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

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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 *O. 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±0.07 *vs.* 0.17±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.

Highlights

► We developed an improved hybrid line of tilapia growing well in brackish water. ► The selection was performed in intensive and extensive environments. ► We studied the improvement of growth traits after 4 generations of selection ► The improvement and the realized heritabilities of the traits are generally high

Keywords : salinity tolerance ; Selection ; Hybridization ; genetic gain ; heritability ; survival rate

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 %.

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 *O. 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 18ppt 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* x *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 showed the same results in another system, as an intensive one.

The aim of the present study was to estimate the response to 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 for through the rearing in brackish water, and possible 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 liter aquaria supplied by gently 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.day^{-1} were registered. The evaluation of salinity tolerance was done by calculating the LS 50% (linear regression), 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 m² intensive rearing conditions with a high stocking density and *ad-libitum* feed) and in ponds (225 m² 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 concerned). Each generation the three heaviest males and the heaviest female within each family were selected

as breeders, measured at five months of age. In addition, the two males and two females that ranked just below the selected breeders for BW were kept as a back-up for selected breeders that failed to spawn in due time or in case of losses due to mortalities or escapes. According to the generation, the environment and the sex of the fish, the selection intensity was ranged from 1.71 to 2.20. Females were manually stripped of their eggs when ready to spawn. The eggs were fertilized by the sperm of three males from another family, randomly chosen after checking that the two families were not too closely related (inbreeding lower than 1.5%). For practical reasons, however, this artificial fertilization was substituted by natural fertilization with one female and one male (in 82 and 83.5% of the cases in generations 0 to 2 in intensive and extensive system, respectively, and in 100% of the cases in generations 3 and 4, whatever the system). Some of the selected females were indeed not ready to spawn in the 20 L aquaria after one month. Consequently, the heaviest female was placed in 9 m² tanks in brackish water with the heaviest male for natural fertilization. In this case, fertilized eggs were removed from the female mouth as soon as possible and then placed in artificial incubators, as were fertilized eggs obtained from artificial fertilization. The data structure (number of sires, as well as the number of families and fish for each generation and each environment) is shown in Table 2. The number of families at the beginning of the selection step (G0) was not 50 as predicted but 46 and 35, in intensive and extensive systems, due to some brackish water fish adaptive and some technical problems. To estimate the genetic gain of the hybrid strain all along the selection step, an internal reference line was added in each family (Basiao et al., 1996). The use of this internal line represented a unique reference for both selected lines (in intensive and in extensive environments). Furthermore, to have a sufficient statistical power, this allows reducing the number of replicates in case of high environmental effects compared to the use of a control line issued from the base line (same line that the selected line at the beginning) (Vandeputte et al, 2002; Basiao and Doyle, 1996). Moreover, it is a way to

estimate the genetic gain on the selected lines through successive generations by removing the environmental effects in the statistical analyses. Consequently, 17 red tilapia fish (showing a good salinity tolerance, which was needed because hybrids selected were reared in brackish water, and easily distinguished from the Molobicus strain) were reared with each hybrid family. The choice of this internal control line is questionable but even if an *O. mossambicus-O. niloticus* red hybrid was chosen this line would not have been isogenic with the Molobicus selected line. Furthermore, Basiao et al (2005) showed the interest of an internal red control line to test for the saline stress effects on the Nile tilapia growth.

The red tilapia founder stocks were reared in a common pond area and to ensure steady supply of fingerlings, some founder stocks were also reared in tanks for back-up purposes. Every time a family of intensive or extensive is produced, red tilapia (with the same size as the produced family) are collected from the ponds or tanks and incorporated in the same environment as that of the selected line during the first month of grow-out culture.

The Red Florida strain was used and reproduction was performed by random selection. At five months, animals were collected and weighed.

Furthermore, for each family and at each generation, the survival rate at 5 months of age was calculated for the selected strain and the internal reference line.

2.2. Statistical analyses

Body weight data were analyzed according to the mixed procedure of SAS 9.3 software (SAS Institute Inc, Cary, NC) for each environment separately. Fixed effects were line (selected or internal reference lines), sex (male or female), generation (0 to 4), interactions between generation and sex, generation and line, sex and line and the triple interaction between generation, line and sex. Random effect was the family of the fish.

The age of measurement was not included as a covariate in the model since this did not significantly change the results. The environment effect was also tested and was significant, explaining the choice to separate the data according to the environment.

Analysis of variance (ANOVA) was done on transformed data, followed by Tukey *post hoc* tests for pairwise comparisons between groups to determine significance levels. Least squares (LS) means and their standard errors were estimated. Differences estimated by the mixed model analysis were considered significant when the *P*-value was lower than 0.05. The relative difference in body weight between hybrids and internal reference fish were calculated as the ratio of the hybrid LS mean to the internal reference line LS mean for the given trait.

The following equation described by Falconer (Falconer, 1989) was used to estimate the realized heritability under a within-family selection:

$$h^2 = \frac{R_w}{i\sigma_p(1-r)\sqrt{\left[\frac{n-1}{n(1-t)}\right]}} \quad (1)$$

where h^2 the heritability of individual values, R_w the observed response to selection (corresponding to the slope of the regression line of the difference between hybrid and internal reference lines between two generations), i the intensity of selection (average value for each generation and environment), σ_p the standard deviation of phenotypic values of hybrid individuals in each generation, r the average genetic relationship within each generation and environment ($r = \frac{1}{4s} + \frac{1}{4d} = \frac{s+d}{4sd}$ (Chevassus et al., 2004) where s and d are the number of sires and dams, respectively), n is the mean number of individuals in the family and t is the intra-class correlation of phenotypic values of members of the families. All the values of the equation (1) were estimated for each generation and environment. Means and standard deviations of each criterion were calculated (with excel software) for the whole selection period.

Survival data were arcsine square root transformed to normalize data. These transformed data were analyzed according to the General Linear Models (GLM) procedure of SAS 9.3 software (SAS Institute Inc, Cary, NC). The following model was used for each environment separately:

$$y_{ijk} = \mu + L_i + G_j + L_i G_j + e_{ijk} \qquad (2)$$

where y_{ijk} is the survival rate of the family *k*, μ the general mean, L_i the effect of the line i (selected or internal reference lines), G_j the effect of generation j (j = 0 to 4), $L_i G_j$ the effect of the interaction between line i and generation j and e_{ijk} the residual pertaining to animal *k*.

3. Results

For the body weight, all effects were significant in intensive and extensive systems (except the interaction between generation and sex in extensive system). For the males, the difference in BW between hybrids and internal reference fish ranged from -2.4 and -1.6 % at the beginning of the selection to 32.3 and 25.1 % at the 4th generation of selection in intensive and extensive systems, respectively (Table 3). For the females, the hybrid fish were 21.5 and 26.1 % lighter than the internal reference fish at the start generation but after 4 generations of selection, the hybrid females were 32.0 and 9.0 % heavier, in intensive and extensive systems, respectively. Whereas internal reference fish (males and females) did not show a significant BW improvement according to generation (1.1 and -3.9 % per generation for males and females, respectively), male and female hybrids showed an increase of 14.3 and 10.6 % per generation, respectively. When all the data were put together (sex and environment), the BW gain was 12.5 g per generation.

In each generation, a significant effect of environment was observed. The fish grew faster in intensive than in extensive system. BW differed by as much as 65.8 and 38.7 % at the last generation in male and female, respectively. In computing the ratio between hybrids and

internal reference fish for the BW in the two environments (Figure 1), a BW gain of 10.58 % and 6.67 % per generation was estimated for intensive and extensive systems, respectively.
In estimating the genetic gain as the difference between successive generations (without using the internal reference line), a reduced determination coefficient of the regression line was seen due to the environmental variability compared to the estimation developed above (from 0.98 to 0.95 in intensive system, and from 0.93 to 0.05 in extensive system, data not shown), and consequently, estimated with less accuracy the genetic gain.
A reduction of the coefficient of variation (CV) of BW was seen according to the generation in females (mean percentage per generation: -1.41 % and -2.27 % for BW in intensive and extensive systems, respectively; data not shown). However, this was not shown in males.

Realized heritabilities of BW were estimated for each environment (intensive or extensive) and each generation separately. The different terms of equation 2, used to estimate realized heritability are averaged for all the generations in Table 4. Realized heritability of BW in intensive systems (0.19±0.07) was significantly different from zero, which was not the case in extensive systems (0.11±0.06).

For the survival rate, all the effects were significant whatever the environment. In intensive and extensive systems, selected lines showed an improvement of the survival rate with generations (Table 5). Furthermore, in intensive system, the difference of survival rate between hybrids and internal reference fish was 10.0 % at the beginning of the selection experiment and 38.0 % at the 4^{th} generation. In extensive system, the difference of survival rate between hybrids and internal reference fish was lower, from 2.6 % at the beginning of the selection experiment and 18.0 % at the 4^{th} generation.

4. Discussion

The aim of the present study was to estimate the impact of genetic selection on growth performance of the hybrid *O. mossambicus* x *O. niloticus* (with one generation of backcrossing with *O. mossambicus*) after four generations of selection for increased BW in brackish water. After four generations of selection, the BW of the hybrid increased by 50 g compared to the internal reference red tilapia, which corresponds to a gain of 12.5 g or 7.3 % per generation. These results are lower than those of a previous study (Bolivar and Newkirk, 2002) using within family selection for 12 generations (12.4 % per generation) but this difference is likely to be due to a lower selection intensity in our study. The selection response estimated in the present study is also similar to the estimations in the GIFT strain between 1991 and 2003 (Khaw et al., 2008). These authors showed that between the base population and the ninth generation of GIFT selection, there was 89.2 g of improvement, which was 9.9 g per generation (or 7.1 %).

The present selection experiment was performed into two distinct rearing environments: an intensive and an extensive system. The impact of selection varied according to the rearing system. The selection response after four generations of selection was 65.8 and 38.7 % greater in intensive than in extensive systems for BW for males and females, respectively. The greater improvement in the intensive system was somewhat expected, as similar results were reported in the literature (Bentsen et al., 1998; Watanabe et al., 2002). In the intensive system, fish were fed *ad libitum* and consequently, could express their maximal growth potential, whereas in the extensive system, earthen ponds were slightly fertilized and fish probably did not have enough feed supply to express an optimal growth. Furthermore, there was a greater influence of the environmental variations due to the relatively uncontrolled environment in

the extensive system, which could probably explain the high variability of the survival difference between hybrids and internal reference fish in this last system.

A significant sex effect for BW was observed in the present study. Five month old females weighed on average 54.4 % less than males. The magnitude of this sexual size dimorphism was comparable with literature estimations (Bentsen et al., 2012; Charo-Karisa, et al., 2006). However, the sexual dimorphism for BW at harvest was larger than for the GIFT strain, in which females weigh 81.4 and 84 % of the male weight according to Nguyen et al. (2007) and Ponzoni et al. (2005), respectively.

Realized heritabilities of BW were estimated separately for each environment and each generation, and were in agreement with the estimation of 0.14 found by Bolivar and Newkirk (2002) in tilapia in outdoor tanks and by Rezk et al. (2003) on channel catfish in earthen ponds. The realized heritabilities estimated in the present study were different between the two environments, with 72.7 % greater h^2 in intensive than in extensive system. The lower realized heritabilities in the extensive system were due to the lower selection response R_w compared to the intensive one. In the same way, Bentsen et al. (2012) showed variations in heritability according to the studied environment, and estimated that heritability was higher in cage than in pond systems (0.47 and 0.27 on average, respectively), which could be explained by genotype by environment interactions. Furthermore, after the first generation of selection, in the intensive as in the extensive systems, an increase of BW was always visible and relatively stable from one generation to the next (from 19.6 to 24.5 g and from 8.2 to 12.8 g of improvement from one generation to the next in intensive and extensive systems, respectively).

According to the formula developed by Falconer (1989), the expected response depends on the selection intensity. This selection intensity was linked to the fertilization method, since in natural fertilization the heaviest male and the heaviest female were used as breeders whereas in artificial fertilization, the 3 heaviest males and the heaviest female were used. The selection intensity was reduced for males using the latter approach. In the present experiment, artificial fertilization was mainly used at generation 0 to 2, whereas natural fertilization was fully used at generation 3 and 4. An improvement of the realized heritability was seen between generations 1-2 and generations 3-4, whatever the environment (+42 % and +109 % in intensive and extensive environment, respectively).

Furthermore, the improvement of the survival rate of the selected fish compared to the internal reference line was in accordance with an adaptation of this hybrid line to their environment. Consequently, it was possible to confirm the hypothesis that by raising fish in brackish water, an improvement of the salinity tolerance (measured by the survival rate) was performed. However, this result was more visible in intensive system than in extensive one. This could be explained by a higher variability of the water quality (for example the water salinity), more complicated to control in earthen ponds than in tanks.

Finally, due to the genetic selection programme improving BW, an increase of the proportion of *O. niloticus* genome at the expense of the *O. mossambicus* genome in the Molobicus strain is hypothesized. It would be interesting to study the proportion of each of the parental genomes in the Molobicus hybrid after 4 or 5 generations of selection and to compare with the original composition of the strain (75 and 25 % of *O. mossambicus* and *O. niloticus* genome, respectively).

5. Conclusion

The aim of the present study was to present a new approach to develop a tilapia strain with both a high salinity tolerance and a high growth performance, and to highlight the impact of the genetic selection on growth in two distinct environments. To our knowledge, the present work was the first to develop a founder hybrid between two tilapia species (one adapted to brackish water and one with high growth capacities) and then implement a genetic selection programme for improving BW and passively salinity tolerance. This selection procedure was simple, and relatively inexpensive, and could easily be transferred to fish farmers in case its efficiency was proven. It was also chosen because within group selection combined with rotational mating is a good way for keeping genetic variability and limiting inbreeding.

The present selection scheme seemed to be satisfactory since response to selection on BW was around 7 % per generation. A solution to increase the genetic gain per generation could be to increase the selection intensity, for example by rearing more fish per family.

Moreover, the hypothesis that rear fish in brackish water could passively improve survival and, in the same way, salinity tolerance, was shown in intensive and extensive systems.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

HdV and MV contributed to data analysis, interpretation of data and manuscript preparation. WR, NM and PM contributed to the experimental design and the data collection. JFB contributed to the manuscript preparation. BC contributed to the experimental design, data analysis, interpretation of data and manuscript preparation.

All authors approved the final version.

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Figure legends

Figure 1: Ratio between LS Mean of hybrid and internal reference fish for each generation according to the environment and the linear regressions

Figure 1

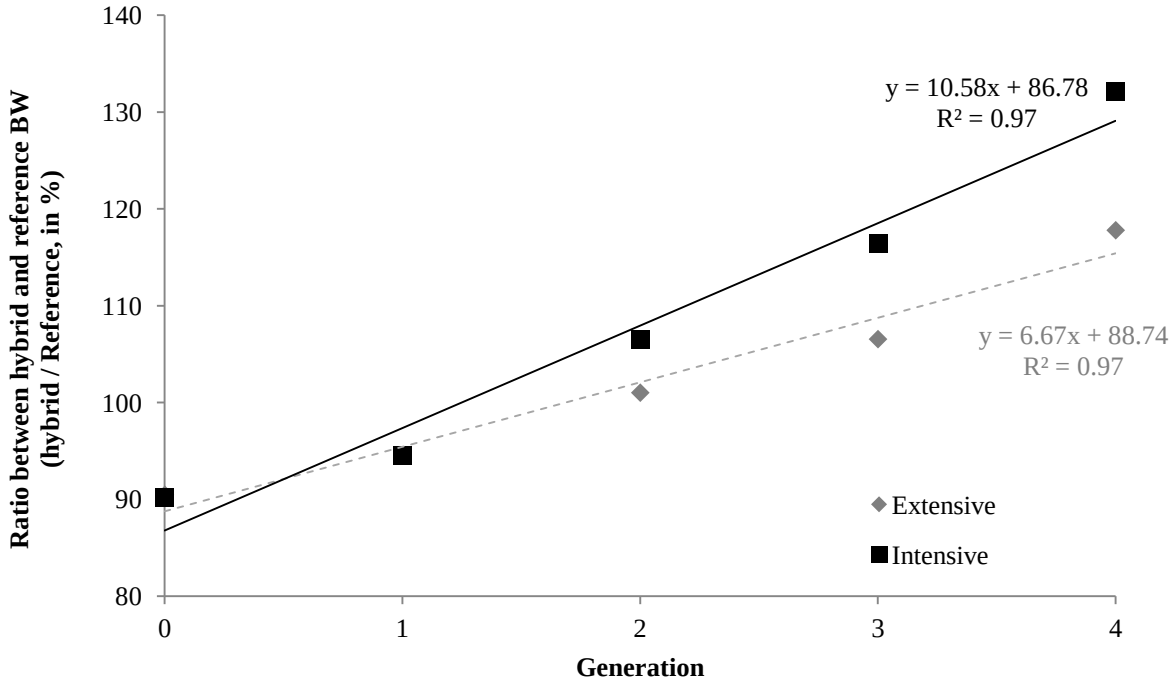

Table 1

Rearing conditions during the selection step in intensive and extensive environments

Month		1	2	3	4	5
Phases		Incub	Fingerlings	Grow out		
Places	Intens	Incub	9 m² service tank	9 m² allocated tanks		
	Extens	Incub	4 x 4m hapa	225 m² earthen ponds		
Number		300 max	300 max+100 red	50 + 17 red		
Density	Intens		44 fish per m²	7.2 fish per m²		
	Extens		25 fish per m²	0.30 fish per m²		
Feed	Intens	X	Prawn fry mash	Prawn fry crumble	Prawn pellets	
	Extens	X	Pond natural food improved by chicken manure			

Incub: Incubation in incubators; Intens: intensive system; Extens: extensive system; each small line in in Month line represent a week.

Table 2

Data structure: number of dams, as well as the number of sires and fish for each generation and each environment

Environment	Generation	Hybrids			Number of control individuals
		Number of dams	Number of sires	Number of individuals	
Intensive	G0	46	58	1688	606
	G1	46	73	1717	417
	G2	41	49	1925	498
	G3	29	29	1330	323
	G4	16	16	734	139
Extensive	G0	35	47	962	347
	G1	33	52	1324	250
	G2	29	29	1134	290
	G3	24	24	860	244
	G4	12	12	564	150

Table 3

LS Means ± standard error of the body weight according to the type of fish (Control or hybrid), the sex (male or female), the environment (intensive or extensive system) and the generation of selection (from G0 to G4)

		Intensive (N=9227)			Extensive (N=6275)		
Sex	Generation	Control	Hybrid	Ratio between Hybrid and Control	Control	Hybrid	Ratio between Hybrid and Control
Female	G0	117.4±6.8	92.1±6.3	-21.5%	157.0±7.8	116.0±7.3	-26.1%
Female	G1	131.1±6.9	113.2±6.3	-13.6%	172.3±8.3	155.4±7.5	-9.8%
Female	G2	146.1±7.0	139.5±6.6	-4.5%	134.7±8.5	128.9±7.8	-4.3%
Female	G3	145.4±8.5	158.5±7.9	9.0%	135.9±9.3	141.0±8.8	3.8%
Female	G4	131.7±11.8	173.8±10.6	32.0%	115.0±13.0	125.3±12.4	9.0%
Male	G0	184.8±6.9	180.3±6.3	-2.4%	159.3±8.0	170.2±7.3	6.8%
Male	G1	199.9±6.9	199.7±6.3	-0.1%	193.7±8.3	190.7±7.4	-1.6%
Male	G2	218.1±7.0	248.4±6.5	13.9%	157.8±8.5	166.6±7.8	5.6%
Male	G3	236.8±8.5	286.4±7.9	20.9%	160.6±9.6	174.9±8.7	8.9%
Male	G4	216.0±11.6	285.6±10.6	32.2%	137.6±13.1	172.3±12.2	25.2%

Table 4

Mean and standard deviation of each criterion used to estimate h^2 of body weight for each environment

	Intensive	**Extensive**
R_w	17.8±7.02	9.39±3.00
i	1.95±0.12	1.92±0.03
σ_p	79.4±8.94	62.4±9.00
r	0.47±0.04	0.48±0.04
n	43.7±3.68	40.2±4.20
t	0.20±0.03	0.54±0.10
h^2	**0.19±0.07**	**0.11±0.06**

R_w, the observed selection response per generation (in g); i, the average intensity of selection (for males and females); σ_p, the standard deviation of phenotypic values of individuals; $r = \frac{1}{4s} + \frac{1}{4d} = \frac{s+d}{4sd}$ where s and d were the number of fathers and mothers, respectively; n, the mean number of individuals in each family and t, the average of the correlation of phenotypic values of members of the families; h^2, the realized heritability.

Table 5

LS Means, minimum and maximum for the survival rate according to the type of fish (Control or hybrid), the environment (intensive or extensive system) and the generation of selection (from G0 to G4). All these results were transformed back from arcsine square root transformation.

	Generation	Control			Hybrid			Percent difference between Hybrid and Control
		LS Mean	Min	Max	LS Mean	Min	Max	
Intensive (N=351[1])	G0	49.8^{c}	5.8	90.3	59.8^{bc}	13.4	84.8	10.0
	G1	45.7^{c}	5.8	90.3	66.0^{bc}	24.1	90.3	20.3
	G2	59.8^{bc}	11.3	90.3	81.1^{a}	17.0	90.3	21.3
	G3	58.0^{bc}	21.9	90.3	81.2^{a}	43.8	90.3	22.4
	G4	46.3^{b}c	11.3	80.0	84.3^{a}	47.0	90.3	38.0
Extensive (N=259[1])	G0	51.9^{cd}	5.8	90.3	54.5^{bcd}	7.8	90.3	2.6
	G1	41.7^{d}	5.8	90.3	69.6^{ab}	35.8	90.3	27.9
	G2	49.2^{cd}	11.3	90.3	66.5^{abc}	30.9	90.3	17.3
	G3	54.2^{bcd}	5.8	90.3	64.1^{abc}	32.5	90.3	9.9
	G4	64.1^{abcd}	41.5	90.3	82.1^{a}	62.6	90.3	18.0

[1]N is the number of observations equal to the number of families in each environment for all the generations

[a-e] Means within each environment (intensive or extensive) with different superscripts are significantly different ($P < 0.05$)