Faculdade de Ciências do Mar e do Ambiente
Masters in Marine Biology
Genetics and Selection

Genetic selection program for improvement of salinity tolerance in a tilapiine fish, *Oreochromis niloticus*

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1. Biology of *Oreochromis niloticus*

Tilapia is a generic term used to designate a group of commercially important food fish belonging to the family Cichlidae. Cichlid fishes have received wide attention from evolutionary biologists for more than 100 years because of their extremely diverse morphology, behavior, and ecology) (Klett & Meyer, 2002). This perciform fish family is one of the most species-rich families of vertebrates with at least 1,300 and perhaps as many as 1,870 species. Tilapias exhibit a wide spectrum of ecological adaptations and tolerances (pH, temperature, salinity).

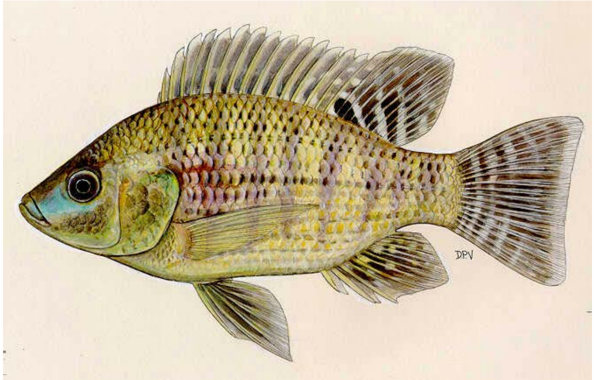

Fig.1 – *Oreochromis niloticus*.

Positive characteristics of tilapiine fish are their high tolerance to poor water quality and that they eat a wide variety of living organisms (Popma & Masser, 1999). Of them the most important cultured fish species is *Oreochromis niloticus* L., also known as the Nile tilapia, and its hybrids (Charo-Karisa, H., 2006).

The Nile tilapia is predominantly herbivorous (Bwanika *et al.*, 2004), with young fry being omnivorous. Phytoplankton becomes progressively more important as the fish grow. Breeding occurs throughout the year in equatorial waters, but peak usually coincides with rain season. They're maternal mouthbrooders, like all fishes in the *Oreochromis* genus. The males construct nests in firm sand at 0,6 - 2 m. Fecundity varies with size: 340 eggs in female of 17 cm TL; and 3706 eggs in female of 57 cm TL. It hybridises with a number of other cichlid species and is used in polyculture with a number of other species. Adults require 28% protein in diet, but maximum growth is obtained at 34% protein. The optimal temperature for growth of most tilapiine species is between 25-28°C. Reproduction stops at 22°C and feeding below 20°C. Tilapia cannot survive temperatures less than 10-12°C for more than a few days (Charo-Karisa *et al.*, 2005). Growth rates are influenced by a variety of factors; water temperature, sex (males grow faster than females), supplemental feeding and stocking density.

2. Culture techniques currently in use for the species

Because of the diversity of culture systems it is impractical to describe all the different operations used for raising tilapia commercially. One of the major problems in tilapia culture is the tendency of females to mature and reproduce at small sizes. This reproduction pattern results in stunted populations because of energy being diverted for gonadal development instead of somatic growth (Pechsiri & Yakupitiyage, 2005). Thus, in order to improve productivity, all male populations are desirable (Tessema *et al.*, 2006; Pechsiri & Yakupitiyage, 2005).

Culture practices of tilapias worldwide are diverse, being probably the most diverse among all aquaculture species and they can suit economic status. *O. niloticus* is commonly cultured in relatively poor quality waters, including sewage fed ponds and primary and secondary treated waste effluents. So far, there have not been any reports of detrimental effects of consumption of fish reared in sewage fed farms on human health even as the practice has been in operation since the 1930s. In integrated-fish culture it was done integration of commercial poultry farming with *O. niloticus* culture, in rice-fish culture. Similarly, the potential of using *O. niloticus* in biogas slurry has been demonstrated with a view to integrating biogas technology in food production (De Silva *et al.*, 2004). Tilapia pond culture practices are, in almost all cases, conducted in static systems, which require water replenishment between grow-out cycles and aeration is rarely used. Cage culture of tilapias is also relatively popular, including sea cage culture.

2.1. Selective breeding of Nile tilapia

A number of selection experiments and breeding programs aiming at increased growth rates in ponds have been conducted for *O. niloticus.* These selective breeding programmes have typically been done in favourable environments where growth is high. In these environments, fish receive high protein supplementary feeds formulated commercially from fish meal and oil. In the presence of genotype by environment interaction (GXE), a strain that has been genetically improved through selection in a favourable environment may not be necessarily good for less favourable conditions (Charo-Karisa, 2006). Large GXE necessitates the development of strains highly adapted to specific environments, and a small interaction permits the development of strains that will perform well in a broad spectrum of environments. Little is known about the degree of GXE in tilapia. Among the techniques used for selection of Nile tilapia are the classical selection methods based on phenotypes, cross-breeding, hybridization, sex manipulation, while others are still being experimented, like gynogenesis, gene transfer (transgenics) and marker-assisted selection (MAS) based on quantitative trait loci (QTLs) (Hulata, 2001).

3. Relevance of *O. niloticus* farming

Tilapias are among the most cultivated fish worldwide. They are second only to Carp which account for 75% of total global aquaculture (Lim & Webster, 2006; De Silva *et al.*, 2004). Tilapias particularly of the genus *Oreochromis* spp. have become one of the most important species in global aquaculture. Native to Africa, several species of tilapia have been introduced to tropical areas of Asia and the Americas to increase supplies of animal protein. World aquaculture production of tilapia now exceeds 1.5 million tons per year (Katagiri, *et al.*, 2005; De Silva *et al.*, 2004).

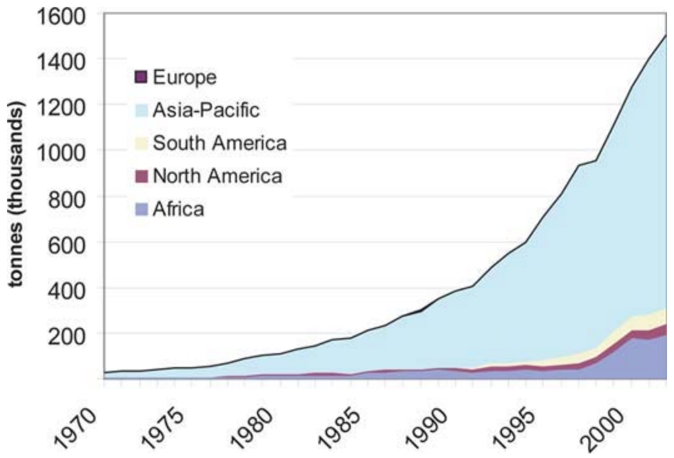

Fig. 2 – Aquaculture production of tilapias worldwide (Source: FAO).

Tilapias have been successful as a cultured species group in the tropical countries due to: ease of culture (and thus relatively less limited by the economic status of the farmer), relevant species exhibiting many of the desirable traits expected of a species suitable for culture, like high growth rate, wide range of tolerance to physicochemical characteristics (Pechsiri & Yakupitiyage, 2005), exhibiting ecological adaptations and high tolerances (Klett & Meyer, 2002; Hassanien & Gilbey, 2005). They are also disease resistant, of easy propagation, having long shelf-life and lending itself to industrial preparations better than most other white fish (Pechsiri & Yakupitiyage, 2005). In addition, most of the commonly cultured tilapias are easily weaned on to artificial feeds. *O. niloticus* is the most cultured species of them, becoming the preferred species for culture in view of its faster growth rate, attractive coloration and supposedly better taste (De Silva *et al.*, 2004).

4. Traits of commercial importance for genetic improvement

Of special interest in the improvement of *O. niloticus* are growth rates, fillet yield (Rutten *et al.*, 2004), the red colouration which leads to better reception by the public, feed conversion and disease resistance. Also of interest are cold temperatures and salinity tolerance. Of all these attention in *O. niloticus* has been focused on growth. Thus, knowledge on the improvement of these other traits is limited. Fillet yield based on body measurements is possible, but correlations can be improved if more accurate methods for measuring body width become available (Rutten *et al.*, 2004).

The Nile tilapia dominates freshwater tilapia culture and has been the subject of recent efforts in genetic improvement but is one of the least salt tolerant tilapia (Kamal & Mair, 2005). Moreover, the inability of tilapia to tolerate low temperatures is of major economic concern as it reduces their growing season and leads to over winter mortality (Charo-Karisa *et al.*, 2005).

The first trait that comes to mind when thinking of fish improvement is growth, in which case mass selection can be implemented easily as you can measure directly the fish. However not all traits have accurate measurement techniques and these could be satisfied with the application of marker assisted selection through QTLs and microsatellites. This kind of techniques is only limited by the lack of high resolution genetic maps for QTLs and markers for the majority of aquaculture species (Sonesson, 2003).

5. Genetics of species

Despite the importance of Nile tilapia as a food fish worldwide, knowledge on the genetic background of natural populations is not very extensive (Hassanien & Gilbey, 2005). No heritability estimates are available for salinity tolerance. Heritability estimates for BW range from 0.38 to 0.60 and the heritability for survival ranged from 0.03 to 0.14 according to Charo-Karisa *et al.*, 2006. Salt tolerance heritability could be estimated by measuring the degree of resemblance between relatives among successive generations, accounting for the quantity of individuals that actually get the desired trait, but it’s necessary to know which individuals to choose and it’s not practical to test fish in different salinities in order to select the best for breeding schemes. This is one trait where MAS can help selection for the trait.

6. Improvement program for salinity tolerance

For indirectly measurable traits such as salinity tolerance, marker-assisted selection proves to be a more efficient mean of selection of the right individuals for breeding programs. A number of simulation studies have compared classical selection programs to those incorporating some form of marker information and phenotypic data. Strategies employing marker information and phenotypic information were always superior to phenotypic selection alone in early generations (Davis & DeNise, 1998). Genomic research and gene mapping of aquatic organisms developed fast during the late 1990s. Novel genetic technologies involving the use of DNA-based tools are under development for a range of aquaculture species and applications, including species/strain identification and pedigree tracing (Hulata, 2001). What is still needed for this technique to be more precise is constructing genetic and physical maps of the tilapia genome (or any other fish to be improved).

How to find these QTLs for salinity tolerance? A genome scan analysis is required. This can be done in a two-step study. First, a genome scan is conducted in an F2 (2^{nd} generation) family that had previously been characterized for segregation for salinity response traits, in order to identify putative QTLs (Cnaani *et al.*, 2004). For this DNA polymorphisms are used as linked or direct markers to detect QTL segregating in particular populations with specific allele frequencies (Davis & DeNise, 1998). The second step involves confirmation of those QTLs by re-analysis of the same markers in another interspecific F2 family, where DNA polymorphisms are used as linked or direct markers to detect QTL segregating in that particular population with specific allele frequencies (Cnaani *et al.*, 2004; Meuwissen, 2003; Davis & DeNise, 1998). Then there is the implementation phase, where linked markers shown to be predictive in a population are used within families and direct markers are used across families to produce a database of genotypes. These data are combined with phenotypic and pedigree information in genetic evaluation for the prediction of genetic merit of individuals within the population (Davis & DeNise, 1998). Salinity responses are thus measured on the fish and designated to each QTL and then the best responses are identified and individuals from different families with the best QTLs are selected for breeding (Cnaani *et al.*, 2004).

For selecting the best individuals for salt tolerance tests could be made with 60 000 fry in brackish-water conditions. Once you know the QTLs involved in this specific trait you can select the individuals better suited for the breeding schemes. GenoMar Supreme Philippines Inc.'s (http://www.genomar.no) already found QTLs for salt tolerance.

After determination of best individuals for breeding schemes by DNA markers, it's time for the production process. There are some available improved strains of tilapia, like GenoMar Supreme Tilapia, part of the GIFT program (Genetic Improvement of Farmed Tilapia). They obtained faster growth in the early life stage and have increased the genetic gain per year with more than 35 % compared to conventional breeding schemes. They produce a new generation every 9 month and the annual genetic gain is more than 15 % for growth rate.

All-male populations should then be created, produced through a breeding scheme involving a series of feminization and progeny testing (Romana-Eguia *et al.*, 2005) for higher growths and population control. GMT or genetically male tilapia as they are called, avoid the creation of high density stocks in the tanks and provide higher growth rates.

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