SALINITY TOLERANCE OF *Oreochromis niloticus* AND *O. mossambicus* F_1 HYBRIDS AND THEIR SUCCESSIVE BACKCROSS

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Abstract

The effect of backcrossing on the salinity tolerance of the offspring of the hybrid *O. mossambicus* (Mo) x *O. niloticus* (Ni) was determined using mean salinity tolerance (MST), median lethal salinity (MLS) and optimum salinity tolerance (OST) as indices of tolerance. The reciprocal Hybrids 1 (H1) (Mo x Ni and Ni x Mo), the first hybridization, were backcrossed with parental *O. mossambicus* in reciprocal crosses (i.e. with *O. mossambicus* as mother or father) to produce the reciprocal Hybrids 2 (H2) (H1 x Mo and Mo x H1), the backcross 1 and reciprocal Hybrids 3 (H3) (H2 x Mo and Mo x H2) and the backcross 2. These reciprocal hybrids and pure parental species were exposed to progressive changes in salinity at 6 ppt intervals until total mortality. Parental *O. mossambicus* showed the highest salinity tolerance, followed by reciprocal H3, H2 and H1, while parental *O. niloticus* had the lowest. There was an increase of salinity tolerance in the offspring as they were backcrossed to the saline-tolerant parent *O. mossambicus*. Maternal inheritance was observed in reciprocal H1 and reciprocal H2, while paternal inheritance was observed in reciprocal H3. H1 had the highest heterosis. Both H2 and H3 stocks had negative and slight positive heterosis, indicating that they are good candidates as base population for selection. Additive inheritance was observed due to their nearly zero heterosis.

Hybrid 1 will not fit as base population for selection due to their high heterosis and aggressive behavior, that might be transmitted in the next generation. Hybrid 3 will not also fit as base population for selection due to the high inbreeding values. Hybrid 2 will best fit as base population for selection due to their low heterosis (additive inheritance), low aggressiveness and zero inbreeding. However, H1 can contribute in the base population for selection, because heterosis is not only governed by dominance-recessive effect but also additive gene effect. H3 can contribute to the base population, due to their high salinity tolerance.

Introduction

Tilapias are naturally distributed in Africa, and the Jordan Valley (Wohlfarth and Hulata, 1981; Pullin and McConnell, 1982; Trewavas, 1982) and were introduced to other places for aquaculture purposes. Tilapias are popular cultured species because of their high environmental tolerant characteristics. Tilapias are euryhaline, and are able to live and reproduce in salinities higher than 30 ppt. Some species like *Tilapia guineensis, Sarotherodon melanotheron, T. zillii and O. mossambicus* can tolerate salinities from 0 to 120 ppt (Trewavas, 1982).

Tilapias possess various characteristics which make them desirable species to culture in brackishwater farms. There are species of tilapias with potential for brackishwater farming. These include *O. niloticus, O. mossambicus, O. aureus, O. spilurus, O. hornorum, S. melanotheron* and the hybrid red tilapia. In the Philippines, development and expansion of tilapia culture in saline waters have greater potential, covering 239,323 hectares of existing brackishwater ponds and about 20 million hectares of marine coastal waters for mariculture production. These waters are of great potential if compared with the most vital freshwater resources of 264,531 hectares of fishponds, lakes, rivers and reservoirs, which are now at different levels of pollution.

Consequently, for many years, tropical aquaculturists have tried to develop tilapia culture in brackish and sea waters. Unfortunately, the true brackishwater tilapias have poor-growing performance while the fast-growing strains are poorly adapted to saline water environment. Recently, scientists of the Molobicus Project, a joint research project of the Bureau of Fisheries and Aquatic Resources - National Integrated Fisheries Technology Development Center (BFAR-NIFTDC), the Center for International Research in Agronomic Development (CIRAD)-France and the Philippine Council for Aquatic and Marine Research and Development - Department of Science and Technology (PCAMRD-DOST), are trying to develop a synthetic strain of saline tilapia that can breed naturally in saline environment with good feed conversion ratio (FCR) and growth. Production of a synthetic strain that naturally breeds in brackishwater environment, can be carried out through a series of repeated backcrossing of the saline tolerant species to the hybrids followed by a selective breeding program.

Information on salinity tolerance and growth performance of F_1 hybrids backcrossed to saline-tolerant parent, e.g., *O. mossambicus*, is lacking (Watanabe, 1996). For further selection, salinity tolerance test is conducted to determine the salinity tolerance and growth performance of the F_1 Hybrid 2 (backcross 1) and F_1 Hybrid 3 (backcross 2) compared with their parental species. This study aimed to provide initial information on the salinity tolerance of these different backcrosses. It was hypothesised in this study that the salinity tolerance of the F_1 hybrids would be higher than *O. niloticus* and closer to that of *O. mossambicus*, in repeated backcrossing, leading to a synthetic saline-tolerant tilapia strain.

Materials and methods

Hybridization protocol

The breeding aim of this study was to associate the fast-growing character of *O. niloticus* and the salinity tolerance of *O. mossambicus*. The hybridization protocol was designed by Bernard Chevassus, a French geneticist and currently, Director of International Network in Research of Agronomy (INRA-France). The first generation of hybrid (F_1H1) was produced from the reciprocal crosses of *O. niloticus and O. mossambicus*. The F_1H1 was then backcrossed to the saline-tolerant parents (*O. mossambicus*) and produced F_1H2. This process was repeated over successive generations until the salinity tolerance of the hybrids was no longer significantly different from the saline tolerant parent (*O. mossambicus*). In each generation, salinity tolerance test was conducted to monitor the progression of salinity tolerance with the first hybridization conducted in freshwater.

Rotational backcrossing procedure

In the first hybridization, three groups of *O. mossambicus x O. niloticus* hybrids and three groups of *O. niloticus x O. mossambicus* hybrids were initially produced. Another six groups of pure *O. mossambicus* were rotationally bred to avoid genetic drift and inbreeding. The *O. mossambicus* groups have been used to backcross in the hybrids. Along the process of repeated backcrossing, use of alternate sexes to backcross was carried out (as counter-check measure) in case inheritance of salinity tolerance is sex-linked.

Salinity tolerance indices

Mean Salinity Tolerance (MST) is the average salinity where the fish dies in a certain salinity level. The population of dead fish in each replicate was multiplied by their respective salinity. The products were summed together and averaged by the total number of experimental fish per replicate. The MST of each replicate was determined using the formula MST = (f1*s1 + f2*s2 +…+ fN*sN)/N; (where f-fish, s-salinity, N- number of individuals observed).). The MST were used as an index for salinity tolerance, to determine the average salinity of a certain group of hybrids or pure species and subjected to analysis of variance in this study.

Median Lethal Salinity (MLS) is the salinity at which survival falls to 50%,during the conduct of salinity tolerance test in a progressive increase of salinity by 6ppt per day. Lemarie (2001, in press) recommended the 6ppt interval to see the wide gap of salinity tolerance between the two species and their hybrids. The 6ppt interval was an accurate and sensitive test, matching the need of a selection program, compared with the previously adapted procedures of Watanabe (1996) and Villegas (1990), where results for the two strains and their hybrids were not significantly different. Linear regression equation (Y = a + bx) was used to predict the exact salinity (ppt) of the MLS.

Optimum Salinity Tolerance (OST) was determined by using the break-line (break point) analysis. The procedures involved in this method are as follows; Plot the values into a graph and determine the formed plateau. These plateaus were values before the start or at initial mortality. Test the significance of the slope of the regression line 1 ($Y = a_1 + b_1x$) of the plateau. If the result is significant, stepwise calculations of linear regression were carried out until the result becomes not significant. The remaining plotted values undergo linear regression 2 ($Y = a_2 + b_2x$), connecting to the first value of the regression plateau. The OST was determined by equating the two linear regression functions ($a_1 + b_1x = a_2 + b_2x$). The intersection of the two regression lines is the break point value. The OST (breakline analysis) was used to determine the maximum salinity at which there was no mortality or low mortality even at longer exposure if the salinity increase will be 6 ppt per day.

Progressive Salinity Increase Tolerance Test

After acclimating the fish at 0 ppt for 20 days in their respective test aquaria, the saline water was administered in the treatments, via water to fish method, to avoid stress. The interval of progressive salinity increase was 6ppt/day (Lemarie, 2001). Daily mortality was recorded before increasing the salinity. The length of dead fish was measured to correlate size and salinity tolerance. Weak fish were observed for any clinical signs before mortality. Salinity was continuously increased until all fish died. Salinity was increased daily up to 126ppt within 22 days.

***Experimental Design for Study 1* (Salinity Tolerance of Different Species and Backcross)**

The treatments were the different strains and hybrids in four replicates as follows:

Treatment 1: *O. mossambicus* (as positive control)
Treatment 2: F_1 Hybrids1 (*O. mossambicus* x O. niloticus)
Treatment 3: F'_1 Hybrids'1 (*O. niloticus* x *O. mossambicus*): Reciprocal F1 Hybrids1 (H1)
Treatment 4: F_1 Hybrids2 (F_1H1 x *O. mossambicus* 1)
Treatment 5: F'_1 Hybrids'2 (*O. mossambicus* 1 x F'_1H'1): Reciprocal F1 Hybrids2 (H2)
Treatment 6: F_1 Hybrids3 (*O. mossambicus* 2 x F_1H2)
Treatment 7: F'_1 Hybrids'3 (F'_1H'2 x *O. mossambicus* 2): Reciprocal F1 Hybrids3 (H3)
Treatment 8: *O. niloticus* (as negative control)

Natural light/natural photoperiod was used in the Study 1 due to the green transparent roof of the hatchery. Complete Randomized Design (CRD) was used in this study.

Experimental Design for Study 2 (Relationship of Size and Salinity Tolerance)

The treatments were the same as in the first experiment without replication. Each group of fishes had variable sizes ranging from <1g to >5g. Stocking density was 0.4g/liter. All sizes were mixed in one container of each group (6 buckets) in a 75-liter capacity bucket, in order to reduce environmental differences. Fish stocks were subjected to salinity increase at 6ppt /day. The standard length of the fish was measured. Fluorescent lamps were positioned above the experimental setup to control the light distribution.

Collection and analysis of data

The MST of each replicate was determined using the formula MST = (f1*s1 + f2*s2 +…+ fN*sN)/N; (where f-fish, s-salinity, N- number of individuals observed). The MLS was determined using linear regression function (Y=a+bx; where Y=MLS). The optimum salinity tolerance index (break-line analysis) was determined by equating the two linear functions ($a_1+b_1x = a_2+b_2x$; where; X=OST). The MST, MLS and OST results were analyzed using one-way ANOVA. Comparison among means was done using Duncan's Multiple Range Test. Regression-Correlation analysis was used to correlate tolerance to size.

Results and discussions

Salinity tolerance indices

Mean Salinity Tolerance (MST). The reciprocal crosses in H1 were significantly different from each other, demonstrating maternal influence on mean salinity tolerance (Table 1 and Table 3) compared to the H2 and H3, which were not significantly different from their reciprocals. The H'1 and H2 were not significantly different with *O. mossambicus* at mean MST of 112.5ppt, 115.5ppt and 118.2ppt, respectively (Table 1). The H'1 and H2 have an *O. mossambicus* mother proving of a bigger share of maternal influence in the salinity tolerance (Table 3). The H'2, H3 and H'3 were not significantly different with each other at mean MST of 110.8ppt, 111ppt and 108.8ppt, respectively (Table 1). These three breeds were not significantly different to *O. mossambicus* and H2. H1 was significantly lower to *O. mossambicus*, H'1, H2, H3 and H'3 at 99.6ppt (Table 1). *O. niloticus* got a 56.8ppt, which was significantly lower than all of the treatments.

*Median Lethal Salinity (MLS)***.** The reciprocal hybrids 1 (H1) were significantly different from each other, exhibiting maternal influence (Table 1 and Table 3) compared to the H2 and H3, which were not significantly different from their reciprocals. The H'1 and H2 were not significantly different to *O. mossambicus* at mean MLS of 111.22 ppt, 112.14 ppt and 115.064 ppt, respectively (Table 1). The H'1 and H2 have an *O. mossambicus* mother proving a bigger share of maternal influence in salinity tolerance (Table 3). The H'2, H3 and H'3 are not significantly different with each other at mean MLS of 109.45 ppt, 109.95 ppt and 108.28 ppt, respectively (Table 1). These three breeds were not significantly different to *O. mossambicus* and H2. H1 was significantly lower to *O. mossambicus*, H'1, H2, H3 and H'3 at 97.33 ppt (Table 1). *O. niloticus* got a 53.87 ppt, which was significantly lower than all of the treatments.

*Optimum Salinity Tolerance (OST)***.** The reciprocal hybrids 1 (H1) were significantly different from each other, exhibiting maternal heterosis at 76.50 ppt for H1 and 101.98 ppt for H'1 (Table 1 and Table 3), compared to the reciprocal H 2 (80.73 ppt for H2 and 76.14 for H'2) and reciprocal H3 (101.94 ppt for H3 and 94.54 ppt for H'3), which were not significantly different (Table 1). H'1 had mean OST of 101.98 ppt and was not significantly different to *O. mossambicus* at 107.64 ppt, where the mother was *O. mossambicus*, thus exhibiting maternal influence (Table 1 and Table 3). The reciprocal hybrids 3 (H3 and H'3), were not significantly different from each other at 101.94 ppt and 94.54 ppt respectively, and with the *O. mossambicus* (Table 1). The non-significant difference of reciprocal H3 was the result of backcrossing. There was an increase in the salinity tolerance of the hybrids in the backcrossing of saline tolerant parent (*O. mossambicus*) to the hybrids. OST of the hybrids increased as they were backcrossed with

Table 1. Mean Salinity Tolerance (MST); Median Lethal Salinity (MLS); Optimum Salinity Tolerance (OST) of the hybrids.

Treatment	**MST**		**MLS**		**OST**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
O. mossambicus	118.2[a]	1.10	115.1 [a]	1.48	107.6[a]	0.29
Hybrid 1	99.6[c]	3.43	97.3 [c]	3.82	76.5[b]	5.56
Hybrid ' 1	112.5[ab]	1.59	111.2[ab]	0.87	102.0[a]	5.89
Hybrid 2	115.5[ab]	5.10	112.1[ab]	1.38	80.7[b]	21.89
Hybrid ' 2	110.8[b]	4.11	109.4 [b]	0.29	76.1[b]	18.63
Hybrid 3	111.0[b]	6.66	110.0 [b]	3.09	101.9[a]	4.22
Hybrid ' 3	108.8[b]	4.53	108.3 [b]	3.64	94.5[b]	14.08
O. niloticus	56.8[d]	4.11	53.9 [d]	3.96	40.8[c]	4.68

Treatment with the same letter notations are not significantly different.

their saline tolerant parent *O. mossambicus*. The close values of the reciprocal hybrids 2 and 3, were due to the reduction of heterosis because of alternate use of sexes during backcrossing. These indicate an additive inheritance, because both parents contribute gene for salinity tolerance of the offspring.

Size and salinity tolerance correlation

There was no significant relationship between size to their survival in elevated salinities in *O. mossambicus, O. niloticus*, reciprocal Hybrid 1, Hybrid 2 and Hybrid 3. However, the Hybrid '2 and Hybrid '3 were significant, showing that bigger fish survives longer in elevated salinities. Linear regression analyses are shown in Table 2.

The regression coefficient (R^2) between size and their survival to elevated salinities in Hybrid 2 and Hybrid 3 were 0.1456 and 0.1564, respectively (Table 2). This means that size influence to survival in elevated salinities gave 14.56% (H'2) and 15.64% (H'3). The remaining percentages were outside factors. The longer survival of bigger fish compared to smaller ones (according to standard length) in H'2 and H'3 may be attributed to the maturation of kidney and gills for osmoregulation and maturation of hemoglobin for transport of oxygen to the cells, since dissolved oxygen (DO) decreases with increasing salinity. Fish requires mature hemoglobin to survive in a DO-devoid environment (Foskett and Scheffey, 1982; Shiraishi *et al.*, 1997; Ayson *et al.*, 1994; Cataldi *et al.*, 1988; Cataldi *et al.*, 1991; Perez and Maclean, 1976; Helms *et al.*, 1987). In the selection phase for growth, there will be an indirect selection for salinity tolerance, due to the significant relationship of size to their survival in elevated salinities in H'2 and H'3.

Only H'3 and H'2 have a significant linear relationship between size and salinity tolerance; in other groups, salinity tolerance was the same in all sizes. However, the slope of regression for H'3 and the slope for regression H'2 were not significantly different. The *O. mossambicus, O. niloticus*, reciprocal H1, the H2 and the H3 had no significant difference in all sizes to salinity tolerance (Table 2). It is an advantage that you can stock these fishes in

Table 2. Relationship of size to salinity tolerance in the different hybrids and pure *O. niloticus* and *O. mossambicus,* where R^2 = Regression coefficient; R = Correlation coefficient; n = population; SE = Standard error; P = probability *(significance).*

Treatment	Linear equation	R^2	R	n	SE	P (sig.)
O. mossambicus	y = 1.0486x + 110.56	0.0602	0.2453	33	5.6171	0.17ns
H1 (Mf x Nm)	y = 5.4013x + 71.17	0.4681	0.2322	14	6.9188	0.34ns
H'1 (Nf x Mm)	y = 5.7486x + 66.91	0.1556	0.3945	21	18.5570	0.08ns
H2 (Mf x H1m)	y = 1.6592x + 106.85	0.1449	0.0004	23	14.6950	0.07ns
H'2 (H'1f x Mm)	y = 6.2823x + 81.59	0.1456	0.3816	27	14.0920	0.05*
H3 (H2f x Mm)	y = -0.0481x + 114.03	0.0004	0.0193	34	3.8192	0.91ns
H'3 (Mf x H'2m)	y = 5.5904x + 84.70	0.1564	0.3954	27	19.8380	0.04*
O. niloticus	y = 0.4937x + 57.17	0.0386	0.1965	40	5.6440	0.22ns

ns - not significant **highly significant * significant

elevated salinities at all sizes (1-8 cm SL). However, the H'3 and H'2 show that bigger sizes survive longer than the smaller ones. Due to the significant linear correlation between size and salinity tolerance, this can be exploited in selection.

Heterotic effect

Heterosis was determined by the formula described by Tave (1990). Heterosis and the difference between the reciprocal hybrids as an indicator of maternal/paternal inheritance are shown in Table 3.

Table 3. Heterosis and the salinity tolerance difference in the reciprocal hybrids.

Hybrids	MST	MLS	OST			
	Heterosis	Diff. of reciprocal s	Heterosis	Diff. of reciprocals (ppt)	Heterosis	Diff. of reciprocals (ppt)
Hybrid 1	21.17	+(12.9)	23.45	+(13.89)	20.23	+(25.48)
Hybrid 2	0.94	+(4.65)	1.02	+(2.69)	-20.32	+(4.59)
Hybrid 3	-5.02	-(2.25)	-3.38	-(1.67)	5.60	-(7.40)

Reciprocal hybrids 1 exhibited the biggest heterosis among the groups in MST, MLS and OST, at 21.17, 23.45 and 20.23, respectively, surpassing the 12% heterosis acceptance for selection. for selection. The reciprocal hybrids 2 have +0.94, +1.02 and -20.32 in MST MLS and OST, respectively. The reciprocal hybrids 3 have -5.02, -3.38 and +5.60 in MST MLS and OST, respectively (Table 3). The negative and the slight positive heterosis (nearly zero heterosis) in the reciprocal H2 and H3 show no dominant effect but additive inheritance. Therefore, the reciprocal H2 and H3 are good candidates as base population in selection. A positive heterosis shows that the salinity tolerance of the reciprocal hybrids is greater than the average of their parents. A slight negative heterosis shows that the hybrids are lower in salinity tolerance than the average of their parents. A nearly zero heterosis is considered as additive inheritance.

Maternal and paternal inheritance

The differences in the salinity tolerance between the reciprocal hybrids as indicator of maternal/paternal inheritance are shown in Table 3. Reciprocal hybrids 1 have the biggest difference with each other at 12.9, 13.9 and 25.5ppt in MST, MLS and OST, respectively. The higher salinity tolerance between the two reciprocal hybrid 1 was shown by H'1 (112.5ppt - MST; 111.2ppt - MLS; 102.0 ppt - OST), where the mother is *O. mossambicus* and father is *O. niloticus*, compared with H1 (115.5ppt - MST; 97.3ppt - MLS; 76.5ppt - OST), where the mother is *O. niloticus* and the father is *O. mossambicus.* These results suggest that there is a strong maternal influence in the transmission of the trait (Table 1). The reciprocal hybrid 2 had the smallest positive difference in the group at +4.65, +2.69 and +4.59ppt in MST, MLS and OST, respectively (Table 3). The higher salinity tolerance between the two reciprocal hybrids was for H2 (112.5 ppt - MST; 112.1 ppt -MLS; 80.7ppt - OST), where the mother was *O. mossambicus* and the father was *O. niloticus,* ,compared with H'2 (110.8ppt - MST; 109.4ppt - MLS; 76.1ppt - OST), where the mother was H'1 and the father was *O. mossambicus*. This also supports the earlier observation of a strong maternal influence in the transmission of the trait (Table 1). The close results of salinity tolerance between the two reciprocal hybrid 2 were due to the alternate use of sexes of *O. mossambicus* during backcrossing.

Reciprocal hybrid 3 got a slight negative difference at -2.25, -1.67 and -7.40 in MST, MLS and OST, respectively (Table 3). The negative results are due to the fact that the group with higher salinity tolerance was the H3 (111.0ppt - MST; 110.0ppt - MLS; 101.9ppt - OST) sired by *O. mossambicus*, compared with H'3 (108.8ppt - MST; 108.3ppt - MLS; 94.5ppt - OST) sired by H'2 (Table 1). This shows that both female and male *O. mossambicus* contribute to the expression of salinity tolerance of the hybrids. However maternal influence is greater than the paternal influence as observed in the consecutive H1 and H2. The higher maternal influence compared with paternal influence may be due to the presence of extra chromosomal genes in the cytoplasm of the eggs (Shikano *et al*., 1997; 1998).

Effects of backcrossing and the effects of alternate use of sexes during backcrossing

As shown in the salinity tolerance of individuals as MST, MLS, OST (Table 1) and their average of reciprocal breeds (Table 4), there were increases in salinity tolerance during backcrossing. This was due to the introduction of more genes of saline tolerant parent (*O. mossambicus*) to the hybrids. The process is called introductory cross (Kirpichnikov, 1981).

Table 4. Average salinity tolerance of offspring and their parents.

	MST Average		MLS Average		OST Average	
Hybrids	Offspring	Parents	Offspring	Parents	Offspring	Parents
Hybrids 1	106.05	87.52	89.24	74.23	104.28	84.47
Hybrids 2	113.18	112.12	78.43	98.43	110.79	109.67
Hybrids 3	109.88	115.69	98.24	93.03	109.11	112.93

The alternate use of sexes during backcrossing reduces possible maternal and paternal inheritance/heterosis because both of the H2 (BC1) and H3 (BC2) exhibit lesser difference in their reciprocal breeds (Table 3). The female parent influenced the salinity tolerance of H2 while H3 was influenced by male parent. Therefore, both of the sexes have the capability to contribute salinity tolerance ability to the hybrids through backcrossing.

Computed inbreeding values

Inbreeding is the pairing of homologous genes in a locus. It is naturally occurring in the environment. It can be obtained through mating of relatives or not relatives as long as homologous genes will pair together. However, there is a bigger chance to produce homologous genes in mating relatives. Inbreeding values (Fx) in the different hybrids were computed through path analysis using the formula described by Tave (1990).

The first hybridization (hybrid 1) has zero inbreeding, because the parents came from different species. The production of *O. mossambicus* 1 (M1) has zero inbreeding because their parents' inbreeding are not known. They were collected in the brackishwater ponds around Lingayen Gulf. The breeders used in the production of H1 were different from the breeders used in M1. The hybrid 2 (H2) was produced from the backcross of M1 to H1. Where the two parents are not related, the H2 have zero inbreeding. The *O. mossambicus* 2 (M2), was produced from the rotational crossing. Whereas the H2 and M2 have common ancestor, the M1. The H2, M1 and M2 were involved in the production of hybrid 3 (H3). Inbreeding values of H3 show that the previous 12.5% heterozygous genes become homozygous. Comparing the three hybrids, H3 will not fit as base population for selection due to its high inbreeding values. The allowable inbreeding values are 5-10% (Tave, 1990).

Transmission of salinity tolerance gene

The heterotic effect shows that transmission of salinity tolerance gene was additive inheritance (polygenic). The nearly zero heterosis of reciprocal hybrid 2 and reciprocal hybrid 3 shows an additive inheritance. However, maternal/paternal inheritance should also be considered due to the difference of salinity tolerance between the reciprocal hybrids, where maternal inheritance was observed in reciprocal H1 and reciprocal H2, and reciprocal H3 was paternal inheritance.

Social behavior of tilapia during salinity increase

During the salinity increase from 0 to 126 ppt, the pure *O. mossambicus*, reciprocal hybrid 2 and reciprocal hybrid 3 were less dominant or had lesser numbers of dominant individuals compared to pure *O. niloticus* and reciprocal hybrid 1. *O. niloticus* and H'1 were increasingly dominant (aggressive) in 30-36 ppt with 10-30% and 10-60% of the population, being dominant respectively. They were more aggressive in this stage compared with H1, which were already docile in this salinity; the reciprocal hybrid 2 and reciprocal hybrid 3 at 10-20% of the population were dominant; and the *O. mossambicus* had no dominant individuals at all. Starting at 48 ppt, there were no dominant fish observed in the reciprocal H2, reciprocal H3 and *O. mossambicus*. While, Hybrid '1 still had residual dominant individuals up to 96 ppt in one of the replicates at one out of 40, which is 2.5%.

Conclusions

The salinity tolerances of offspring were lower than their saline-tolerant parental stocks but increased through backcrossing with their saline-tolerant parent. *O. mossambicus* had the highest salinity tolerance, while the parental *O. niloticus* had the lowest. There was an increase in salinity tolerance of the offspring when they were backcrossed to the saline tolerant parent *O. mossambicus*. The average salinity tolerances of the hybrids measured using MST were 106.5ppt for H1; 113.2ppt for H2 and 109.9ppt for H3 and as MLS were 89.2ppt for H1; 78.4ppt for H2 and 98.2ppt for H3 and as OST were 104.3ppt for H1; 110.8ppt for H2 and 109.0ppt for H3 (Table 4, 5, 6). The reciprocal H1 were significantly different with each other, while the H2 and H3 were not significantly different to their reciprocals. The OST values for H3 were not significantly different from the saline-tolerant parent *O. mossambicus*, indicating that there was an increase of salinity tolerance during backcrossing.

There was a significant linear relationship between size and their salinity tolerance in the H'3 and H'2, that larger fish survives longer in elevated salinities. In selection for growth rate, salinity tolerance might be indirectly selected, due to high correlation of the two traits.

All hybrids displayed heterosis. H1 showed the highest heterosis. The H2 and H3 had a negative and slight positive heterosis. A heterosis closer to zero (additive inheritance) is a good candidate for selection. On this basis, H2 and H3 are good candidates for selection. However, we cannot rule out H1 to contribute its share in the base population, because not all heterosis is governed by dominance-recessive effects, but also additive and epistatic effects which is very important in quantitative selection. Furthermore, heterosis is not only additive effect, but multiplicative effect.

Hybrids 1 have zero inbreeding value because they are the result of crosses between two different species of *Oreochromis*; Hybrids 2 have a zero inbreeding, because there is no common ancestor; Hybrids 3 have 12.5% computed inbreeding values, due to the common ancestor. These results show that hybrids 3 will not fit as base population for selection. However, we cannot rule out H3 as base population for selection because of their high salinity tolerance and considerable heterosis. Furthermore, not all inbreeding produces bad traits; there are positive contributions of inbreeding in the development of stocks. Actually, selection process is a type of directed inbreeding.

Reduced dominance of dominant individuals was observed at higher salinities. At 30-36ppt, the *O. niloticus* and H1 were increasingly aggressive. The H1 hybrids still had residual dominant individuals until 96ppt. For other groups – H2 and H3 hybrids and pure *O. mossambicus*, dominance disappeared at 48ppt. The dominance of some individuals in *O. niloticus* and H'1 resulted to early mortality, because subordinant individuals were not able to feed well and were stressed. The H2 and H3 hybrids were peaceful and had their share of food that resulted to longer survival.

Hybrids 1 will not fit as base population for selection due to their high heterosis and aggressiveness. The aggressiveness of Hybrids 1, creates stress to other fish and prevents them from feeding. Aggressive individuals utilize more energy that will result to slow growth. This character might be transmitted to the next generation during selection.

Prior to mortality, there was loss of dominance among dominant individuals, cessation of feeding /minimal food intake, sunken eyes and abdomen as a sign of the fish' inability to osmoregulate. Color changes from silver to intensified barring or black coloration were observed. Some fishes were swimming upside down; others were gasping air in the corner of the aquaria while others were resting in the bottom of the aquaria. A minute before they died, a darting motion in an unpredicted direction was observed. Contrary to other reports, hyperplasia, septicemia and swollen eyes were not observed.

Finally, there was increase in the salinity tolerance of the hybrids as they were backcrossed to the saline tolerant parent (*O. mossambicus*). Reciprocal H1 had the highest heterosis compared to H2 and H3. H1 still had dominant individuals up to 96 ppt, compared to H2 and H3 where dominance disappeared at 48 ppt. H2 and H3 hybrids were found to be good candidates as base population for selection.

Recommendations

1. Reciprocal Hybrids 1, reciprocal Hybrid 2 and reciprocal Hybrid 3 can be used as base population for selection. Option 1: Hybrid 2 = 70%, Hybrid 3 = 20%, Hybrid 1 = 10% of the base population of selection; Option 2: Hybrid 2 = 60%, Hybrid 3 = 40% of the base population for selection; Option 3: Hybrid 3 = 100% of the base population for selection; Option 4: Hybrid 2 = 100% of the base population for selection.

2. Size and salinity tolerance was significantly correlated in H'2 and H'3. Selection for growth is recommended; salinity tolerance might be indirectly selected in growth selection.

Acknowledgement

The following agencies are acknowledged: BFAR; PCAMRD for awarding me the scholarship under the PCAMRD Graduate Research Fellowship Program (PGRFP); UPV-CFOS; CIRAD – France for its support in the tilapia genetics (Molobicus) project at BFAR in Bonuan-Binloc, Dagupan City; the BFAR-National Integrated Fisheries Technology

Development Center (NIFTDC) and BFAR-National Freshwater Fisheries Technology Research Center (NFFTRC) for providing me with the working space, materials and equipment as well as experimental fish; the SEAFDEC-AQD for its good collection of books and references in fisheries.

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