Aquaculture 430 (2014) 159–165

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

# Response to selection for growth in an interspecific hybrid between *Oreochromis mossambicus* and *O. niloticus* in two distinct environments

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ARTICLE INFO

*Article history:*
Received 29 November 2013
Received in revised form 31 March 2014
Accepted 31 March 2014
Available online 12 April 2014

*Keywords:*
Salinity tolerance
Selection
Hybridization
Genetic gain
Heritability
Survival rate

ABSTRACT

The development of a saline tolerant tilapia strain able to grow fast is of importance in the Philippines, where 240,000 ha of brackish water ponds are available. To this end, founder hybridization between *Oreochromis niloticus* (with favorable growth traits) and *Oreochromis mossambicus* (with favorable salinity tolerance traits) was performed and followed by backcrossing with *O. mossambicus* to develop a strain highly tolerant to saline environments. Genetic selection for growth performance was subsequently conducted. The aim of the present study was to estimate growth performance and survival of the hybrid in two rearing environments following four generations of genetic selection.

A comparison between the hybrid and an internal reference line was performed in two distinct environments: one intensive (tanks: feed and high stocking density) and one extensive (fertilized earthen ponds: without feed and low stocking density) system. The selection experiment consisted of a within group or within family selection with a random mating system. The selection criterion was body weight at five months, and salinity tolerance was passively selected by rearing fish in brackish water.

After four generations of selection, the average body weight of the hybrid had increased by 50 g compared to the red tilapia internal reference line, which corresponds to a gain of 12.5 g or 7.3% per generation. Average weight in the intensive system was greater than in the extensive system (65.8 and 38.7% at the last generation in male and female, respectively). Realized heritability of body weight was only significant in the intensive system ($0.19 \pm 0.07$ vs. $0.17 \pm 0.06$ in the extensive system).

This selection scheme was performed in brackish water, allowing a passive selection on salinity tolerance, as shown by the improvement of the survival rate according to the generation in intensive and extensive systems.


## 1. Introduction

In 2010, the Philippines was the 10th largest aquaculture producer of fish, crustaceans and mollusks (FAO, 2012) with a total of around 745,000 Mt. With a production estimated at 260,000 Mt, tilapia is, along with milkfish (*Chanos chanos*), one of the most important fish commodities in the country. Tilapia production in brackish water ponds accounts for 5.4% of national tilapia production. In Central Luzon, one of the most important aquaculture areas of the country, tilapia production in brackish water ponds is currently increasing and contributes to 7% of the total production in a farming environment where it didn't exist at all only 10 years ago.

In the Philippines, brackish water ponds used as fish farming systems cover some 240,000 ha across coastal tidal areas. These areas produce mostly milkfish and shrimps; however, tilapia production is now gaining significance as a brackish water aquaculture species.

Farmers produce tilapia in three distinct brackish water farming systems: i) in association with intensive shrimp farming to contribute in part to the production of green water in reservoirs supplying good quality water to the shrimp ponds; in this case, tilapia is a by-product of shrimp production with little direct impact on profit (Danaher et al., 2007; Kamal and Mair, 2005), ii) extensive polyculture with shrimp: farmers use tilapia to clean ponds of macrophytes such as *Hydrilla verticillata* and iii) intensive monoculture in large brackish water ponds where the salinity is below 20‰.

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http://dx.doi.org/10.1016/j.aquaculture.2014.03.051
0044-8486/

Generally, tilapias can endure a wide range of salinities (El-Sayed, 2006; Kamal and Mair, 2005; Watanabe et al., 1985) with a large variability according to the species. As an example, the optimum salinity for *Oreochromis mossambicus* was shown to be around 32 ppt whereas for *Oreochromis niloticus* it was lower (ranging from 0 to 10 ppt; Villegas, 1990). For upper salinity tolerance, *O. mossambicus* endure 27 ppt in direct transfer and 120 ppt by gradual transfer whereas *O. niloticus* can only endure 18 ppt and 36 ppt in direct and gradual transfer, respectively (Al-Amoudi, 1987; Whitefield and Blaber, 1979). These two tilapia species are found in the Philippines, but neither of them have both salinity tolerance and fast-growing traits. Tilapia species with fast growth such as *O. niloticus* cannot endure high salinity and conversely, species with high salinity tolerance such as *O. mossambicus* or *Tilapia zillii* do not grow fast (El-Sayed, 2006). A possible solution to this problem could be the development of terminal hybrids between a fast growing species and a highly salinity tolerant species, as has been done in some studies (Bartley et al., 2001; Behrends et al., 1990). This method allows combining traits of interest of two parental species, taking advantage of species complementarity and potential heterosis effects. Moreover, hybridization between two intergeneric tilapia species *O. niloticus* and *Sarotherodon melanotheron* (highly salinity tolerant) showed segregation in accordance with Mendelian expectations, allowing the creation of a new fertile strain (Bezault et al., 2012) and relevant use of quantitative genetics theory for improvement of this strain. Although several studies have shown positive impacts in terms of salinity tolerance and growth with the use of the *O. mossambicus* × *O. niloticus* terminal hybrid (Kamal and Mair, 2005; Lutz et al., 2010; Tayamen et al., 2002; Villegas, 1990; Watanabe et al., 1985), such a strategy present large disadvantages: i) obligation for the fish farmer to produce or to buy F1 hybrids, ii) necessity to maintain and control the pure parental stocks, iii) impossibility to combine the parental traits of interest otherwise than by a 50:50 ratio, which is not always optimal, and iv) necessity to perform selective breeding on both parental species if hybrid performance has to be improved, which is complex and time-consuming.

A potential alternative to reduce these disadvantages is to perform founder hybridization and then to select these fertile fish on desired characteristics to produce a fast growing red tilapia strain, as proposed by Behrends et al. (1990). This is the option we chose to create a salinity tolerant tilapia possessing high growth characteristics. This has been named the "Molobicus" (term derived from *MOssamBIcus* and *niLOtiCUS*) programme in the Philippines. This research programme was realized by several partners, Philippine Council for Aquatic and Marine Research and Development (PCMARD) and Bureau of Fisheries and Aquacultural Resources — National Integrated Fisheries Technology Development Center (BFAR-NIFTDC) in the Philippines, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and Institut National de la Recherche Agronomique (INRA) in France. The basic principle of this programme was to create an artificial pool of genes for salinity tolerance and growth characteristics in an interspecific hybrid tilapia population and then to selectively breed these fish based on their growth performance in brackish water. The first step of this programme was to create an interspecific hybridization between *O. niloticus* (with favorable growth traits) and *O. mossambicus* (with favorable salinity tolerance traits) and then to perform several backcrossing with *O. mossambicus* to introgress high salinity tolerance traits (Mateo et al., 2004). The second step began when salinity resistance of the hybrid population was judged sufficient to rear the fish in brackish water.

Furthermore, this second step was performed in brackish water to passively improve salinity tolerance, non-adapted fish being expected to die or at least experience slow growth.

The selection procedure was performed in two different environments: in tanks and in earthen ponds (Rosario et al., 2004). This permitted some security in case of accident (rearing or climatic problem for example) and to better anticipate possible changes in the aquaculture practices e.g. a strain selected in extensive system could not show the same results in another system, as an intensive one.

The aim of the present study was to estimate the response to the selection of the genetic selection programme on the growth performance of the hybrid in the two rearing environments over four generations of selection but also to estimate survival which is directly selected through the rearing in brackish water, and possibly also indirectly through the selection practiced for increased growth.

## 2. Material and methods

### 2.1. Fish and rearing conditions

The first step of the Molobicus programme has been previously described by Mateo et al. (2004). At the beginning, 80 *O. niloticus* fish issued from the 7th generation of the GIFT (Genetically Improved Farmed Tilapia) were used (with a balancing sex-ratio) for the experiment. Moreover, 300 *O. mossambicus* were collected from the wild and 80 fish (with a balancing sex-ratio) were kept (mainly according to their health) to be used for the experiment. The first generation of hybridization (generation H1) was produced from the reciprocal crosses (by artificial fertilization in freshwater) of *O. niloticus* and *O. mossambicus* (wild-caught in the Philippines), with a rotational mating scheme. The largest individuals of each family of H1 hybrids were then backcrossed with *O. mossambicus* to produce H2 hybrids. This process was repeated a second time in order to improve salinity tolerance to a level similar to that of *O. mossambicus*. All along the process of repeated backcrossing, use of alternate sexes to backcross was done in case inheritance of salinity tolerance was sex-linked. At each generation, a salinity tolerance test developed by Lemarié et al. (2004) was performed to monitor the progression of salinity tolerance. This comparative evaluation of the salinity tolerance was performed on 40 fish from each generation and each cross (foundation broodstock, first backcrossed hybrids (H2) and second backcrossed hybrids (H3)). Fish were stocked in 20 L aquaria supplied by gentle aeration (ten fish per aquarium). The experiment was done using four replicates and one control (in freshwater). During the test, half the water in each aquarium was replaced each day and replaced by water with higher salinity in such a way that the salinity was increased by 3 ppt. The mortalities of the various pure species and hybrid strains through a progressive increase of the salinity of 3 $ppt \cdot day^{-1}$ were registered. The evaluation of salinity tolerance was done by calculating the LS 50% (linear regression), and the salinity level at which 50% of the fish were dead.

It was shown that one generation of introgressive hybridization (hybrid H2 possessing 75 and 25% of *O. mossambicus* and *O. niloticus* genome, respectively) was sufficient to reach a salinity tolerance similar to *O. mossambicus*, and greater than that of *O. niloticus* (Mateo et al., 2004; Rosario et al., 2004). The LS 50% of *O. mossambicus*, H2 and H3 fish was on average 106 ppt whereas for *O. niloticus*, it was 50 ppt. Furthermore, in terms of growth performance in brackish water, this H2 hybrid was not significantly different from *O. niloticus* after 120 d of rearing. The H2 hybrid was consequently the more appropriate to begin the second phase of the programme, selective breeding (H2 hybrid was consequently the G0 in the selection step). This step consisted of within-family or within group selection combined with random mating, with body weight (BW) at 5 months as a selection criterion. A total of 50 H2 hybrid families were created and then separated and maintained (50 families in each environment) in two different environments in tanks (9 $m^2$ intensive rearing conditions with a high stocking density and ad libitum feed) and in ponds (225 $m^2$ extensive rearing conditions with fertilization of the ponds, no feed input and with a low stocking density; rearing conditions are described in Table 1) in brackish water (variable and fluctuating salinities associated with rainfall, typhoons and salinity variations due to seasons but on average 14.9 ± 6.93 ppt and 21.6 ± 7.66 ppt in extensive and intensive systems). Each family was reared in separate tanks or ponds (according to the environment