Salinity Tolerance of *Oreochromis niloticus* and *O. mossambicus* F1 Hybrids and Their Successive Backcross

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Significance of the Study

- **Freshwater now becoming a scarce resource, with competing use for:**
  - **Domestic or household, agriculture and power generation.**

- **Future prospect in aquaculture:**
  - **Expansion to saline waters, unfit for domestic/household and agricultural uses.**
  - **Fish cage culture in saline waters.**
  - **Alternative species for brackishwater pond culture.**

- **Tilapias are popular cultured species due to their high environmental tolerances.**
- **Tilapias posses various characteristics which make them desirable species for brackishwater farming.**

- **Consequently, for many years, tropical aquaculturists have tried to develop saline tilapia culture.**
- **Unfortunately, the true brackishwater tilapias (e.g. *O. mossambicus*) have poor-growing performance while the fast-growing strains (e.g. *O. niloticus*) are poorly adapted to saline water environment.**
- **The usual practice of using F1 hybrids of the foregoing species failed.**

Why F1 hybrids failed?

- Difficult to maintain two pure species; small production due to incompatibility of breeders; and unsustainable mass production.

- With the foregoing reasons, there is a need to produce tilapia strains that can be bred in brackishwater.

- The creation of a synthetic strain can be produced through repeated backcrossing of the saline tolerant parent to their offspring.

Why Backcrossing?

- Through backcrossing of saline tolerant parent to their hybrids, the salinity tolerance of the offspring is significantly increased.

- It creates a true breeding population that can be exploited in a selection process.

General Objective of the Study

- **To determine the salinity tolerance of the different hybrids and their pure parental species:**
  - ***Oreochromis mossambicus***
  - ***Oreochromis niloticus***
  - **Reciprocal Hybrids 1**
  - **Reciprocal Hybrids 2**
  - **Reciprocal Hybrids 3**

Specific Objectives of the Study

1. To determine an increase of salinity tolerance of hybrids as they are backcrossed to their saline tolerant parent *O. mossambicus*.
2. To determine the relationship of size and salinity tolerance.

METHODOLOGY

BREEDING STRATEGY

- Combining two species with different desirable traits:
  - *Oreochromis mossambicus* (salinity tolerance), and
  - *Oreochromis niloticus* (fast growth rate).

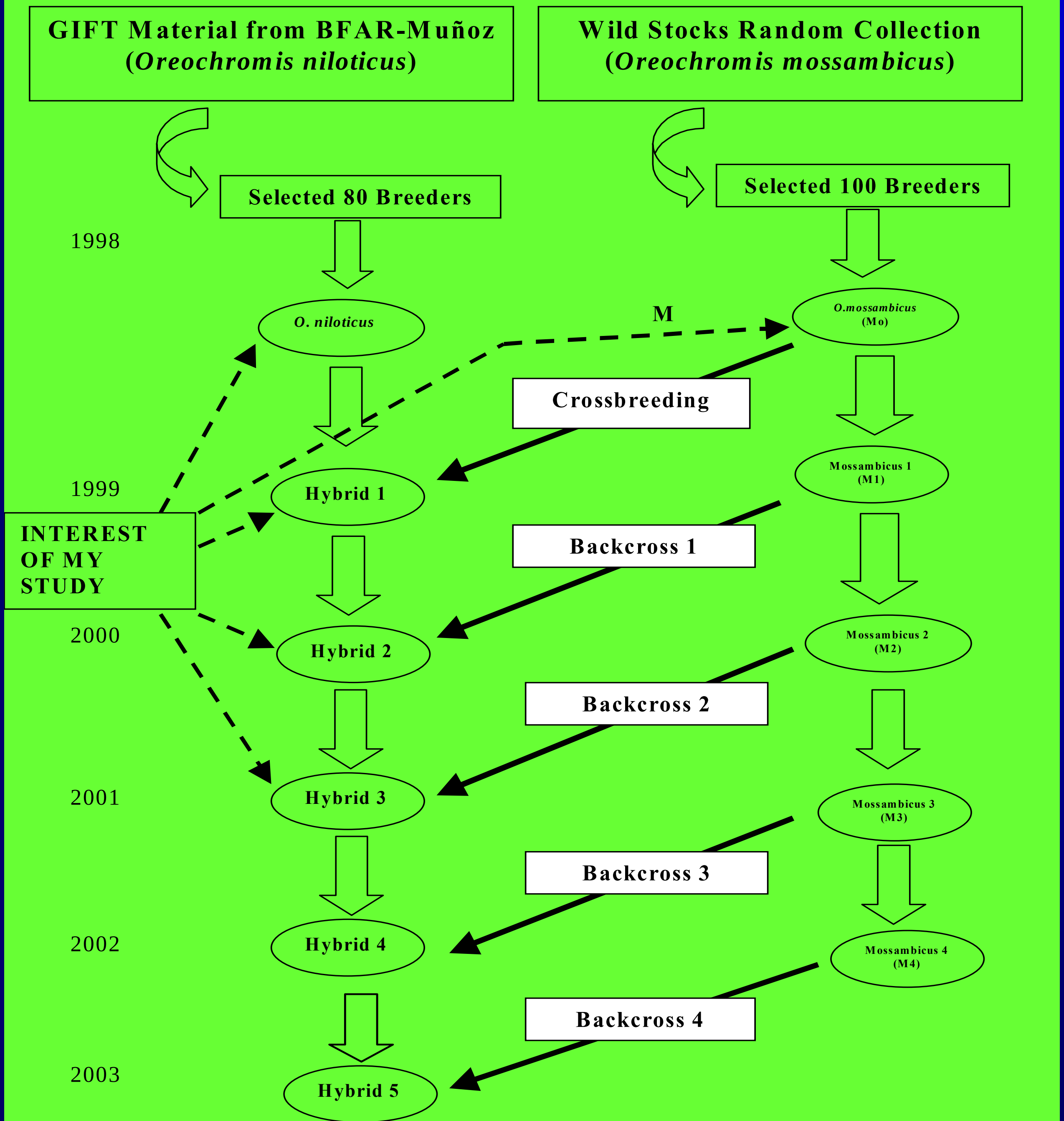


Figure 1. *Schematic diagram of Molobicus Project*

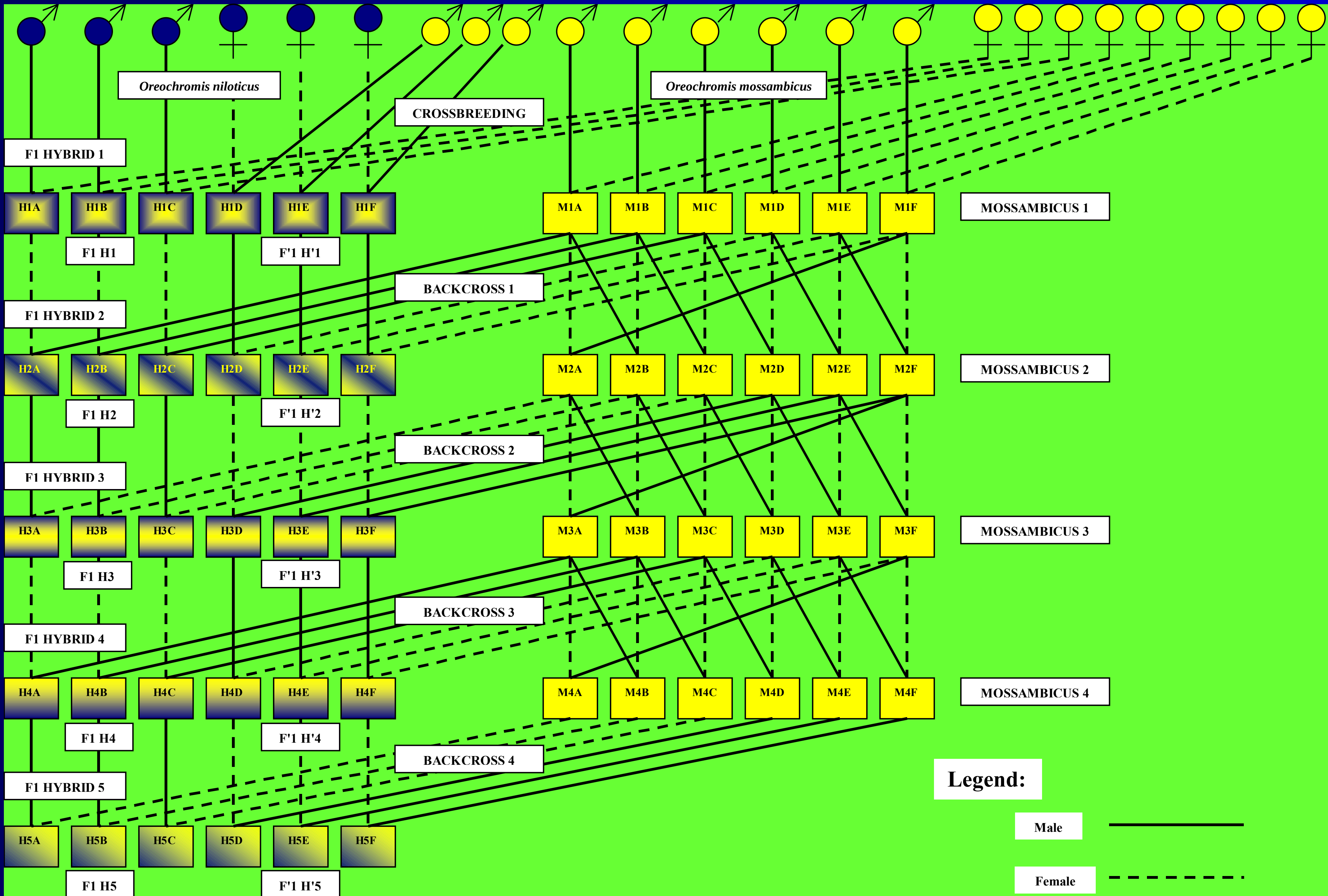


Figure 2. ***Rotational backcrossing scheme to develop a saline-tolerant tilapia***

Methodology

Set-up to produce experimental fish

Fish were Produced
in 1 x 1.5 x 6 m hapa
& 500-L aquaria

Methodology

Study 1: Salinity tolerance of the different treatments

- •8 treatments
- •4 replicatess
- •3 cm size (2 months old)
- •1 aquaria for the reserved fish in each treatment
- •20 liter water
- •21-liter capacity aquaria

Methodology

Study 2: Size and salinity tolerance correlation

- 8 treatments
- mixed sizes 1-6 cm
- standard length
- 0.4 g / liter
- 75 liter water
- 100-liter capacity containers

The Treatments	
1	*O. mossambicus*
2	Hybrid 1 (*O. mossambicus* φ *X O. niltocus* σ)
3	Hybrid '1 (*O. niltocus* φ *X O. mossambicus* σ)
4	Hybrid 2 (Hybrid 1 φ *X O. mossambicus* σ)
5	Hybrid '2 (*O. mossambicus* φ *X* Hybrid '1 σ*)*
6	Hybrid 3 (*O. mossambicus* φ *X* Hybrid 2 σ*)*
7	Hybrid '3 (Hybrid '2 φ X *O. mossambicus* σ)
8	*O. niloticus*
Legends: φ - female; σ - male	

Data Analysis

- Mean Salinity Tolerance = (f*1+f*2+…+fN*sN)/N (where f-fish; s-salinity; N-number of individuals)
- Median Lethal Salinity (using linear regression) $Y = a + bX$
- Optimum Salinity Tolerance (using break-line analysis) $a_1+b_1X = a_2+b_2X$
- Heterosis (Douglas Tave)
- Maternal/Paternal Inheritance (Douglas Tave)
- Analysis of Covariance (initial wt as covariant)
- Duncan's Multiple Range Test (DMRT)

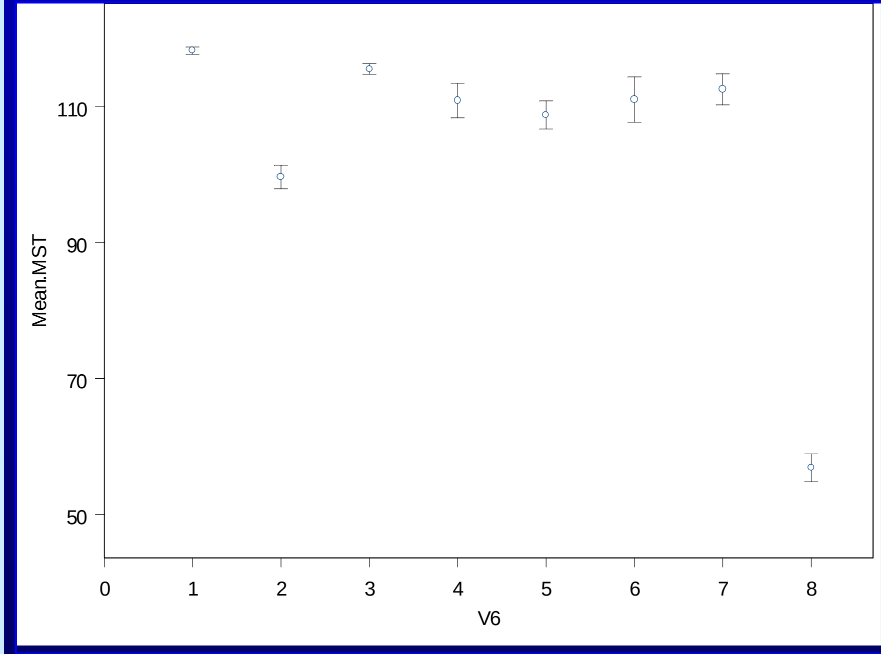
Note: Sigma Plot was used in Regression Analysis; SPSS10 was used in ANCOVA and DMRT.

Results and Discussions

Salinity Tolerance Index

Median Lethal Salinity (MLS)

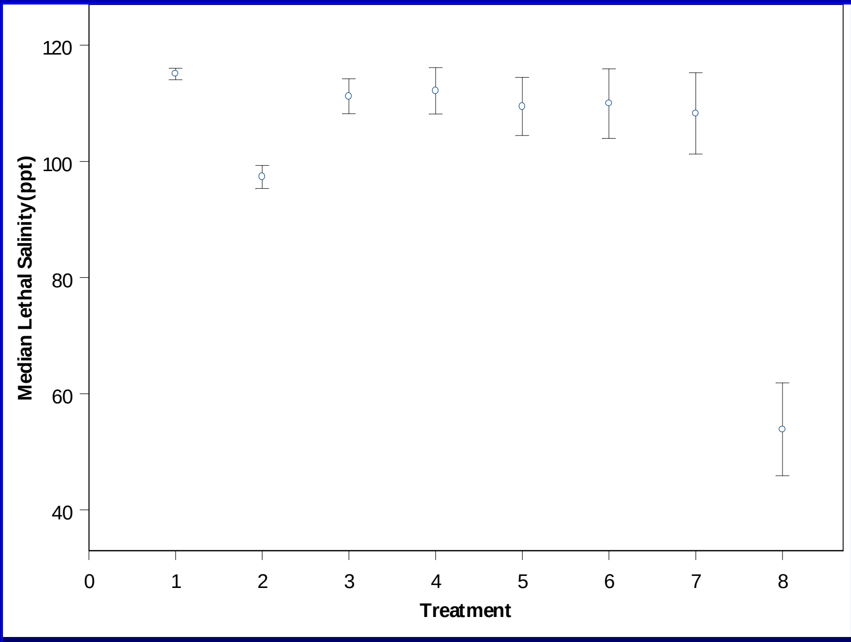
Treatment	**Mean**	**Std. Dev.**
O. mossambicus	115.06 a	1.48
Hybrid 1	97.33 c	3.82
Hybrid ‘1	111.22 ab	0.87
Hybrid 2	112.14 ab	1.38
Hybrid ‘2	109.45 b	0.29
Hybrid 3	109.95 b	3.09
Hybrid ‘3	108.28 b	3.64
O. niloticus	53.88 d	3.96



Salinity Tolerance Index

Mean Salinity Tolerance (MST)

Treatment	**Mean**	**Std. Dev.**
O. mossambicus	118.20 a	0.5477
Hybrid 1	99.60 c	1.7146
Hybrid ‘1	112.50 ab	0.7937
Hybrid 2	115.50 ab	2.5500
Hybrid ‘2	110.85 b	2.0549
Hybrid 3	111.00 b	3.3317
Hybrid ‘3	108.75 b	2.2650
O. niloticus	56.85 d	2.0549



Salinity Tolerance Index

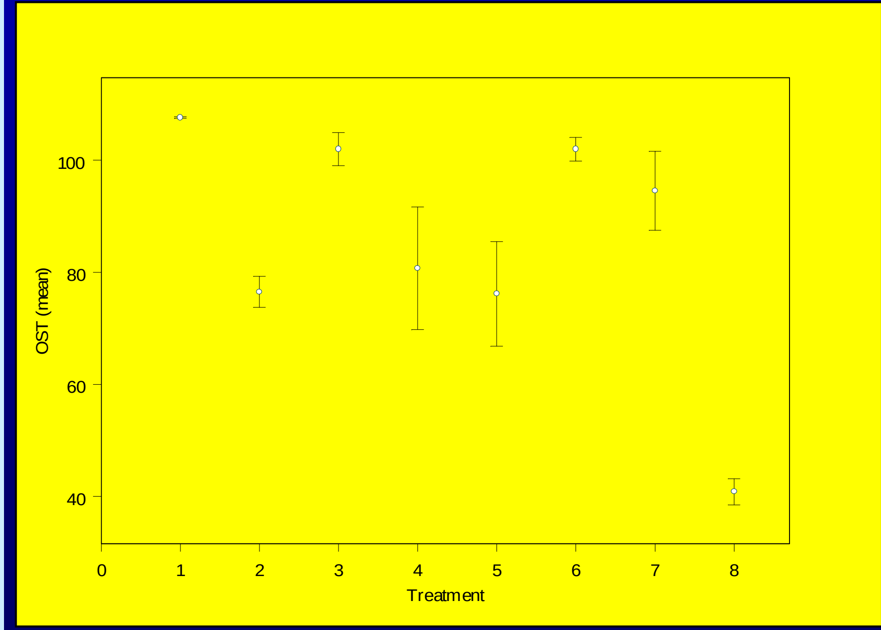
MLS & MST

- ***O. mossambicus*** **got the highest salinity tolerance.**
- ***O. niloticus*** **got the lowest.**
- **Hybrid 1 got next to the lowest (mother: *O. niloticus*).**
- **H'1, H2, H'2, H3 and H'3 were not significant to each other.**

Salinity Tolerance Index

Optimum Salinity Tolerance (OST)

Treatment	**Mean**	**Std. Dev.**
O. mossambicus	107.63 a	0.29
Hybrid 1	70.50 b	5.56
Hybrid ‘1	101.98 a	5.89
Hybrid 2	80.73 b	21.89
Hybrid ‘2	76.14 b	18.63
Hybrid 3	101.94 a	4.22
Hybrid ‘3	94.54 ab	14.08
O. niloticus	40.83 c	4.68



Salinity Tolerance Index

Optimum Salinity Tolerance (OST)

- ***O. mossambicus*, H3, H'3 and H'1 got the highest salinity tolerance.**
- **H1, H2, H'2 and H'3 were the next group of highest salinity tolerance.**
- ***O. niloticus was* the lowest.**

Salinity Tolerance Index

- **The results show that there was an increase in salinity tolerance as they were backcrossed with *O. mossambicus*.**

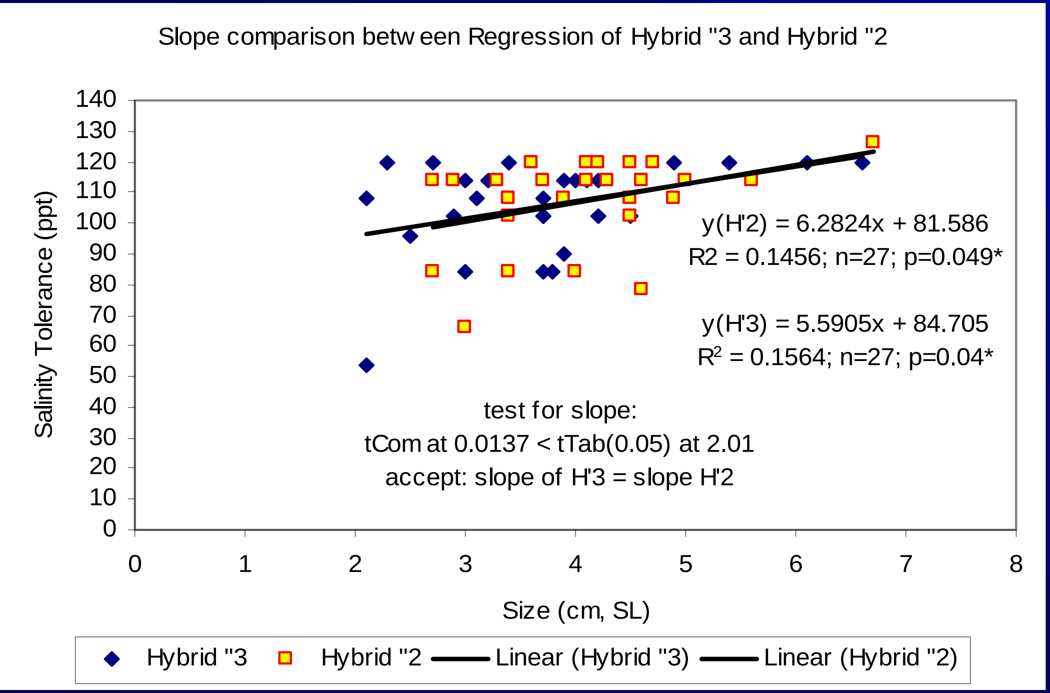
Size and Salinity Tolerance Correlation

Treatment	Linear equation	R^2	R	n	SE	P (sig.)
O. mossambicus	y = 1.0486x + 110.56	0.06	0.24	33	5.62	0.17ns
H1 (Mf x Nm)	y = 5.4013x + 71.17	0.46	0.23	14	6.92	0.34ns
H'1 (Nf x Mm)	y = 5.7486x + 66.91	0.16	0.39	21	18.56	0.08ns
H2 (Mf x H1m)	y = 1.6592x + 106.85	0.14	0.00	23	14.69	0.07ns
H'2 (H'1f x Mm)	y = 6.2823x + 81.59	0.15	0.38	27	14.09	**0.05***
H3 (H2f x Mm)	y = -0.0481x + 114.03	0.00	0.02	34	3.82	0.91ns
H'3 (Mf x H'2m)	y = 5.5904x + 84.70	0.16	0.39	27	19.84	**0.04***
O. niloticus	y = 0.4937x + 57.17	0.04	0.19	40	5.64	0.22ns

Influence of Size to Survival in elevated salinities showed 15% (H'2) and 16% (H'3).

Size and Salinity Tolerance Correlation

Slope Comparison Between Hybrid'2 &Hybrid'3



There was no significant difference in the slope comparison between H'2 and H'3.

Size and Salinity Tolerance Correlation

- Larger tilapias survived longer than smaller ones.
- This is due to the more matured osmoregulating parts like gills and kidney and matured hemoglobin, so more efficient in an environment with lower DO level like seawater.

Heterotic Effect

Heterosis of the different Hybrids			
Hybrids	OST	MLS	MST
Hybrids 1	20.23	23.45	21.17
Hybrids 2	-20.23	1.025	0.94
Hybrids 3	5.60	-3.378	-5.02

- **Hybrid 1 had the largest heterosis.**
- **Hybrids 2 and 3 had slight positive and negative heterosis.**
- **Note: Nearly zero heterosis is considered as additive inheritance.**

Heterotic Effect

- From the results of heterosis, Hybrids 2 and 3 are good candidates as base population for selection.

Maternal / Paternal Inheritance

Difference of Salinity Tolerance on their Reciprocal Breeds			
Hybrids	OST	MLS	MST
Hybrids 1	+(25.48)	+(13.89)	+(12.9)
Hybrids 2	+(4.59)	+(2.69)	+(4.65)
Hybrids 3	-(7.4)	-(1.67)	-(2.25)

- Positive results show maternal inheritance.
- Negative results show paternal inheritance.
- Hybrid 1 had the biggest positive results showing a strong maternal influence.

Maternal / Paternal Inheritance

- Hybrid 2 got lower positive results than Hybrid 1 but still showing maternal inheritance.
- Hybrid 3 got negative results showing paternal inheritance.
- Both sexes contributed to the salinity tolerance of the hybrids as observed in H2 and H3.

Maternal / Paternal Inheritance

- Maternal inheritance was greater than paternal inheritance as shown in H1 and H3 because it is a fact that eggs carry more extra-chromosomal genes in their cytoplasm as compared to the sperm of male.

Effects of Backcrossing

Treatment	MLS	MST	OST
O. mossambicus	115.06 a	118.20 a	107.63 a
Hybrid 1	97.33 c	99.60 c	70.50 b
Hybrid ‘1	111.22 ab	112.50 ab	101.98 a
Hybrid 2	112.14 ab	115.50 ab	80.73 b
Hybrid ‘2	109.45 b	110.85 b	76.14 b
Hybrid 3	109.95 b	111.00 b	101.94 a
Hybrid ‘3	108.28 b	108.75 b	94.54 ab
O. niloticus	53.88 d	56.85 d	40.83 c

Results show that there was an increase of salinity tolerance of the F1 hybrids as they were backcrossed to their parent with a high salinity tolerance (*O. mossambicus*)

Effect of Backcrossing

	MLS Average (ppt)		OST Average (ppt)	
Hybrids	Offspring	Parents	Offspring	Parents
Hybrids 1	104.28	84.47	89.24	74.23
Hybrids 2	110.79	109.67	78.43	98.43
Hybrids 3	109.11	112.93	98.24	93.03

Increase in salinity tolerance is due to the introduction of more genes from the saline tolerant parent (O. *mossambicus*) to the hybrids. The process is called INTRODUCTORY CROSSING.

Effect of Alternate Use of Sexes

Heterosis in the different Hybrids			
Hybrids	OST	MLS	MST
Hybrids 1	20.23	23.45	21.17
Hybrids 2	-20.23	1.025	0.94
Hybrids 3	5.60	-3.378	-5.02

- Alternate use of sexes during breeding reduced heterosis.

Effect of Alternate Use of Sexes

Difference of Salinity Tolerance on their Reciprocal Breeds			
Hybrids	OST	MLS	MST
Hybrids 1	+(25.48)	+(13.89)	+(12.9)
Hybrids 2	+(4.59)	+(2.69)	+(4.65)
Hybrids 3	-(7.4)	-(1.67)	-(2.25)

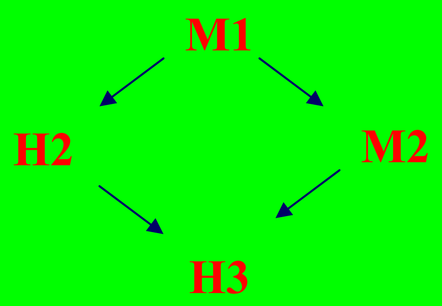
- Alternate use of sexes reduced the difference of salinity tolerance of the reciprocal breeds.

Social Behavior of Tilapia During the Salinity Tolerance Test

- **Dominant behavior of fish have been reported to:**
  - **Impose stress to other fish,**
  - **Prevent other fish to eat,**
  - **Utilize more energy by chasing and attacking other fish, and**
  - **Result in slow growth.**

- **Observed Social Behavior During the Salinity Test**
  - **O. niloticus and Reciprocal Hybrids 1 were more aggressive than the other groups of fishes.**
  - **Therefore, Hybrids 1 are not good candidates as base population for selection due to their aggressiveness which can be transferred from parents to offspring.**

Inbreeding Values



$F_x = [(0.5)^3]$

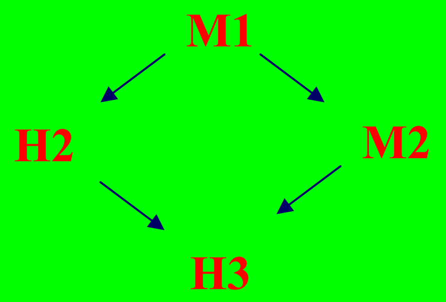
3 - # of ancestors involved

$F_x = 0.125$ or 12.5 %

Simplified Path Diagram

- Three ancestors were applied in the production of H3.
- Inbreeding value of H3 was 0.125, so a previous 12.5% heterozygous genes became homozygous.

Inbreeding Values



$$F_x = [(0.5)^3]$$

3 - # of ancestors involved

F_x = 0.125 or 12.5 %

Simplified Path Diagram

- Therefore H3 is not fitted as base population for selection due to its high inbreeding value of 12.5%.
- Note: Allowable value is 5-10%.

Conclusions

- Salinity Tolerance
  - **There is an increase in the salinity tolerance of hybrids as they were backcrossed to *O. mossambicus* (high salinity tolerant parent).**

- Size and Salinity Tolerance
  - **Larger fish are more tolerant than smaller fish.**
  - **This may be due to the more matured osmoregulating parts and hemoglobin for oxygen distribution (respiration).**

- Heterosis
  - **Hybrids 2 and 3 are good candidates as base population for selection in terms of HETEROSIS.**

- Maternal and Paternal Inheritance
  - **Both sexes of *O. mossambicus* contributed salinity tolerance to the Hybrids.**
  - **Maternal inheritance is greater than paternal inheritance which may be due to extra-chromosomal genes and environmental influence from the mother.**

- Effect of Backcrossing and Alternate Use of Sexes
  - **Increased salinity tolerance in backcrossing.**
  - **Alternate use of sexes in backcrossing reduced heterosis and resulted to manageable maternal and paternal inheritance.**

- Inbreeding Values
  - **Hybrids 1 & 2 have zero (0) inbreeding values.**
  - **Hybrids 3 have 12.5% inbreeding values.**
  - **Therefore, Hybrids 3 will not fit as base population for selection in terms of inbreeding values.**

- Social Behavior during the Salinity Tolerance Test
  - ***O. niloticus*** **and Hybrid'1 are more dominant than the other groups. (This behavior might be transferred to the offspring.)**
  - **Hybrids 1 will not be good candidates as base population for selection in terms of their aggressiveness.**

- **General Conclusion**
  - **Reciprocal Hybrids 2 will be best fitted among the hybrids as base population for selection due to:**
    - low heterosis,
    - low aggressiveness, and
    - zero inbreeding values.

Recommendations

- Size and salinity tolerance were significantly correlated in H’2 and H’3.
- Therefore, selection of fast growing ones is recommended as breeders because size and salinity tolerance are significantly correlated.

- Options as base population for selection
  - Option 1: for large hatcheries
    - 70 % Hybrids 2
    - 20% hybrids 3
    - 10% hybrids 1
  - Option 2: for medium hatcheries
    - 60 % Hybrids 2
    - 40% hybrids 3
  - Option 3: for small hatcheries
    - 100% Hybrids 2
  - Option 4: for small hatcheries
    - 100% Hybrids 3

- For hatcheries using freshwater, 5-6 cm (SL) fingerlings are ideal for stocking.
- For better growth of fry, hatcheries must be in elevated salinities, i.e. 15-28 ppt.
- If freshwater hatcheries are used, rear the fry in elevated salinities.
- Conduct qualitative genetics to hasten selection process through biotechnology,
  - e.g. identification of gene responsible for salinity tolerance.

Thank You!

Introduction

Expected Outputs

1. As hybrids were backcrossed to their saline tolerant parent O. mossambicus, hybrids increased their salinity tolerance

2. Creation of a synthetic saline tolerant tilapia that can be bred and exploited in selection

Methodology

Data collected

Initial weight before salinity test
Daily mortality with their corresponding salinity
Mean Salinity Tolerance (MST)
Median Lethal Salinity (MLS)
Optimum Salinity Tolerance (OST)
Standard length (for experiment 2 only)
Dominant behavior
Clinical signs before mortality

Methodology

Oreochromis mossambicus

- Original stocks were collected in the BW ponds around Lingayen gulf
- Undergo rotational crossing to prevent further inbreeding
- Naturally breeds in hapas or in

Methodology

Oreochromis niloticus

- Fry were from the Genetically Enhanced Tilapia (GET) of BFAR-NFFTRC, CLSU, Muñoz, N.E.

Methodology

Reciprocal Hybrids 1

Hybrid 1 (H1), hybridization between female **O.mossambicus** X male **O. niloticus**

Hybrid "1(H'1), hybridization between female **O.niloticus** X male **O. mossambicus**

Methodology

Reciprocal Hybrids 2

Hybrid 2 (H2), hybridization between female **Hybrid 1** X male **O. mossambicus**

Hybrid "2(H'2), hybridization between female **O.mossambicus** X male **Hybrid '1**

Methodology

Reciprocal Hybrids 3

Hybrid 3 (H3), hybridization between female **O. mossambicus** X male **Hybrid 2**

Hybrid "3(H'3), hybridization between female **Hybrid '2** X male **O. mossambicus**

Methodology

Salinity Tolerance Test

Acclimation

Light (12L:12D)

Feeding (ad libitum)

H_2O Quality (NO-3, PO-4, DO, pH, NH-3)

Reserved fish (to replenished in case mortality before the test)

Methodology

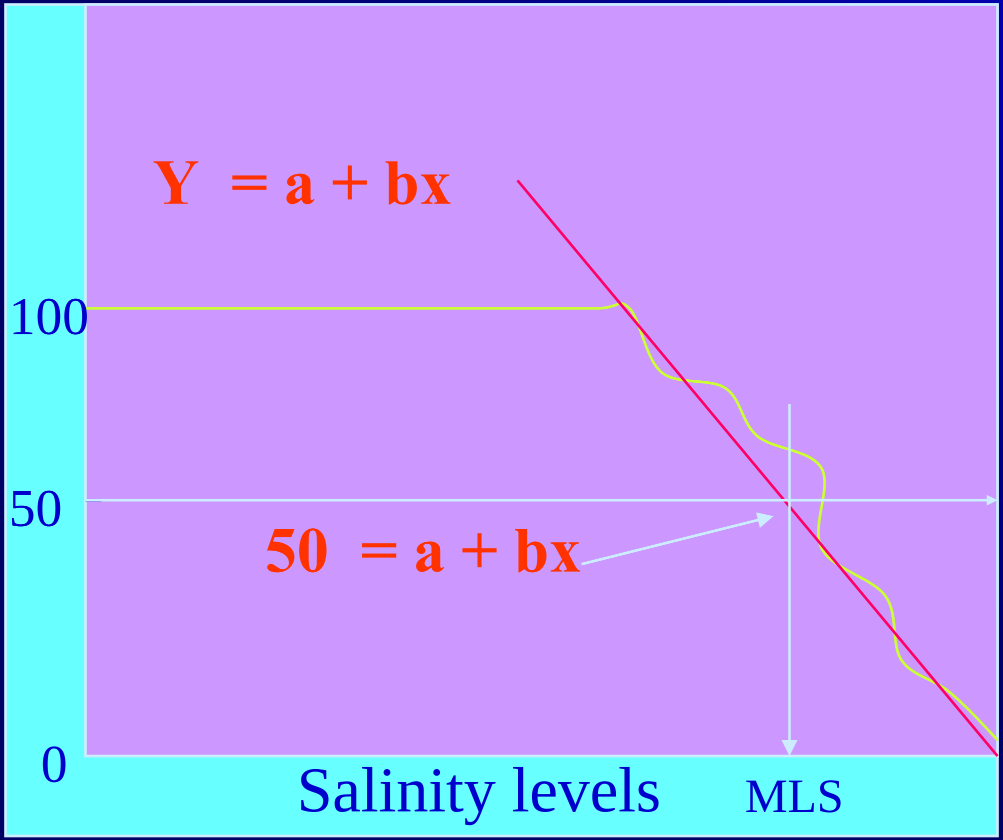
Salinity Levels Preparation

Artificially Prepared *N1 V1 = N2 V2*

Day	ppt	Day	ppt	Day	ppt	Day	ppt	Day	ppt	Day	ppt
1	0	5	24	9	48	13	72	17	96	21	120
2	6	6	30	10	54	14	78	18	102	22	126
3	12	7	36	11	60	15	84	19	108		
4	18	8	42	12	66	16	90	20	114		

Methodology

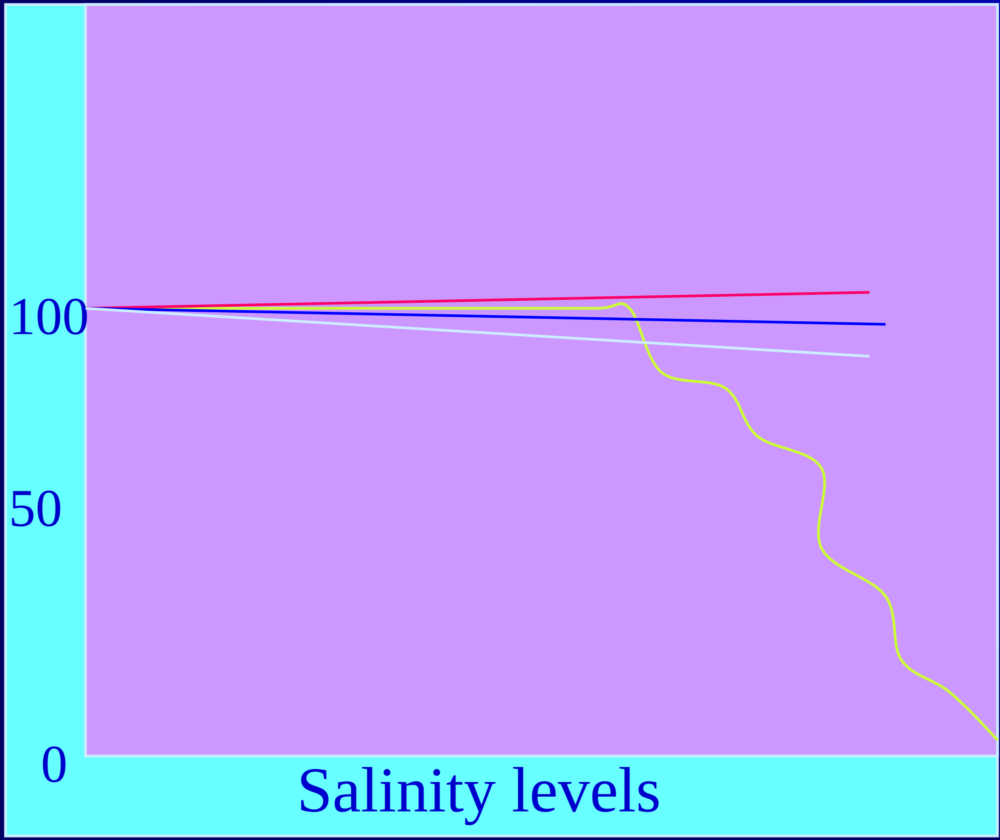
MLS Determination



- Plot the survival data in a graph
- Obtain the regression equation from the 100% to 0% survival
- Substitute the Y to 50
- Compute MLS

Methodology

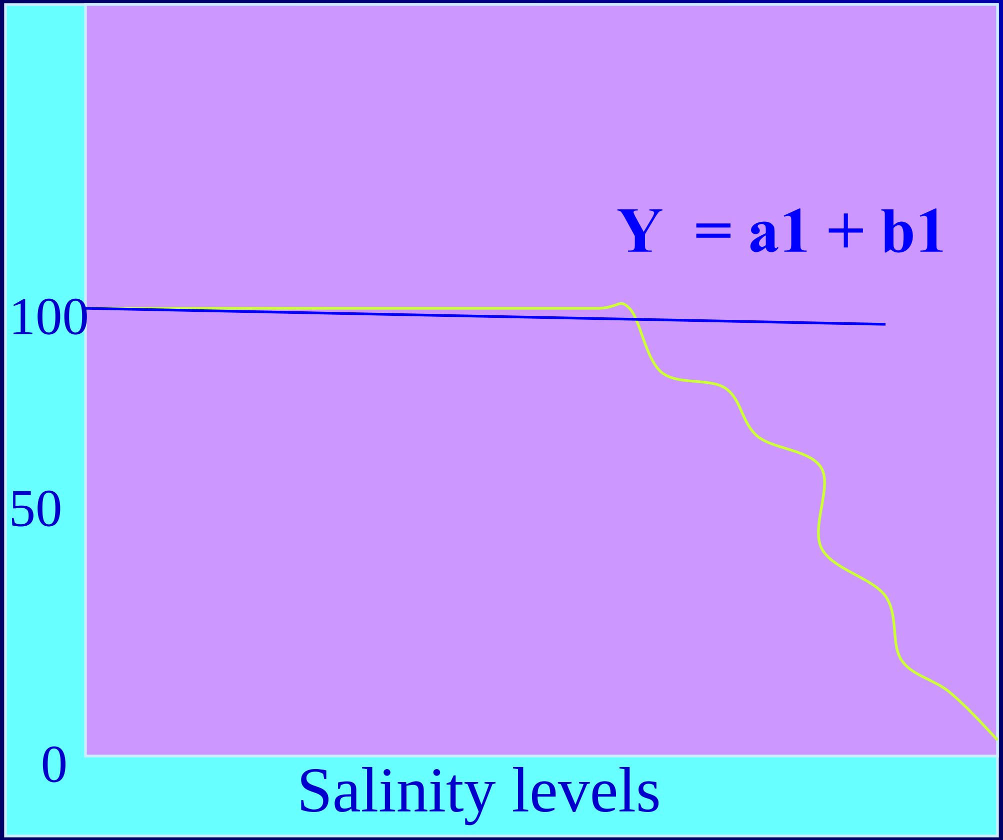
OST Determination



- Try different regression of the plateau until reaching a significant one
- Test the significance of the regression plateau using stepwise regression

Methodology

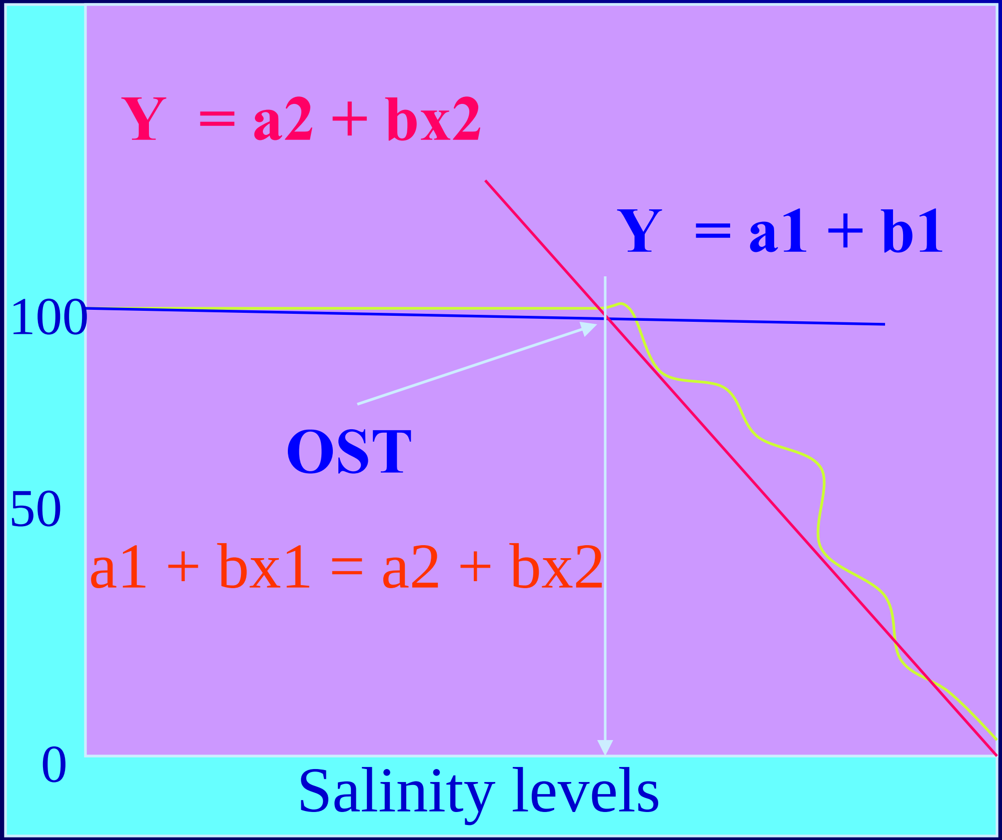
OST Determination



- Choose the regression line that is significant
- The regression equation were marked as Regression line 1

Methodology

OST Determination



- Obtain the regression line 2 connecting to the last value of the regression line 1
- Equate the two equation to obtain the value of X (OST)

Methodology

Heterosis Determination

$$\textbf{Heterosis} = \left[\frac{\text{Average of Offspring} - \text{Average of Parents}}{\text{Average of Parents}} \right] \times 100$$

Methodology

Maternal/Paternal Inheritance Determination

Difference of Reciprocal Breeds	**=**	**Salinity Tolerance Offspring with a mother that has a high salinity tolerance**	**-**	**Salinity Tolerance Offspring with a father that has a high salinity tolerance**

Methodology

Inbreeding Values Determination

$$Fx = \Sigma [(0.5)^N]$$

F_x	=	The inbreeding of an individual
Σ	=	The symbol of "sum of" or "add"
N	=	The numbers of individuals in a path that is determined by tracing a path from one parent back to the common ancestors and forward from the common ancestors to the other parent. If more than one ancestor exists, the term "$(0.5)^N$" is repeated for each common ancestor. If more than one path exists between the individual and a common ancestor, the term "$(0.5)^N$" is repeated for each unique path.
F_A	=	The inbreeding of a common ancestor.

Results and Discussions

Social Behavior of Tilapia During Salinity Tolerance Test

- **Recognition of Dominant Behavior**
  - **Guarding a territory**
  - **Guarding the source of food**
  - **Chasing and Driving other fish away from the territory.**
  - **Change in Coloration**

Results and Discussions

Social Behavior of Tilapia During Salinity Tolerance Test

- **Observed Social Behavior**
  - **Hybrid 1 are docile at 30-36 ppt**
  - **Reciprocal Hybrid 2 and Reciprocal Hybrid 3 has 10-20% of the population are dominant until 48 ppt**
  - **At 98 ppt, H’1 still had dominant individual. one out of 40 (2.5%)**

Results and Discussions
Inbreeding Values

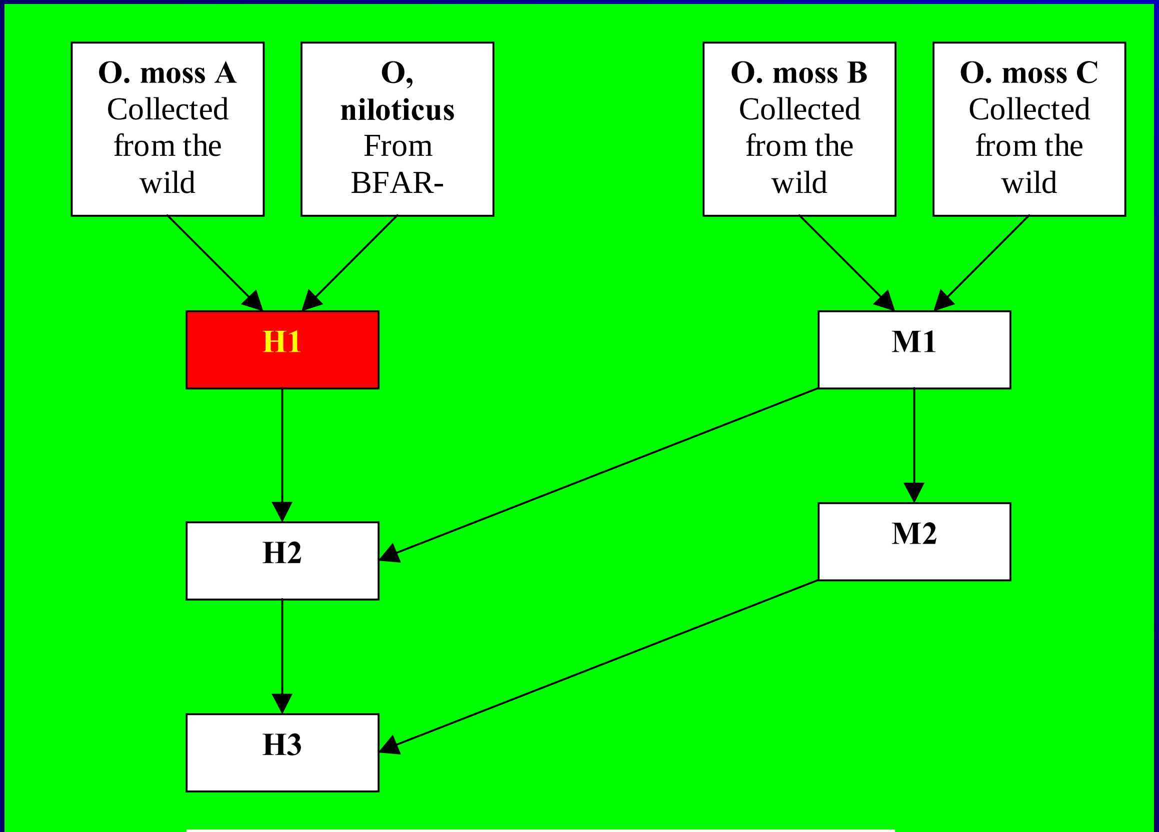


Figure x. Path diagram of the different hybrids

- **H1 has a zero inbreeding because it is a result of hybridization of two different species**

Results and Discussions

Inbreeding Values

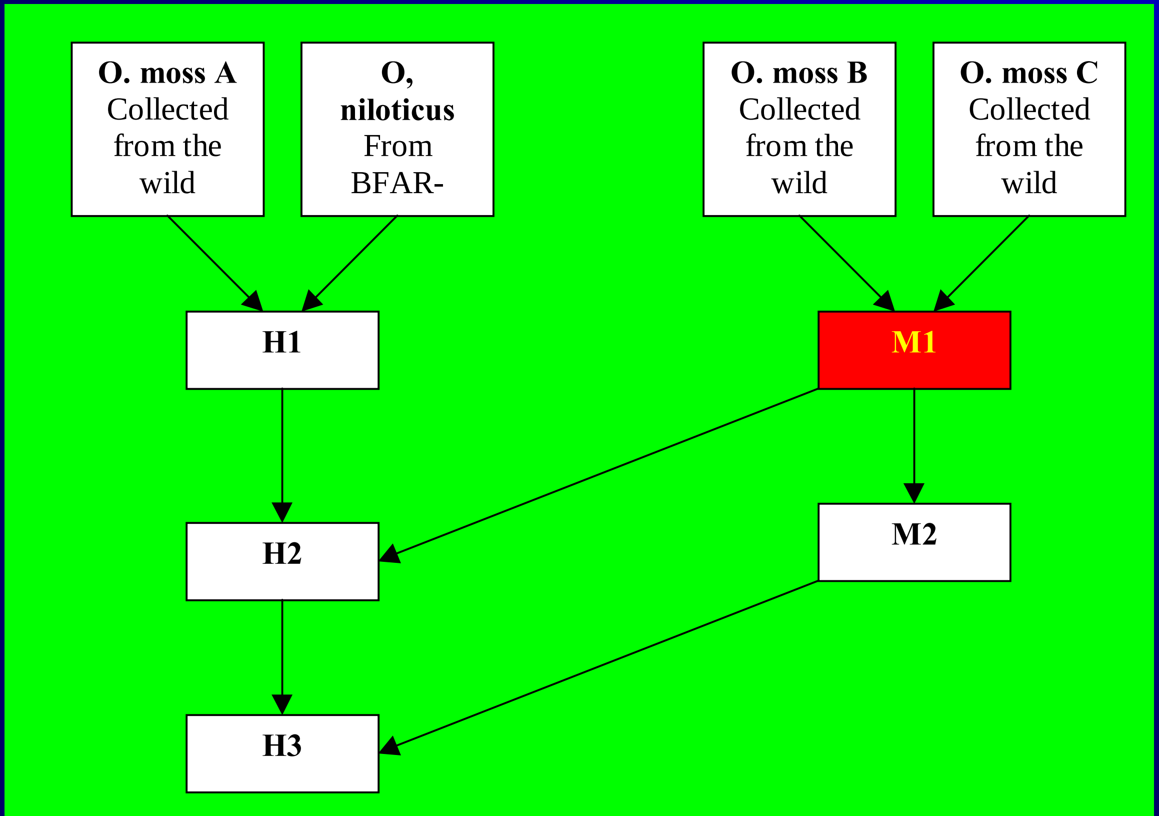


Figure x. Path diagram of the different hybrids

- M1 assumed to have a zero inbreeding, because the parents are collected in the wild

Results and Discussions

Inbreeding Values

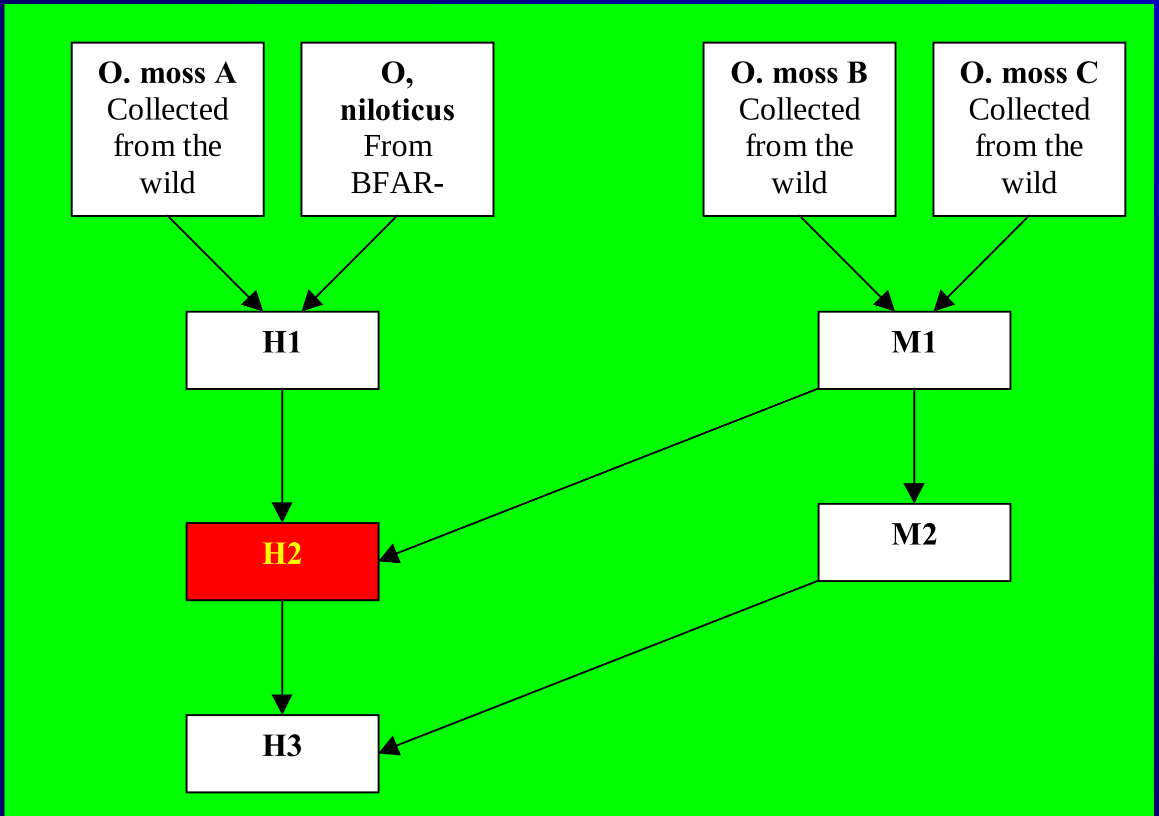


Figure x. Path diagram of the different hybrids

- **H2 has a zero inbreeding because the parents did not come from a common ancestor**

Results and Discussions

Inbreeding Values

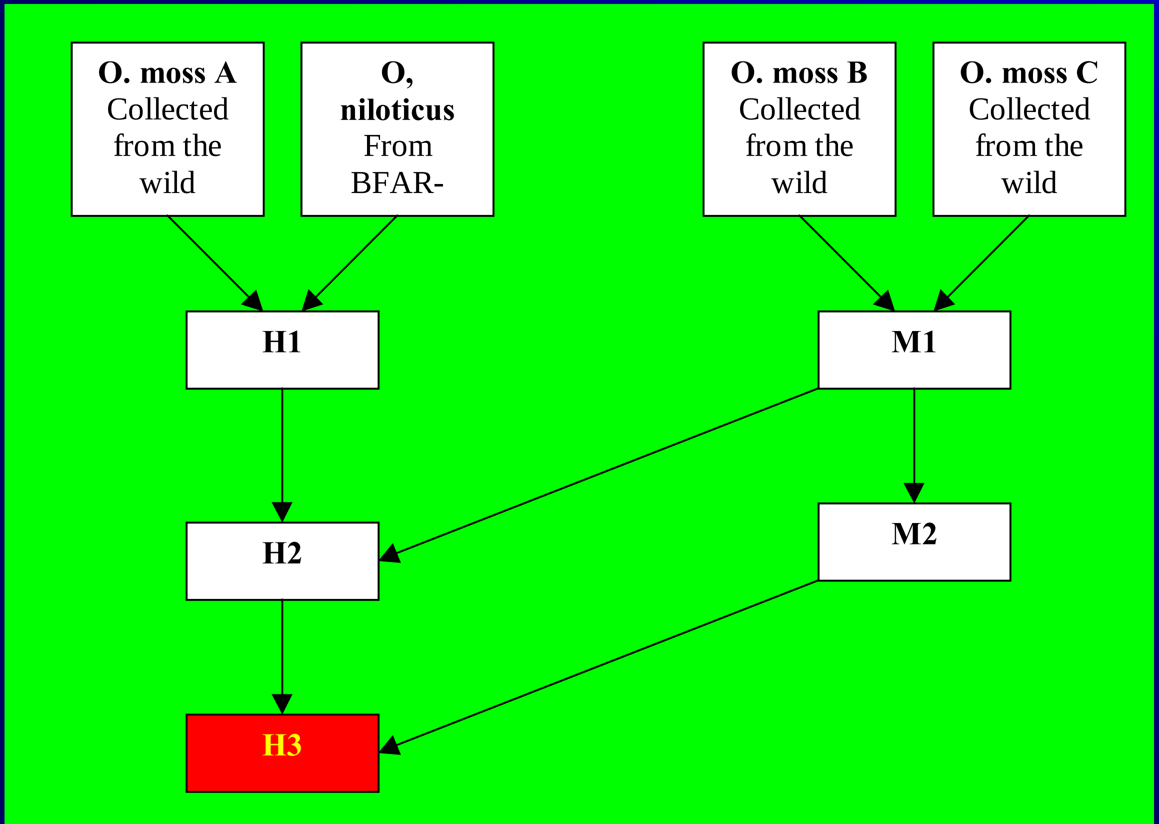


Figure x. Path diagram of the different hybrids

- **H3 has a theoretical computed inbreeding of 0.125, because the parents are both descendant of M1**

Results and Discussions

Fish Behavior Before Mortality

Results and Discussions

Fish behavior Before Mortality

- **Loss of dominance for the dominant fish**
- **Sunken eyes and abdomen indicating its inability to osmoregulate**
- **Coloration change from normal silver color to intensified body barring or to total black**
- **Resting in tank bottom or gasping air**

Results and Discussions

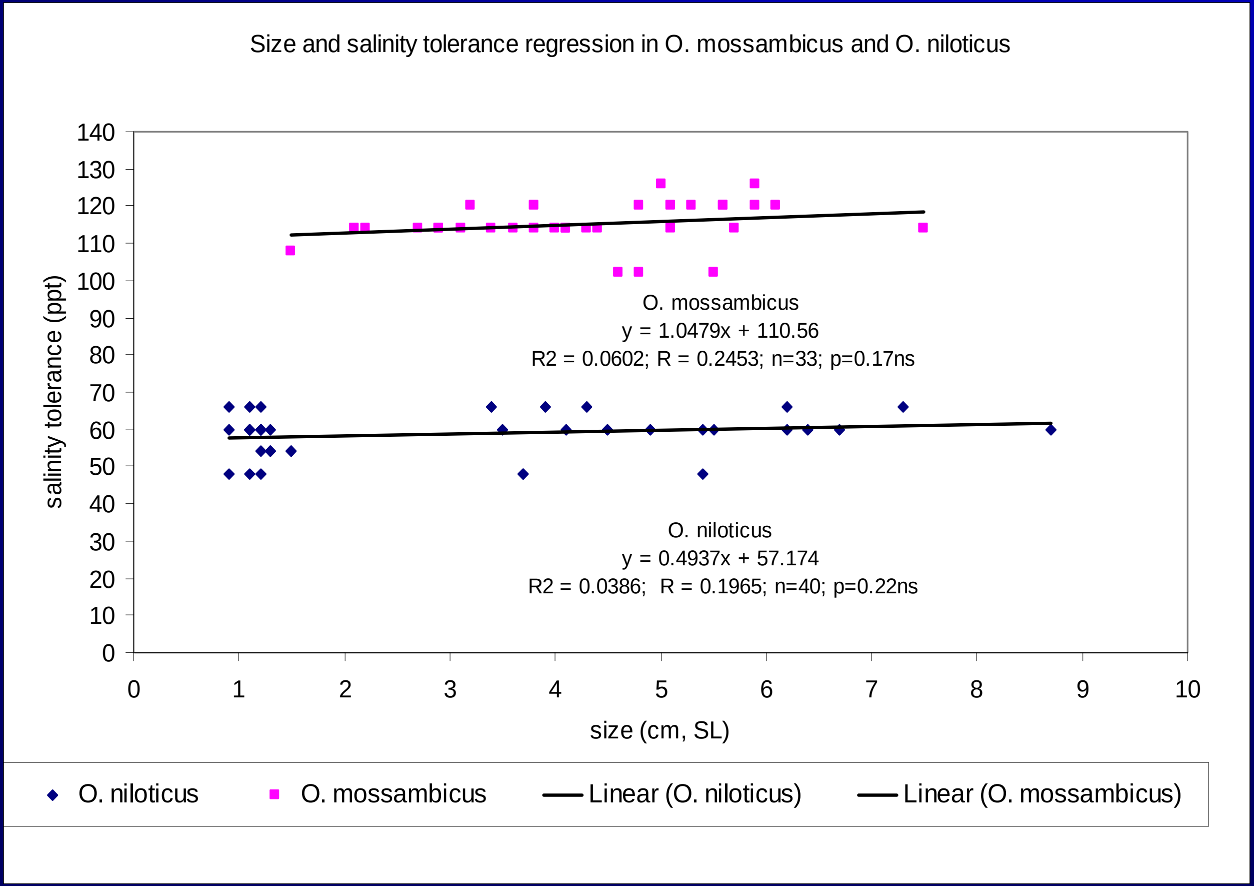
Fish Behavior Before Mortality

- **A minute before they die, some fish exhibit erratic swimming in a darting motion to an unpredicted direction**
- **Some are to weak to counteract water movement**
- **Contrary to other reports, there is no hyperplasia, swollen belly, septicemia and swollen eyes.**

Results and Discussions

Size and Salinity Tolerance Correlation

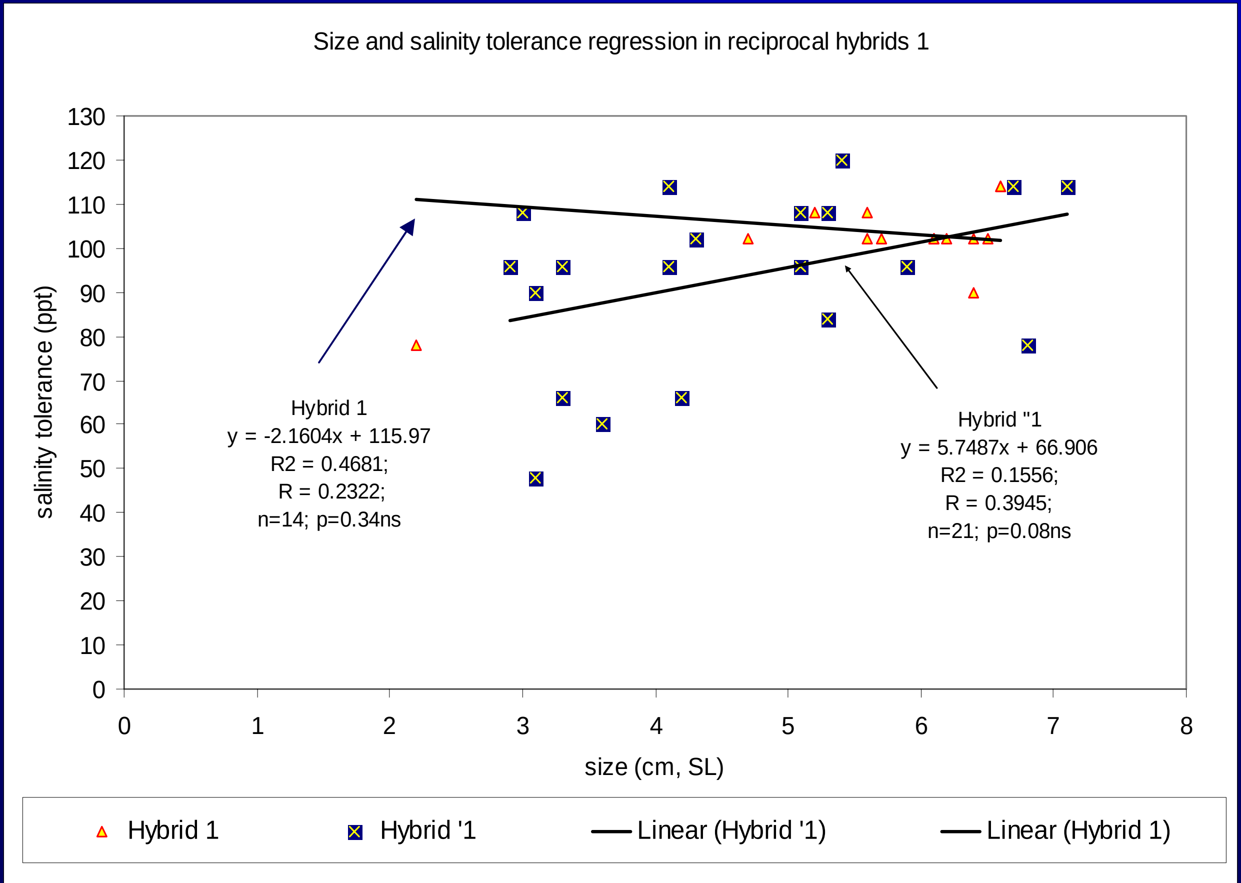
O. mossambicus & O. niloticus



Results and Discussions

Size and Salinity Tolerance Correlation

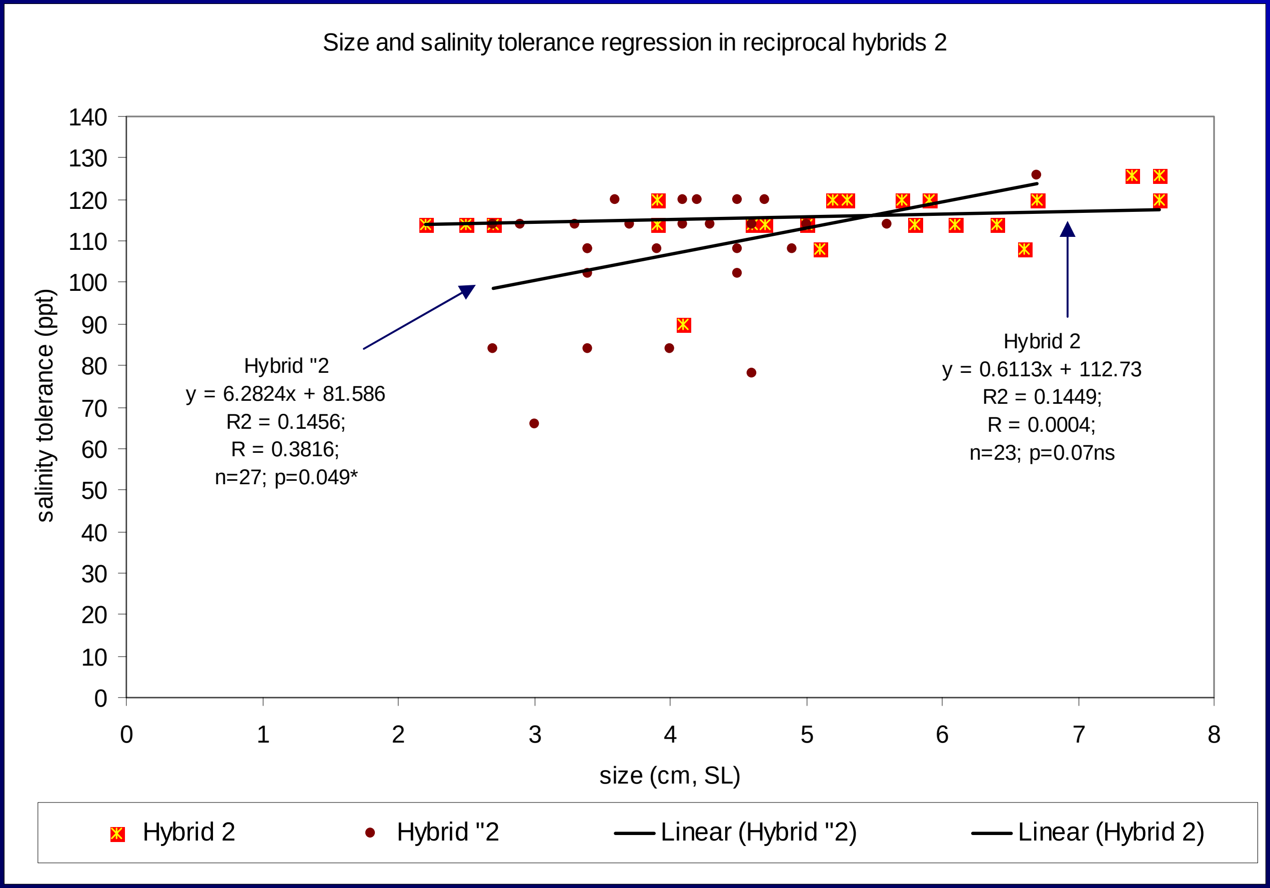
Hybrid 1 & Hybrid '1



Results and Discussions

Size and Salinity Tolerance Correlation

Hybrid 2 & Hybrid ‘2



Results and Discussions

Size and Salinity Tolerance Correlation

Hybrid 3 & Hybrid ‘3