Selection from an Interspecific Hybrid Population of Two Strains of Fast Growing and Salinity Tolerant Tilapia

Westly Rosario, Bernard Chevassus-Au-Louis, Pierre Morissens, Nerafe Muyalde, Angelito Dela Cruz, Cyril Georget, Jean-Paul Poivey and Efren de Vera

MOLOBICUS PROGRAM

Bureau of Fisheries and Aquatic Resources
NATIONAL INTEGRATED FISHERIES TECHNOLOGY DEVELOPMENT CENTER
Bonuan Binloc, Dagupan City

Background

1. Many tilapia species and H1 hybrids had been tested in saline environments in the 80's and 90's:
   - West African coastal lakes (CIRAD).
   - Jamaica (Watanabe)
   - Kenya and Persian Gulf (Sterling University)…etc
     - ➢ mainly leading to failure…

2. In 1991, Dr. B. Chevassus suggested to investigate the possibility to associate hybridization (to create wide pools of genes) and selection.

- To validate this approach, successful attempts were conducted to produce an intergeneric highly saline-tolerant hybrid (*O.niloticus* x *S.melanotheron*). On the other hand, a study was conducted to check if genes in tilapia hybrid populations were associating and combining as they do in a pure species (Bezault *et. al.*, CIRAD, 2000-2001).

Background

It was found out that …

- In intergeneric tilapia hybrids (*S. melanotheron* x *O. niloticus*), the genes of the two species are associating and combining as they would in the progeny of pure species (Bezault, 2000).
- Unlike most animal and plant inter-specific hybrids, tilapia hybrids are fertile.

  This finding allows the selection from an interspecific hybrid population (synthetic strains) showing an association of characters one would not find in a pure species.

Why grow tilapia in saline environment?

- Saline tilapia can optimize production in more than 200T has of brackishwater ponds in the Philippines.
- The demand for saline tilapia could be huge considering the area of brackishwater fishponds in the country.
- The prospect of producing large tilapia for *fillet* business will prosper with the use of BW tilapia.
- The problem on high cost of tilapia feeds can be addressed by the production of the NIFTDC saline tilapia - Molobicus (extensive) hybrid.
- The culture of tilapia in saline environment could discourage conversion of areas otherwise used for rice and crop production.

Why MOLOBICUS?

Two tilapia species are used in the MOLOBICUS program:

O. mossambicus

O. niloticus

MOLOBICUS

What is it?

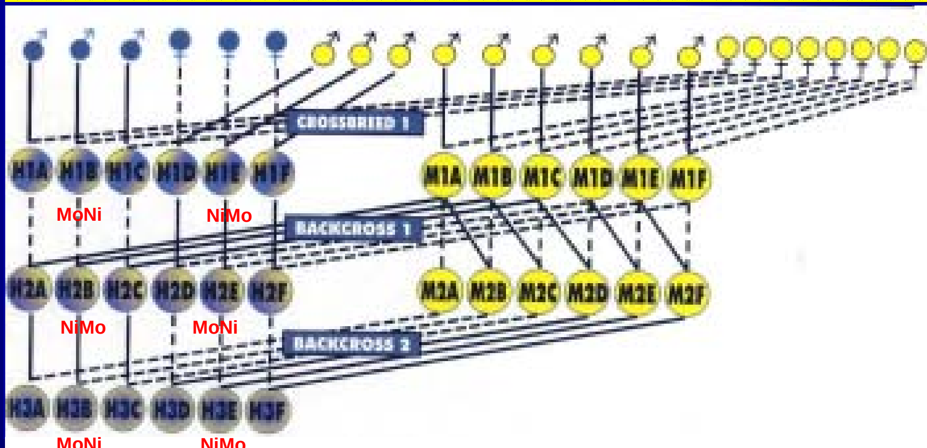
- **The program aims to produce a new strain of tilapia that grows fast in high saline environment.**

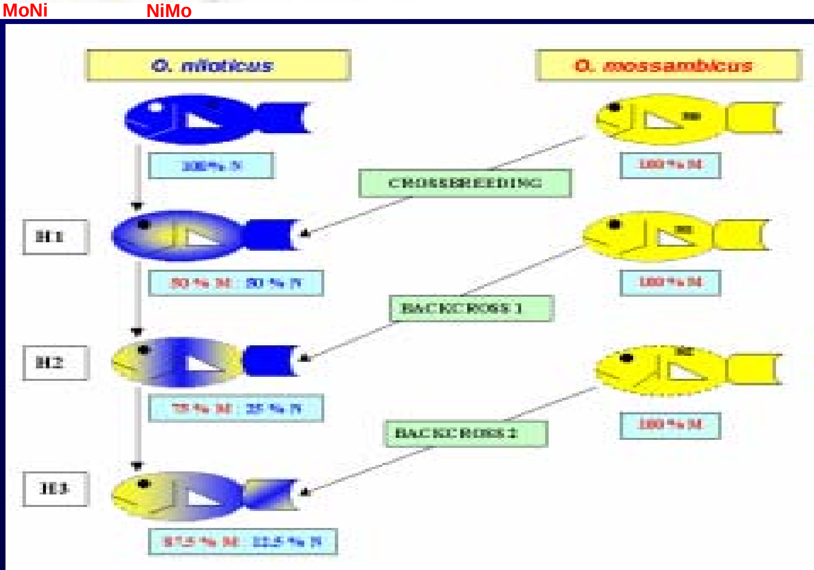
How?

- **By hybridization and selection.**

Phase 1

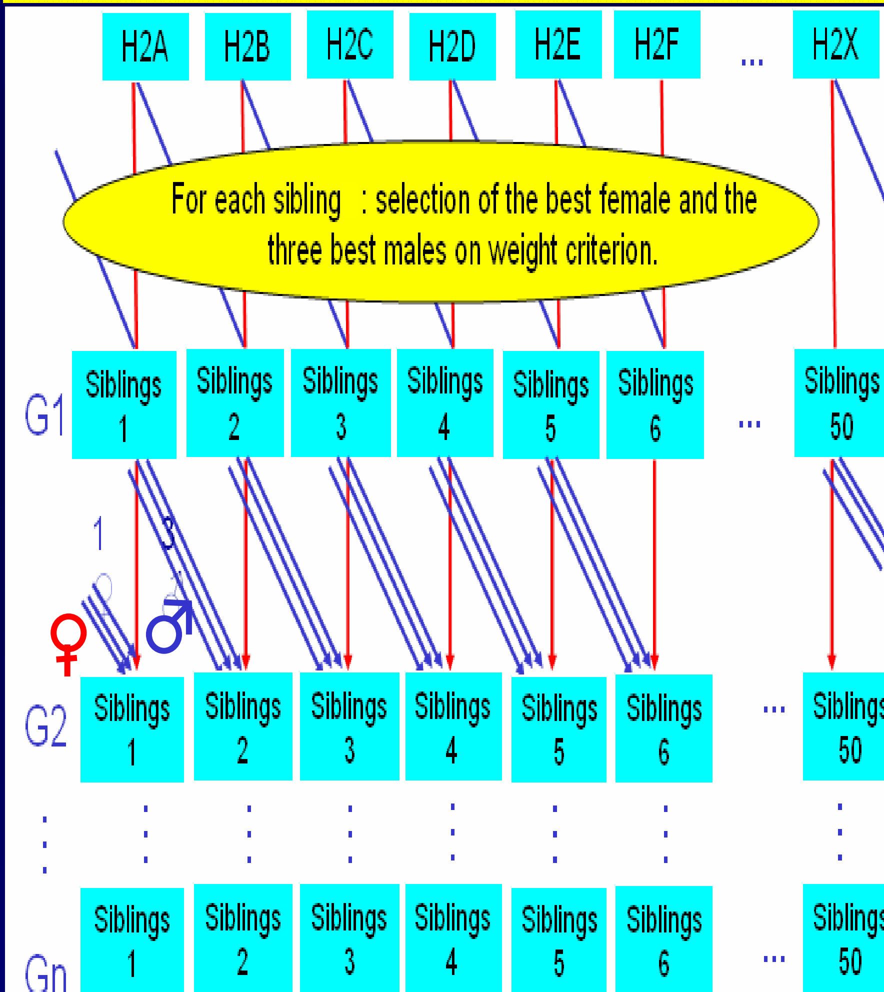
Rotational Backcrossing Scheme to Develop Saline Tolerant Hybrids





Phase 2

Rotational Crossing Scheme to Preserve Genetic Variability



Phase 1

Families Produced (1998 - 2002)

H2 :

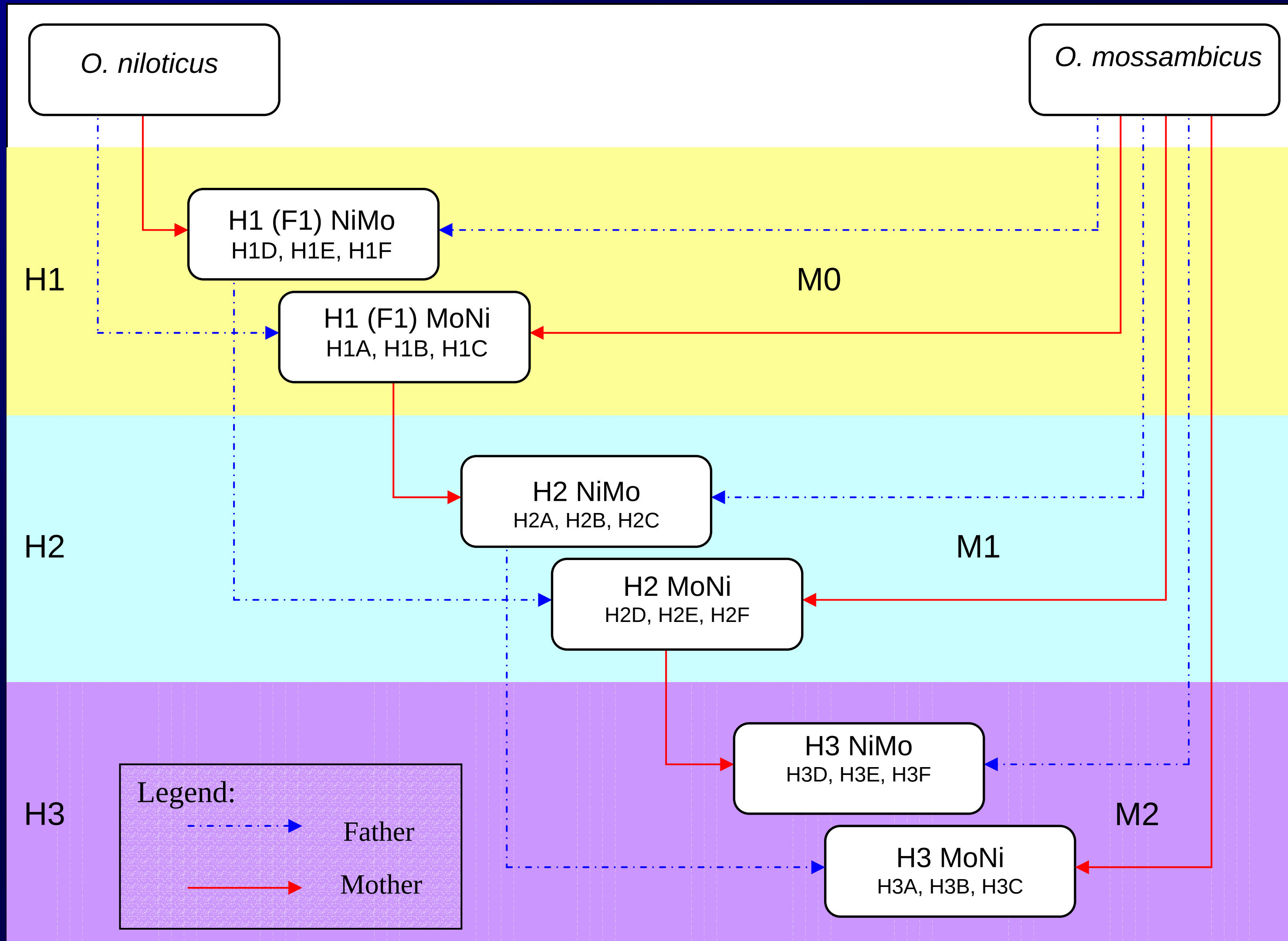
6 families,

27 sub-families

H3 :

6 families,

58 sub-families



SHORT TERM SALINITY TEST

Aim:

- **Determine the salinity resistance of the successive hybrid generations using *O. niloticus* and *O. mossambicus* as reference species.**

Why evaluate the salinity resistance of each hybrid generation?

- **To determine which hybrid generation would be used in Phase 2 : Growth Selection Program**

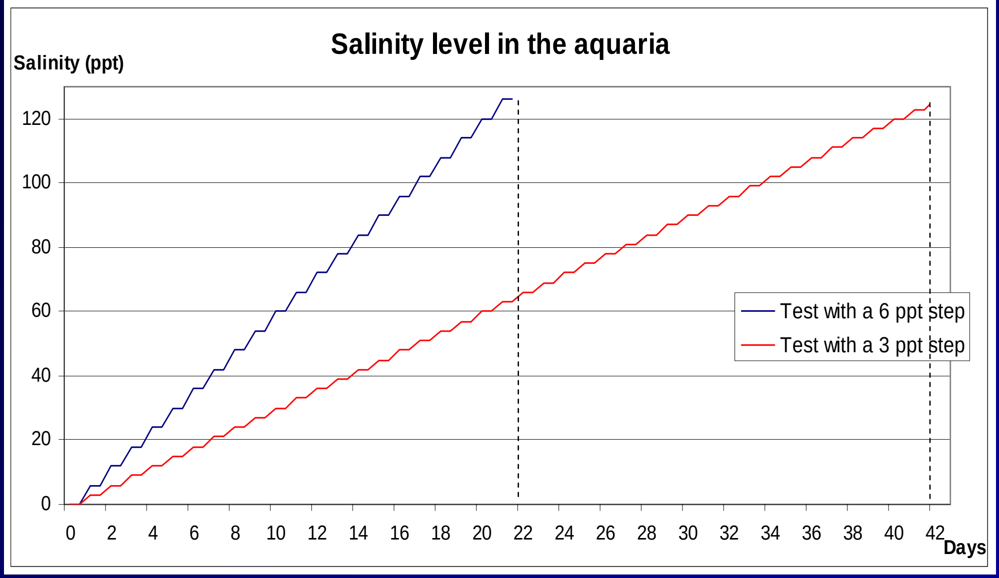
Several short term tests were tried:

- **Direct transfer to saline environment, from 20 to 35 ppt during 96 hours (Watanabe protocol), to determine the most segregating salinity.**
- **Subject fish to daily increase of salinity (6ppt and 3ppt), following the Lemarie protocol.**

SHORT TERM SALINITY TEST
(6 ppt and 3 ppt daily increase)

Protocol:

- 10 fish per aquarium, 4 replicates and 1 control (FW) for each strain
- Fish were fed twice daily
- Water temperature, DO and salinity were checked daily
- Increased salinity daily (6 ppt and 3 ppt)

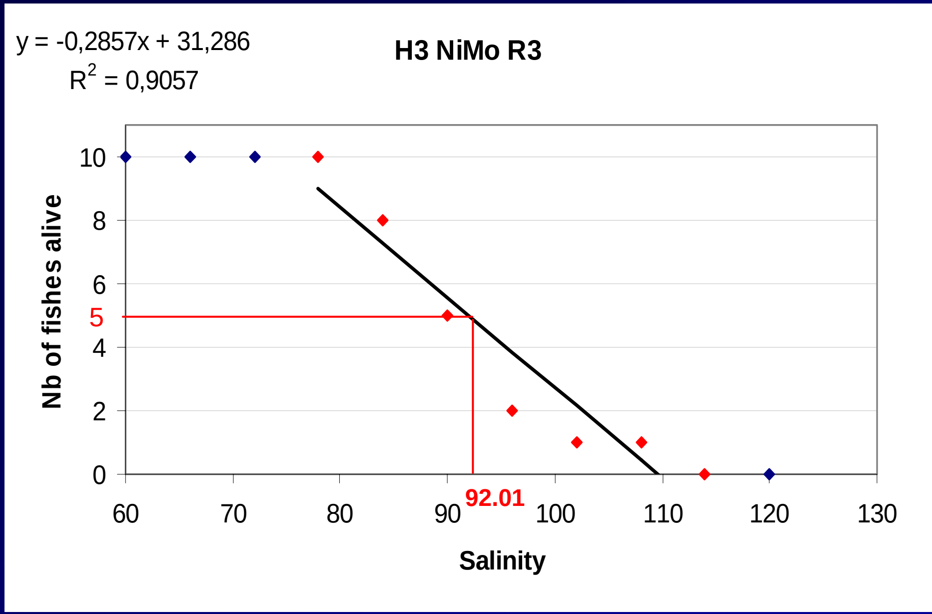


SHORT TERM SALINITY TEST
(6 ppt and 3 ppt daily increase)

Protocol:

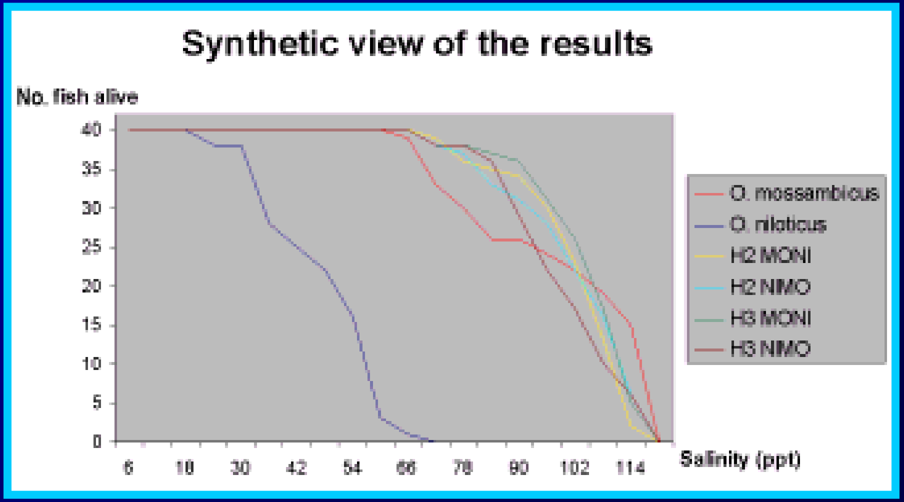
- Recorded mortalities daily
- Evaluated salinity resistance by calculating the LS-50

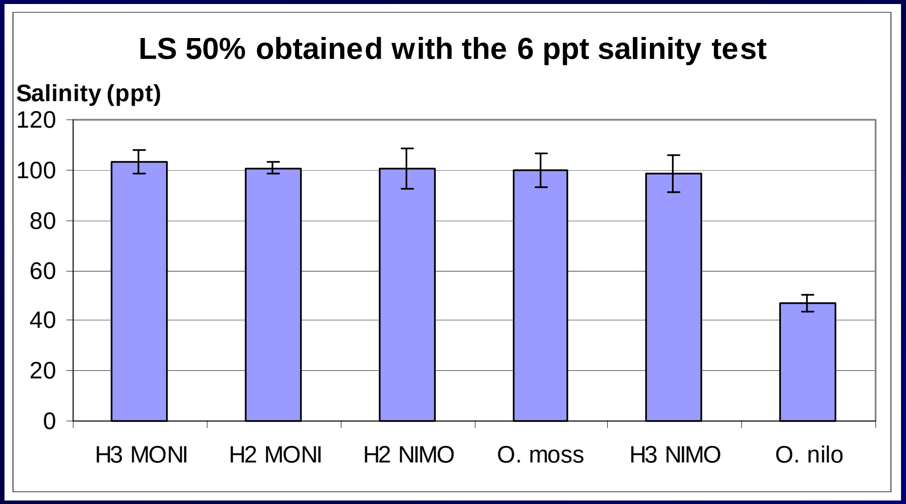
Species		*O. mossambicus*				*O. niloticus*				H2 MoNi				H2 NiMo				H3 MoNi				H3 NiMo			
		R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
d 1-15	0 ppt	A C C L I M A T I O N																							
16	6 ppt																								
17	12 ppt																								
18	18 ppt																								
19	24 ppt						2																		
20	30 ppt																								
21	36 ppt					3	2	2	3																
22	42 ppt						1	1	1																
23	48 ppt						1	1	1																
24	54 ppt					2	1	1	2																
25	60 ppt					4	2	4	3																
26	66 ppt	1				1	1																		
27	72 ppt	1	4		1			1			1					2		1	1				2		
28	78 ppt			1	2					1	1		1			1									
29	84 ppt	2		2									1			3	1	1						2	
30	90 ppt												1	1		1		1					4	3	
31	96 ppt	1	1							1		2	1	3				1		2	2			3	4
32	102 ppt	1	1							3	2	1	1	2	3		1	2	2		1	2		1	2
33	108 ppt		2		1					2	3	4	1	2	1	1	2	3	1	2	3	3	3		1
34	114 ppt		1	2	1					3	3	1	4	1	5	1	3	1	3	5	3	2	1	1	
35	120 ppt	4	1	5	5							2		1	1	1	3		3	1	1	3			3
TOTAL		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10



SHORT TERM SALINITY TEST
(6 ppt and 3 ppt daily increase)

6ppt step





Results:

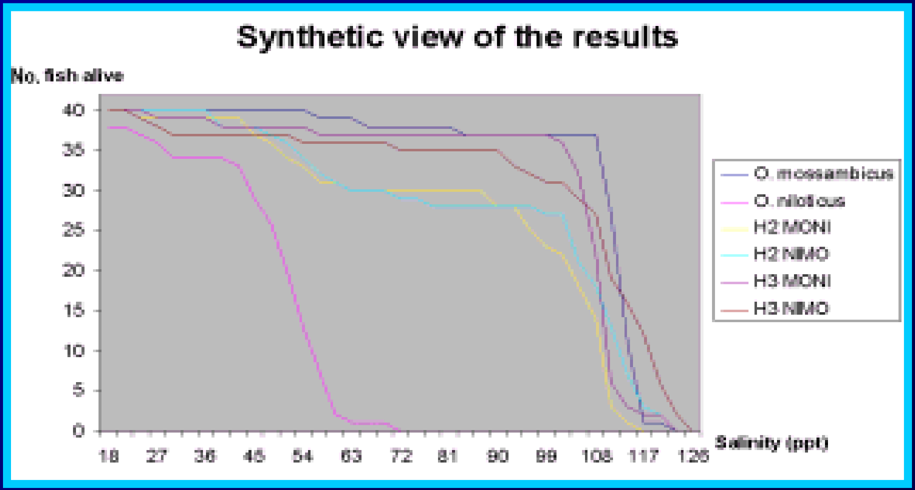
- The hybrids had a salinity resistance significantly higher than *O. niloticus*.
- No significant differences could be found between the hybrids, and between the hybrids and *O. mossambicus*.

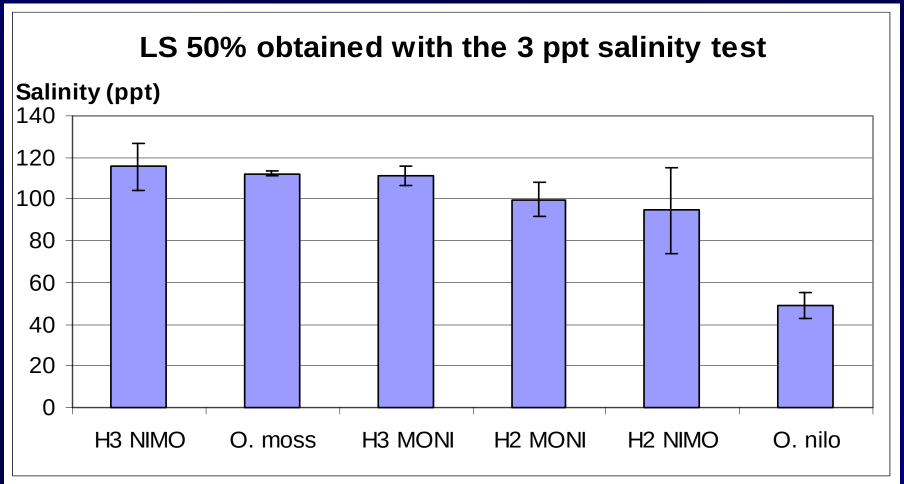
Conclusion:

- The test failed to segregate the tolerance characteristics of the hybrids
- Therefore, another test with a 3ppt daily increase of salinity was conducted.

SHORT TERM SALINITY TEST
(6 ppt and 3 ppt daily increase)

3ppt step





Results

- The hybrids had a salinity resistance significantly higher than *O. niloticus*.
- No significant differences between the hybrids, and between the hybrids and *O. mossambicus*

Conclusions

- The test at 3 ppt was more segregating than the 6 ppt test
- H3 seemed to have slightly higher resistance to salinity than H2
- Males and females had no significant difference with regards to resistance to salinity
- No significant heterosis effect appeared in the hybrids

SHORT TERM SALINITY TEST

(6 ppt and 3 ppt daily increase)

3ppt step

Comparison of resistance to salinity between males and females

- Dead fish were sexed starting at 87 ppt
- Criteria to choose replicates used for the analysis:
  - 70% of the fish still alive at 87 ppt; at least 2 fish for each sex

Result : No significant differences (p-value: 0.74) (1-factor ANOVA)

	O. niloticus	*O. mossambicus*	H2 MoNi	H2 NiMo	H3 MoNi	H3 NiMo
No of replicates used	0	3	4	3	4	3

Check for an eventual heterosis effect

	H2 MoNi	H2 NiMo	H3 MoNi	H3 NiMo
Theoretical LS 50%	96.4	96.4	104.3	104.3
Observed LS 50%	99.7 ±12.9	94.7 ±32.9	111.4 ±7.4	115.6 ±17.6
p-value	0.535	0.893	0.078	0.175

Result: No significant heterosis effect

SHORT TERM SALINITY TEST
(6 ppt and 3 ppt daily increase)

Discussion about the methodology

- The test was proven to be simple and economical in terms of number of fish, facilities and manpower.
- The test at 3 ppt increase was proven to be more segregating than the test at 6 ppt. Therefore, the accuracy of the test could be improved by reducing the salinity daily increment. Maybe better results could be derived when daily increase of 1 to 2 ppt is adopted.

LONG TERM SALINITY TEST

Aim:

- **Compare and evaluate the growth performance and the resistance to salinity of the hybrids in true farming conditions.**

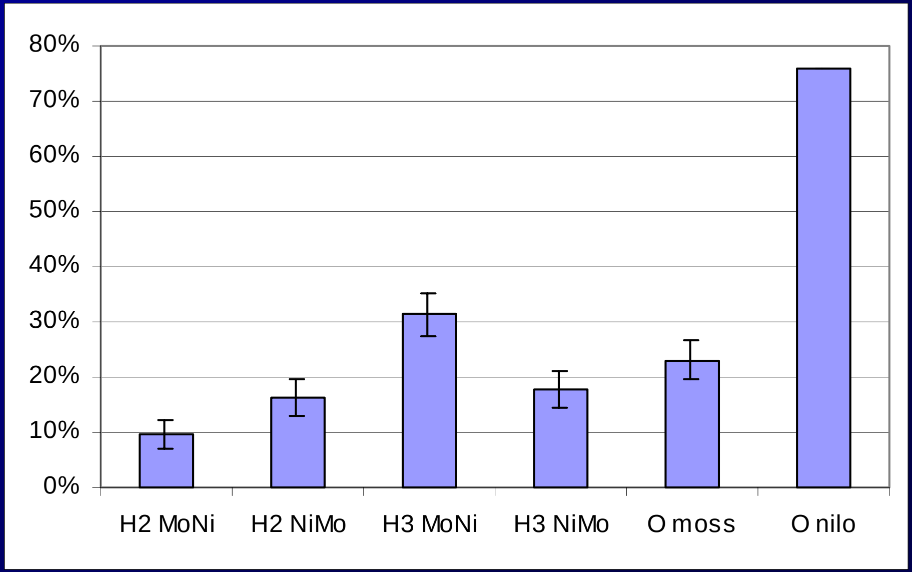
LONG TERM SALINITY TEST

Protocol:

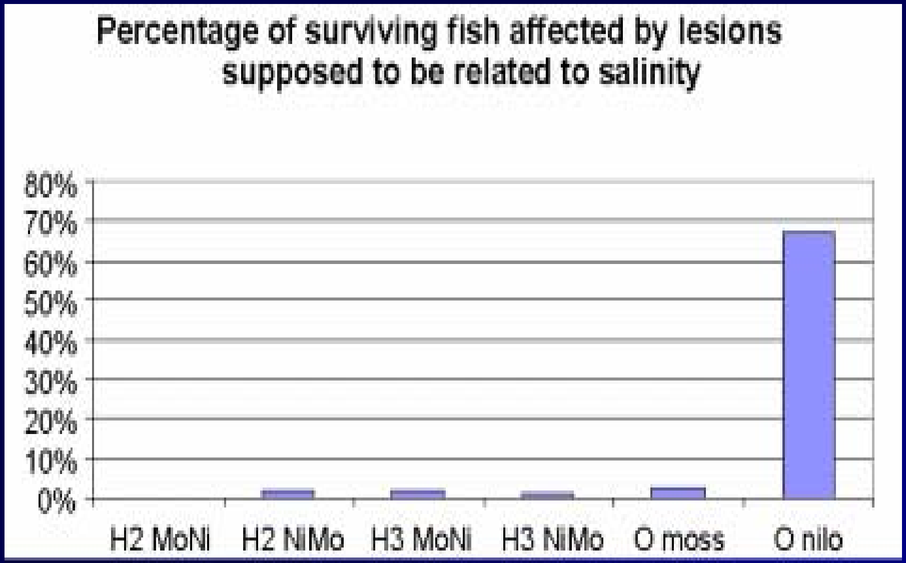
- Stocking density : 1 fish/m^2. 1500 tagged fish (250 each of *O. niloticus*, *O. mossambicus*, H2 NiMo, H2 MoNi, H3 NiMo, H3 MoNi).
- Rearing was conducted in a brackishwater pond under real farming conditions.
- Salinity started at 26 ppt (16 days) up to 35 ppt (104 days).
- Feeding management was *ad libitum* with 29% CP commercial feeds given 3 times daily.
- Pond was harvested after 120 days.
- Mortalities and the growth were analyzed.

LONG TERM SALINITY TEST

Mortalities



Resistance to salinity



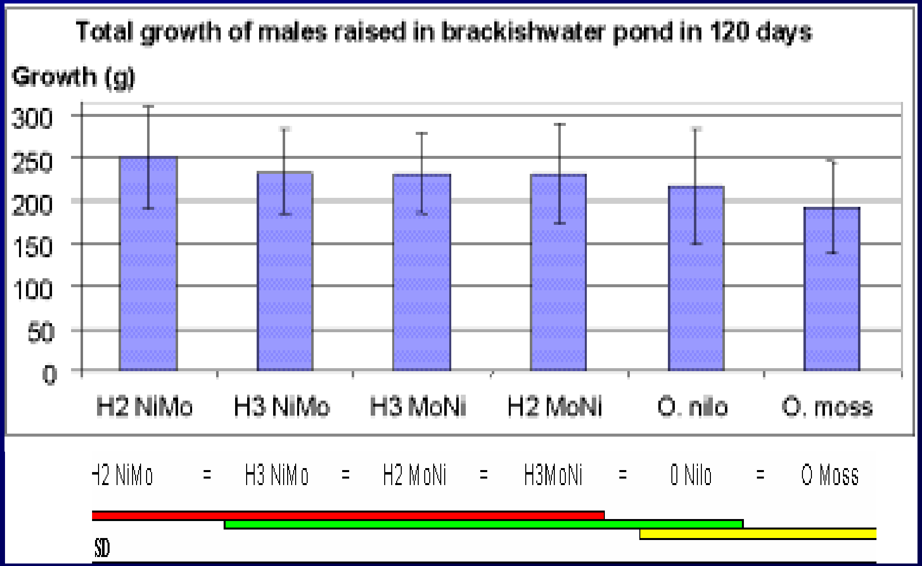
Results:

- **H2 generation showed the least mortality than H3 and *O. mos***
- **Hybrids (H2 and H3 generations) were least affected by lesions**

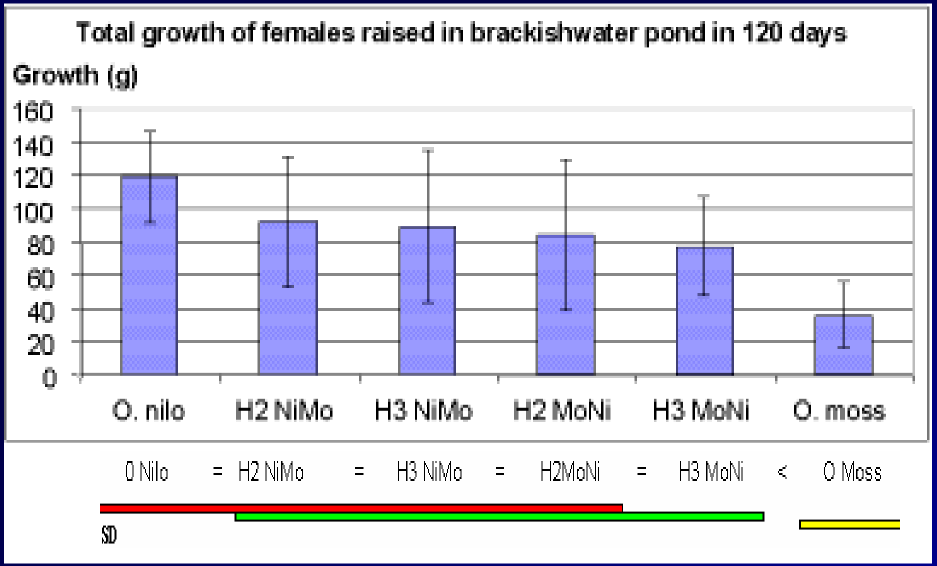
LONG TERM SALINITY TEST

Growth Comparison

Male



Female

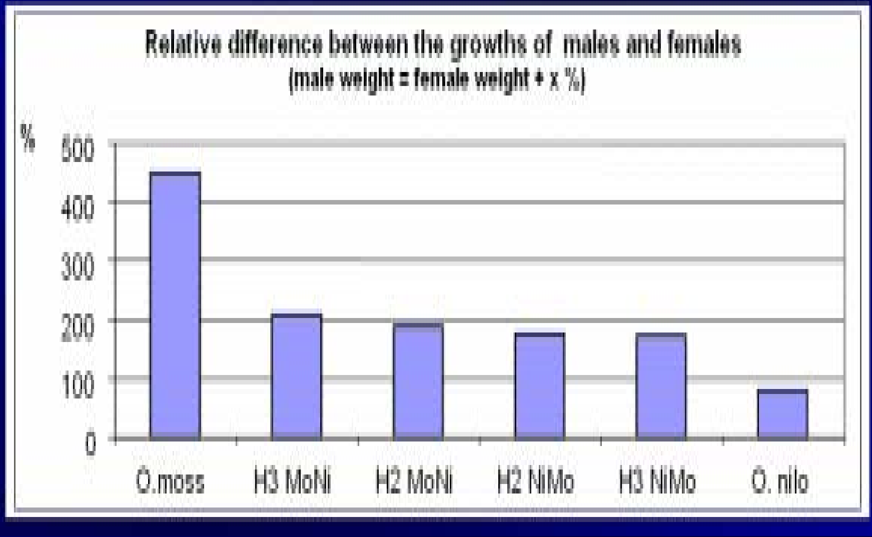


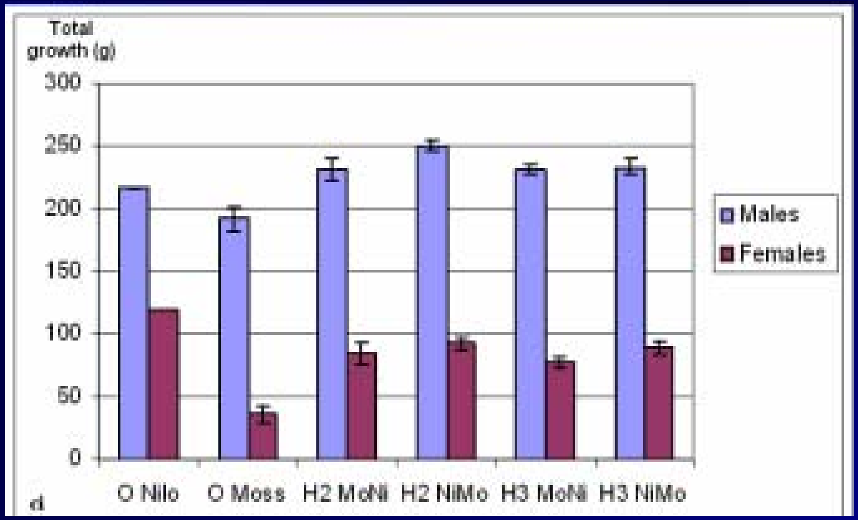
Results:

- Males and females of the hybrids were significantly better than *O. mos*
- No significant difference was found between the hybrids and *O. nil*

LONG TERM SALINITY TEST

Growth Comparison



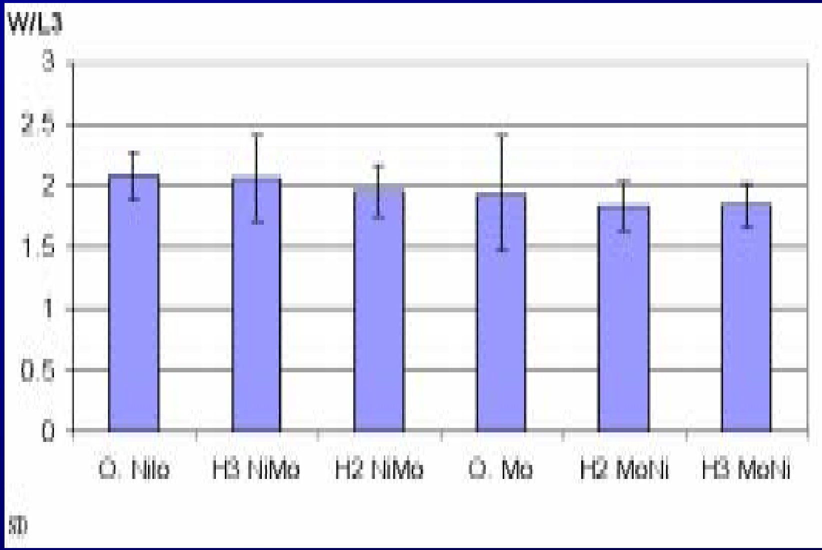


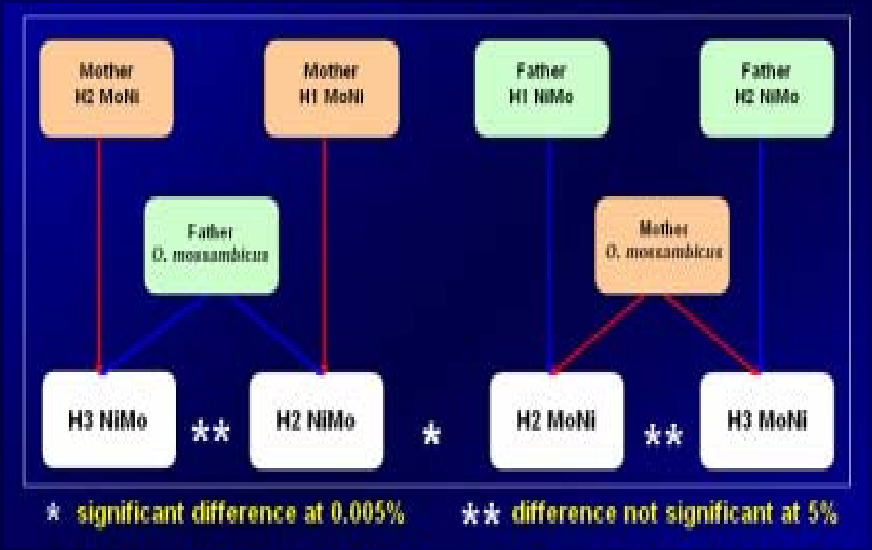
Results:

- Relative difference in growth of *O. nil* males and females was lower than in the hybrids
- Growth of hybrids were significantly better than *O. mos* (both males and females)

LONG TERM SALINITY TEST

Highlight of a possible maternal effect on the W/L^3 ratio





Results:

- Negative maternal effect from *O. mos*
- The hybrids MoNi have a smaller W/L^3 ratio than the hybrids NiMo
  Since consumers prefer short and deep fish, NiMo hybrids could have a slight advantage over the other hybrids
- No significant difference was found between the hybrids on growth performance
- However, it seemed NiMo hybrids grew faster and had a better W/L^3 ratio than the MoNi hybrids

LONG TERM SALINITY TEST

CONCLUSIONS

- The results show that the hybrids have reached a resistance to salinity high enough under common culture condition.
- There is no significant difference in growth between the hybrids and *O. niloticus*.
- NiMo hybrids seem to grow slightly faster and have a bigger W/L^3 ratio than MoNi hybrids.

LONG TERM SALINITY TEST

CONCLUSIONS

Salinity

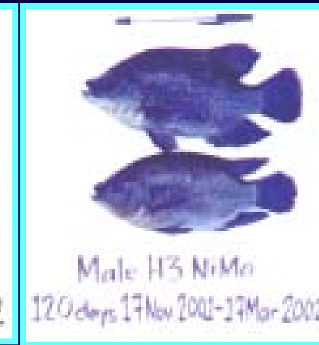
- No significant difference found between hybrids.
- H3 hybrids seem to have a slightly better resistance to salinity than the H2 hybrids.
- H2 and H3 hybrids seem to have reached resistance to salinity, high enough to begin the selection phase.

Growth

- No significant difference on growth found between the hybrids.
- NiMo hybrids seem to grow faster and attain a better W/L^3 ratio than MoNi hybrids.

LONG TERM SALINITY TEST

CONCLUSIONS



Choice of a hybrid generation for selection

Considering the following factors:

a. salinity tolerance

b. growth performance

c. percentage of *O. niloticus* genome in the hybrids

- H2 - 25% of *O. niloticus* genome
- H3 - 12.5% of *O. niloticus* genome

H2 generation was chosen for the growth selection phase of the MOLOBICUS program.

Phase 2

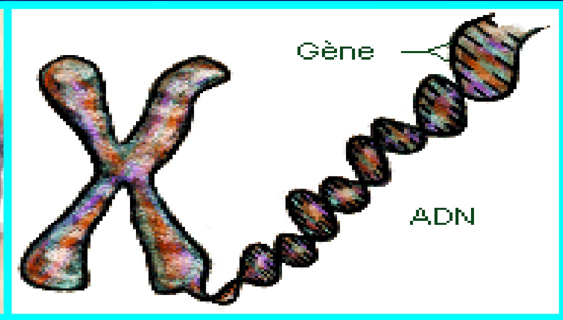
Rotational Crossing

(ON-GOING)

Phase 2

Within Families Selection

P (phenotype) = A (additive value) + E (environment effects)



- *Growth heritability $(h)^2 = Av/Pv = 0.40$ → Selection should be efficient*

Advantages:

- *Simple and efficient (10% increase on growth per generation expected considering the high heritability)*
- *Not expensive*
- *Most important : Selection process can be decentralized to private hatcheries*

Phase 2

Molobicus Selection Phase

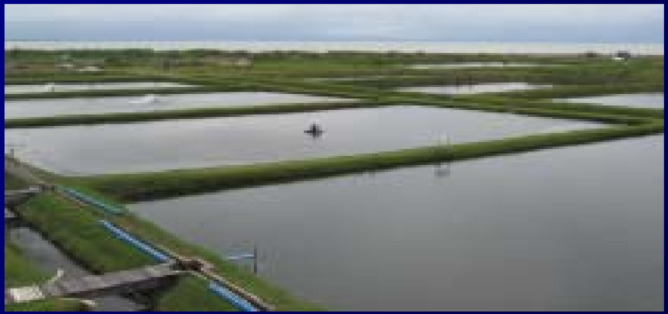
Protocol

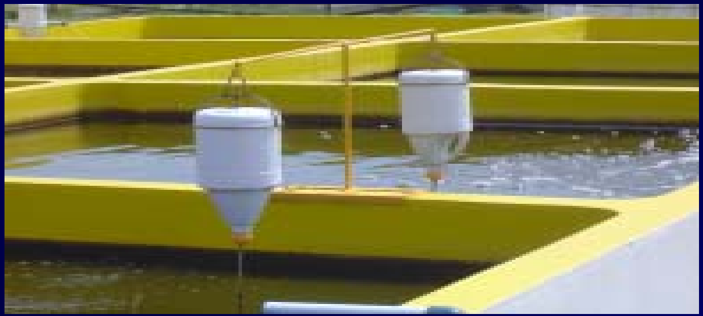
- ***Selection in brackishwater (passive selection on salinity tolerance)***
- ***2 selection environments : Intensive (tanks) and Extensive (ponds) in order to produce 2 different strains***
- ***Wide genetic base : 100 families (50 fish per family)***
- ***Selection criterion : Weight at 5 months (active selection on growth criterion)***
- ***2 generations per year (5 months cycle)***
- ***Rotational crossing to limit inbreeding effects***

Phase 2

Molobicus Selection Phase

Selection in two environments : 2 different strains





	EXTENSIVE	INTENSIVE
Stocking density	0.3 fish/m2	7.5 fish/m2
Area	225m2	9m2
No. of fish	67 (50 Mol & 17 rt)	67 (50 Mol & 17 rt)
Feeding management	Natural Food (no feeding)	Commercial feeds (*ad libitum*)

- **Aim of selection in extensive :** ***Selection of a fast growing tilapia strain specifically adapted to extensive BW farming.***
- **Aim of selection in intensive:** ***Selection of a fast growing tilapia strain adapted to intensive farming in a saline environment.***

Phase 2

Extensive Rearing (in Ponds)

Acclimatization — **Nursing** — **Grow-out**

1st sampling

Harvesting :

Selection of the best breeders

(5 f & 3 m)

7 — **15** — **40** — **150** — Time (days)

300 larvae — **Random selection** → **50 fingerlings (0.3 / m²)**

Rotifers — **Pond natural production (plankton, macroalgae)**

Predator: *Seabass* (1:10)

Phase 2

Intensive Rearing (in Tanks)

Acclimatization

Nursing

Grow-out

Harvesting :

Selection of the best breeders

(5 f & 3 m)

7 — **40** — **150** → Time (days)

300 larvae → **Random selection** → **50 fingerlings (7.5 / m²)**

Phase 2

Calculation of Genetic Gain

Why?

- *To know the genetic value of selected product.*
- *To evaluate the genetic progress*

How? (Various possibilities)

- *Breeders sperm preservation*
- *Conservation of unselected molobicus strain line*
- *Use of the internal control technique.*

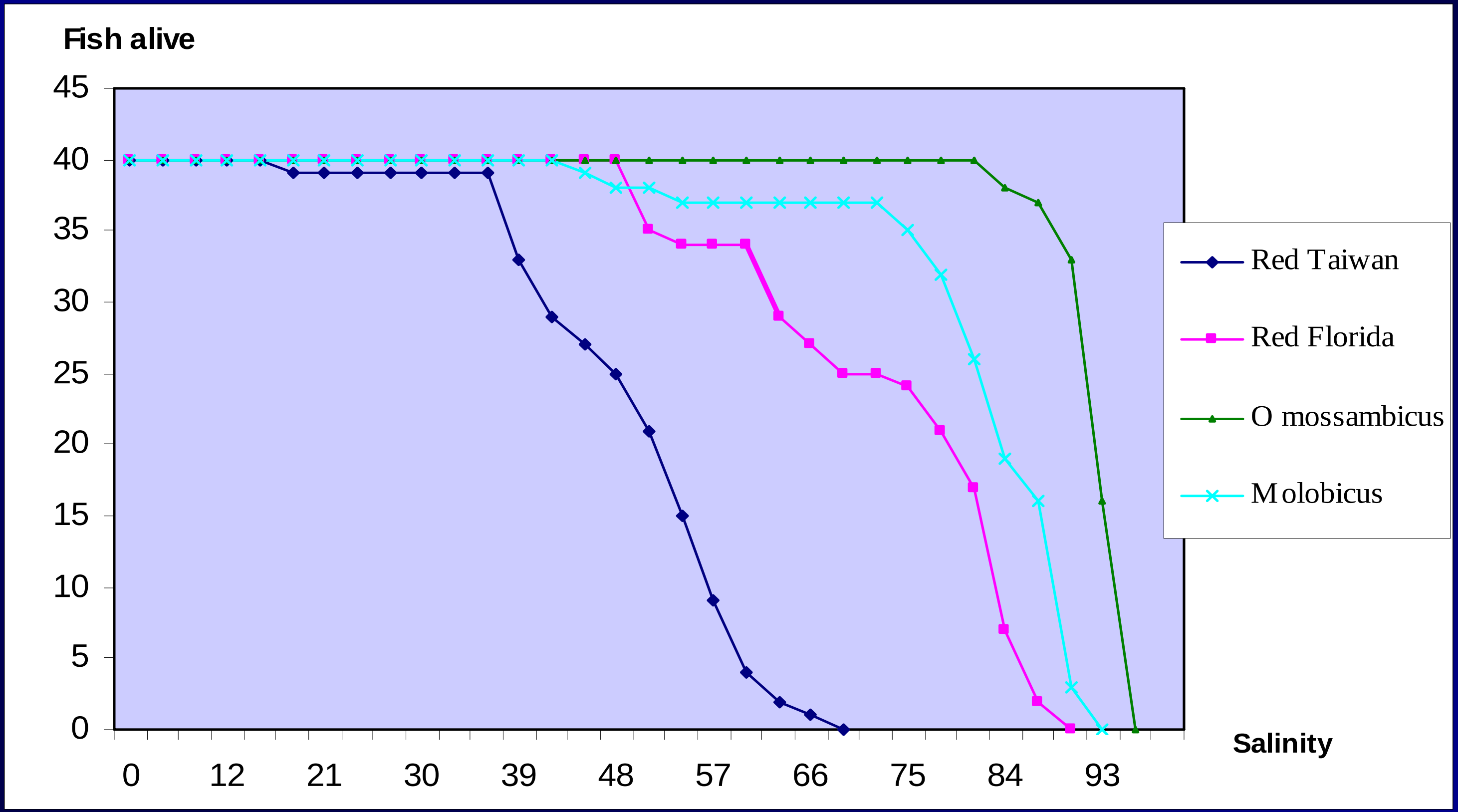
Phase 2

Concept of the Internal Control

- ***The control fish (non-selected "Red Tilapia") is a way to estimate the gain on the hybrids through successive generations.***
- ***17 Red Tilapia are mixed and reared with every group of 50 molobicus siblings in each environment.***
- ***Internal control could remove environmental effects in statistical analyses.***

Phase 2

Comparison of Salinity Tolerance of Red Taiwan, Red Florida, *O. mossambicus* and MOLOBICUS in Aquaria

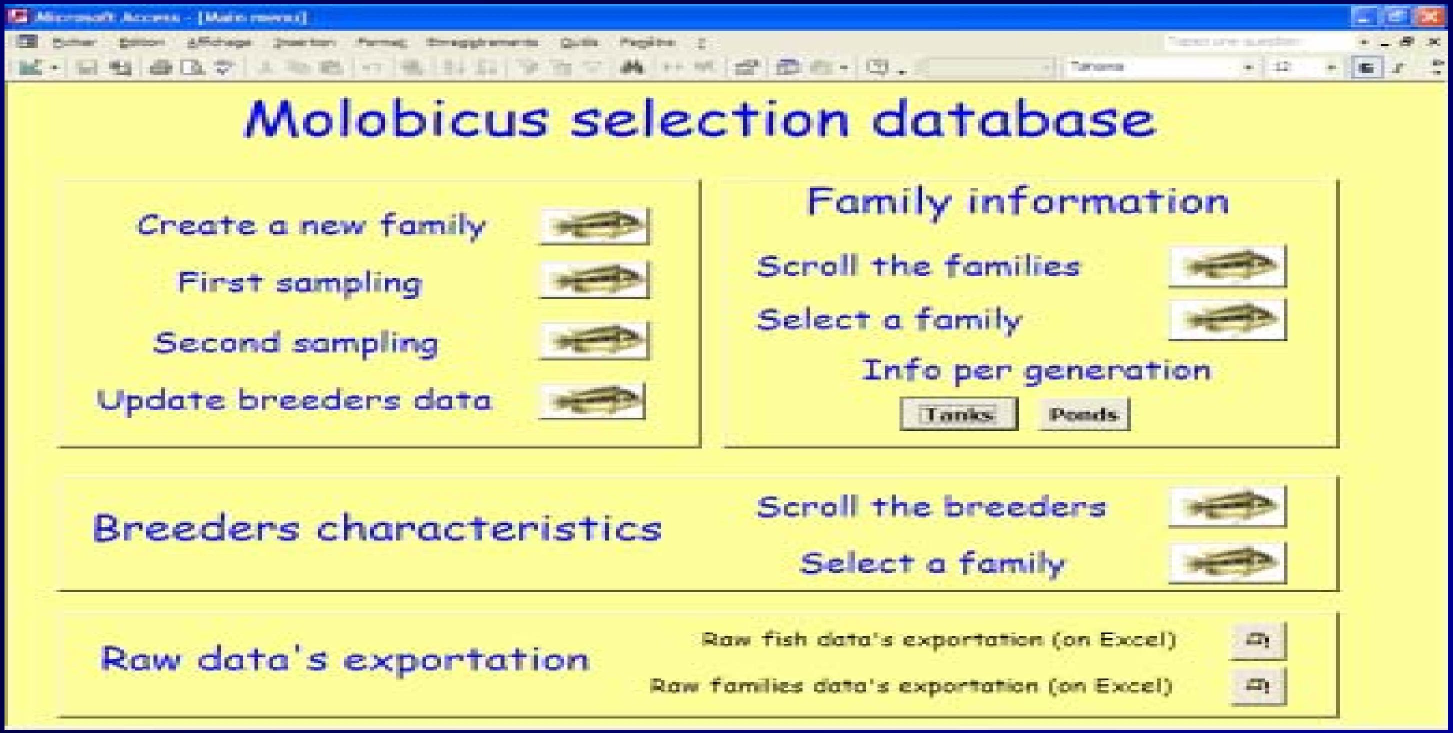


3 ppt daily increase of salinity (Lemarie protocol)

The Database : A Management tool to the Molobicus Program

Aims:

- **To encode data related to the creation of families and the selection phase (more than 50,000 fish will be sampled)**
- **To have an interactive and useful tool which can provide us with data sheets about generations, families, feeding, breeders …**
- **Data are exportable to statistical software for further analysis**





Thank You

ACKNOWLEDGEMENT

The authors wish to thank the following for making this work possible: The Philippine Council for Aquatic and Marine Research and Development (PCAMRD) for the logistic support; the Bureau of Fisheries and Aquatic Resources (BFAR) for the provision of experimental facilities; Dr. Bernard Chevassus for the scientific advise on genetics; Dr. Jerome Lazard for helpful advise; Mr. Cyril Georget for helping in the statistical analysis of the data and CIRAD for providing assistance in the implementation of the Molobicus program. **Mabuhay!**

FUTURE

Additional experiments will be conducted prior to the start of the MOLOBICUS program phase II:

- Experiment on the growth performance of hybrids in brackish and freshwater to:
  - validate initial results
  - check for an eventual heterosis effect.
- Experiment on the maturation and fecundity of hybrids in brackish and freshwater.

THEN, the H2 generation will be chosen for the growth selection process. This will allow full expression of growth potential (contributed by *O. niloticus* genome) in the hybrids.

Conclusion and Prospects

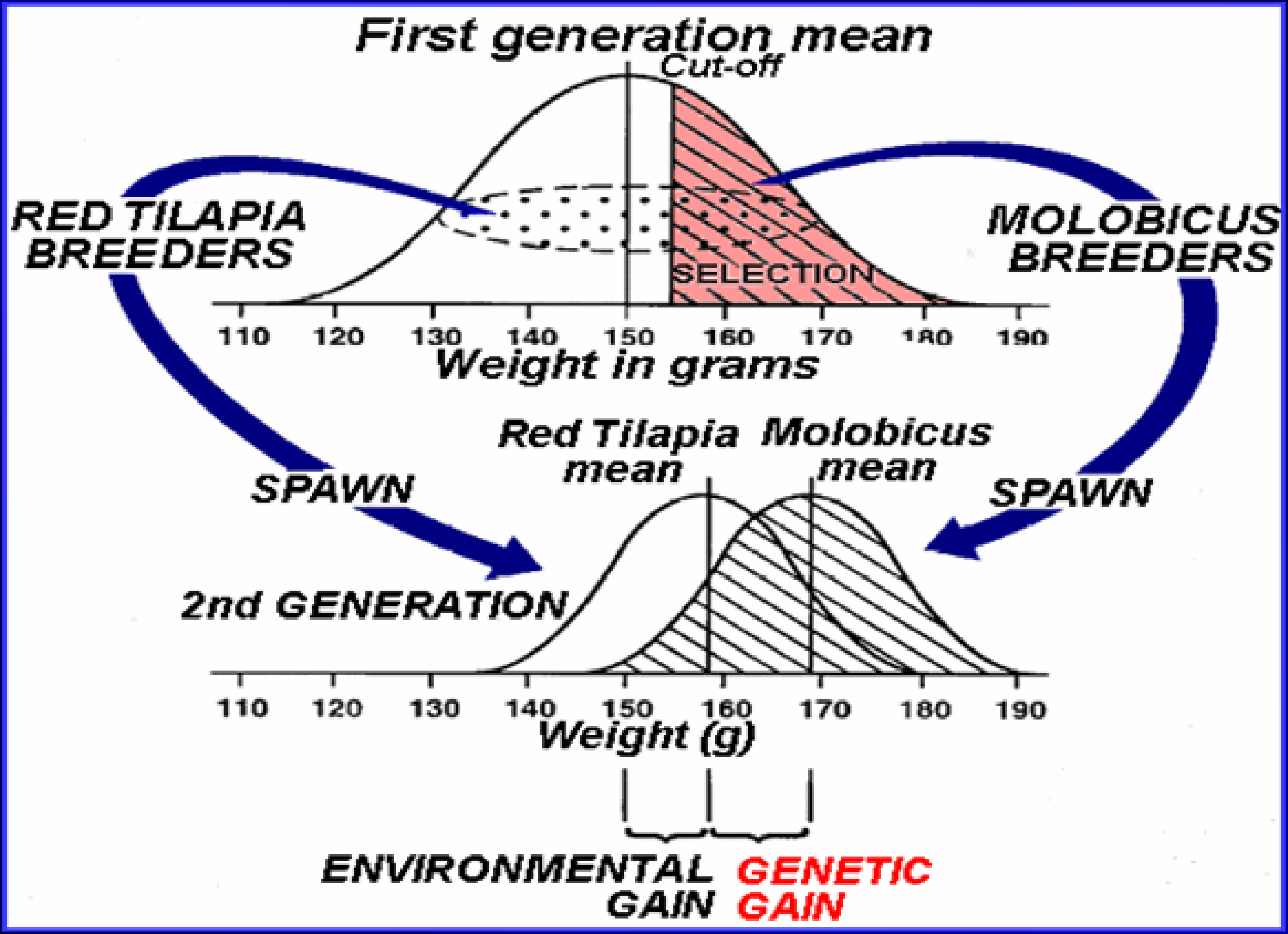
Advantage

- **A tilapia produced at low cost offers great development prospects for both export and local market.**

Practically, today, BW Tilapia could become some form of low cost by-product of the prawn industry

Phase 2

Computing the genetic gain : Inclusion of an internal control



LONG TERM SALINITY TEST

Analysis of the lost tags

Hypothesis : for each type of hybrid and for *O. mossambicus*, the number of unidentified dead fish is proportional to the number of identified dead fish

	O. niloticus	*O. mossambicus*	H2 MoNi	H2 NiMo	H3 MoNi	H3 NiMo	**Total**
Number of tagged fish alive at harvest	60	115	128	173	152	148	**776**
Number of tagged fish found dead during the test	118	17	7	12	23	13	**190**
Number of fish found dead during the test with lost tag	0 ***	4 *	2 *	3 *	5 *	3 *	**17**
Number of fish alive harvested with lost tag	0 ***	77 **	98 **	36 **	20 **	58 **	**289**
Number of fish missing	72 **	37 *	15 *	26 *	50 *	28 *	**228**
Total	**250**	**250**	**250**	**250**	**250**	**250**	**1500**

***	Estimated number related to the percentage of observed mortalities**
****	Estimation (data's deducted from other data's)**
*****	The O. niloticus could be identified without tag**

LONG TERM SALINITY TEST

Check for the Hypothesis

	Males	Females
Theoretical mean weight of the lost tags	233.5 g ± 7.0 g *	82.5 g ± 6.5 g *
Observed mean weight of the lost tags	231.2 g	84.4 g

* Confidence intervals at 5% risk

LONG TERM SALINITY TEST

Influence of the lost tags on the growth estimation

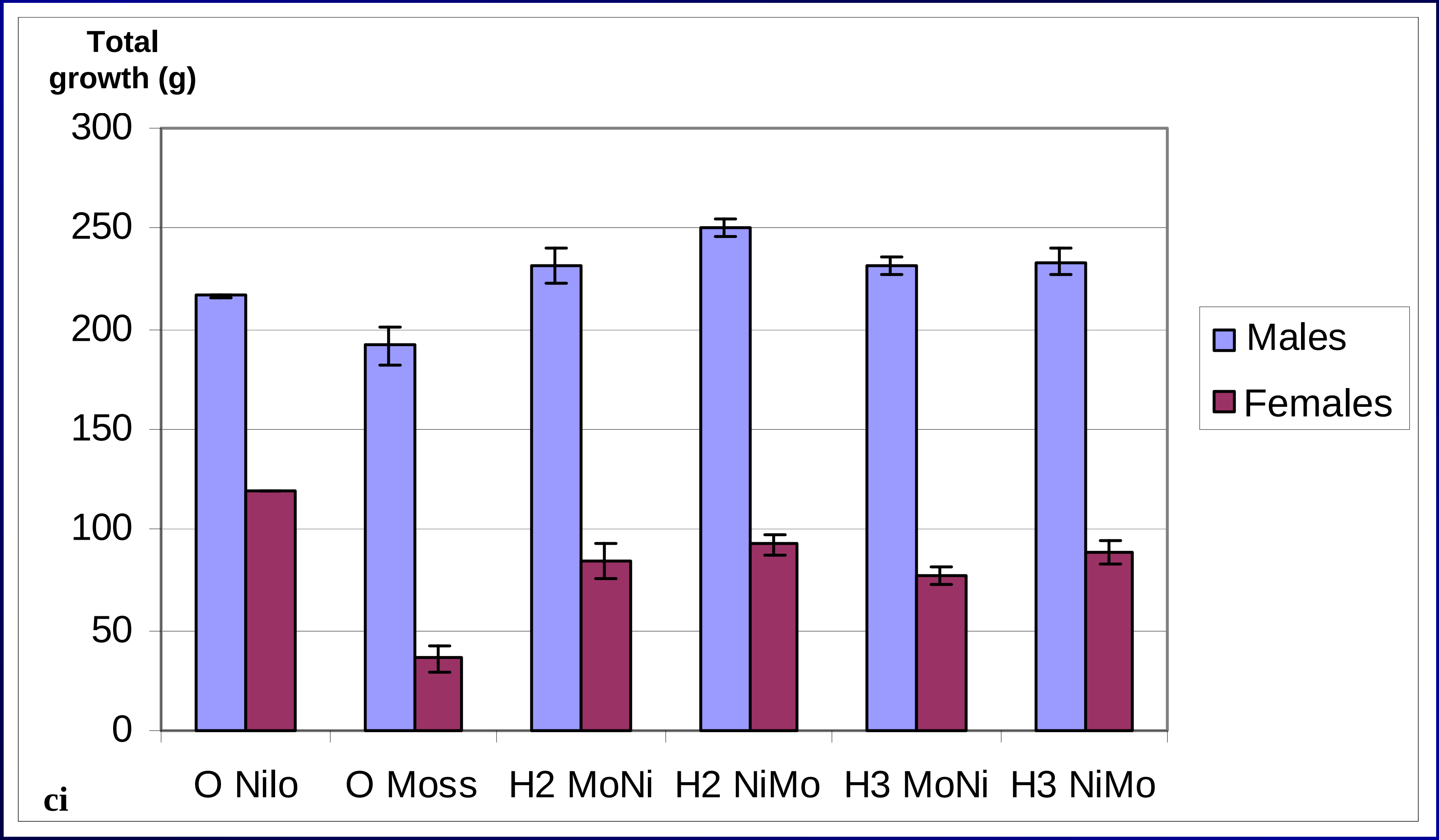
Confidence intervals of the mean initial and final weight and of the total growths

	O. niloticus		*O. mossambicus*		H2 MoNi		H2 NiMo		H3 MoNi		H3 NiMo	
Sex	m	f	m	f	m	f	m	f	m	f	m	f
Width of the confidence interval of the initial weight at 5% risk (g)	0.40		0.21		0.23		0.16		0.18		0.16	
Width of the confidence interval of the final weight at 5% risk (g)	0	0	9.0	6.5	7.9	8.2	4.0	4.8	4.0	3.9	6.1	5.8
Width of the total confidence interval of the growth at 5% risk (g)	0.40	0.40	9.21	6.71	8.13	8.43	4.16	4.96	4.18	4.08	6.26	5.96

The results on the growths are valid only for the fish alive and identified at the end of the test.

LONG TERM SALINITY TEST

Total growth and confidence interval at 5% risk



HETEROSIS EFFECT ON THE GROWTH

The evaluation of the heterosis on the growth was not calculated since:

- Between the salinities of 26 to 35 ppt, *O. niloticus* failed to show normal growth performance and therefore cannot be used as reference.
- With mortalities between 10 to 30% for the hybrids and *O. mossambicus* and 75% for *O. niloticus*, the confidence intervals on growth jeopardize the comparison between the observed weight and the theoretic weight used in the calculation of heterosis effect. In addition, it was assessed that the ability to live in salt water depends on the individual weight (Chervinski, 1982 in Lemarié 2001).
- Lost tags and missing fish aggravate the impact of mortalities on confidence intervals.

GROWTH MODEL FITTING

$$\sqrt{Growth} = Type\ of\ hybrid + Sex + Type{:}Sex$$

Analysis of Variance Table

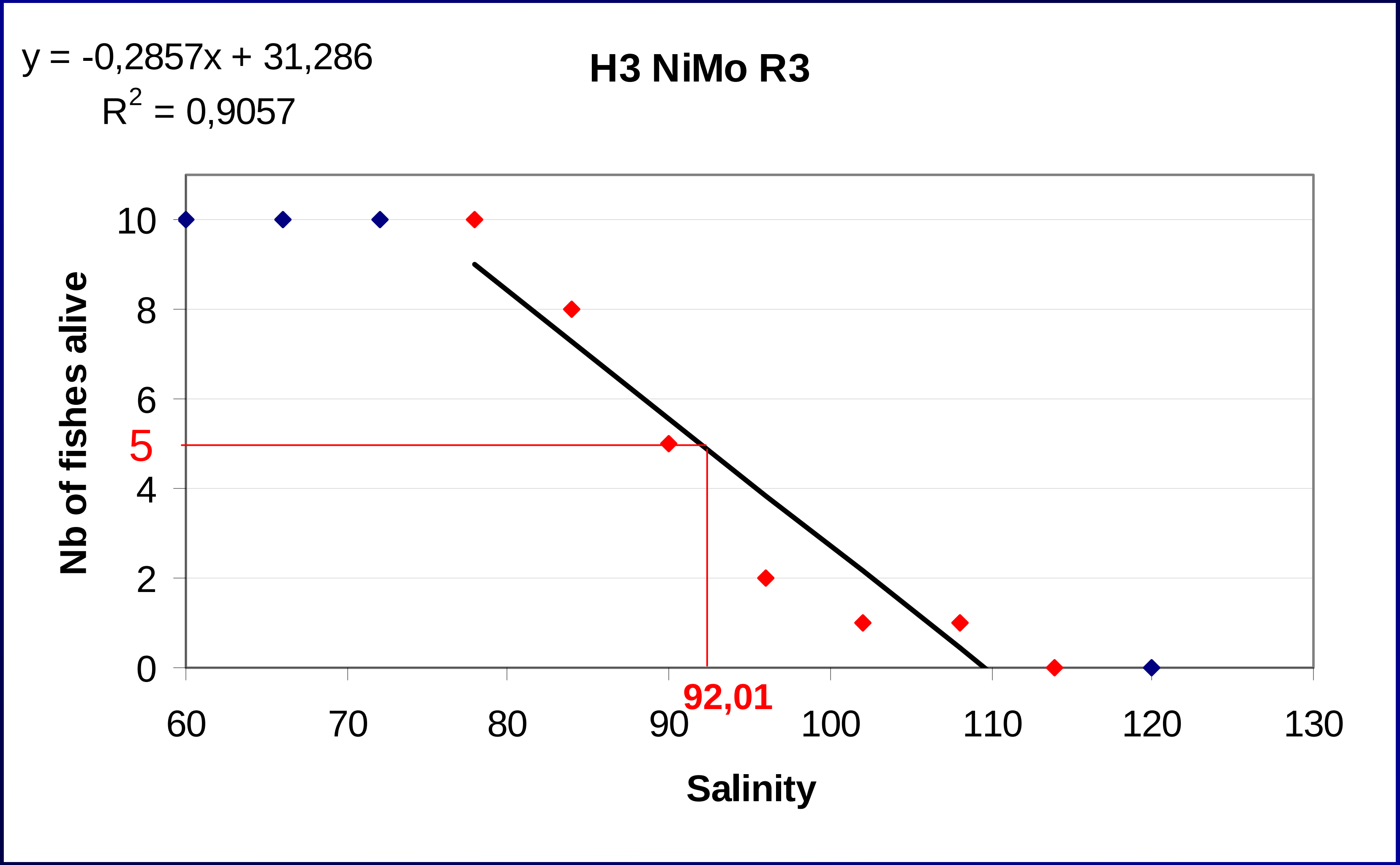
Response: sqrt(weight)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
type	5	1265.1	253.0	68.8952	< 2.2e-16 ***
sex	1	6898.6	6898.6	1878.4366	< 2.2e-16 ***
type:sex	5	124.2	24.8	6.7651	3.493e-06 ***
Residuals	764	2805.8	3.7		

Signif. codes: 0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` '

SHORT TERM SALINITY TEST

Calculation of the salinity at which 50% of fish died (LS 50%)

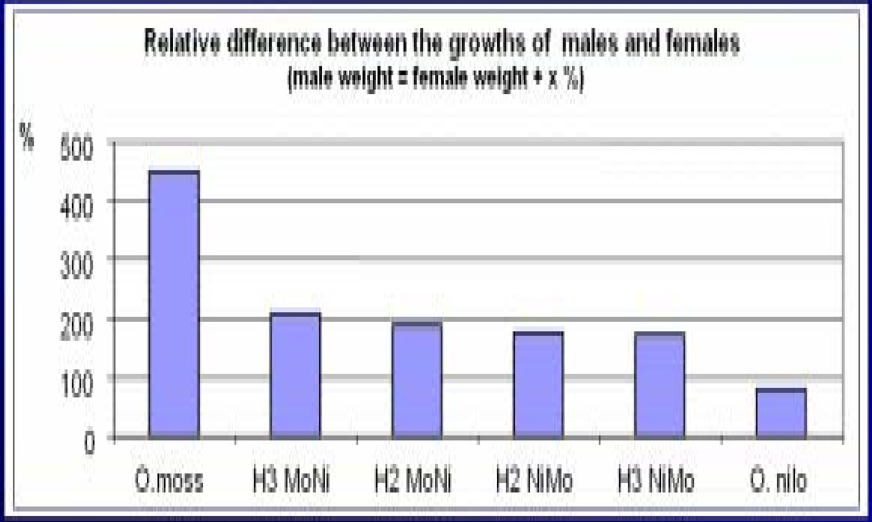


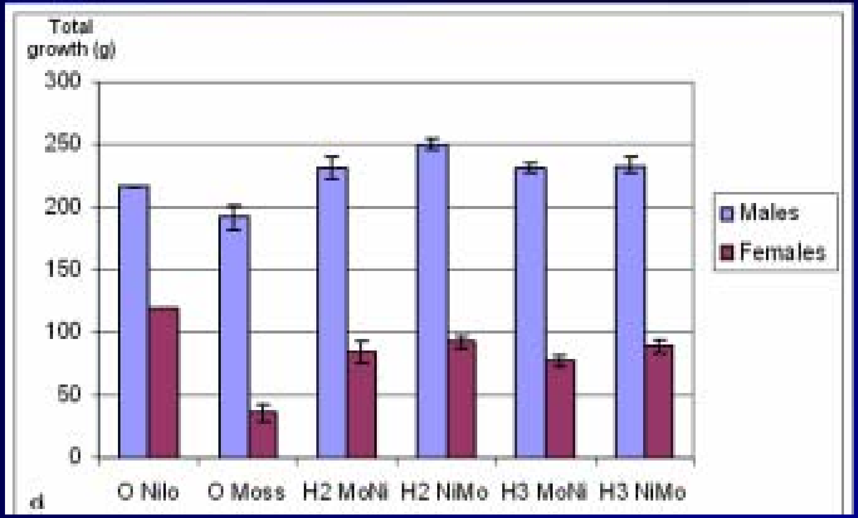
LONG TERM SALINITY TEST

Growth Comparison

Model:

$$\sqrt{Growth} = Type\ of\ hybrid + Sex + Type{:}Sex$$





Results:

- Relative difference in growth of *O. nil* males and females was lower than in the hybrids
- Growth of hybrids were significantly better than *O. mos* (both males and females)

ADDITIONAL INFORMATIONS

LONG TERM SALINITY TEST

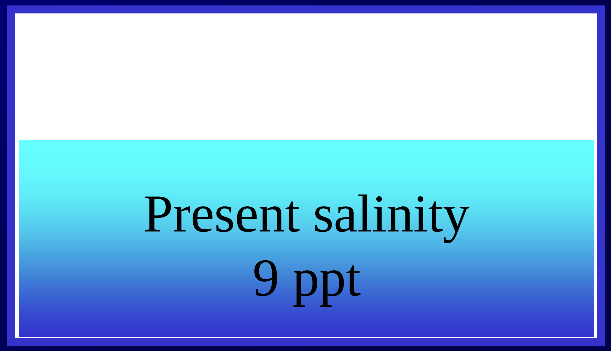
- Analysis of the Lost Tags
- Check for the Hypothesis
- Influence of the lost tags on the growth estimation

HETEROSIS EFFECT ON THE GROWTH

GROWTH MODEL FITTING

CALCULATION OF THE SALINITY AT WHICH 50% OF FISH DIED (LS 50%)

Present salinity + 2 x the gap



Bukas dapat 12 ppt

Solution : 9 + (2 x 3) = 15 ppt

THANK YOU