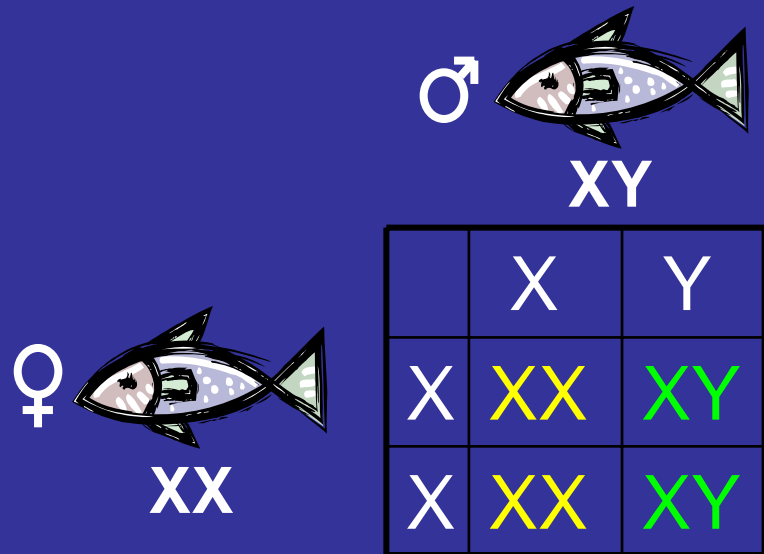


Developing a Theoretical and Experimental Framework for the Trojan Y Chromosome Eradication Strategy

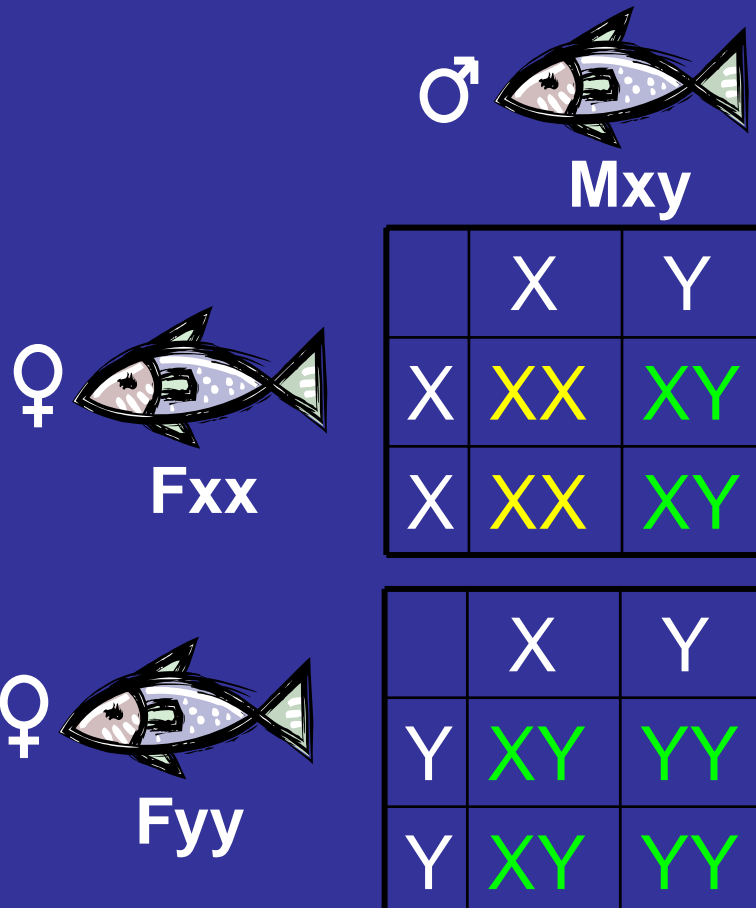
John Teem, Florida Department of Agriculture
and Consumer Services
Division of Aquaculture
Juan Gutierrez, University of Georgia

XY Sex-Determination

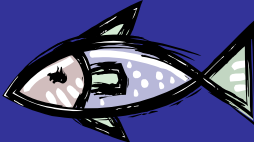


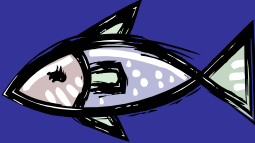
Males/Females
Ratio 1:1

Females with Two Y chromosomes Produce Only Male Progeny, Half of Which are Myy

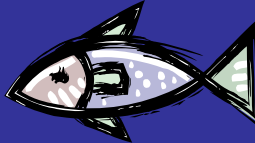


Myy males are viable and produce only male offspring

♀  **Fxx**

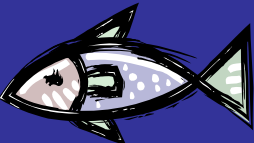
♂  **Mxy**

	X	Y
X	XX	XY
X	XX	XY

♂  **Myy**

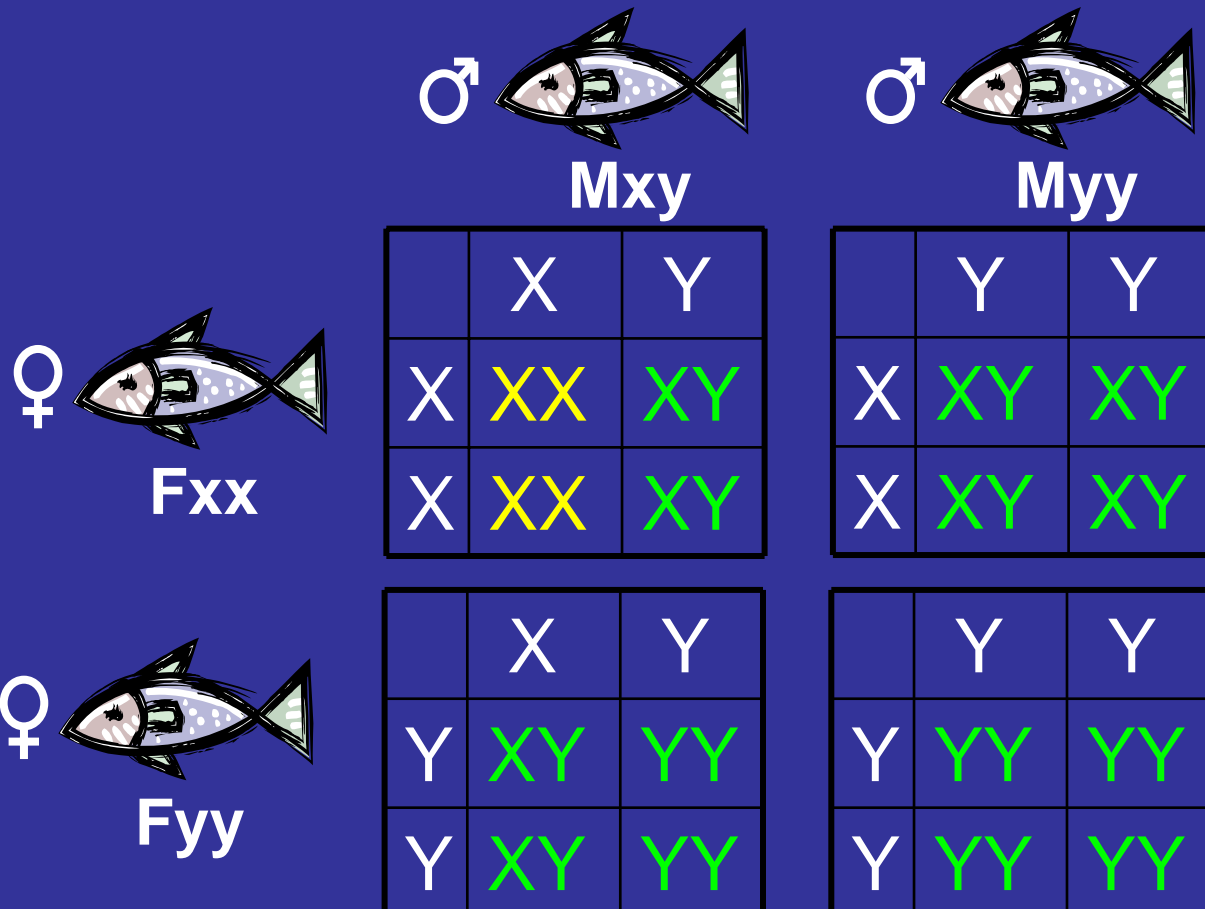
	Y	Y
X	XY	XY
X	XY	XY

Males/Females
Ratio 1:0

♀  **Fyy**

	X	Y
Y	XY	YY
Y	XY	YY

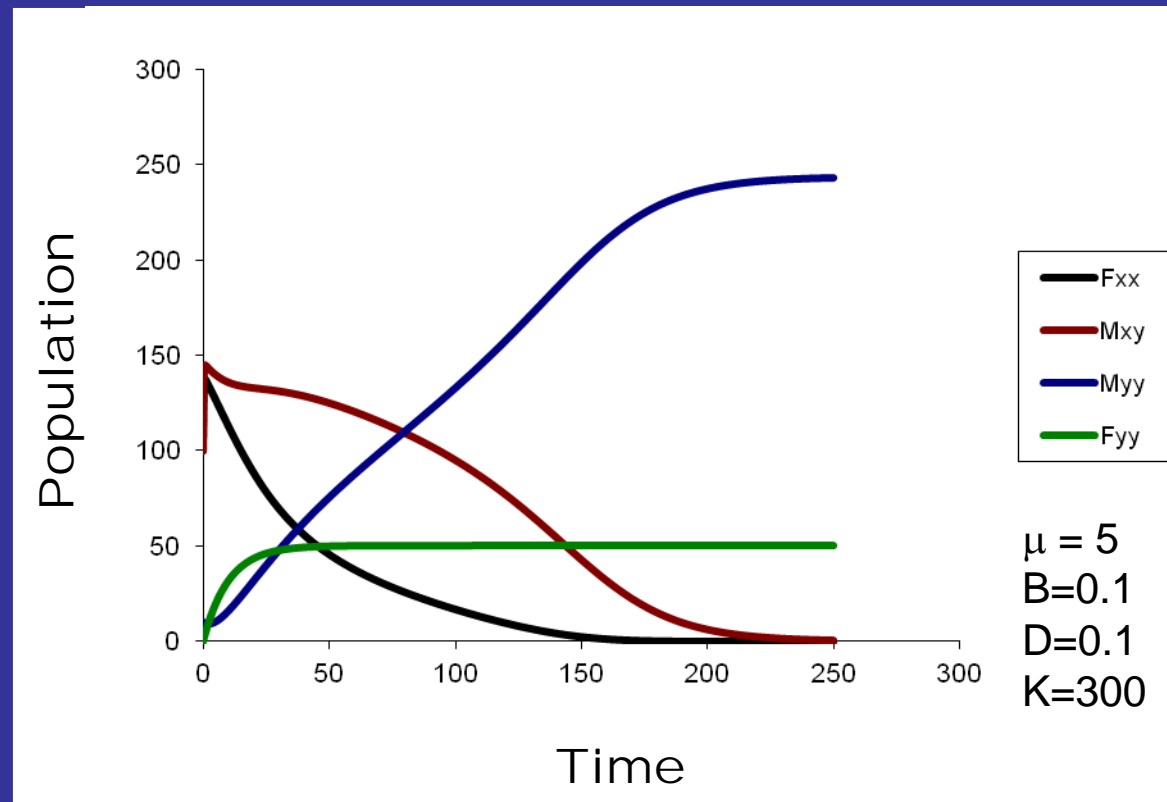
Four different matings are possible, leading to increased male production



Males/Females
Ratio 7:1

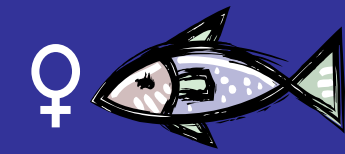
Male/Female ratio will
increase over time if
Fyy added.

The addition of a Trojan Y female (Fyy) to a target population will cause females (Fxx) to go to extinction over time.

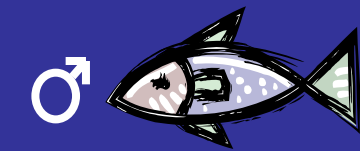


The carrying capacity of the system becomes occupied by Myy fish (males with two Y chromosomes).

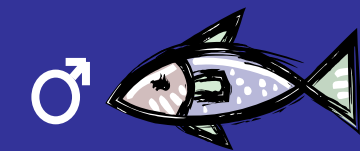
Modeling the Trojan Y Chromosome System Using Differential Equations



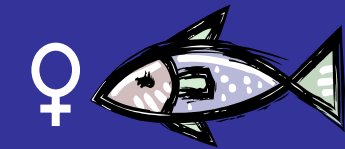
♀ **100** F_{xx}



♂ **100** M_{xy}



♂ **0** M_{yy}



♀ **10** F_{yy}

$$\frac{d}{dt} F_{xx} = 0.5BF_{xx}M_{xy}L - DF_{xx}$$

$$\frac{d}{dt} M_{xy} = (0.5BF_{xx}M_{xy} + 0.5BF_{yy}M_{xy} + BF_{xx}M_{yy})L - DM_{xy}$$

$$\frac{d}{dt} M_{yy} = (0.5BF_{yy}M_{xy} + BF_{yy}M_{yy})L - DM_{yy}$$

$$\frac{d}{dt} F_{yy} = \mu - DF_{yy}$$

$$L = \left(1 - \frac{F_{xx} + F_{yy} + M_{xy} + M_{yy}}{K} \right)$$

B = Rate of births. Is proportional to the fecundity.

D = Rate of deaths. Takes into account the life span.

K = Carrying capacity of the ecosystem.

μ = **Fyy Trojan fish added**

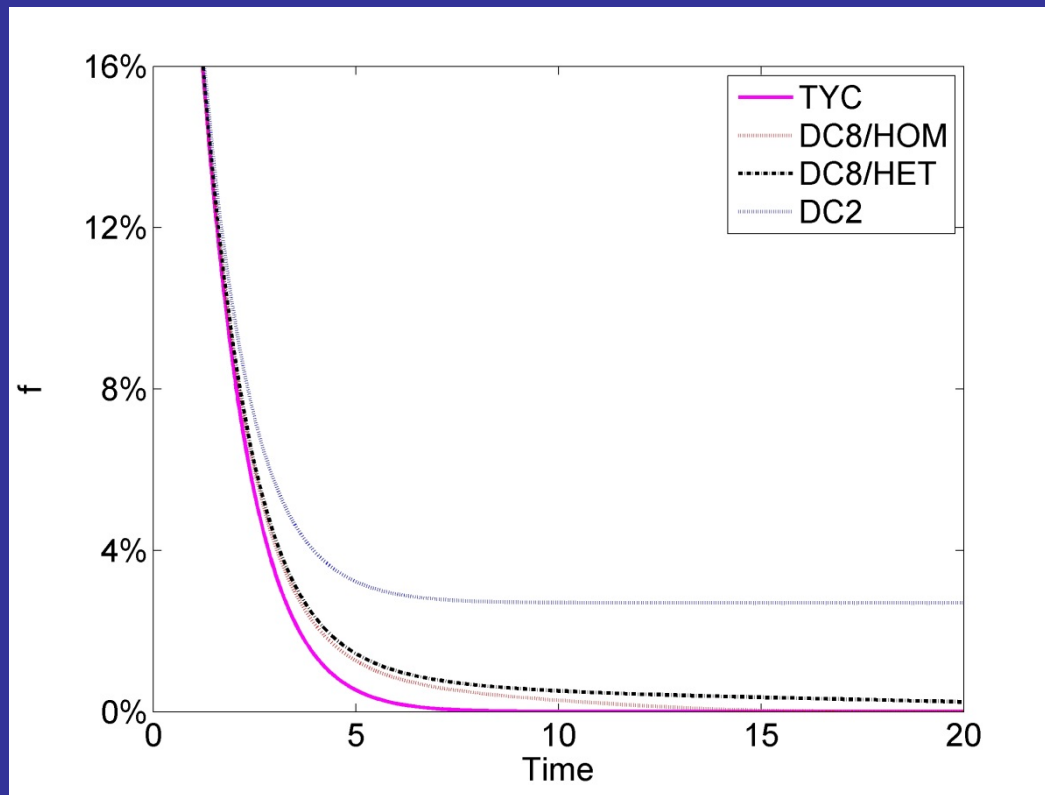
$B=[0.01, 10]$

$D=[0.1, 3]$

$K= 300$

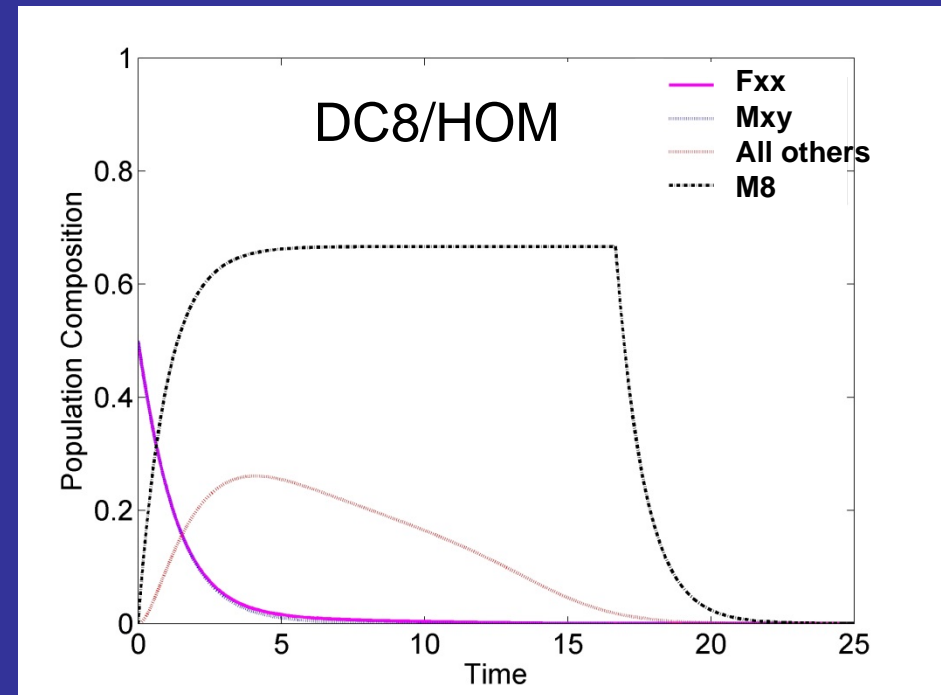
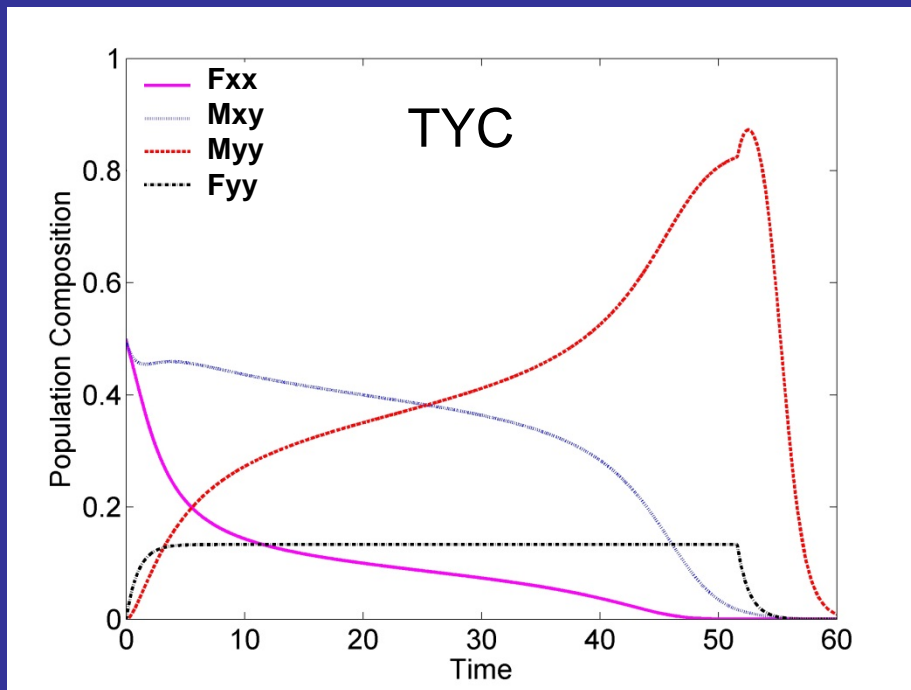
$\mu= [0, 100]$

Elimination of Fxx females occurs more rapidly with the TYC strategy as compared to Daughterless Carp strategies



Eradication can be achieved with the TYC strategy at a lower stocking rate (about 1.33% of the population) as compared to the DC strategy (about 6.66% of the population).

Eradication is achieved when the carrying capacity of the system is replaced by males as a result of the addition of the autocidal fish



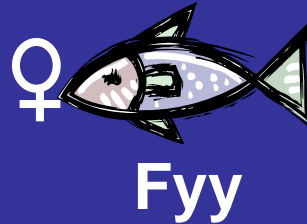
In the TYC system, this replacement is generated primarily by mating of the autocidal fish. In the DC system, the replacement is primarily a result of physical substitution.

Extinction of females occurs more rapidly with a Trojan Y Chromosome eradication model as compared to a Daughterless Carp eradication model.

The TYC strategy additionally requires fewer fish to achieve female eradication.

Reduced fitness of the autocidal fish is a potential problem in each system and could have a large impact on the dynamics of female extinction.

YY Broodstock



The production of YY fish requires selective breeding and the use of hormone-induced sex reversal techniques.

YY genotypes are verified by test crosses and evaluation of the sex distribution in progeny.

Sex-specific DNA markers can greatly reduce the time required to generate YY fish by allowing YY genotypes to be detected by DNA analysis (instead of test crosses).

For some fish, sex-specific DNA markers have been identified by using the RAPD PCR method.

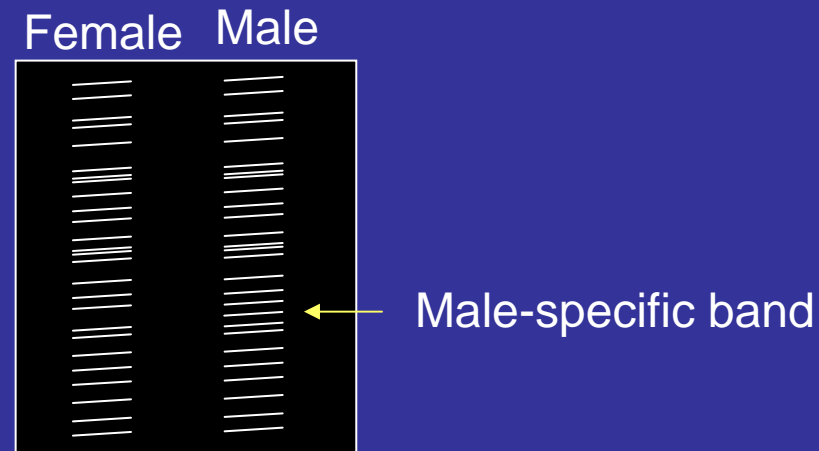
RAPD PCR

Create a DNA pool from only females and another from only males.

Test each pool with PCR using a collection of short DNA primers that will amplify sequences at different locations in the genome.

For each primer, compare female-specific DNA amplified products with male-specific amplified products using gel electrophoresis.

Find a primer that gives a band in one DNA pool, but not the other.



Three invasive fish species were screened for sex-specific DNA markers using RAPD PCR.

Nile Tilapia



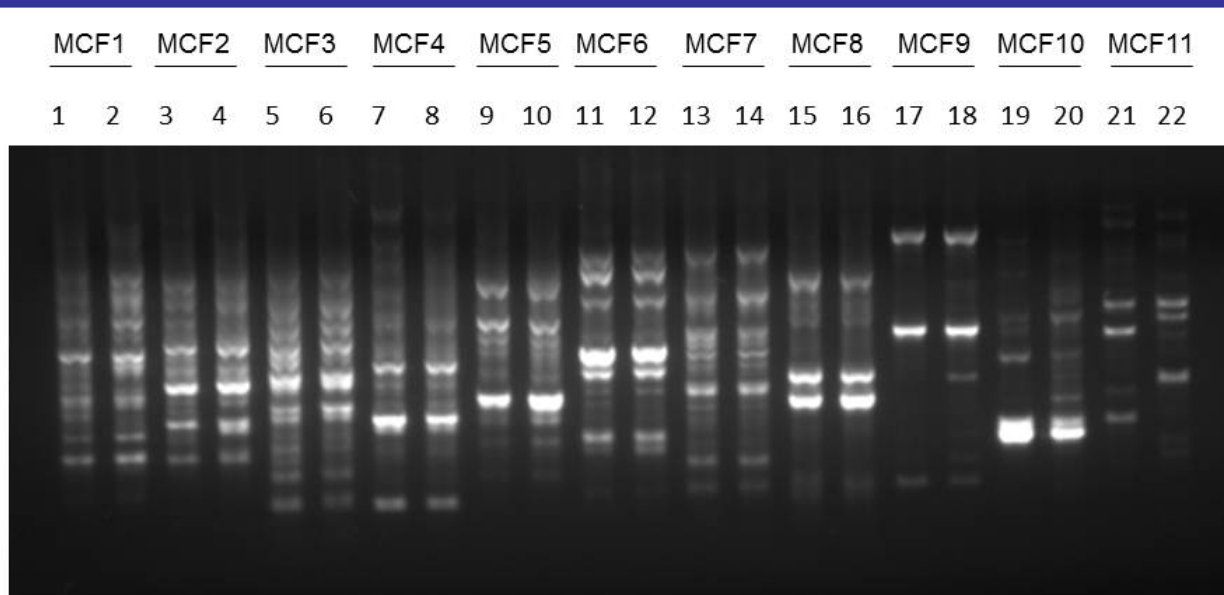
African Jewelfish



Silver Carp



PCR Screening for Sex-specific DNA Markers in African Jewelfish



Odd # lanes = male-specific African jewelfish DNA pool 1 (M2-M9)

Even # lanes = female-specific African jewelfish DNA pool 1 (F2-F9)

DNA fragments from PCR reactions using RAPD primers MCF1-MCF11 are separated on a 1.5% agarose gel.

Conclusions

Screening for sex-specific DNA markers has been done with African Jewelfish, Nile Tilapia and Silver Carp.

African Jewelfish have been the first priority because broodstock are being developed for this species by USGS.

No sex-specific markers have been identified as yet for any of the three species.

Experiments to determine the sex-determination system for African Jewelfish are in progress in collaboration with USGS.