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The domestication of *Oreochromis mossambicus* in a breeding program in Mozambique

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Master of Sciences in Aquaculture

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Abstract

This study describes the establishment of a baseline and a base camp for the development of a genetic enhancement program for the Mozambique tilapia, *Oreochromis mossambicus*, with the scope of utilising both additive and eventually non-additive genetic effects as well as maternal effects. The selection program is carried out at CEPAQ (Centro de Pesquisa em Aquacultura, translated as the Research Centre in Aquaculture) located in Chókwè, Gaza province, in the southern region of Mozambique for the development of the aquaculture industry on this region and with especial focus on the "Terra Morta" (dead soil) around Chókwè area and the costal regions of the country. This project comprises the collection of suitable broodstock of wild pure *O. mossambicus*, the setup of a breeding scheme, management of breeders, pond management, mating strategies, production of full sib and half sib groups, tagging, recording of traits and tissue samplings for DNA-typing and genotyping. In summary 2055 breeders were collected from 12 different lagoons and rivers and grouped in 5 strains. Out of them 428 successfully contributed to produce a total of 418 families and 10350 fry following a full diallel crossbreeding scheme of 25 combinations. After a grow-out period of 103 days the average body weight (ABW) was 85,9 g with a survival rate of 73,6%. The ABW of males was 105,9 g while the ABW of females was 61,6 g. The 2272 largest fish at harvest were selected, tagged and sampled a total of 1128 males and 1144 females. The ABW for the selected males was 131,7 g and 78,4 g for the selected females Preliminary results from the genetic analysis shows that the different subpopulations of *O. Mossambicus* collected along the country can be sorted as 3 different genetic groups, It was also possible to develop a pedigree assignment for the evaluation of the strains. The best growth potential is shown by the 2 strains from the south, A and C, and their crosses.

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Introduction

Aquaculture is a traditional food production system that during the last decades has become increasingly important for global food supply and is growing faster than any other animal production (FAO, 2018). Tilapias are the most common fish species cultured in the world (FAO, 2018), mostly in tropical and subtropical regions, where natural conditions allow the production in a wide range of systems, from extensive and semi extensive systems based on green water and low-quality feed, to more intensive ones with formulated feed and water treatment by either aeration or flow-through.

Tilapia is the common name used for many cichlids species native from the African continent compiled mostly in three genus, *Tilapia, Oreochromis*, and *Sarotherodon,* (Nelson, 2006; Chapman, 1992). Three species and their hybrids are by far the most commonly used for aquacultural purposes, the *Oreochromis niloticus, Oreochromis mossambicus* and *Oreochromis aureus* (Chapman 1992). Those species have been widely used, both inside and outside of their natural range of distribution but many times they have been intentionally introduced on natural water systems or they have escaped from the farms establishing feral populations in the natural environment and thus have become a major threat for biodiversity (Canonico *et al*, 2005). The introduction of alien species, together with habitat loss, are the major threats for biodiversity nowadays, according to World Wildlife Found (WWF) and the United Nations (UN), due to displacement of native species, habitat and resources competition, predation and, in the case of the Tilapias in African regions, by hybridization with other native tilapia species (Rhymer *et al*, 1996).

The actual policies of the international community, as the ones compiled on the Sustainable Development Goals of the United Nations, and the advice of international experts, recorded i.e. in the Nairobi declaration (Kenya, 2002), points out that development of human welfare and creation of new industries must only occur together with the protection of the environment and its biodiversity (FAO 2018). In the case of many African regions, where aquaculture may play an important roll to cover the demand of affordable high quality protein to reduce hunger, malnutrition and poverty (FAO 2013,FAO 2014), the use of local available tilapia species that may have a potential for food production should be considered. This may be possible by

implementing genetic enhancement programs for local species based in prior well-documented methods, such as demonstrated in the GIFT program (Acosta, B.O. *et al*, 1997, Ponzoni *et al.,* 2007) and others (Workagegn & Gjøen, 2011). This may bring forth fast growing fish strains in a relative short period of time, which could suit the interests of commercial and non-commercial projects and, at the same time, comply to the ethical and ecological policies mentioned above.

There are several ways to develop genetic enhancement programs to utilise the potential genetic diversity of the different species. The most common genetic enhancement programs utilised for fish until now are the ones based on utilising only additive genetic effects, in which, through selection of the best individuals of each generation, an accumulative genetic gain is obtained in every new generation, leading to better performances, step by step. Other breeding systems are focusing more on utilising non-additive genetic effects. Those compromise the identification and production of different genetic lines that, afterwards, are finally crossbred to produce mix lines with better characteristics than the average of their ancestors. It is typically used in plants and livestock animals like poultry and pigs, but not so much, until now, in fish, except some carps, e.g. (Gjerde, B. *et al*, 2002; Linhart, O. *et al*, 2002), and are for the appropriate species commercially interesting as this can provide a certain genetic lock, by what by some is called F2-breakdown, to protect economic investments.

There are different strategies to test the potential of the different subpopulations, or strains, at the beginning of a breeding program, and this will give very valuable information to improve both short and long term results. Those may be considered according to the practicalities of each program as they may further suggest different strategies to achieve those results. The simplest approach is to only compare the pure strains, i.e. no crossbreeding, whereas the use of full diallel reciprocal cross breeding schemes allows also the identification of non-additive genetic effects, as heterosis and epistasis, and reciprocal effects, i.e. maternal effects, that can add genetic gains to the traditional programs, which often are based only on additive genetics. Also the use of modern biotechnology tools as genotyping together with the use of reliable tagging systems, usually PIT-tags, that are getting more and more affordable every day, provide improvement on the accuracy of the breeding value estimation, and may thus add new

opportunities by e.g. crossbreeding or faster genetic gain in this kind of programs, as described above.

For the establishment of such programs it is first necessary to get enough genetic material with a wide genetic diversity. This will ensure the presence of genetic variation for desirable traits, as fast growth or disease resistance, allowing long-term selection response, if inbreeding is avoided by appropriate measures like restriction of selected per family or the use of optimal contribution procedures (Skaarud *et al*, 2011). Although a considerable number of strains (>30) were sampled at the start-up of the Norwegian breeding program for salmon (Gjedrem *et al*, 1991), the GIFT-program in the Philippines (8 strains) (ref. used earlier) and also computer simulations (Holtsmark, 2007) has shown that a sufficient number of different strains, or subpopulations, needed to ensure the success of a genetic enhancement program more likely can be as low as 4, given that the genetic diversity of each of the different strains are wide enough (Holtsmark, 2007). However, it may happen that different subpopulations of a species may have different levels of genetic diversity according to their history and degree of isolation (Lande, R. 1988). For instance farmed strains with a poor control of their breeding history or wild populations that may have gone through adverse natural events, as droughts, may have led into what is called a "bottle neck effect", where the whole population may be descendant of very few individuals. In such cases the genetic variation may have been eroded, resulting in very high levels of homozygosis due to continued inbreeding. High inbreeding levels are considered as non-desirable as it can also result in higher percentages of hereditable diseases, malformations, loss of fitness and lower yields in production traits (Akinoshun, 2015).

In the particular case of Mozambique, The Mozambique tilapia, as it name suggests, is a very common specie in the country, naturally present in most of the water systems, from small lagoons to rivers and their estuaries, in a wide range of water parameters and conditions (Skelton 2001). Its natural spreading range comprises several river basins of South-East Africa, from the Eastern Cape (South Africa) in the south, to the Zambezi River basin in the northern part of its range (Skelton PH 2001). It has many desirable characteristics for it use in aquaculture, e.g. high capacity to adapt to new environments or different farming systems, early sexual maturation, high fecundity and fast growth

potential, among others (Gupta, M.V. *et al.* 2004). It is also an euryhaline species and has a remarkable capacity to thrive in very different degrees of salinities, from fresh to full sea water (Trewavas *et al*, 1983) and even hyper saline conditions (Robins *et al* 1991). This makes it very attractive for aquaculture, especially in areas where the salinity of the available soil or water makes it unproductive for e.g. agricultural purposes.

Aside of being listed as one of the 100 worst world invasive alien species by the International Union of Conservation of Nature (IUCN) it is also classified as ‘‘Near Threatened’’ on the IUCN Red List in its natural spread range, mostly because of its hybridization with the widely introduced *O. niloticus*, native to the Nile-Sudanic region (Cambray *et al* 2007). In Mozambique, the introduction of *O. niloticus* and its hybridization with the *O. mossambicus* is well documented, at least from the 90´s, in the Limpopo river basin (D´Amato *et al* 2007, Van der Bank *et al*, 2007). Since then, successive introductions have been reported (Van der Waal B, *et al*, 2000). Even the Mozambican authorities have tried to develop small-scale aquaculture and to improve the quality of their inland fisheries stocks by releasing *O. niloticus* fingerlings in different water systems, mostly in the southern provinces of Maputo, Gaza and Inhambane, but also further north. These introductions are not well documented and the real status of the wild populations of *O. mossambicus* nowadays remains unknown. But presumably, due to the still limited distribution of the aquaculture industry in the country and the difficult access to large unspoiled and remote areas, it is still possible to find several rivers and lagoons that have remained isolated and not influenced by such planned or unplanned distribution of *O. niloticus* where pure populations of *O. mossambicus* still persist (Firmat C. *et al*, 2013).

The goal of this study is to establish a base camp and a base line at CEPAQ for the evaluation of different locally available wild populations of *O. mossambicus* to develop commercially reliable strains for aquaculture to promote the industry in the country avoiding the dispersion of alien species.

Materials and Methods

The facilities for the development of the breeding program (CEPAQ)

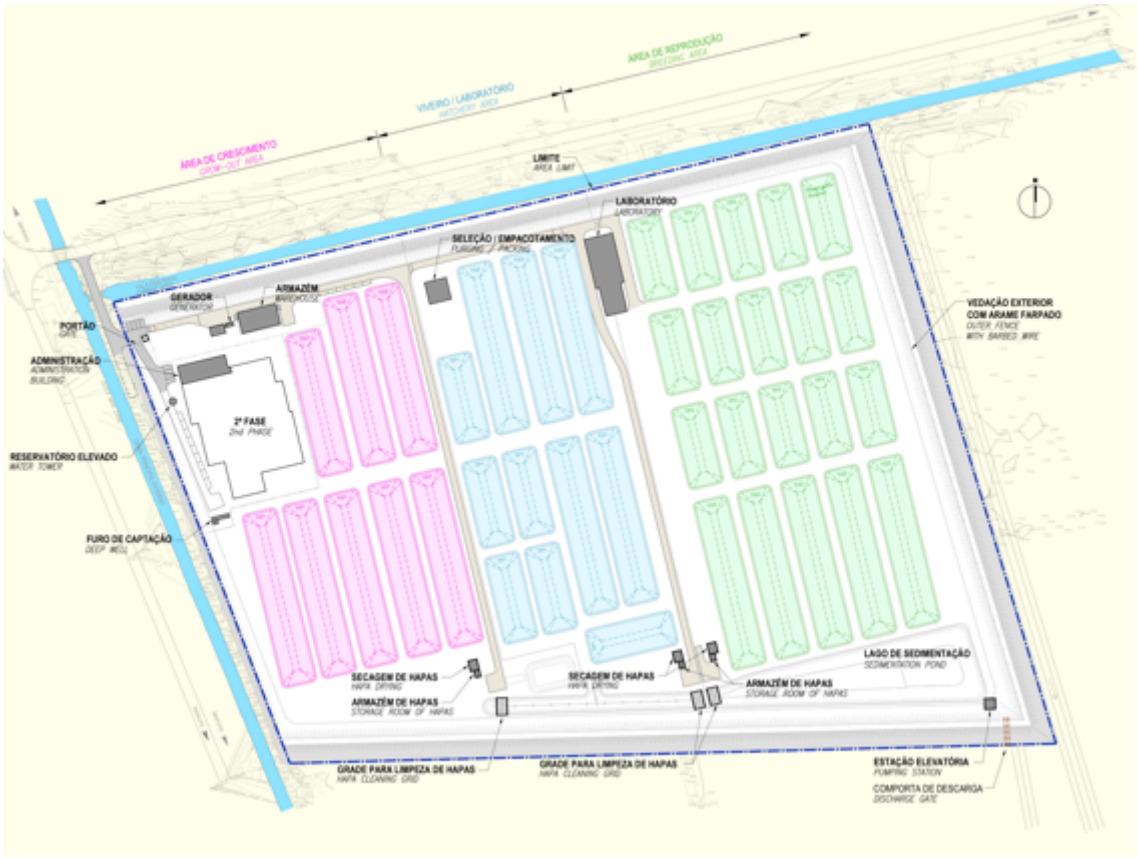

Figure 1- CEPAQ layout

The Centro de Pesquisa em Aquacultura (CEPAQ), translated as The Research Centre in Aquaculture, is a facility promoted and founded by the governments of Norway, Iceland and Mozambique in the municipality of Mapapa, on the district of Chókwè in Gaza province, on the southern region of Mozambique, to develop the aquaculture sector in the country with special focus on the "Terra Morta" area, translated as "Dead soil", which refers to an area of approximately 10 500 Ha irrigated land not suitable for agriculture due to high levels of salinity on the soil but suitable for tilapia pond farming, and eventually the coastal regions of the country.

The centre has a surface of approximately 14 Ha and its connected to an irrigation canal which supplies fresh water to the Chókwè area coming from the Massingir

Dam located on the Rio dos Elefantes (Olifants River). The centre is also having its own supply of underground water, but this is salty, with around 8 g of salt per litre.

The centre is divided in three areas, the Genetic Enhancement Area, designated to conduct a program for genetic enhancement of the *Oreochromis mossambicus,* a Hatchery Area, to produce sex reversed fingerlings, and a Grow-out Area for development of protocols and as training area (see Figure 1).

The Genetic Enhancement Area at CEPAQ has a surface of approximately 4,5 Ha. It contains 20 earthen ponds where 15 of them have an average capacity of 500 m^3 and are used for mating, nursery and grow-out. The remaining 5 have an average capacity of 1162 m^3 and are used for holding the breeders. It also has a building with 2 offices, 1 lab room, that is now used for several purposes as making hapas and as a resting area for the workers, 1 feeding room and 1 hatchery room which contains an artificial incubator with 40 individual jars of 250 ml (see figure 2)

Figure 2 – Artificial Incubator (AI) at the Genetic Enhancement Area

Collection of the strains

Figure 3 – Map of collection

For the establishment of the genetic enhancement program of *O. mossambicus* at CEPAQ, one of the biggest challenges was the process of collecting breeders of pure *O. mossambicus* from the wild environment. It implied literature review and interviews

with local entities, communities and aquaculture companies to identify areas where the presence of *O. niloticus* was confirmed and others where the chances of finding pure *O. mossambicus* where higher, as they were without signs of any kind of aquaculture activity or releasing of fingerlings. Also, this phase of the project was conducted in the middle of a drought, which lasted for several years, thus many of the lagoons where it was expected to still find pure *O. mossambicus* were dry or nearly collapsing. It was decided that, by preference, sexually mature fish should be collected from at least 5 different locations, to ensure better genetic quality, as they had gone through natural selection, and to also speed up the process of mating. Finally, sexually mature specimens of *O. mossambicus*, but also some juveniles on some of the places, were collected from 12 different locations belonging to 5 different hydric basins of the southern and central regions of Mozambique from October 2016 until June 2017 see figure 3)

Figure 4 – Collection of wild O. mossambicus

The fish were collected using different methods like seine nets, gill nets, fish traps and hooks, depending on the different social, technical and natural conditions of each one of

the locations. We needed to adapt to the policies and traditions of the local communities, as well as the local fauna, as e.g. crocodiles and hippos were present in some on the places. The protocol was to hire local fishermen, in coordination with the local community leaders, to collect the fish, as they knew the area, its risks and the behaviour of the fish and other relevant animals. The fishermen were taught briefly and effectively about how to handle the fish to keep them alive, to reduce stress and to increase the survival rate during transport and later acclimation at CEPAQ. Most of the advices were related to how to avoid damage on the fish mucus, scales and gills when releasing them from the nets, among others.

On 3 of the collection places, 3 different local private companies related to aquaculture (Aquapesca Lda, Agropecus Lda and Xibaha Lda) provided fish collected from their surrounding area. The total numbers of fish collected per location are shown in table 1.

Table 1 – Number of fish collected per location and strain assignment

Strain	Origen	Province	Number of Males	Number of Females	Total Number of Fish per Origen	Total Number of Fish per Strain
A	Sotiva	Maputo	184	162	346	346
B	Aquapesca Lda	Zambezia	227	125	352	594
	Bons Sinais	Zambezia	75	167	242	
C	Catuane	Maputo	105	114	219	219
D	Agropecus Lada Sumbanene	Inhambane	116	66	182	538
	Marrangua	Gaza	207	150	356	
E	Govuro	Inhambane	46	35	81	357
	Xibaha Lda	Inhambane	13	11	24	
	Govuro 3	Inhambane	23	13	36	
	Nhawanza	Inhambane	51	31	82	
	Ximite	Inhambane	65	65	130	
	Makuri	Inhambane	1	3	4	
5	12	4	1113	942	2055	

Transport of fish

The transport of the fish was done mostly in a closed water tank of 500 l, placed at the back of a 4x4 pickup and filled with water from the collection place and continuously aerated by an air-blower and air stones powered by a portable petrol generator. To fill the tank it was used a portable electric pump powered by the petrol generator and a hose. A few times this procedure was done manually with buckets due to technical issues. Water parameters in the tank, as dissolved oxygen and temperature, were continuously monitored from inside the car by using a portable multiparameter device with a sounding line. Ice blocks were added to low down water temperature when needed. Also a rectangular piece of expanded polystyrene floating on the tank was used to avoid waves.

In a few occasions, fish were transported by using plastic bags filled with 1/3 of water and 2/3 of pure oxygen closed with elastic rubber bands. We used double bags with some newspaper sheets in between them. It helped to reinforce the plastic, to reduce the stress on the fish and, at the same time, as an indicator if any leakage appeared on the inner bags.

Figure 5 – Transport of fish

Acclimating of fish

Fish newly collected were stocked in 8 concrete tanks of 4 m^3 for 7 to 10 days, filled with underground water (3-8 ppt of salt) continuously aerated. During this time, fish were monitored and adapted to eat artificial feed and as well to the presence of people. Once the fish were in good condition (healed, normally eating artificial feed and following the feeder around the tank) they were transferred to holding hapas in earthen ponds at the genetic enhancement area of CEPAQ.

Holding and management of fish at Genetic Enhancement Area

Fish at the genetic enhancement area were held in hapas of 2,5 m^3 in earthen ponds with a maximum density of 3kg/m^3 per hapa. The different strains were hold in separate ponds. Fish were sorted by sex and size to avoid reproduction and to monitor growth. They were sampled monthly for feeding adjustments, to control health status and performance. Fish were fed with commercial artificial feed according to their body weight (BW). The smallest ones (<60g) were given 5-6% of their BW per day, while the biggest ones (>250g) were fed only 1-1,5% of their BW per day to avoid too big differences in sizes at the moment of mating. The rest were fed with 3% of their BW.

Tagging and recording of traits

Out of the 2055 wild breeders that were stocked at the genetic enhancement area, 921 were selected randomly from the 5 strains among the ones that were inside of the desirable size range for reproduction (>60 g) and had optimal health status. They were tagged using PIT-tags. For that procedure, the fish were anesthetised using a solution of clove oil and ethanol (10ml/l) mixed with water in a 10 l basin continuously aerated (5-6ml/l). Once the fish had lost conscience (loosing the floating line, turning the belly up, and not giving any sign of movement when handled), the PIT-tags were inserted intramuscularly by using an injector with a needle on the left side of the fish, in parallel to the middle line under the 2nd or 3rd scale at the 6th-9th dorsal fin ray counting from the head. Before and after the tagging, the area of injection was disinfected using a commercial solution of povidone Iodine (10%) in gel applied with a piece of cotton. The needles were also disinfected in between each fish by sinking them in pure ethanol. After the tagging, the fish were measured and then transferred to a basing with clean fresh water well aerated for recovery.

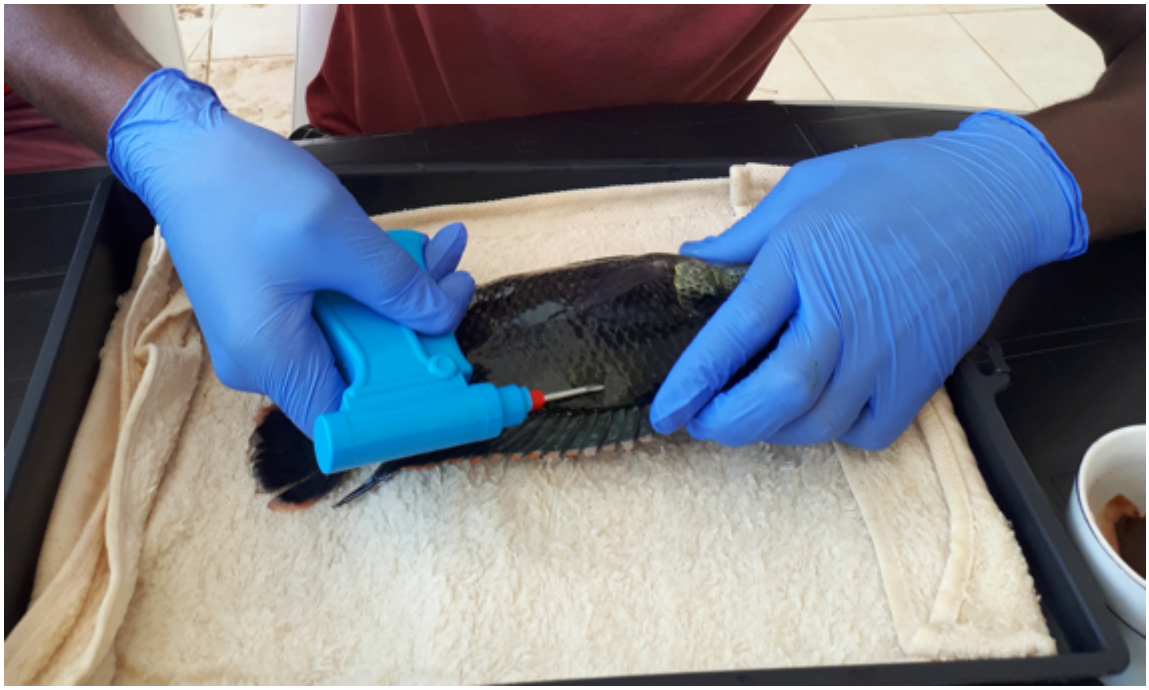

Figure 6 – Tagging

Table 2 - Number of fish tagged per strain

Strains	Nº of Males	Nº of Females
Strain A	83	71
Strain B	96	93
Strain C	105	102
Strain D	73	88
Strain E	106	104
Total	463	458

For every fish the following traits were recorded:

-Sex (Male/Female)

-Weight (g)

-Total Length (cm)

-Standard Length (cm)

-Head Length (cm)

-Body Depth (cm)

-Body Width (cm)

Genetic evaluation of the strains

It was planned to test the fish to confirm that just pure *O. mossambicus* would be used for reproduction before to start mating but, due to technical issues, the test was delayed so the fish from 8 of the locations were selected and grouped into 5 different strains for mating according to the phenotypic traits and the information available according to their origins as follows (see table 3).

Table 3 - Strain assignment

Strain	Origen
A	Sotiva
B	Bons Sinais
C	Catuane
D	Marrangua
E	Govuro
	Govuro 3
	Ximite
	Makuri

After mating, tissue samples from the caudal fin of all successful breeders were collected, preserved in pure ethanol and stored in a freezer until they were sent for genotyping to AgResearch Ltd. on New Zealand. The same have been done with 100 samples of *O. niloticus* from the GIFT strain located at the Department of Animal and Aquacultural Sciences of the Norwegian University of Life Sciences (NMBU), Aas, Norway. Those results will be later confronted to determine if there is any kind of genetic relation that may confirm the hybrid degree or the pureness of fish.

Mating Design

A full diallel reciprocal cross design was set to test 5 different strains (see table 4). A total of 25 combinations, 5 pure and 20 crossbred, were targeted. 250 males and 250 females were planned to be used. All breeders, males and females, were allowed to mate with 2 different partners (3 in some cases) from different strains in different batches to improve the accuracy of their genetic values. A total of 20 families of each of the 25 combinations (500 families in total) were targeted (see also Appendix).

Table 4- Breeding matrix

Females\Males	Strain A	Str. B	Str. C	Str. D	Str. E
Strain A	AA	AB	AC	AD	AE
Str. B	BA	BB	BC	BD	BE
Str. C	CA	CB	CC	CD	CE
Str. D	DA	DB	DC	DD	DE
Str. E	EA	EB	EC	ED	EE

Mating procedure

In order to maximise the utilization of the facilities a system of batches was chosen to produce the 500 families that were targeted. A total of 10 batches were produced where each of them consisted in 50 hapas where 2 replicas of each of the 25 combinations were represented. 1 male and 1 female were set on each of the hapas. Rotation of 3 sets of 50 hapas in 3 ponds of 500 m^3 was used (see figure 7). The mating period of each batch had a maximum duration of 3 weeks since the moment where the mating couples were set in the mating hapas until the last female was removed from the mating hapa and the fry transferred to the rearing hapas.

Figure 7– **Breeding ponds with the three sets of 50 mating hapas installed**

Each batch was set more or less weekly from October 2017 to January 2018 according to technical issues (see table 5).

Table 5 – Date of setting the fish on the mating hapas

Batch Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Date of setting	11-Oct	19-Oct-	26-Oct-	02-Nov	13-Nov	23-Nov	27-Nov	21-Dec	31-Dec	09-Jan

After being conditioned for 2 weeks where the feed rate was slightly increased to 4% and the feed was mix with some vegetable oils, 50 males and 50 females were set into breeding hapas of 1 m^3 with a sex ratio 1:1 in earth ponds of 500 m3. The selection criteria for breeders at the moment of mating were random on males and according to the physical signs of readiness for spawning on females (reddish and big genitals, bloated belly) (see figure 8).

Figure 8- Male (up) and Female ready to spawn (down). Note the reddish genital papilla.

On the first 5 batches all breeders were eligible, while on the last 5 batches only fish that successfully contributed on the first round were allowed to mate again, with a different partner from a different strain that also had successfully contributed (in some cases new fish were allow to spawn due to lack of successful breeders available). Some individuals were mated up to 3 times.

To avoid mortality on females, the upper libs of the males were surgically removed (under anaesthesia) and few pieces of PVC pipe were set into each hapa in order to provide refugee and protection. In case of dead fish, they were replaced with another one. If a male was too aggressive (killing more than 2 females) it was also replaced.

Since the moment of setting, the couples had 2 weeks allowance to spawn. The hapas were checked for females carrying eggs on their mouth every 3 days. When a female carrying eggs was found, the male was removed leaving the female alone in the hapa. By preference, eggs were naturally incubated by the females. In cases where females spitted the eggs or females were in low condition, the eggs were collected with a fine mesh scoop-net and transferred to the artificial incubator (AI), where they were clean and rinsed and transferred to individual jars.

After 2 weeks from the setting, all fish were removed from the breeding hapas, just allowing the females carrying eggs to finish the incubation for one more week.

Nursery

From every family, 25 fry were collected once they could swim (except on batch 1 where only 20 fry were collected per family) and transferred to rearing hapas of 1 m^3 where a mix of fry o the same age was reared. Another 50 fry were collected as a backup and split into another 2 hapas.

Once all fry from the same batch were collected, they were transferred to a communal hapa installed on the grow-out pond where they will be raised. After 1 week, all fingerlings were counted and released on the pond.

Grow-out

The grow-out period of each batch run for 103 days on average. On each batch around 1000 fry was stoked on earth ponds of 554 m3 where green water was promoted by applying inorganic fertilizer (Urea and NPK). Commercial artificial feed was used following feeding protocols provided by consultants based on *O. niloticus* performance.

On early stages, when the fish were still very small and was not possible to use floating feed, they were fed always on the same spots, two to three per pond, where a square piece of white fine mess hapa was set underwater tied to four sticks. It allowed to the feeders to spot the fish and also give time to the fish to find the feed (see figure 9)

Figure 9– Feeding fingerlings on the grow-out ponds with detail of the feeding spot

To try to avoid bird predation, the ponds were covered with nylon lines with pieces of feeding bags hanging on them. The lines were tied to a thicker rope that was set on the perimeter of each of the ponds.

Around 100 fish were sampled every 2 weeks on each pond, starting after the 8th week of the grow-out period, for feeding adjustments. To do that, the pond was partially seined to conduct the fish into a big hapa where they were trapped (see figure 10). Then the fish were collected in big buckets and transferred to the sampling area located at the back of the hatchery room.

Figure 10- Partial harvest for sampling on the grow-out ponds

Harvest and Selection

At the moment of harvest, the pond was partially drained and then all fish were collected with a seine net. The seining could be repeated several times. After most of the fish were harvested, the pond was drained completely and the remaining fish were collected by hand.

For selection, fish were counted and sorted by sex and weight on different basins continuously aerated. The fish were sorted by weight according with the last sampling, setting 3 categories, big, medium and small fish either for males and females. After the first round of selection, a second one and even a third one could be done to get the exact numbers needed for tagging. After that, the selected fish were hold in hapas for resting for 1 or 2 days until tagging. The non-selected fish were culled and distributed among the workers.

Tagging and DNA sampling

The best 115 males and 115 females from each batch were measured and tagged with PIT-tags. DNA samples were collected from the caudal fin (see figure 11). The samples were kept on small tubes on 12x8 trays filled with pure ethanol and then stored on a freezer.

Figure 11– Fin sampling setup and caudal fin after sampling

Holding of selected fish

The selected fish from all batches were sorted by sex and split into 2 groups with same numbers of individuals and released into 2 ponds of 1162 m^3 divided by double nets to avoid reproduction and covered with nylon lines to avoid bird predation..

Results

Mating results

The mating process ran from October 2017 until January 2018. Of the 500 families targeted, 418 were successfully produced, i.e. 84%. (See table 6)

Table 6 – Number of successful mattings and families produced per batch

Batch Nº	1	2	3	4	5	6	7	8	9	10
Successful spawns out of 50	41	41	44	37	41	42	43	40	42	47
Success (%)	82%	82%	88%	74%	82%	84%	86%	80%	84%	94%

Nursery results

A total of 10350 fry were collected from the mating hapas. The survival rate after the nursery stage was higher than 90 %, but the count of fingerlings shows that there were some errors while counting fry, at least in batch 3, 5 and 9 (survival higher than 100 %). (See table 7)

Table 7 - Number of fry collected and fingerlings stocked per batch

Batch Nº	1	2	3	4	5	6	7	8	9	10
Total fry collected	850	1025	1100	1000	1025	1050	1075	1000	1050	1175
Total fingerlings stocked	776	978	1102	991	1357	1028	982	948	1080	1105
Survival (%)	91,3	95,4	100,2	99,1	132,4	97,9	91,4	94,8	111,2	97,9

Grow-out results

A total of 10347 fingerlings were stock into 10 ponds (around 1000 per pond). The average body weight was 0,32 g at the moment of stocking. (See table 8)

Table 8 – Average Body Weight

Batch Nº	1	2	3	4	5	6	7	8	9	10
Date of Stocking	13 - Nov	19 - Nov	28 - Nov	05 - Dic	16 - Dic	26 - Dic	30 - Dic	25 - Jan	5 - Feb	11- Feb
Number of fingerlings	776	978	1102	991	1357	1028	982	948	1080	1105
ABW (g)	0,23	0,33	0,38	0,51	0,27	0,28	0,22	0,40	0,21	0,43

The average days of culture was 103 while the average survival was 73,6 %.(See table 9)

Table 9 - Days of culture of each batch and survival

Batch Nº	1	2	3	4	5	6	7	8	9	10
Days of culture	114	114	112	112	108	104	107	91	85	84
Number of fish Harvested	650	800	921	695	1018	606	866	544	554	732
Survival (%)	89,2	81,8	80,8	70,1	75,0	79,2	86,8	59,4	49,8	63,7

The ABW at the end of grow out was 85,9 g. The ABW of males was 105,9 g while the ABW of females was 61,6 g. (See table 10 and figure 12)

Table 10 - Average Body Weight of all fish from each batch

Batch Nº	1	2	3	4	5	6	7	8	9	10
ABW(g) Both sexes	95,7	106,9	90,8	78,5	78,3	85,6	91,6	74,9	62,9	93,9
ABW(g) Males	110,4	132,1	113,0	96,8	94,5	110,1	119,7	89,6	82,1	111,1
ABW(g) Females	76,7	76,2	65,8	59,4	61,1	60,3	59,4	54,9	39,9	62,1

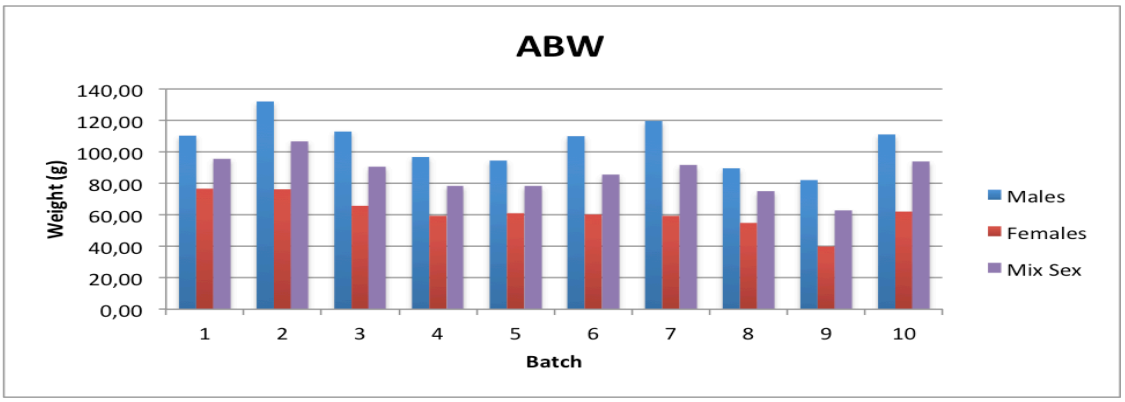

Figure 12– Graphic of total ABW

Selection of potential parents for the next generation

From every batch around 115 males and 115 females were selected based on their BW. In total 2272 fish were selected and tagged, 1128 males and 1144 females. The ABW for the selected fish was 104,9 g. The ABW of selected males was 131,7g and the ABW of selected females was 78,3 g (See table 11). The ABW of each batch is shown on the table 12 and figure 13.

Table 11 – Total number of selected fish per sex and ABW

Sex	Total Number	ABW (g)
Males	1128	131,7
Females	1144	78,4
Total	2272	105,0

Table 12 – ABW of each sex per batch

Batch Nº	1	2	3	4	5	6	7	8	9	10
ABW (g) Both sexes	111,0	127,7	115,6	88,1	99,1	100,8	126,9	92,1	72,9	115,1
ABW (g) Males	129,5	158,1	142,3	104,3	119,8	130,1	170,1	115,1	98,9	148,5
ABW (g) Females	92,2	97,0	88,7	72,9	78,4	72,8	83,6	69,4	47,7	81,7

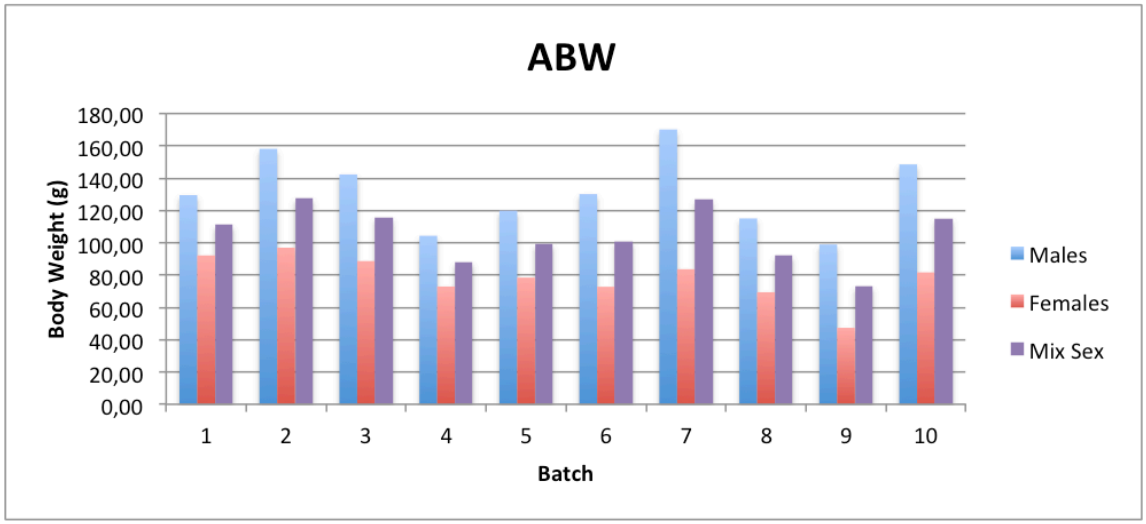

Figure 13– Graphic of ABW of the selected fish

Genotyping results

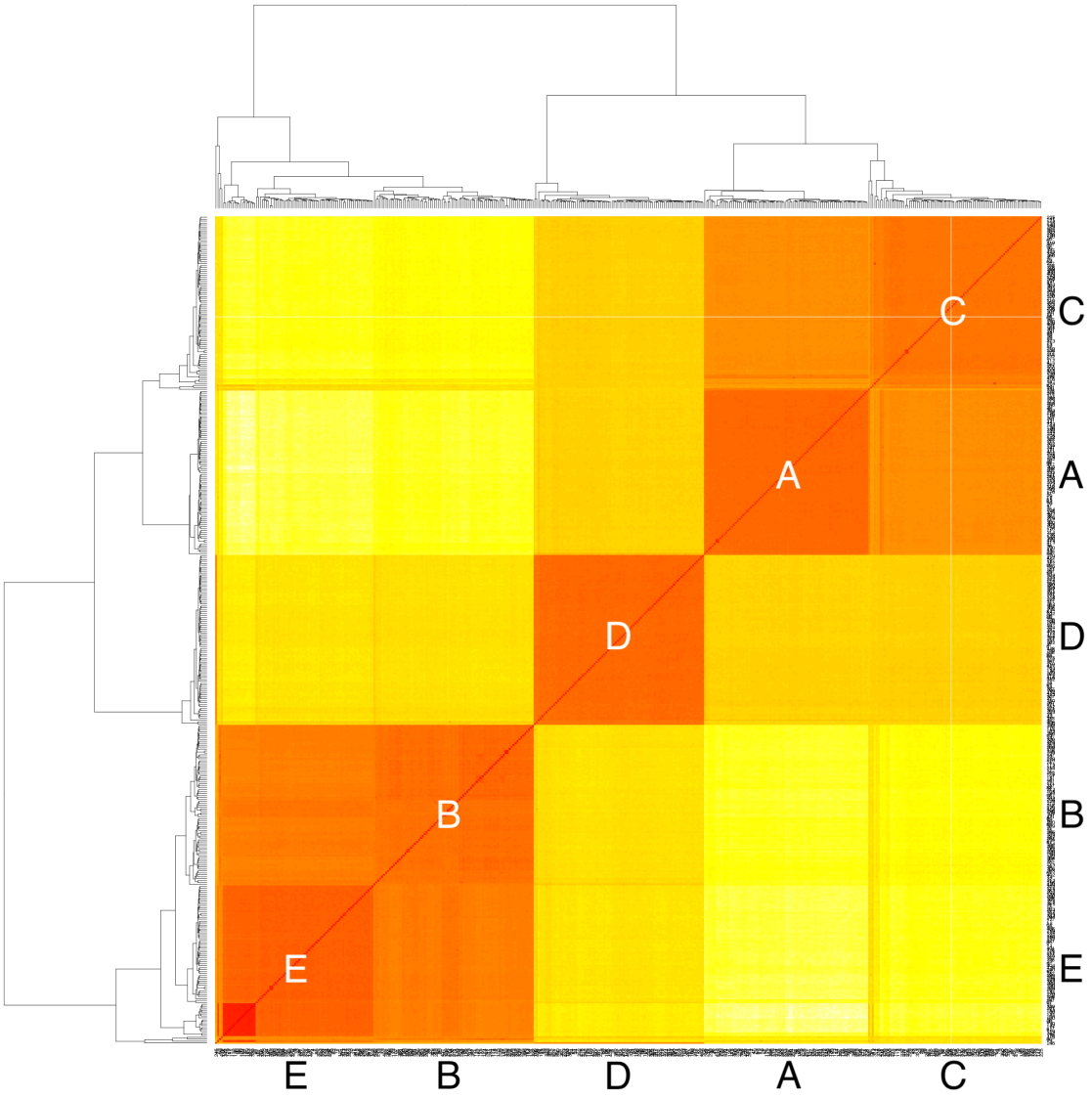

Figure 14 - Heat-map over the G-matrix with the genetic information of the successful breeders from the wild strains clustered according to their genetic distance (note the left and top margins). Red colours show high relationship while light yellow shows low relationship

The first batch of results from the genotyping belonging to the 428 wild breeders used to produce the first generation arrived in June of 2018 and they show the genetic distance of the different individuals. As can be seen from the figure, each strain can be distinguished or separated from the other, since the individuals cluster well within each strain. There were identified 36922 SNPs, but with a relatively low *Call Rate* of 0,55, which means that only 55% of called/analysed SNPs per sample/individual could be analysed. This is much lower than expected and targeted, and caused some challenges in the preceding genetic analysis. The main reason for this is believed to be the relatively large distance among the strains. It was produced a heat-map (see figure 14) where all genetic information from each individual tested was compared with all the other fish,

and according to the degree of similarities among the allelic information from the SNPs, it is possible to identify the genetic distances and assign them to different genetic groups. It is clear that the genetic distance between some of the strains is quite high, and thus easy to identify the existence of at least 3 different genetic groups.

The second batch of results, from the 2272 selected individuals from the first round of selected individuals from the F1 was available mid-August of 2018. There were identified 34722 SNPs, but with an even lower Call Rate of 0,42. With that information it was still possible to produce another heat-map to develop a pedigree assignment and also assign Breeding Values to all individuals in order to rank their genetic potential as parents for the next generation.

Strain evaluation

In all the 10 batches the fish were selected according to their BWs and only the ones with better performance were selected. That means that not all combinations were present among the selected ones in equal numbers, and this gives an idea about which strains have the best potential for growth. Thus, more than 35% of the selected individuals belonged to only two strains, A and C, and their crosses AC and CA. The total number of individuals per family and combination are given on the figures 15,16 and 17.

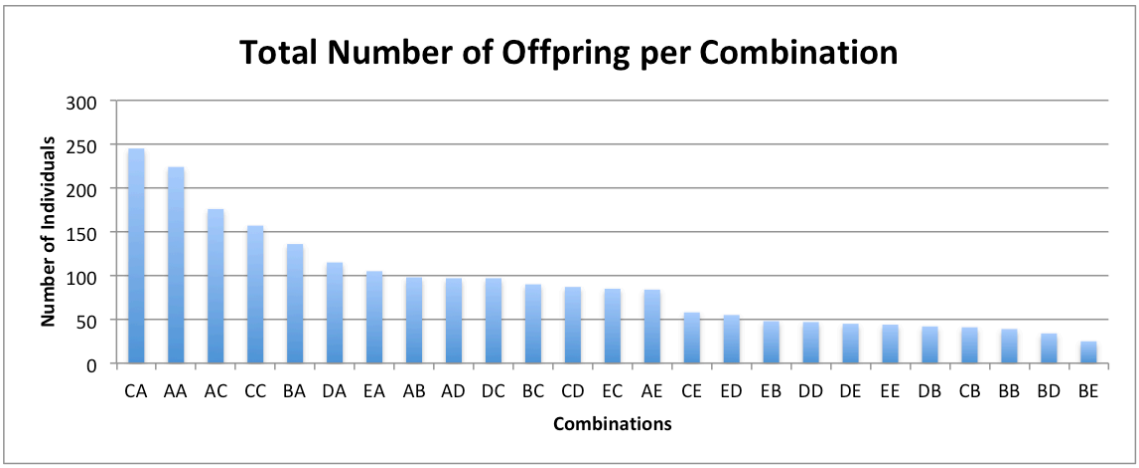

Figure 15– The 25 combinations ordered by number of individuals among the selected

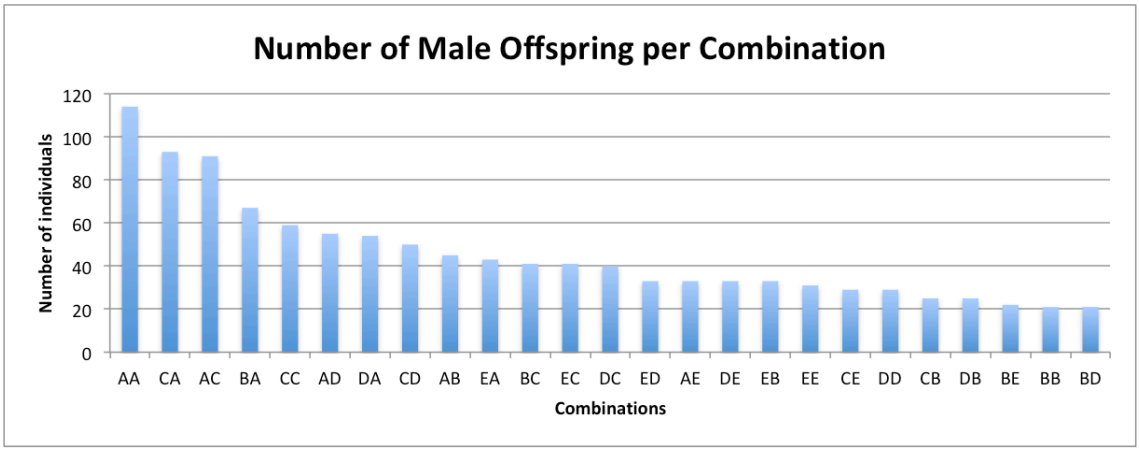

Figure 16- The 25 combinations ordered by number of Males among the selected

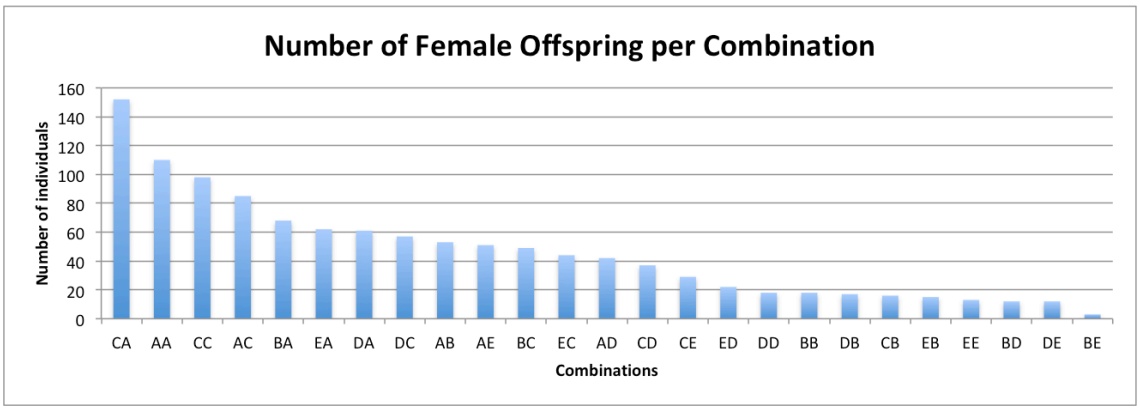

Figure 17- The 25 combinations ordered by number of Females among the selected

Conclusion

This thesis documents the process to generate the first generation employed to conduct a genetic enhancement program for pure *O. mossambicus* at CEPAQ, Mozambique. The program is based on sampling from five different locations, and the genetic study has revealed that these cluster into three fairly clear genetic groups, to be used in the proceeding analysis.

The results show that the sampling procedures have working satisfactory, as have the protocols for handling, rearing and mating the fish. Whether or not a crossbreeding scheme will be feasible are to be clearified in an associated MSc thesis that are being generated in the coming months. At the moment of delivering this work it was not possible to give a clear answer about the presence of hybrids within alien species among the wild strains of *O. mossambicus* collected and neither heterosis among the strains, so much effort should be spent on this area in order to create a solid genetic base to protect the investments and the future of the program.

Suggested further research is an evaluation of all the different populations of wild *O. mossambicus* among the different river basins inside the area of influence of CEPAQ to determine their genetic status and potential. This may be important in order to plan future strategies that may consider the inclusion of new strains on the program, the protection of certain areas or even the development of different genetic lines in order to protect the natural genetic reservoirs if the genetic distances between different populations are too high.

Also the development of feeding protocols adjusted for this new species is a must, to check their nutritional efficiency, growth potential and economic proficiency.

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Appendix

Breeding scheme for genetic enhancement of *Oreochromis mossambicus* at CEPAQ

5 strains of wild *O. mossambicus*.
250 males + 250 females.
10 batches.
Setting a new batch every week.
Rotating 3-4 breeding ponds of 1000 m^2.
Nursery of fry in 1 pond of 1000 m^2.
Stocking of fish for grow out in 10 ponds of 1000 m^2.
Holding of breeders in 1 to 5 ponds of 2000 m^2.