culture, tank culture, the use of predators, high density stocking, sterilization, intermittent/selective harvesting, and the use of slow maturing tilapia species, among others. However, these population control methods have their limitations; e.g. the use of reproductive inhibitors, such as irradiation, chemosterilants has disadvantages which are: expensive technology, hatchery facilities and skilled labour are required and hormones are expensive and difficult to obtain. Hence there is need to examine less expensive and appropriate technology to control tilapia recruitment in ponds using natural reproductive inhibitory agents in some plants.

O. niloticus is a maternal mouth brooder and becomes sexually matured in 4-5 months at small size (10 cm; 20-50 g) in ponds; each female lays about 1,500-2,000 eggs/spawning and 3 spawnings/year (Balarin and Hatton, 1979). The objective of this study was to investigate the effects of varying dietary inclusion levels *A. vera* latex on some reproduction traits (gonad development stages, fecundity, egg size (length and diameter), histology of gonads) in *O. niloticus* fed for 60 days.

MATERIALS AND METHODS

A. vera leaves were cut with a sharp, clean knife and the bitter yellow latex were collected and stored in a dry, clean, air-tight transparent plastic container, labelled and refrigerated at -20^0. Feedstuffs were purchased from a local feedstuff market and were separately milled to small particle size (< 250 µm). A basal diet (D1, 350g crude protein and 18.5MJ gross energy/kg diet) was prepared as formulated in Table 1. Four test diets (D2, D3, D4, D5) were formulated by adding 0.5, 1.0, 1.5, or 2.0ml of *A. vera* latex (AL) to 1 kg of basal diet, respectively. The feedstuffs were thoroughly mixed in a Hobart A-200T mixer. Hot water was added at intervals to gelatinize starch. The five diets were pelletized using a die of 8 mm diameter and air-dried at ambient temperature for 72 hours; broken, sieved into small pellet sizes, packed in air-tight containers, labelled and stored.

Table 1: Ingredient composition of basal diet

	g/kg diet
Menhaden fish meal	280
Soybean meal	370
Corn meal	250
Cod liver oil	30
Corn oil	20
Vitamin-mineral mix	30
Corn starch	20

O. niloticus fingerlings, obtained from a single spawn, were acclimated for 14 days in concrete tanks during which they were fed with a commercial diet. After acclimation, 5 male and 5 female *O. niloticus* (mean wt., 30.38 $\pm$0.16g) were stocked in each of 15 glass tanks (75cm x 40cm x 40cm) supplied with 60 litres of fresh water (water temperature, 27 ^{o}C; pH, 7.3; alkalinity, 50 ppm; dissolved oxygen, 7.6-7.9 mg/L). Continuous aeration was provided using a blower and air stones (Tecas air pump AP-3,000; 2 ways). The treatments were replicated thrice. Fish were fed at 4% body weight/day of the basal diet in two instalments at 0900-0930 h and 1700-1730 h for 60 days; after which they were removed, sorted by sex and weighed. Sex determination was done through visual examination of the gonad. Fish mortality was monitored daily.

Six male and six female *O. niloticus* samples were randomly taken from each treatment, dissected, and the testes and ovaries removed and weighed. Gonad development stages in male and female *O. niloticus* were classified according to Kronert *et al*. (1989) and Oldorf *et al*. (1989), respectively. Fecundity was estimated from gonads of six fish from each treatment in the final maturation stage from a sample representing at least 50% of ovary weight then reported to the total weight of the ovary. Thirty (30) eggs were measured using a microscope eye-piece graticule for length (L) and width (H) (Rana, 1985). Short and long axis of two egg samples from each spawn (treatment) were measured using light microscope containing a calibrated eye piece graticule.

Mean egg diameter was calculated from each spawn (treatment) as follows:

$$\text{Mean egg diameter (mm)} = \frac{\text{length of long axis + length of short axis}}{}$$

The gonads were sectioned, fixed for 24 hours in formalin-saline solution made of equal volumes of 10% formalin and 0.9% NaCl solution. Histological sections of 8µ thickness were prepared following standard procedures. Photomicrographs were taken with Leitz (Ortholux) microscope and camera.

Statistical comparisons of the results were made using the one-way Analysis of Variance (ANOVA) test. Duncan's New Multiple Range Test was used to evaluate the differences between means for treatments at the 0.05 significance level (Zar, 1996).

RESULTS AND DISCUSSION

Reproduction traits and histology of testes in *O. niloticus* fed varying inclusion levels of *Aloe vera* Latex (AL) diets. In fish fed with the control diet, milt motility from its initial level of 84.84% for O. *niloticus* dropped to 17.88%, for those fed with 2.0ml AL/kg diet for 60 days. In a similar study, Lohiya and Goyal (1992), Lohiya *et al.* (1999a, 1999b) reported that the chloroform extract, the benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate sub-factions and the isolated compounds of *Carica papaya* showed significant effects on sperm parameters by oral administration in rats and rabbits. Also, milt count dropped from its initial level of 163,600 to 138,000 in *O. niloticus* fed with 2.0ml AL/kg diet for 60 days. This was corroborated by Sadre *et al.* (1983) who studied male antifertility activity of neem on mice, rats, rabbits, and guinea pigs by daily oral administration of a cold-water extract of fresh green neem leaves and reported infertility effect in treated male rats, as there was a 66.7% reduction in fertility after six weeks, 80% after nine weeks, and 100% after 11 weeks. There were no inhibition of spermatogenesis, no decrease in body weight and no manifestation of toxicity, but there was a marked decrease in the motility of spermatozoa. The infertility in rats was reported not to be associated with loss of libido and that the animals maintained normal mating behavior.

Milt morphology was also evaluated using the evaluation criteria suggested by World Health Organization (WHO) (1999) and examined under Olympus microscope model CX 40. The milt showed normal milt morphology; the oval head and distinctive and well defined tail. At low treatment dosage i.e. 0.5ml or 1.0ml AL/kg basal diet, no appreciable visible changes was noticed in the milt morphology, while at high treatment dosage 1.5ml or 2.0ml AL/kg basal diet, deleterious changes in the plasma membrane of the head was observed, consequently suggesting that the milt were infertile (Farnsworth and Waller, 1982).

Histological sections of testes in *O. niloticus* fed 0ml AL/kg diet (basal diet) showed normal tissue architecture and spermatids distribution (Table 2). Fish fed 0.5ml AL/kg diet showed no visible alterations in the testicular architecture and cystic seminiferous tubules. In fish fed 1.0ml AL/kg diet, there was atrophy, while fish fed 1.5ml AL/kg diet showed cystic seminiferous tubules and atrophy. In fish fed 2.0ml AL/kg diet, there was severe tissue atrophy, spermatids disintegration and necrosis.

Table 2: Histological description of male *O. niloticus* fed *Aloe vera* latex (AL) diets.

Treatments (ml AL/kg diet)	Histological description
0	normal testicular tissue architecture and normal spermatids distribution
0.5	no visible alterations in the testis architecture and cystic seminiferous tubules
1.0	Atrophy
1.5	cystic seminiferous tubules and atrophy
2.0	severe tissue atrophy, spermatids disintegration and necrosis

a, b, c, d – Mean values in a column followed by dissimilar letters are significantly different ($P<0.05$).

This result corroborate that reported by Udoh *et al* (2001) that oral intubation of ethanol extracted *Momordica charantia* at 1.3mg/kg treated guinea pigs shows degeneration of tubules from connective tissue. Also in a related study Jegede *et al*. (2008a) obtained similar histological effects (severe alteration in the testicular architecture and necosis) in male redbelly tilapia (*Tilapia zillii*, fed varying dietary inclusion levels (0.5-2.0 g/kg diet) of neem (*Azadirachta indica*) leaf meal (NLM). Also, in a similar study by Verma and Chinoy (2002) on male albino rats administered intramuscularly Papaya seed extract at a dose of 0.5mg/kg/day for 7 days, a much severe decrease in the contractile response of epididymal tubules was obtained when compared with the control.

Reproduction traits and histology of ovaries in *O. niloticus* fed varying inclusion levels of *Aloe vera* Latex (AL) diets.

The inclusion of *A. vera* latex (AL) at varying levels in the diets of *O. niloticus* fed for 60 days treatment period, revealed no deleterious changes in the shape of the eggs when observed under an electronic microscope. Normal oval shape of eggs was observed. This was corroborated by Arrignon (1998) who reported that the normal egg shape of tilapia is oval. Egg size and fecundity decreased (2.25 ± 7.07mm - 2.13 ± 3.54mm and 228 ± 4.24 - 150 ± 1.41 respectively) as the inclusion level of *A. vera* latex (AL) increases (Table 3). This result agrees with Coward and Bromage (2004) who reported that eggs produced by mouth brooders (*O. niloticus*) normally exceed 2mm in diameter and that fecundity is usually less than 350 in mouth brooders. In *O. niloticus* fed with the basal diet (0ml AL/kg diet), typical bilateral lobes of the ovaries were evident; and the normal olive green colour was still maintained.

Table 3: Egg sizes (mm) and fecundity of *Oreochromis niloticus* fed ALM diets

	ALM diet treatments (ml/kg)				
	0	0.5	1.0	1.5	2.0
Egg size	2.33 ± 1.77	2.20± 0.00	2.20± 0.00	2.13± 3.54	2.18± 3.54
Fecundity	258.0 ± 2.12	228.0*± 4.24	218.0*± 2.12	203.0*± 1.41	198.0*± 2.12

*The mean difference is significant to control at the 0.05 level

Sections of ovaries in *O. niloticus* fed with the basal diet showed normal ovary histology. No pathological lesions was observed, atretic follicles were less visible (Table 4). Also in fish fed low AL/kg basal diets (0.5 and1.0 ml AL/kg diet) no visible changes were noticed, normal ovarian colour was maintained; ovary histology was similar to that of the control except for few pockets of leisons. In fish fed 1.5 and 2.0ml AL/kg diet, ovirectomy reveals a change in colour of ovaries, while histology reveals ruptured follicles, inflammation of the granulomatous in the interstitium, evidences of abnormal gonadal development and necrosis.

Table 4: Histological description of female *Oreochromis niloticus* fed *Aloe vera* latex (AL) diets.

Treatments (ml AL/kg diet)	Histological description
0	normal histology and less visible atretic follicles
1.0	ovary histology was similar to that of the control except for few pockets of lesions; normal ovarian colour was maintained;
2.0	change in colour of ovaries was noticed, ruptured follicles, inflammation of the granulomatous in the interstitium, evidences of abnormal gonadal development and necrosis.

a, b - Mean values in a column followed by dissimilar letters are significantly different (P<0.05)

Similar histological effect was reported by Jegede (2010) where the damage done to tissues of the testes and ovaries were minimal at lower dietary *Hibiscus rosa sinensis* leaf meal (HLM) levels (1.0 or 2.0 g/kg diet), and at higher dietary HLM levels (3.0 or 4.0 g/kg diet), it caused disintegration of many more cells, rendering the testes and ovaries devoid of spermatids and oocytes, respectively.

Although *A. vera* gel had been reported to provide evidence of anti-genotoxic against mutagenicity induced by alkylating agent ethyl methanesulfonate (Stanić 2007), nothing of such had been reported on *A. vera* latex. This indicates that *A. vera* latex at high levels of concentration causes histological damage to the gonads of both male and female O*. niloticus*, thereby potentially impairing reproduction.

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MORPHOMETRIC AND MERISTIC CHARACTERISTICS AND THEIR VARIATIONS BETWEEN TWO DIFFERENT STRAINS (GIFT & GIFU) OF NILE TILAPIA, *Oreochromis niloticus*

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Abstract

To investigate the morphological variations, 12 morphometric characters viz. total length, standard length, head length, pre-orbital length, post-orbital length, eye diameter, inter-orbital length, snout length, mouth gape, maximum body circumference, minimum body circumference, body depth and 11 meristic characters viz. dorsal fin spines, dorsal fin soft rays, pectoral fin soft rays, pelvic fin spines, pelvic fin soft rays, anal fin spines, anal fin soft rays, caudal fin rays, branchiostegal rays, scale above lateral line, scale below lateral line were studied for 100 specimens of traditional strain GIFT and recently developed strain GIFU of Nile tilapia, *Oreochromis niloticus*. The value of the co-efficient of correlation showed that the relationship between total length with other body measurements were highly significant at 5% level. Again the relationship between head length and other characters of head were also highly significant at 5% level. GIFU showed faster linear growth on body depth, maximum body circumference and minimum body circumference where GIFT showed faster linear growth on standard length and head length. On meristic characters notable variations were observed in case of scale above and below the lateral line, where GIFU individuals showed greater number of scales in both parameters. The total length and body weight relationship of the fish was found to be a straight line in logarithmic scales. The mean values of condition factor (K) have been found to be 1.671 for GIFT and 1.711 for GIFU and the mean values of relative condition factor (Kn) have been obtained as 1.001 for GIFT and 1.002 for GIFU, which represents the better condition of GIFU individuals during the study period. Findings of the present study suggested the superiority of strain GIFU over GIFT in most important parameters.

MATERIAL AND METHODS

100 specimens of GIFT and 100 specimens of GIFU of same age and total length ranging from 115 mm to 257 mm were collected from earthen ponds of Zubin Agrobased Industries Limited, Noakhali. Total length (TL), standard length (SL), head length (HL), pre-orbital length (PreOL), eye diameter (ED), post-orbital length (PostOL), inter-orbital length (IOL), snout length (SnL), mouth gape (MG), maximum body circumference (Max BC), minimum body circumference (Min BC) and body depth (BD) of fish were measured to the nearest mm using fish measuring board. The fishes were weighed on tanetag, KD-160 balance having one gm precision. The body characters viz. SL, HL, PreOL, ED, PostOL, IOL, SnL, MG, Max BC, Min BC, BD were expressed as percent to total length of the fish as done by Carlender and Smith (1954) and Hile (1948). Regression of various body parts against TL of fish were drawn by least square method. Length-weight relationship was calculated by cube law as given by Le Cren (1951).

$\log W = \log a + b \times \log L$

Where, W is weight, L is length of fish and 'a' and 'n' are constants.

$$K = \frac{W \times 10^5}{L^3}$$

Where, K is condition factor, W is observed body weight of fish and L is observed length of fish

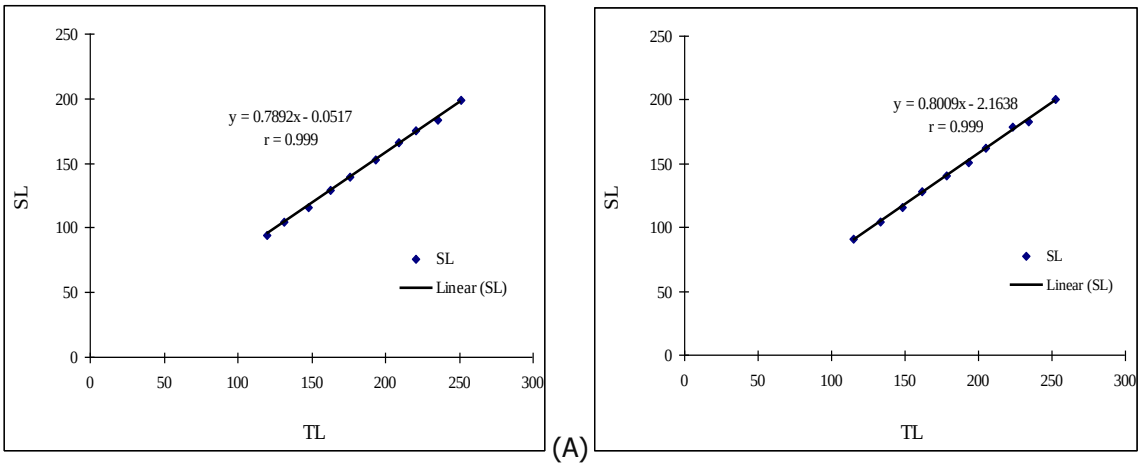
$$Kn = \frac{W}{W'}$$

Where, Kn is relative condition factor, W is observed body weight of fish (g) and W' is calculated body weight of fish (g)
Regression of morphometric characters were compared between GIFT and GIFU by Snedacor (1956).

RESULT AND DISCUSSIONS

From morphometric characters it was observed that mean total length of GIFU (176.73) was slightly larger than the GIFT (173.88). Again the mean body weight of GIFU (103.63) was also larger than the GIFT (97.03). Other morphometric measurements for GIFT and GIFU were standard length 137.34 & 139.14, head length 49.81 & 48.45, pre-orbital length 16.4 & 15.45, eye diameter 9.98 & 9.72, post-orbital length 23.3 & 23.39, inter-orbital length 34.62 & 35.46, snout length 15.03 & 15.67, mouth gape 19.14 & 18.41, maximum body circumference 129.91 & 135.55, minimum body circumference 46 & 48.3, body depth 52.79 & 54.65 respectively.

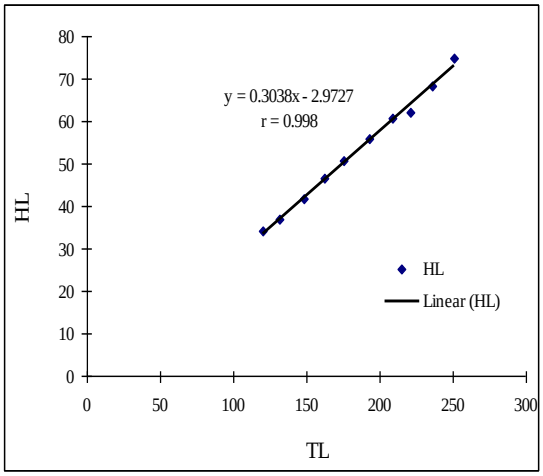
Highly significant (p<0.05) relationships were found between total length and other variables *viz.* standard length, head length, pre-orbital length, eye diameter, post-orbital length, inter-orbital length, snout length, mouth gape, maximum body circumference, minimum body circumference and body depth of both GIFT and GIFU. Again the relationships between head length and other head parameters *viz*. pre-orbital length, eye diameter, post-orbital length, inter-orbital length, snout length, mouth gape were also highly significant (p<0.05).



(A)

(B)

Figure 1. Relationship between standard length (SL) and the total length (TL) of (A) GIFT and (B) GIFU of *Oreochromis niloticus*



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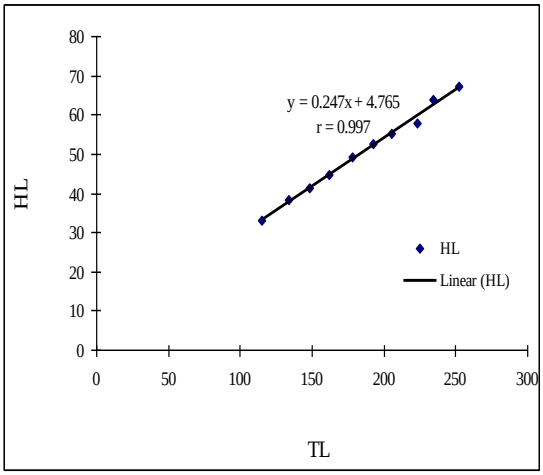
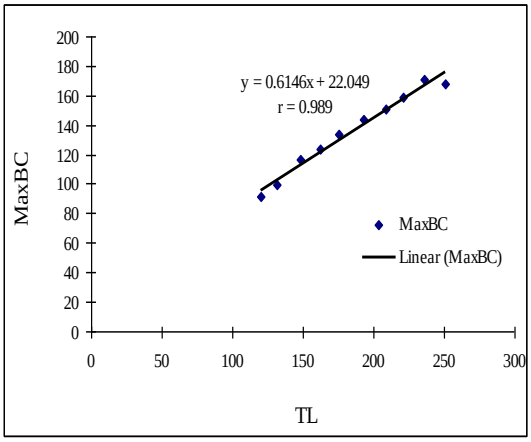


(B)

Figure 2. Relationship between head length (HL) and the total length (TL) of (A) GIFT and (B) GIFU of *Oreochromis niloticus*



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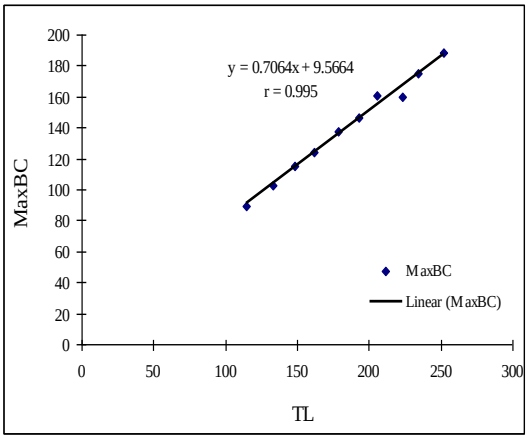
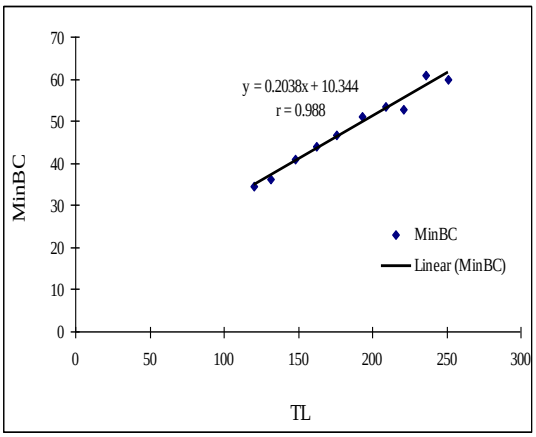


(B)

Figure 3. Relationship between maximum body circumference (MaxBC) and the total length (TL) of (A) GIFT and (B) GIFU of *Oreochromis niloticus*



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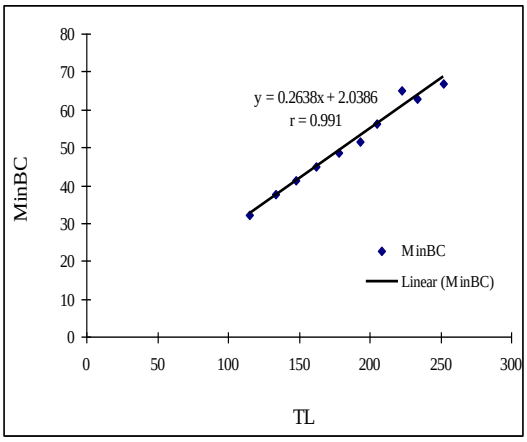
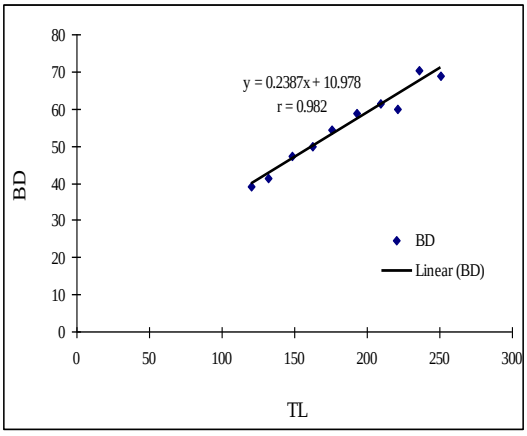
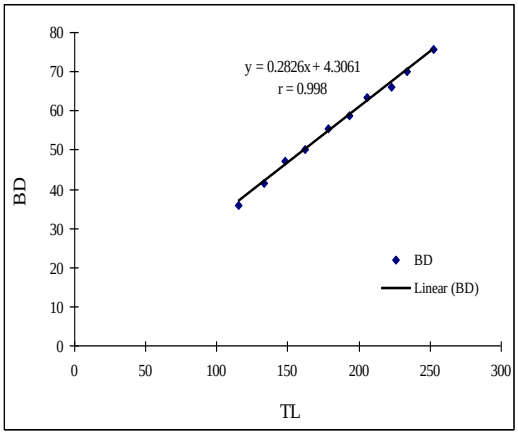


(B)

Figure 4. Relationship between minimum body circumference (MinBC) and the total length (TL) of (A) GIFT and (B) GIFU of *Oreochromis niloticus*



(A)



(B)

Figure 5. Relationship between body depth (BD) and the total length (TL) of (A) GIFT and (B) GIFU of *Oreochromis niloticus*

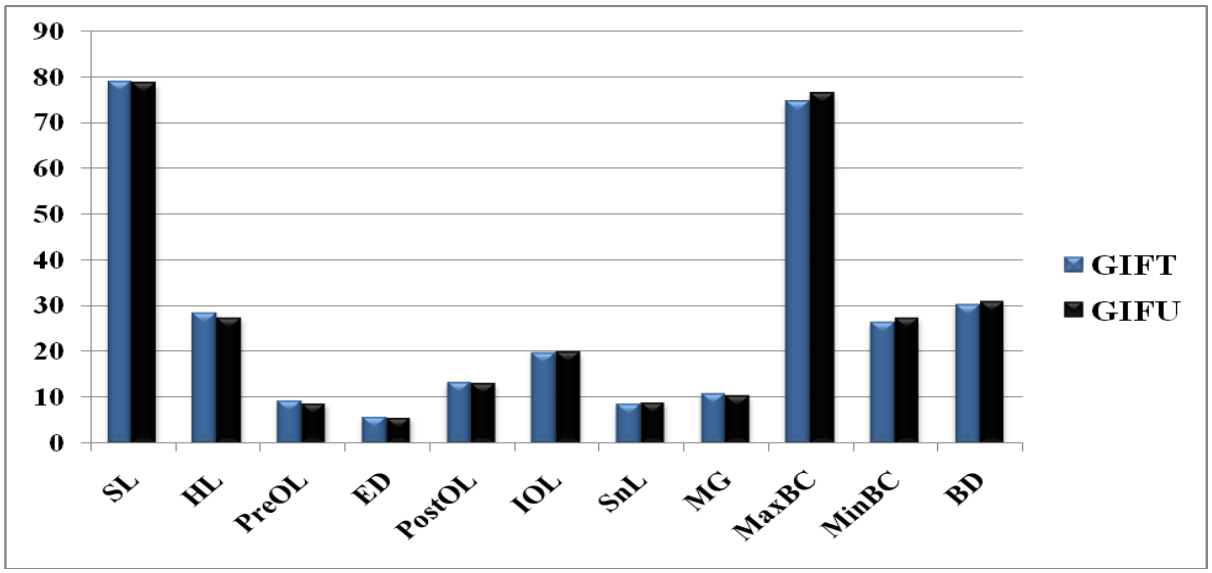


Figure 6. Growth of different morphometric body parts of GIFT & GIFU in relation to the total length (TL) of the fish on a percentage basis

Percentage values (Figure 6) of standard length (78.99) of GIFT was slightly higher than the standard length (78.73) of GIFU. Again, the value of body depth at pectoral fin-base in GIFU (30.92) was higher than that of GIFT (30.36). A close examination of values of the characters revealed a strong heterogeneity between GIFT and GIFU. The GIFU were broader in anterior part of the body at pectoral fin than the GIFT where GIFT (28.65) having longer head than the GIFU (27.41). The maximum body circumference and minimum body circumference of GIFU (76.7 & 27.33 respectively) are higher than that of GIFT (74.71 & 26.46 respectively). Regarding other characteristic like standard length of GIFT was longer than that of GIFU. Thus it may be inferred that GIFU shows faster linear growth on body depth, maximum body circumference and minimum body circumference where GIFT shows faster linear growth on standard length and head length. Simply it may be said that GIFU is fatter or heavier than GIFT which allows more flesh. Devi *et al.* (1991) reported the value of head length (24.91) of males to be higher than that of females (22.91) and the value of depth of body at pectoral fin-base in females (21.09) to be higher than that of males (19.50) in *Rita rita*. This finding is similar to the present study, only the difference is that the variation here is conducted within two different strains. This heterogeneity of body characters may be due to their strain variation. Such phenomenon was also reported by Khumar (1985).

In case of dorsal fin spines (16-17), dorsal fin soft rays (11-13), pectoral fin soft rays (13-14), pelvic fin spines (1), pelvic fin soft rays (5), anal fin spines (3), anal fin soft rays (9-11), caudal fin rays (16) and branchiostegal rays (3) the meristic variations were observed merely. Siddique *et al.* (2007) found the number of dorsal fin spines ranged from 16 to 17, dorsal fin soft rays from 11 to 15, pectoral fin soft rays 15, pelvic fin spines 1, pelvic fin soft rays 5, anal fin spines 3 and anal fin soft rays from 8-11 which is almost similar to the present findings. Only the notable variation in the present study was observed in case of scale above and below the lateral line. The range of the scales above lateral line is 4.5-5.5 in both GIFT and GIFU but greater number of individuals of GIFU obtained 5.5. In case of scale below lateral line the range of GIFU (11.5-15.5) was greater than the range o f GIFT (10.5-11.5). This variation may be due to that GIFU individuals showed greater body depth and body circumference which allowed more scales than the GIFT individuals.

The length weight relationship of GIFT and GIFU were not significantly different. The regression equation is expressed as:

LogBW (GIFT) = 2.6932 LogTL - 4.0895
LogBW (GIFU) = 2.7221 LogTL - 4.1421

The value of coefficient of correlation showed that the relationship between length and weight of the fish was highly significant (P<0.05). The value of exponential in the length-weight equation ($W = aL^b$) was found to be 2.6932 and 2.7221 for GIFT and GIFU respectively which were within the range from 2.0 to 4.0 mentioned by LeCren, 1951. Various workers calculated the values of regression coefficient (b) in different fish species and found the value of b>3. Narejo *et al.* (1999) from Pakistan and Al-Baz and Grove (1995) from Kuwait calculated value of regression coefficient b in *Tenualosa ilisha* (3.0246 for males and 3.0345 for females) and (2.68 for males and 3.16 for females) respectively. Azadi and Naser (1996) reported the values of regression coefficient to be 3.16 for males and 3.20 for females in *Labeo bata* and Quddus (1993) reported value of regression coefficient to be 3.40 in *Gudusia chapra* from Bangladesh. Hile (1936) and Martin (1949) observed that the value of regression coefficient (b) usually lies between 2.5 and 4.0 in cisco, *Leochthys artedi*. However, a variation in 'b' value may occur due to species variation, strain variation, stock variation, differences in environmental factors, sex variation etc.

The values of condition factor (K) were found to vary from 1.343-1.871 for GIFT and 1.385-1.825 for GIFU and the mean values were 1.671 and 1.711 for GIFT and GIFU respectively. The values of relative condition factor (Kn) ranged from 0.897-1.06 for GIFT and 0.876-1.097 for GIFU and the mean values were 1.001 for GIFT and 1.002 for GIFU. From this finding it can be stated that the higher value of condition factor (K) and relative condition factor (Kn) of GIFU expresses the better condition of GIFU individuals over GIFT individuals during study period.

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GENETIC STOCK IMPROVEMENT OF THE GIFT STRAIN IN BANGLADESH

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Abstract

Tilapia is globally recognized as one of the most important aquaculture species of the 21st century. Culture of tilapias has expanded rapidly under a wide range of farming environments from extensive to intensive scale in both fresh and brackish water in Asia and many other countries of the world including Bangladesh. The world's total tilapia production was forecasted that it would be reached 3.70 million tones by the end 2010. In case of Bangladesh, it is estimated that the tilapia production in 2010 would be more than 1.00 million tones. Tilapias will be the prime culture species in different water ecosystems at various production scales (small, medium and commercial scales) in Bangladesh. This has led to a great demand in terms of both quantity and quality of tilapia seeds in the country. To meet this growing demand, Bangladesh Fisheries Research Institute (BFRI) has undertaken research work to further improve performance of the GIFT strain (Genetically Improved Farmed Tilapia) through combined family selection with the technical assistance of The WorldFish Center, Malaysia.

In the present study, we report growth performance of the GIFT strain after four generations of selection for increased body weight at BFRI, Bangladesh. Founder stock comprising 300 individuals from 30 families of the GIFT strain were introduced from The WorldFish Center, Malaysia, in March 2005. They were reared in 100 m^2 hapa for three months, and then individually tagged using Passive Integrated Transponder (PIT) tags at a mean weight between 30 and 40g. After tagging, all the fish were communally grown out in pond until harvest. Breeding values (EBVs) for body weight were estimated using SAS and ASREML. Based on EBV ranking, the best 40 females and 40 males from the founder stock were then selected to produce progeny of the first generation (G1) in 2007. From each family 25 female and 25 male fingerlings were sampled and tagged using Passive Integrated Transponder (PIT). A total of 2,000 tagged fish from 40 families were stocked in a pond (1000 m^2) for a continuation of the selection program. The same production and selection procedures were practiced in subsequent generations in 2008, 2009 and 2010 (corresponding to generations G2, G3 and G4). In addition to the mainstream selection program, surplus fish after tagging were also reared together with progeny of the founder stock in cisterns (G1) and in earthen ponds (G2 to G4) for growth evaluation. Our preliminary analysis showed that the upgraded fish had 7.2, 13.1, 23.2 and 30.3% greater harvest weight than that of the founder population (non selected population) in G1, G2, G3 and G4 generations, respectively. Due to fast growth of GIFT, the strain is widely sought and cultured in Bangladesh. To date BFRI has supplied seed to about 100 hatcheries, which in turn multiply and distribute GIFT fry to fish farmers throughout the country. The paper also discusses strategies for future expansion of GIFT culture in Bangladesh.

Key words: Stock Improvement, Genetic selection, GIFT strain.

INTRODUCTION

The last three decades have seen significant developments in farming of tilapias worldwide. In view of the increasing commercialization and continuing growth of tilapia industry, the commodity is not only the second most important farmed fish globally, next to carps but is also described as the most important aquaculture species of the 21st century (Shelton 2002). The fish is being farmed in about 85 countries worldwide, and about 98% of tilapia produced in

these countries is grown outside their original habitats (FAO 2002). In INFOFISH Tilapia 2010 Conference it was forecasted that the world's total tilapia production would be reached 3.70 million tones by the end 2010. The main culture industries are in the Far East but they are increasingly being farmed in Caribbean, Latin America and recently, in temperate countries where warm water through artificial means (thermal effluents or geothermal springs) are also available.

The development of Genetically Improved Farmed Tilapia (GIFT) technology that is based on traditional selective breeding as a means to improve commercially important traits of tropical farmed fish is a major milestone in the history of tilapia aquaculture (Azhar, *et al*. 2004). The GIFT was developed by WorldFish Center through several generations of selection from a base population involving eight different strains of Nile tilapia (Eknath *et al*. 1993 and 1998). Bangladesh Fisheries Research Institute (BFRI) received GIFT strain in 1994 and again 116 families in 1996 through WorldFish Center (Formerly ICLARM). In on-station and on-farm trials of BFRI, the GIFT strain was reported to show 35-57% superior growth than that of the existing strain of the country (Hussain *et al*. 2000). Further stock improvement of GIFT through mass selection was initiated in 1998. Through mass selection, six generations (F-1 to F-6) were produced. Through combined selection technology, the F-6 generation of GIFT strain achieved 33.7% growth over the existing GIFT strain. The rate of genetic gain in weight of fish was greater up to third generation but it decreased gradually after that and up to sixth generation. The reason behind such a decrease in genetic gain in particularly for body weight might have been the accumulation of inbreeding. Therefore, the genetic improvement strategy for GIFT was re-designed. Now the stock improvement program is being implemented through family selection protocol under the technical assistance of WorldFish Center. In this paper we report results of growth evaluation of G1, G2, G3 and G4 generations of improved GIFT strain in Bangladesh.

MATERIALS AND METHODS

Stock improvement through Family selection

Origin of stock

Founder stocks comprised of 30 families having 300 individuals of GIFT strain were introduced from Malaysia through WorldFish Center in March 2005. The founder stocks were reared in 100 m^2 hapa for three months. The fish were fed with SABINCO feed (28% crude protein) at the rate of 6% of estimated biomass. After three months rearing, the mean weight of female and male were 41.18±5.41 and 30.42±3.47g, respectively.

Tagging of founder stock

Then the female and male were tagged by using Passive Integrated Transponder (PIT). A PIT tag was injected into the peritoneal cavity of a fish and the number of tag was recorded. After tagging all the fish were transferred to a pond having 1000 m^2 area.

Rearing in pond

During rearing period, the fish were fed with supplementary feed six days in a week (28% crude protein) at the rate of 4-5% of estimated biomass. Fish were sampled at fortnightly interval to assess the growth and feed adjustment. Water was supplied once in a week to maintain water depth at 1.0 meter. Pond was fertilized fortnightly with Urea and TSP at the rate of 12.5 and 25.0 kg/ha, respectively. After four months rearing, the fish were recaptured through seine netting and pond drying. The final body weight, sex and tag number of all harvested fish were recorded.

Estimation of breeding value

Breeding value was estimated for individual fish in a full pedigree, using SAS (SAS Inc, 1997) and ASREML (Gilmour *et al*. 1999).

Breeding in hapa for G1 (Generation 1) production

On the basis of breeding values of the founder stock, the best 40 males from 30 families were crossed with 40 best females (from 30 families) for the production of F-1 generation. For breeding, 40 breeding hapas ($1.0m^3$) were set up in a pond with bamboo poles. A pair of female and male breeders (1:1) was stocked in each breeding hapa. After 12 days of stocking, fertilized eggs were collected from brooding females. After that, collected eggs were transferred to the hatchery for incubation. Immediately after hatching, the larvae were shifted to a series of trays and were kept until their yolk sac resorption stage.

Nursing in hapa

After resorption stage, 300 fry from each family were transferred to 40 individual fine mesh nursery hapas (2.0 m^3) in pond. The progeny were fed with nursery feed containing 30% protein at the rate of 30% of estimated body weight. After 45 days nursing, the mean weight of the fry was 2.80±0.42g.

Rearing in hapa

Subsequently, 150 fry from each progeny group were transferred to 40 individual rearing hapa (2.0 m^3 in size). Supplementary feed (Nursery feed) was applied in all the hapas at the rate of 15% of estimated biomass. After two months of rearing, the weight range of male and female were 36-43 and 28-32g, respectively.

Tagging

From each progeny group 25 male and 25 female fish were selected and tagged using Passive Integrated Transponder (PIT). Tagged fish from 40 families (2000 fishes) were stocked in a pond having $1000m^2$ area for communal rearing. Supplementary feed (25% crude protein) was supplied regularly at the rate of 6% of estimated biomass. After six months of grow-out in pond, the fish were harvested, and tag number, weight, sex, body depth were recorded. After harvesting, breeding values of G1 generation were estimated from the complete data set, tracing back to the foundation population (F0).

Evaluation of Growth performances G1 generation of GIFT strain

This trial was conducted to compare growth performance between G1 progeny of the selected fish and progeny of the non-selected population (founder stock) in cisterns (2.0 m^3) for a period of four months during April to July 2007. Progeny of the selected fish were produced from 40 single pair matings in separate hapas. Family rearing of the selection progeny was as described above. After tagging, surplus fish were sampled for this experiment. By contrast, the non-selected population (200 breeders) was stocked in a 300 m^2 pond for mass breeding. After 40 days of stocking, 6,000 fry were collected and reared in a $10m^3$ hapa for a period of 3 weeks. From this population, fry samples were taken for growth evaluation. The initial mean weight of the selected fish and of the founder population (non selected population) was 2.95±0.65 and 2.65±0.82g, respectively. There were two treatments with three replicates. Before stocking the cisterns were cleaned and filled up with deep tube well water at the depth of 1.0 meter. Fry of GIFT strain were stocked at a density of 5 fish/m^3.

The fry of both treatments were fed twice a day in six days in a week with supplementary feed (28% crude protein) at 5-8% body weight. During grow out period, first and second months, feed was given at the rate of 8% and 7% of body weight, respectively, Then subsequently, 6% and 5% feed were given to the fish in the 3rd and 4th month, respectively. Fish sampling was done at monthly interval to assess the growth, and feeding ratio was adjusted on the basis of estimated weight of fish biomass. In every week cisterns were cleaned through siphoning and 80% water changed with deep tube well water. Average water depth was maintained in all the cisterns at 1.0 m during the experimental period. After five months rearing, all the fishes were harvested. After harvest, body weight was measured on individual fish. Statistical analysis was carried out to test significant differences in growth between the F1 generation fish and the founder stock.

Production of G2 *(Generation 2)* generation of GIFT strain

On the basis of breeding values of G1 generation, the best 60 males were crossed with 60 females. For breeding, 60 breeding hapas ($1x1x1m^3$) were set up in a pond. The range of breeding values of selected males were 8.23 to 16.13, while in case of females, the values were 5.01 to 14.47. A pair of female and male breeders (1:1) was stocked in each breeding hapa. After 21 days of stocking, 300 larvae from each progeny group were shifted to a series of hapas in a pond. The progeny were fed with nursery feed containing 30% protein at the rate of 30% of estimated body weight. After 30 days nursing, the mean weight of the fry was 2.46±0.81g. Each progeny group, 150 fry were transferred to 70 individual 2.0 m^3 size rearing hapa. Supplementary feed (Nursery feed) was applied in all the hapas at the rate of 10-15% of estimated biomass. After 1.5 months of rearing, the weight ranges of fingerlings were 15-22g.

Tagging and communal rearing

From each progeny group 20 male and 20 female fish were tagged them by using Passive Integrated Transponder (PIT). Tagged fishes from 60 families (2400 fishes) were stocked in a pond ($1000m^2$) for communal rearing in 1 July 2008. The tagged fishes were reared in the pond. Supplementary feed (25% crude protein) were supplied regularly at the rate of 6-8% of estimated biomass. After four months rearing, fish were harvested and tag number, weight, sex, body depth were recorded. Then data were analyzed through statistical analysis for breeding value estimation.

The same production and selection procedures were followed in subsequent generations G3 and G4.

Evaluation of growth performances between upgraded GIFT strain (G2) and founder population in pond

This trial was conducted to evaluate the growth performances of upgraded GIFT strain (G2) and founder population of GIFT strain in a pond for a period of five months during June to November 2008. A pond having an area of $1000m^2$ were selected for growth performances evaluation. Prior to the evaluation, the pond was cleaned and limed at the rate of 250 kg/ha. After three days of liming, pond was fertilized with urea and TSP at the rate of 12.50 and 25.0 kg/ha, respectively.

Fry of upgraded GIFT strain generation (G2) were stocked together with the progeny of the founder stock in a pond for communal rearing. In each group, 600 hundred fry were stocked. The initial mean weight of upgraded GIFT strain (G2) and founder Population were 4.81±0.65 and 4.72±0.82g, respectively. Fry of founder population of GIFT strain were marked through cauterization of pelvic fin. After stocking fry were fed with nursery feed contained 28% crude

protein at the rate 5-10% of estimated body weight. Fry were sampled at fortnightly interval to know the growth as well as feed adjustment. In the first month, fry were fed at the rate of 10% of estimated body weight and the consecutive months feed ration was reduced to 8, 6 and 4% in the 2nd, 3rd and 4th month, respectively. After four months of rearing, fish were harvested through repeated netting followed by pond drying. The same protocol was practiced in subsequent generations in 2009 and 2010 (corresponding to generations G3 and G4) for growth evaluation.

RESULTS AND DISCUSSIONS

A total of 2000 fish (1000 males and 1000 females) of the first generation were harvested and measured of body weight in June 2007. General linear model analysis indicated that there was significant difference ($P < 0.001$) in body weight between the two sexes, where the males were substantially heavier than the females (278 vs. 156 g) (Table 1). The effect of sex on size and growth is often found in aquaculture species (Ponzoni et al. 2005, Nguyen et al. 2007).

Table 1: Body weight of male and female

Sex	Number of records	Weight (g)
Male	**1000**	**277.76±29.77**
Female	**1000**	**156.05±30.26**

Therefore, the statistical model included sex as the fixed effect and the additive genetics of individual fish as the random term to estimate breeding values (EBV) of all animals in the pedigree. Based on EBV ranking, the best 60 females and 60 males from 40 families were selected to produce progeny for the second generation (G2). The EBV range for the selected males and females were 4.17- 9.70 g and 4.24-9.36 g, respectively (Table 2).

Table 2: Breeding values of selected male and female breeders

Sex	Number of animals	Breeding Values
Male	**60**	**4.17 - 9.70**
Female	**60**	**4.24 - 9.36**

The body weight data of the upgraded (selected) and founder stock (non selected population) were measured at different months. The initial mean weight was 30.23±0.41 and 31.70±0.60g for the upgraded (selected GIFT) and founder stock, respectively. Month wise sampling data showed that growth rate of the upgraded GIFT strain was always higher than the founder stock. After four months rearing, the final cumulative mean weights were recorded at 168.67±3.51 and 157.33±2.52g for the selected and founder stock, respectively.

Table 3 also presents net gain and daily gain for the F1 and founder stocks. The net gains for weight estimated for the selected GIFT was significantly ($P< 0.05$) higher than that of the founder stock (138.4 vs. 125.6 g). The final weight of the selected GIFT was 7.20% higher than that of the founder stock. In regard to survival rate, hundred percent survivals were obtained in both the stocks.

Table 3: Growth rate of the GIFT strain tested in cistern ecology at BFRI

Population	No of records	Net gain (g)	Daily gain (g)
Selected GIFT	30	138.43±3.40	1.15±0.03
Founder stock	30	125.50±3.30	1.04±0.02

Evaluation of growth performances of upgraded GIFT strain (G2, G3 and G4)

The harvesting mean weight of upgraded GIFT strain (G2) and founder GIFT strain were 142±4.18 and 125±3.97g, respectively. During sampling, the upgraded GIFT strain (G2) showed higher growth rate than founder GIFT strain in all the events. The mean weight of the upgraded GIFT was 13.60% higher than that of the average GIFT strain. The upgraded GIFT strain showed higher survival rate than founder GIFT which were 91 and 88%, respectively.

Table 4 presents the weight gain for the generations of G2, G3 and G4 and founder stocks. The upgraded GIFT of G3 and G4 were found 23.21 and 30.30% higher growth over founder generation (Fig. 1). The G2, G3 and G4 upgraded groups attained 7%, 8% and 7% cumulative weight gain, respectively over three generations. The average gain per generation across four generations of selection for growth performance has been found at 8%.

Table 4: Growth rate of the upgraded GIFT in communal rearing in pond ecology

Generation	**Weight (g)**	
	Upgraded GIFT strain	**Founder GIFT strain**
G2	141.60±19.01	125.72±16.54
G3	151.74±17.29	123.39±17.35
G4	168.25±23.25	129.17±15.50

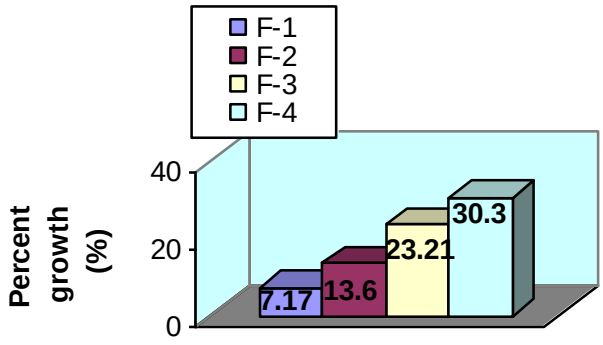

Fig 1: Generation wise percent weight gain of BFRI-GIFT strain

The results showed that the upgraded GIFT strain had a significant higher growth than the foundation stock, after four generation of selection. BFRI has initiated stock improvement program for GIFT strain through mass selection in 1998. Through mass selection, G1 generation of GIFT showed 5% higher growth over average GIFT strain, which was introduced from ICLARM (Now WorldFish Center), Philippines in 1994. Subsequent generations (F-2 to F-6) were produced in the same manner. Through combined mass selection technology, the F-6 generation of GIFT strain achieved 32.7% growth over existing GIFT strain (Annual Progress Report, 2007). In the present study, we applied family selection protocol, and an approximately 8% genetic gain was achieved after four generation.

Dissemination of GIFT germplasm

Due to fast growth and high survival of GIFT strain, the strain is widely cultured throughout the country in both fresh and brackish water as well as in cage culture and rice field ecosystem. Presently, in Bangladesh, over 200 tilapia hatcheries are established in the last couple of years and producing about 2.0 billion fry of tilapia. Aquaculture production in Bangladesh has been dominated by GIFT strain which commenced in 2003. Last 7 years (2003 – 2010) a tremendous progress in tilapia farming in Bangladesh. Presently, Tilapia production of Bangladesh is more than 1.0 million tonnes. This was due to the development of monosex seed production technology and grows out technique(s) for farming of GIFT tilapia in ponds and cages. Bangladesh should include in the list of top eight tilapia producer states in Asia in near future.

But it is alarming that, a large number of hatchery operators are not producing quality fry due to unavailability of good quality brood fish. So farmers are not getting expected production rather sometimes they become frustrated. Bangladesh Fisheries Research Institute (BFRI) as a center of excellence has given thrust to produce quality seeds as well as stock improvement program of GIFT strain. In the last year (2010), BFRI distributed 0.60 million fry to 100 tilapia hatcheries. When these GIFT fry attaining maturity, the hatchery operators producing millions of monosex fry and sale to the farmers for the production of table size fish. It is expected that in near future, generically improved GIFT strain will be the prime culture species next to riverine cat fish ie. Pangasius spp. and carp spp. in Bangladesh.

Dissemination Strategy:

The demand of GIFT strain is increasing day by day. In every year 40-50 tilapia hatchery have been established in this country. It is not possible at all to meet up the nation wide demand of quality GIFT fry from Freshwater Station of BFRI. Bangladesh Fisheries Research Institute (BFRI) authority is thinking to produce mass scale quality germplasm production through applying rotational breeding program in the satellite breeding station and sub stations. Moreover, improved germplasm of GIFT strain will be also handed over to the live gene banks of Department of Fisheries (DoF). These gene banks will take initiative to produce quality seed of GIFT strain and distribute to the hatcheries and farmers.

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PRODUCTIVE PERFORMANCE AND MUSCLE GROWTH OF THREE DIFFERENT STRAINS OF NILE TILAPIA, *Oreochromis niloticus,* DURING THE INITIAL DEVELOPMENT

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The aim of this study was to compare the productive performance and muscle growth (fiber hyperplasia and hypertrophy, and gene expression of the myogenic regulatory factors) of three strains of Nile tilapia (GIFT, Supreme and Thai), fed commercial diet containing or not the hormone 17α-methyltestosterone (MT), during the phase of sexual differentiation. The experiment was conducted in a factorial scheme (3x2), making up six treatments with four replicates each. Fish growth was evaluated along the experiment, as well as the survival rates, masculinization rates and homogeneity of the animals at the end (29 days). For the morphometric analysis of the muscle fibers, samples (n = 8 per treatment) were taken at 4, 19 and 33 days post-hatching (dph) and transversal sections of the epiaxial muscle were obtained. The diameter (*d)* and the area of 200 muscle fibers were measured in each sample, and the diameters were classified as follow: *class 10* = $d \leq 10$ µm, *class 20* = $10 < d \leq 20$ µm, *class 30* = $20 < d \leq 30$ µm, *class 40* = $30 < d \leq 40$ µm, and class 50 = $d > 40$µm. The analysis of gene expression of MyoD and Myogenin were performed by real time PCR (sampling at 4 and 33 dph). The "Supreme" strain showed better growth performance, survival rates, and size homogeneity. All strains displayed masculinization rates above 97%. The use of MT affected the productive performance of Nile tilapia larvae, and the effects were different among the strains. The development of the muscle fibers of the juvenile of Supreme strain that did not receive MT was characterized by more intense hyperplasia of the muscle fibers than the Thai strain. Preliminary results on the expression of MyoD and myogenin showed no effect of MT in juveniles of the Thai strain. In general, tilapia juveniles that received the hormone showed higher frequency of fibers of higher diameter classes, indicating intense participation of hypertrophy. However, this trend did not accompany increasing in weight, suggesting possible adverse effects on fish's physiology.

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SECTION IV
NUTRITION and FEEDS

Chair: Professor Wing-Keong Ng
Universiti Sains, Malaysia

Effects of saponin fractions from *Trigonella foenum-graecum* and *Balanites aegyptiaca* on gene expression of GH, IGF-1 and their respective receptors, growth, nutrient utilization, body composition, oxygen consumption and plasma IGF-1 in Nile tilapia (*Oreochromis niloticus*, L.).

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Abstract

Saponins in aquaculture feedstuffs are generally considered anti-nutritional. However, in several experiments it was shown that low level (150 ppm) supplementation with saponins from *Quillaja saponaria*, the South American soap bark tree, yielded several beneficial effects. Among them were improved growth rates, feed conversion efficiency, protein utilization and reduced oxygen consumption per unit body mass gain in common carp (*Cyprinus carpio*). In Nile tilapia, *Oreochromis niloticus*, supplementation levels of 300 ppm showed similar beneficial effects as the 150 ppm inclusion in carp diets.

Based on the above mentioned results an experiment was conducted in which saponin fractions (eluated with 60% or 80% methanol) from two different saponin rich plants, fenugreek (*Trigonella foenum-graecum* L.) and a methanol extract from the Egyptian desert date (*Balanites aegyptiaca* L.) were fed at different concentrations to 15 individually stocked Nile tilapia (19.1 ± 0.6 g, mean ± SD) in a respirometer system. Five treatments, namely a control diet (no saponin), three fenugreek saponin diets and one desert date saponin diet were fed to three replicates each.

Every week the fish were weighed and feed allowance was calculated accordingly. At the end of the eight week experiment the fish were anaesthetized and killed. IGF-1 levels in plasma were determined using a radio-immuno-assay, expression of genes encoding for IGF-1, GH and their receptors were determined using semi-quantitative reverse transcriptase real time PCR and proximate composition determined.

Fish fed with 60% *T. foenum-graecum* saponins at a concentration of 300 mg kg^{-1} showed the highest performance. Their expression levels of GH and IGF-1 genes were highest followed by control. The other groups had a significantly lower expression of GH and IGF-1. These results were also reflected in the numerically best growth and feed utilization parameters and the lowest oxygen consumption.

On the contrary, all other saponin supplementations resulted in reduced performance with considerably higher oxygen consumptions for fish fed 600 mg kg^{-1} 60% fenugreek saponins.

Results of gene expression levels strongly correlated with other performance parameters.

The obtained results suggest that the 60% MeOH eluated *Trigonella foenum-graecum* saponin fraction has a potential as natural growth promoter depending on applied concentration.

Key Words: Saponin, Nile tilapia, growth promoter, GH and IGF-1 gene expression

Introduction

With the rapidly growing aquaculture industry the demand for high quality fish feeds is increasing. At current and expected aquaculture growth rates the demand will eventually outgrow the availability of fish meal as highly digestible protein source (Hardy 2010). As a consequence the inclusion levels of plant derived proteins are increasing for formulated fish and crustacean feeds. However these plant derived ingredients have, in comparison to fish meal, a serious drawback since they always contain one or more anti-nutrients like protease inhibitors, lectins, gossypol, phytic acid, tannins or saponins (Francis *et al*. 2001a).

While saponins are generally considered as anti-nutrient, it has been shown in several experiments that low concentrations of *Quillaja saponaria* (South American soap-bark tree) saponins in the diet are improving the oxygen consumption, nutrient utilization and growth performance of carp, *Cyprinus carpio* and Nile tilapia, *Oreochromis niloticus*, respectively. When fed to common carp (*Cyprinus carpio*) at 150 mg kg^{-1} in the diet, *Quillaja saponaria* saponin supplementation resulted in significantly increased final body mass, reduced the oxygen uptake per unit body mass gain and improved the protein and energy utilizations compared to the control (Francis *et al*. 2002a, b). Nile tilapia fed with 300 ppm *Q. saponaria* saponins in their diet showed a significantly higher body mass gain and increased energy retention when compared to the control fish (Francis *et al*. 2001b).

Saponins are glycosidic compounds mainly produced by plants that are often activated after tissue damage and act for instance as antimicrobial defense substances (Gus-Mayer *et al.* 1994). Saponins can be found in a great variety of different plants, including many cultured plants like soybean which is the most common plant protein source for aquaculture. Some marine invertebrates like starfish also produce saponins most likely as a chemical defense against predators (Rio *et al.* 1965).

Saponins consist of a steroidal or triterpenoidal core structure called aglycone or sapogenin and one or more sugar side chains. Due to the large variations in either the aglycone or the sugar moiety they produce very diverse biological effects in animals. A detailed review of the biological actions of saponins is given by Francis *et al.* (2002c).

Saponin fractions were derived from two different plants, one being Fenugreek, *Trigonella foenum-graecum*, the other one being the Egyptian desert date, *Balanites aegyptiaca*. Both are frequently occurring and cultivated in the Middle East and are rich in different saponins (Marker *et al*. 1947, Dawidar and Fayez 1969, Hosny *et al*. 1992, Kamel 1998, Murakami *et al.* 2000). Both plants are commonly used in traditional folk medicine in the Middle East. Ethanol extract of *T. foenum-graecum* was considered an excellent alternative to a well known anti-diabetic drug as tested in artificially induced diabetic rats (Eidi *et al*. 2007). Diosgenin, a sapogenin present in fenugreek, did stimulate ion transport in human cortical neuronal cells (Wang *et al.* 2006).

The desert date is traditionally used as an anti-diabetic drug in folk medicine in Egypt and other parts of northern Africa and the Middle East. Kamel *et al.* (1991) were able to show that aqueous extracts of the desert date mesocarps and its fractions reduced the blood glucose levels significantly. A totally different application of the desert date was demonstrated by Chapagain *et al*. (2008) who used saponins extracted from a root derived callus as a larvicidal agent against the mosquito *Aedes aegypti*, the major vector for dengue fever and dengue hemorrhagic fever.

The saponins were added in low concentrations to the diets of Nile tilapia. To test whether saponin fractions derived from Fenugreek and the desert date yield similar results as obtained for carp and tilapia by Francis et al. (2001b, 2002a, b), an eight week feeding experiment with Nile tilapia was conducted. Based on previous trials two eluates from fenugreek (one in two concentrations) and a methanol extract from the desert date were chosen and tested for their effects on gene expression of GH, IGF-1 and their receptors, IGF-1 plasma levels, growth performance, oxygen consumption, nutrient utilization and chemical composition.

Material and Methods

Experimental set-up

A total of 20 male Nile tilapia, *O. niloticus*, with a body mass of 19.0 ± 0.5 g (mean ± SD) were divided in two groups. At the start of the experiment five fish were killed by a sharp blow on the head and immediately frozen at -20°C for subsequent analysis of chemical composition. The fish were obtained from the University of Göttingen, Department of Aquaculture and Water Ecology.

The other 15 fish were individually stocked in 12-L chambers of a fully computer controlled respirometric system (Focken *et al.* 1994).

The flow rates were adjusted to 0.3 L min^{-1} and the temperature was kept at 27°C. The light cycle was set to 12/12 light/dark and water quality was analyzed once per week. Once weekly the fish were weighed to the nearest 0.1 g and the feed ration adjusted accordingly. After the individual weighing the fish were kept for 5 to 10 minutes in a bucket with well aerated water while the respective respirometer chamber was cleaned. The feed rations were calculated as four times (14 g $kg^{-0.8}$ day^{-1}) the daily energy maintenance requirement (3.5 g $kg^{-0.8}$ day^{-1}) on metabolic body mass basis.

A standard diet was prepared according to Table 1 which also served as control diet. The different saponin fractions were added to the standard diet in different concentrations (Table 1) resulting in four saponin supplemented diets termed according to the included fraction and its concentration, for example 60TS600 refers to the 60% methanol extracted *Trigonella* saponin eluate or fraction included at 600 ppm while BA stands for *Balanites* saponin. The five diets were randomly assigned to the 15 chambers in triplicates.

At the end of the eight week feeding period all experimental fish were anaesthetized with 200 ppm MS 222, weighed, blood drawn from the caudal vein and killed with a sharp blow to the head. Afterwards, brain, liver and muscle samples were taken and stored on liquid nitrogen for later gene expression analysis while the carcasses were kept at -20°C for later proximate composition analysis. For the chemical analysis the fish were chopped while still frozen, autoclaved for 30 minutes at 120°C, homogenized with an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany), refrozen and freeze dried. Water content was calculated by difference from body mass at slaughter and dry matter mass after freeze drying. Basically the chemical analysis

was conducted according to AOAC (1990) on each individual fish. In brief, dry matter was determined by drying over night to constant mass at 105 °C, ash was determined by ashing over night at 500°C, crude lipid (CL) was determined by a modified Smedes method (Smedes 1999, Schlechtriem *et al*. 2003). Crude protein (CP) was determined using a C/N-analyzer (C/N VarioMAX, Elementar Analysensysteme GmbH, Germany) and N x 6.25 = CP.

Saponins were extracted from fenugreek seeds (*T. foenum-graecum* L.) seeds generally according to Marston and Oleszek (2000). Ethanol extracts were fractionated using a reversed phase HPLC and different consecutive methanol/water solutions (v/v, 40/60, 60/40, 80/20) resulting in three saponin eluates or fractions (40, 60 and 80%) of which the 40% eluate was discarded. An 80% methanol extract of *Balanites aegyptiaca* was produced by grinding 5 g of seeds to a fine powder and mixing with 80% methanol over night. Afterwards the extract was centrifuged (5 minutes at 2400 g) and the supernatant collected and evaporated in a rotary evaporator at 40°C. After another MeOH washing step with 80% MeOH and subsequent centrifugation the extract was washed with 10 ml butanol, centrifuged, butanol phase incubated over night at 6°C and next morning evaporated at 45°C. The material was dissolved in aqua dest. and freeze dried before use.

Calculations

The following parameters were calculated as shown:

Metabolic Body Mass (MBM ($kg^{0.8}$)	(Live body mass (g) / 1000)$^{0.8}$
Metabolic Growth Rate (MGR (g $kg^{-0.8}$ day^{-1})	Live body mass gain (g) / average metabolic live body mass ($kg^{0.8}$) / experimental period (Dabrowski *et al*. 1986)
Specific Growth Rate (SGR (% day^{-1}))	100 x [(ln final mass - ln initial mass) / days of experiment]
Routine Metabolic Rate (RMR)	mean oxygen consumption in 24 h (mg) / metabolic body mass ($kg^{0.8}$) x 24
Energy Expenditure (EE (kJ))	Oxygen uptake (g) x 14.86 (kJ g^{-1} O_2, Huisman 1976)
Energy Retention (ER (kJ))	Final gross energy (kJ) of fish – initial gross energy (kJ) of fish
Metabolizable Energy (ME), (kJ)	ER (kJ) + EE (kJ)
EE (% of GE fed)	EE (kJ) x 100 / Feed energy intake (kJ)
ER (% of GE fed)	ER (kJ) x 100 / Feed energy intake (kJ)
ME (% of GE fed)	ER (kJ) + EE (kJ) x 100 / Feed energy intake (kJ)
AUE (% of GE fed)	100 - EE (%) - ER(%)

O_2 consumption (g) / protein gain (g)	Total oxygen consumption (g) / total protein gain (g)
EE (kJ) / protein gain (g)	Total EE (kJ) / total protein gain (g)
Protein Efficiency Ratio (PER)	Live body mass gain (g) / feed protein intake (g)
Protein Productive Value (PPV (%))	Total protein gain (g) x 100 / total protein fed (g)
Apparent lipid conversion (%)	Total lipid gain (g) x 100 / total lipid fed (g)
Feed Conversion Ratio (FCR)	Feed consumption (dry matter) / live body mass gain (g)

Radio-Immuno-Assay

Blood was drawn from each fish with a heparinized 1 ml syringe from the caudal vein after anaesthetizing the fish with 200 ppm MS 222. The blood was centrifuged at 4°C and 2,500 g for 5 minutes and the plasma was frozen at -20°C. For the determination of the plasma levels of IGF-1 a fish IGF-1 RIA kit from GroPep [including Anti Barramundi IGF-1 Polyclonal Antiserum (Rabbit) and Recombinant Barramundi IGF-1 (*Lates calcarifer*)] (catalogue nos. PAF1 and YU100 respectively) was used following the basic methodology of Claus and Weiler (1996) with some minor changes.

Isolation of total RNA

Extraction of total RNA from brain (including pituitary), liver and muscle tissues of Nile tilapia was carried out using TRIzol® Reagent (cat#15596-026, Invitrogen, Germany) according to the manufacturer's instructions with minor modifications. Tissue samples were homogenized in 1 ml of TRIzol® Reagent per 50 mg of the tissue. RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water.

Total RNA was treated with 1 unit of RQ1 RNAse-free DNAse I (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water and quantified photospectrometrically at 260 nm. Purity of total RNA was assessed by the 260/280 nm ratio which was between 1.8 and 2.1. Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for reverse transcription (RT) otherwise they were stored at -80°C.

Reverse transcription (RT) reaction

The complete Poly(A)$^+$ RNA isolated from Nile tilapia tissues was reverse transcribed into cDNA with a total volume of 20 µl using RevertAid^{TM} First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) according to manufacturers instructions. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with a denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until subjected to DNA amplification using the quantitative Real Time polymerase chain reaction (RT-qPCR).

Quantitative Real Time-Polymerase Chain Reaction (RT-qPCR)

An iQ5-BIO-RAD Cycler (Hercules, CA, USA) was used to determine the Nile tilapia cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense and antisense primers, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated

to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 50 cycles in which each cycle divided to 3 sub-steps: (a) at 95.0°C for 15 sec; (b) at 60°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each RT-qPCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each analysis included a non template control.

The sequences of specific primers of the genes used and sequence references are listed in Table 2. The quantitative values of RT-qPCR of GH, IGF-1 and their receptor genes were normalized to the bases of ·-actin gene expression.

Statistical Analysis

All data was analyzed using SPSS version 10.0 (IBM SPSS, Chicago, IL, USA). All data is presented as mean ± SEM if not stated otherwise. To test for homogeneity of variance a Levene test was applied while the test for normal distribution was conducted with a Kolmogorov-Smirnov test. To test for significant differences between the groups all data was subjected to an ANOVA with a subsequent Scheffé post-hoc test. Pearson's correlation coefficient was used to check for correlations among parameters. Statistical significance level was $p < 0.05$.

Results

Observations, growth performance, oxygen consumption and nutrient utilization

All fish accepted the respective diets and ate the provided feed during the first two minutes. No abnormal behavior or signs of stress were observed.

Over the experimental period all groups gained similarly in protein, lipids and subsequently in energy (Table 3). Fish fed with either control or 60TS300 feed showed a numerically higher apparent lipid conversion compared to the other saponin fed groups (Table 4).

Fish fed with 60TS300 grew numerically best in terms of body mass gain and final body mass compared to all other groups. The other saponin fed groups showed the lowest growth response while the control fed fish grew close to the 60TS300 group (Table 4).

The same results can be observed in all measured and calculated growth performance and nutrient utilization parameters. Fish fed with 60TS300 always showed better or equal numerical values compared to the control group while the 60TS600 group exhibited the lowest performance. Strong positive correlations were found between feed utilization (FCR, PER and PPV) and growth performance (MGR, SGR, FBM and BMG) ($p < 0.01$).

Somewhat different to the growth and nutrient utilization performances was the oxygen consumption. It was highest for fish fed with 600 mg kg^{-1} of the 60% *Trigonella* fraction fed fish while it was lowest in fish fed with only 300 mg kg^{-1} of the same fraction. It can be seen from Fig. 1 that the oxygen consumption in the 60TS600 treatment increases from week 4 onwards together with a dramatically increasing standard error of mean. This is caused by a dramatically increasing oxygen consumption of one replicate in the respective treatment. The control and the other two saponin fed groups had an oxygen consumption between those two groups (Fig. 1). The oxygen consumption per gram of protein gain was lowest for fish fed with 60TS300 followed by control fed fish, while it was highest for fish fed with 60TS600 (Table 5). Logically, a similar result is gained for the heat dissipation (energy expenditure) since it is calculated from the oxygen consumption (Huisman 1976). Negative correlations were found for the energy expenditure per unit of protein gain and growth performance (MGR, SGR, FBM and BMG) showing that lower oxygen consumptions resulted in higher growth performance ($p < 0.05$).

Gene expression and IGF-1 plasma level

Expression of GH in brain and pituitary was highest for fish fed 60TS300 followed by control while the other saponin fed groups showed a significantly reduced expression of GH. A similar result was obtained for the expression of GHR-2 expression in brain and pituitary but not in liver and muscle tissue. IGF-1 expression was significantly lower for all saponin treated groups compared to control except 60TS300 which was numerically even higher than control. No differences were found between groups and tissues in expression of GHR-1, IGF-1 R_a and IGF-1 R_b. and between plasma levels of IGF-1. Expression levels of GH did strongly correlate to growth related parameters like BMG ($r = 0.99$, $p < 0.01$), MGR ($r = 0.99$, $p < 0.01$), SGR ($r = 0.99$, $p < 0.001$), nutrient utilization parameters like FCR ($r = -0.98$, $p < 0.005$), PER ($r = 0.98$, $p < 0.005$), PPV ($r = 0.97$, $p < 0.01$) and ER ($r = 0.96$, $p < 0.01$). Similar correlations but not as strongly pronounced were found between expression of IGF-1 and performance related parameters (see Table 6).

Discussion

To our knowledge, this is the first experiment where a traditional growth trial has been combined with proximate composition analysis, respirometry and gene expression to evaluate the effects of supplementation with saponin fractions in Nile tilapia. Furthermore this is the first time that saponin extracts have been fractionated to be tested as possible natural growth promoters.

While the observed differences between growth, oxygen consumption and proximate composition between control and treatments were not statistically significant due to low number of replicates (which is limited by the number of boxes of the respirometer system), the expression of GH, GHR-2 and IGF-1 showed significant differences between treatments. The expression of GH in the pituitary and brain and the expression of IGF-1 in the liver were significantly reduced for all saponin treatments except 60TS300 which had numerically even higher expressions of these genes than the control. In our experiment the expressions of GH and IGF-1 genes directly reflected the overall performance including the growth rates and the various evaluated feed utilization and metabolic parameters of the respective treatments. All results point towards a performance depression in all saponin supplemented treatments except the 300 ppm supplementation with the 60% methanol fractionated saponin eluated from *T. foenum-graecum* seeds. High expression levels of GH and IGF-1 genes resulted in numerically highest growth rates and best nutrient utilization while significantly reduced gene expressions of GH and IGF-1 resulted in numerically lowest growth and inferior nutrient utilization.

Generally an over-expression of GH in transgenic fish results in significantly higher growth rates as reported for coho salmon (Devlin *et al*. 2004), Atlantic salmon (Du *et al*. 1992), Arctic charr (Pitkänen *et al*. 1999), rohu (Venugopal *et al*. 2004), common carp (Hinits and Moav 1999), channel catfish (Dunham *et al*. 1999) and Nile tilapia (Rahman and Maclean 1999).

In an *in vitro* study it has been shown that saponins extracted from Fenugreek significantly stimulated GH release in rat pituitary cells. The most potent substance proved to be a crude methanol extract of *T. foenum-graecum* (~ 22-fold higher GH release compared to control) while dioscin (~18-fold) and fenugreek saponin I (~13-fold) where also highly potent (Shim *et al*. 2008). Although no direct growth promotion was observed in GH transgenic tilapia, a significantly improved feed utilization was reported by Martínez *et al*. (2000). The differences between the different treatments compared to control, although we did not measure GH release, were far less pronounced as the above mentioned results. Feeding common carp with 150 mg per kg diet for eight weeks with crude *Quillaja saponaria* saponins resulted in significantly increased final body mass and improved oxygen consumption while a similar effect was yielded in tilapia when the saponin inclusion level was raised to 300 mg kg^{-1} diet (Francis *et al*. 2001b, 2002 a, b). The two main differences between those experiments and our experiment where the nature of the saponins on one side and the degree of saponin fractionation on the other side. *Q.*

saponaria saponins are of triterpenoid nature (Guo & Kenne 2000) while *T. foenum-graecum* (Marker *et al*. 1947, Murakami *et al*. 2000) and *B. aegyptiaca* (Marker *et al*. 1947, Dawidar and Fayez 1969) saponins are of steroidal nature. The goal of this experiment was to test if saponins derived from two common and widespread Middle Eastern plants can be used as environmentally friendly growth promoters. To do so a fractionation was conducted with the future goal in mind to identify and purify a single compound responsible for a growth promoting effect as experienced in the experiments of Francis and his colleagues. However, evidence exists that the biological activity of saponins is not the consequence of one single biologically active saponin or sapogenin which can be extracted and purified but rather that saponin mixtures exhibit the highest biological activity. As mentioned above, the highest release of GH in rat pituitary cells was measured after stimulation with crude methanol extract derived from fenugreek (Shim *et al*. 2008) and Kamel *et al*. (1991) showed that single saponins derived from *B. aegyptiaca* showed no anti-diabetic activity while different mixtures of single extracted saponins showed significant anti-diabetic activity. Also Francis *et al*. (2001b, 2002a, b) used crude *Quillaja* saponin mixtures yielding far more pronounced effects than we observed in our experiment. Nevertheless we found a tendency that certain saponin fractions were less detrimental for growth and nutrient utilization (60% methanol fraction) while another fraction of the same plant seemed to exhibit stronger anti-nutritional activity (80% methanol fraction) when applied in the same concentration of the diet. Furthermore the concentration of the saponin matters since 600 ppm of the 60% MeOH extract from Fenugreek in the diet yielded similar bad results as 300 ppm of the 80% MeOH extract from Fenugreek. The variance in the oxygen consumption experienced in the 600 ppm treatment of the 60% Fenugreek methanol fraction is starting to increase from the 4^{th} week onwards. The cause is a strongly increasing oxygen consumption of a single fish while the other two fish's metabolic rates only increase slightly which could be explained by increasing body mass. That might point towards a rising inability of the fish's metabolism to cope with higher amounts of this specific saponin fraction.

Despite a potential application of saponins as growth promoter in aquaculture they might also be used to influence the sex ratio *in vivo*. This is especially interesting for tilapia since the commercial tilapia production depends on male monosex cultures which are at the moment mainly produced by application of a synthetic androgen, 17-▪-methyletestosterone.

Commercial extracts of *Tribulus terrestris*, known as Gokshura, had a significant effect on sex ratio and in the highest dose also on growth of the African catfish, *Clarias gariepinus* (Turan and Çek 2007). Furthermore, the same extract was able to increase the sex ratio of male convict cichlids (*Cichlasoma nigrofasciatum*) (Çek *et al*. 2007).

Gokshura is reported to be rich in steroidal saponins but with varying compositions depending on origin (Dinchev *et al*. 2008).

Up to now only very little attention has been paid to effects and potential of saponins as feed additives in aquaculture feeds. While saponins are in use in terrestrial livestock feeds for example to control ammonia and odor (Cheeke 1999) they are generally still considered anti-nutrients for aquatic animals.

We conclude that the saponin fractions derived from Fenugreek and the desert date in the applied concentrations are no possible alternative for prohibited antibiotics as growth promoters in Nile tilapia production. However, a certain potential as growth promoter might be found in the 60% MeOH saponin fraction from Fenugreek or in crude saponin extracts and that the effects of the saponins are likely to be concentration depending.

More studies are needed to test a possible optimum dietary concentration of that saponin fraction (60TS). Furthermore, the decreased performance of other saponin treated fish, including

the higher concentration of 60TS, points toward anti-nutritional effects. The combined results are in good coherence since high expression levels of GH and IGF-1 genes positively correlated with parameters related to growth, nutrient digestion and metabolic efficiency.

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Figures and Tables

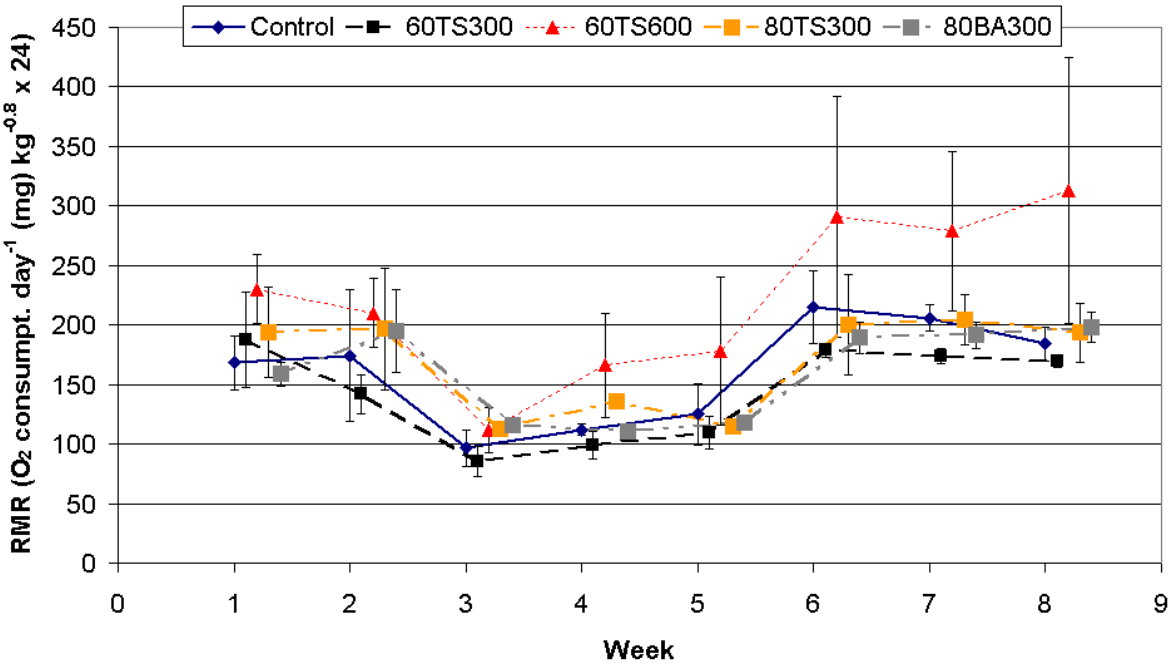
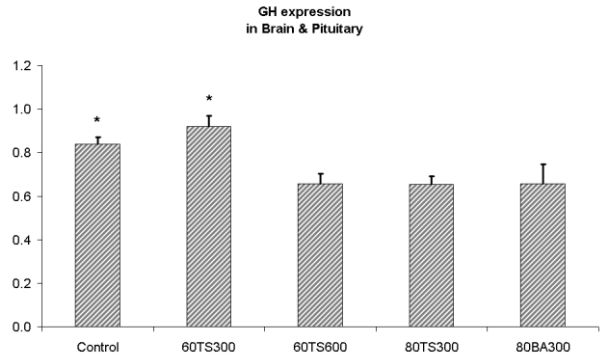


Figure 1: Average weekly routine metabolic rate over the experimental period. Values are presented as mean ± SEM, n = 3



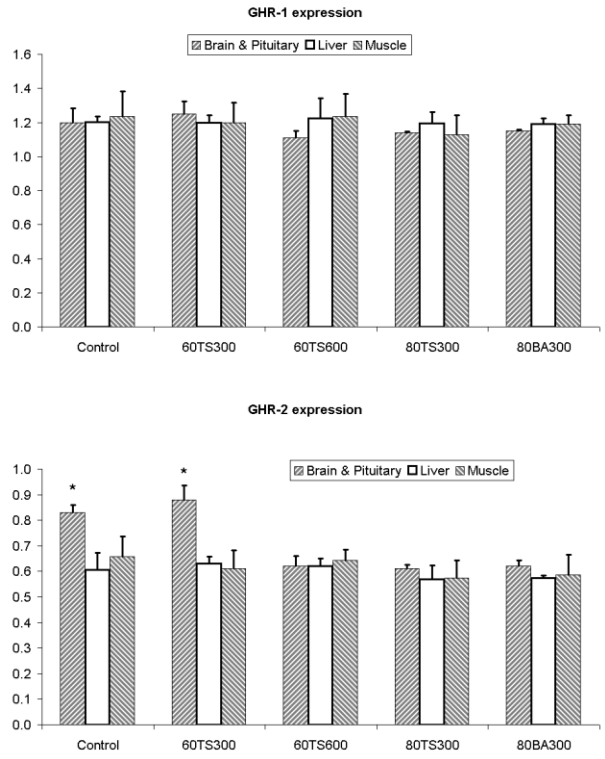
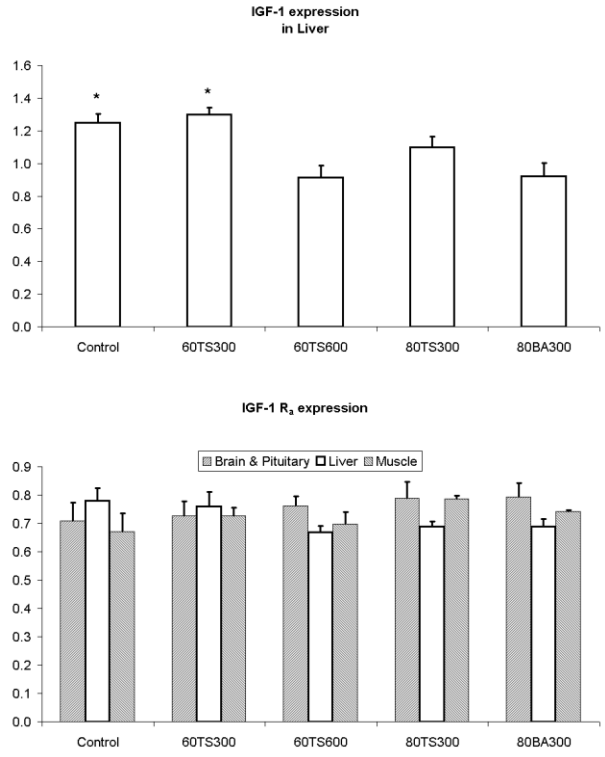


Figure 2: Expression of GH in brain and pituitary and GH receptors 1 and 2 in brain & pituitary, liver and muscle, respectively. Values are presented as mean ± SD, n = 3, *: $p < 0.01$



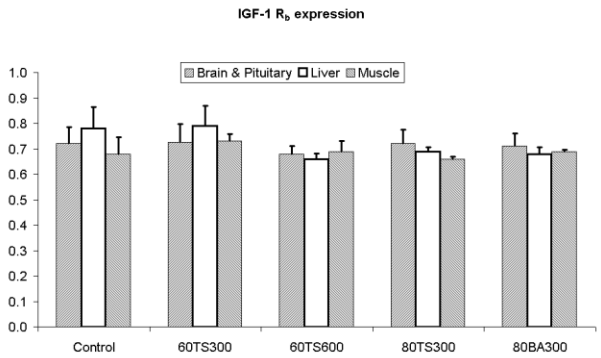


Figure 3: Gene expression of IGF-1 in liver and IGF-1 receptors a and b in brain & pituitary, liver and muscle, respectively. Values are presented as mean ± SD, n = 3, *: $p < 0.01$

Table 1: Ingredients of the basal and experimental diets and chemical composition of the standard diet

	Diet				
Ingredient	Control	60TS300	60TS600	80TS300	80BA300
Trigonella 60% Fraction (mg kg^{-1})		300	600		
Trigonella 80% Fraction (mg kg^{-1})				300	
Balanites 80% Fraction (mg kg^{-1})					300
Fish meal[a] (g kg^{-1})	500	500	500	500	500
Whole wheat meal (g kg^{-1})	420	420	420	420	420
Sunflower oil (g kg^{-1})	40	40	40	40	40
Vitamin premix[b] (g kg^{-1})	20	20	20	20	20
Mineral premix[b] (g kg^{-1})	20	20	20	20	20
Standard diet	DM (%)	CA (g kg^{-1} DM)	CP (g kg^{-1} DM)	CL (g kg^{-1} DM)	GE (kJ g^{-1} DM)
	93.2	11.9	41.9	12.6	20.8

DM = dry matter, CA = crude ash, CP = crude protein, CL = crude lipids, GE = gross energy

[a]Norwegian fish meal obtained from Wuerttembergische Zentralgenossenschaft, Germany.
[b]Prepared after Gaye-Siessegger *et al.* (2004).

Table 2. Primer sequences used for RT-qPCR

Gene	Primer sequence (5′–3′)[a]	Sequence references
GH	F: GAA CTG ATG CCA GCC ATG A R: AGC TAC AGA GTG CAG TTT G	Ber and Daniel (1992)
GHR-1	F: CCA TCA GAT GAG CAA CTT CTG AAA AGT R: ACT TCC TGG TGA ATC AGC CTT A	Jiao *et al.* (2006)
GHR-2	F: CAC AGA CTT CTA CGC TCA GGT CA R: TGA GTT GCT GTC CAG GAG ACA	Kajimura *et al.* (2004)
IGF-1	F: GTC TGT GGA GAG CGA GGC TTT R: AAC CTT GGG TGC TCT TGG CAT G	Schmid *et al.* (2003)
IGF-1 R_a	F: CTAAGGGCGTGGTTAAGCAC R: TTGTTGGCGTTGAGGTATGC	Greene and Chen (1999)
IGF-1 R_b	F: AGG GAC GAG CCA GAG ACG R:TTC AGA GGA GGG AGG TTG	Greene and Chen (1999)
β-actin	F: GTG ATG TGA CGC TGG ACC AAT C R: CCA TGT CAT CCC AGT TGG TCA CAA T	Hwang *et al.* (2003)

[a] F: forward primer; R: reverse primer.

Table 3: Initial and final proximate chemical analysis of control and experimental fish on fresh matter basis.

	Initial group	Control	60TS300	60TS600	80TS300	80BA300
Crude protein (%)	11.8 ± 0.17	15.2 ± 0.50	15.1 ± 0.15	15.3 ± 0.21	14.8 ± 0.23	15.3 ± 0.10
Crude lipids (%)	3.3 ± 0.10	6.9 ± 0.28	6.8 ± 0.18	6.1 ± 0.36	6.3 ± 0.28	6.2 ± 0.62
Crude ash (%)	4.0 ± 0.12	4.5 ± 0.14	4.4 ± 0.10	4.4 ± 0.07	4.4 ± 0.11	4.4 ± 0.11
Dry matter (%)	22.9 ± 0.19	27.4 ± 1.06	27.1 ± 0.12	26.1 ± 0.23	25.9 ± 0.37	26.1 ± 0.80
Gross energy (kJ g^{-1})	4.51 ± 0.12	6.08 ± 0.35	5.94 ± 0.12	5.62 ± 0.14	5.63 ± 0.20	5.67 ± 0.24

values are expressed as mean ± SEM, n = 3

Table 4: Growth performance, nutrient utilization and IGF-1 plasma levels of tilapia.

	Control	60TS300	60TS600	80TS300	80BA300
Initial body mass (g)	19.2 ± 0.34	18.9 ± 0.32	18.7 ± 0.26	18.9 ± 0.41	19.3 ± 0.06
Final body mass (g)	52.0 ± 6.25	54.0 ± 6.21	44.3 ± 2.17	45.8 ± 4.93	47.0 ± 3.04
Body mass gain (g)	32.8 ± 6.03	35.1 ± 6.38	25.6 ± 2.41	27.0 ± 5.09	27.2 ± 2.98
Growth (%)	270 ± 29.3	286 ± 35.0	238 ± 14.8	244 ± 27.9	243 ± 15.0
SGR (% day^{-1})	1.75 ± 0.19	1.85 ± 0.23	1.54 ± 0.11	1.56 ± 0.22	1.58 ± 0.11
MGR (g $kg^{-0.8}$ day^{-1})	6.47 ± 0.81	6.86 ± 0.98	5.52 ± 0.44	5.66 ± 0.88	5.74 ± 0.46
Feed conversion ratio	1.13 ± 0.13	1.12 ± 0.17	1.33 ± 0.09	1.35 ± 0.22	1.31 ± 0.10
Protein efficiency ratio	2.17 ± 0.29	2.21 ± 0.29	1.81 ± 0.13	1.85 ± 0.27	1.85 ± 0.14
Protein productive value (%)	34.4 ± 3.33	34.7 ± 4.29	29.5 ± 1.31	28.6 ± 3.16	30.0 ± 1.98
Apparent lipid convers. (%)	62.5 ± 7.04	62.1 ± 4.66	46.3 ± 5.43	48.5 ± 2.13	47.8 ± 5.18
Feed intake (g DM)	35.6 ± 1.76	37.3 ± 2.20	33.6 ± 0.92	34.3 ± 1.95	35.5 ± 1.18
IGF-1 plasma level (ng ml^{-1})	23.8 ± 2.68	23.1 ± 3.63	22.5 ± 3.46	25.3 ± 2.29	23.5 ± 5.65

Values are expressed as mean ± SEM, n = 3

Table 5: Energy balance for all five groups.

	Control	60TS300	60TS600	80TS300	80BA300
Initial fish GE (kJ)	86.8 ± 1.55	85.4 ± 1.43	84.3 ± 1.15	85.1 ± 1.85	87.0 ± 0.29
Final fish GE (kJ)	314 ± 32.4	321 ± 38.3	249 ± 16.1	256 ± 18.9	265 ± 11.5
Ingested feed GE (kJ)	739 ± 36.6	775 ± 45.6	698 ± 19.1	711 ± 40.5	738 ± 24.4
ER (kJ)	227 ± 32.0	235 ± 38.8	165 ± 17.3	171 ± 19.7	178 ± 11.4
EE (kJ)	201 ± 32.7	186 ± 20.7	258 ± 59.2	198 ± 12.7	197 ± 4.35
ME (kJ)	429 ± 64.7	422 ± 54.7	423 ± 45.9	369 ± 15.5	376 ± 14.6
AUE (kJ)	310 ± 30.7	353 ± 9.25	275 ± 64.9	342 ± 35.7	362 ± 15.7
ER (% of GE fed)	30.5 ± 2.85	30.0 ± 3.45	23.6 ± 2.04	23.9 ± 1.66	24.2 ± 1.23
EE (% of GE fed)	27.0 ± 3.14	23.9 ± 1.63	37.5 ± 9.68	28.2 ± 2.14	26.8 ± 0.29
ME (% of GE fed)	57.5 ± 5.99	53.9 ± 4.11	61.1 ± 8.46	52.1 ± 2.57	50.9 ± 1.24
AUE (% of GE fed)	42.5 ± 5.99	46.1 ± 4.11	38.9 ± 8.46	47.9 ± 2.57	49.1 ± 1.24
Cons. O_2 (g) / protein gain (g)	2.61 ± 0.13	2.36 ± 0.26	4.34 ± 1.30	3.45 ± 0.74	3.00 ± 0.23
EE (kJ) / protein gain (g)	38.7 ± 1.93	35.0 ± 3.87	64.5 ± 19.4	51.2 ± 11.0	44.6 ± 3.48

Gross Energy (GE), Energy Retention (ER), Energy Expenditure (EE), Apparently Unutilized Energy (AUE), Metabolizable Energy (ME) and Oxygen Consumption and Energy Expenditure per gram Protein gain. Values are expressed as mean ± SEM, n = 3