Trojan Y Chromosome Eradication: Sex-specific DNA Markers for Invasive Fish

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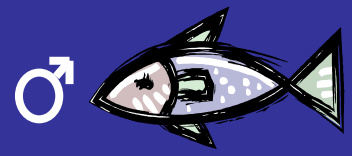
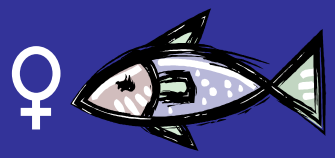
XY Sex-Determination



XY

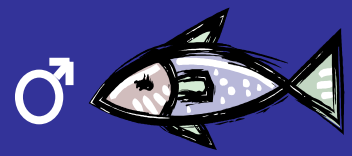


XX

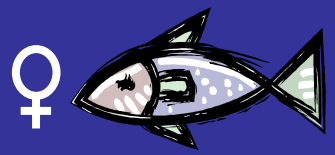
	X	Y
X	XX	XY
X	XX	XY

Males/Females
Ratio 1:1

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

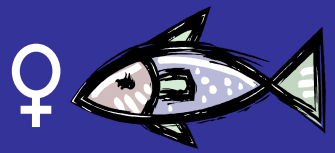


Mxy



Fxx

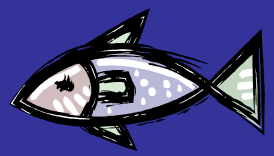
	X	Y
X	XX	XY
X	XX	XY



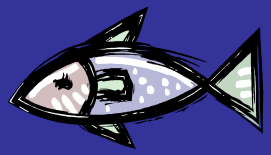
Fyy

	X	Y
Y	XY	YY
Y	XY	YY

Myy males are viable and produce only male offspring

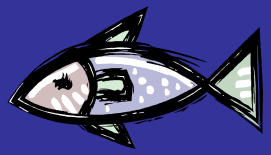
♀ 

Fxx

♂ 

Mxy

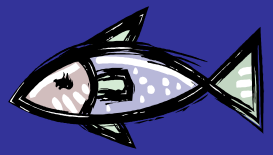
	X	Y
X	XX	XY
X	XX	XY

♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

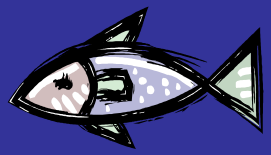
Males/Females Ratio 1:0

♀ 

Fyy

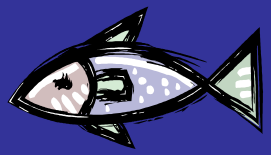
	X	Y
Y	XY	YY
Y	XY	YY

Four different matings are possible, leading to increased male production

♂ 

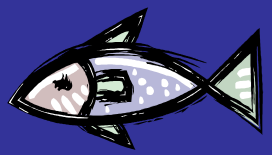
Mxy

	X	Y
X	XX	XY
X	XX	XY

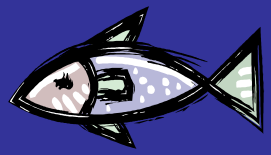
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

♀ 

Fxx

♀ 

Fyy

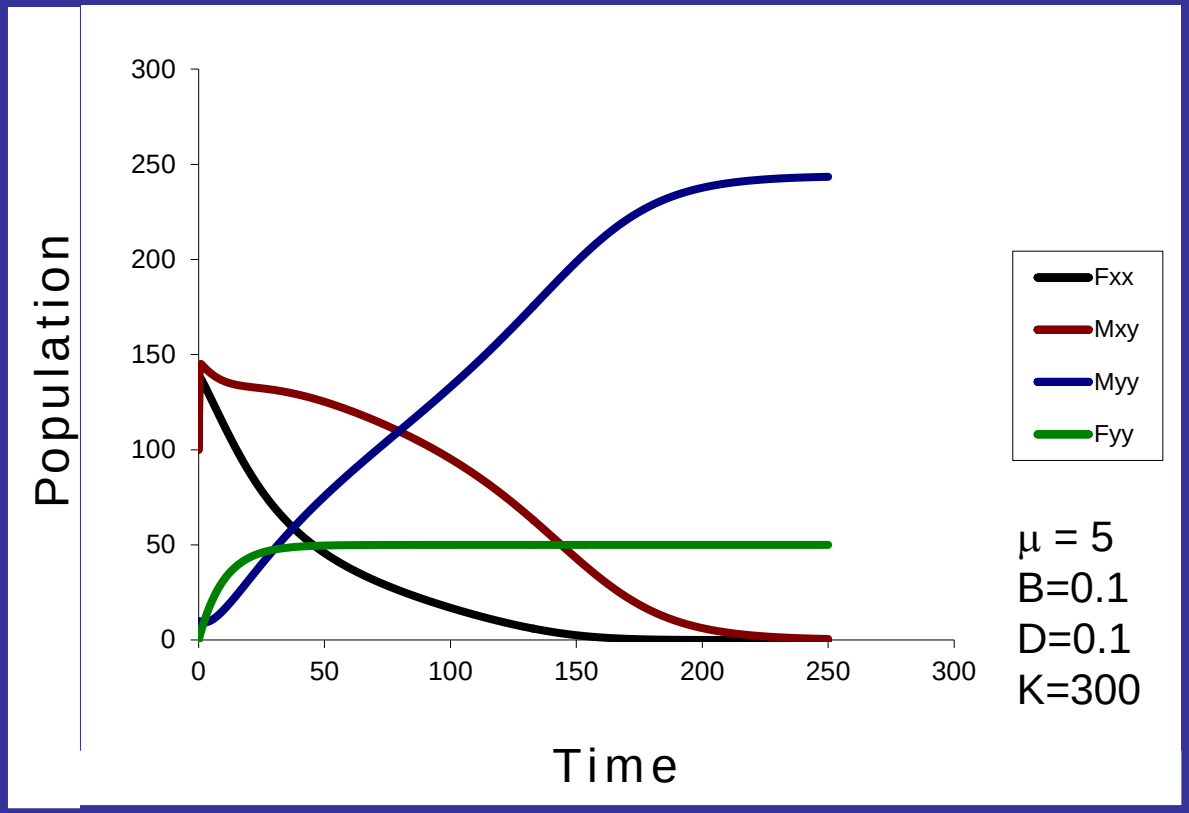
	X	Y
Y	XY	YY
Y	XY	YY

	Y	Y
Y	YY	YY
Y	YY	YY

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).

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

Nile Tilapia

African Jewelfish

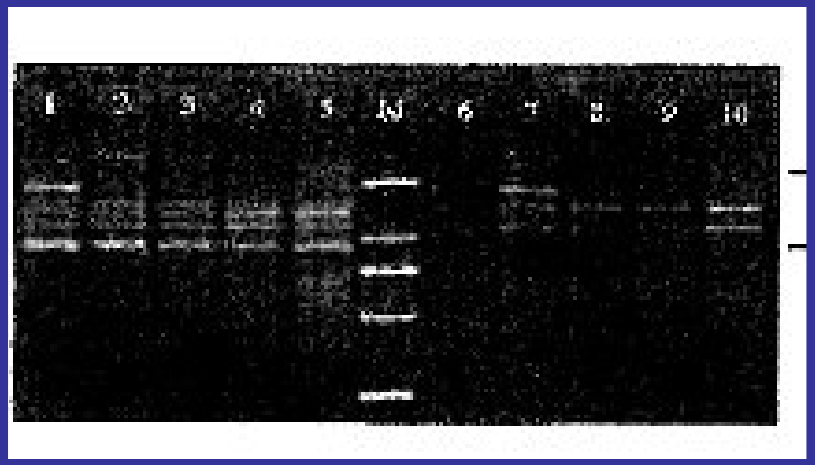
Silver Carp

A Male-specific DNA Marker for Common Carp

Male

Female

Male-specific DNA band

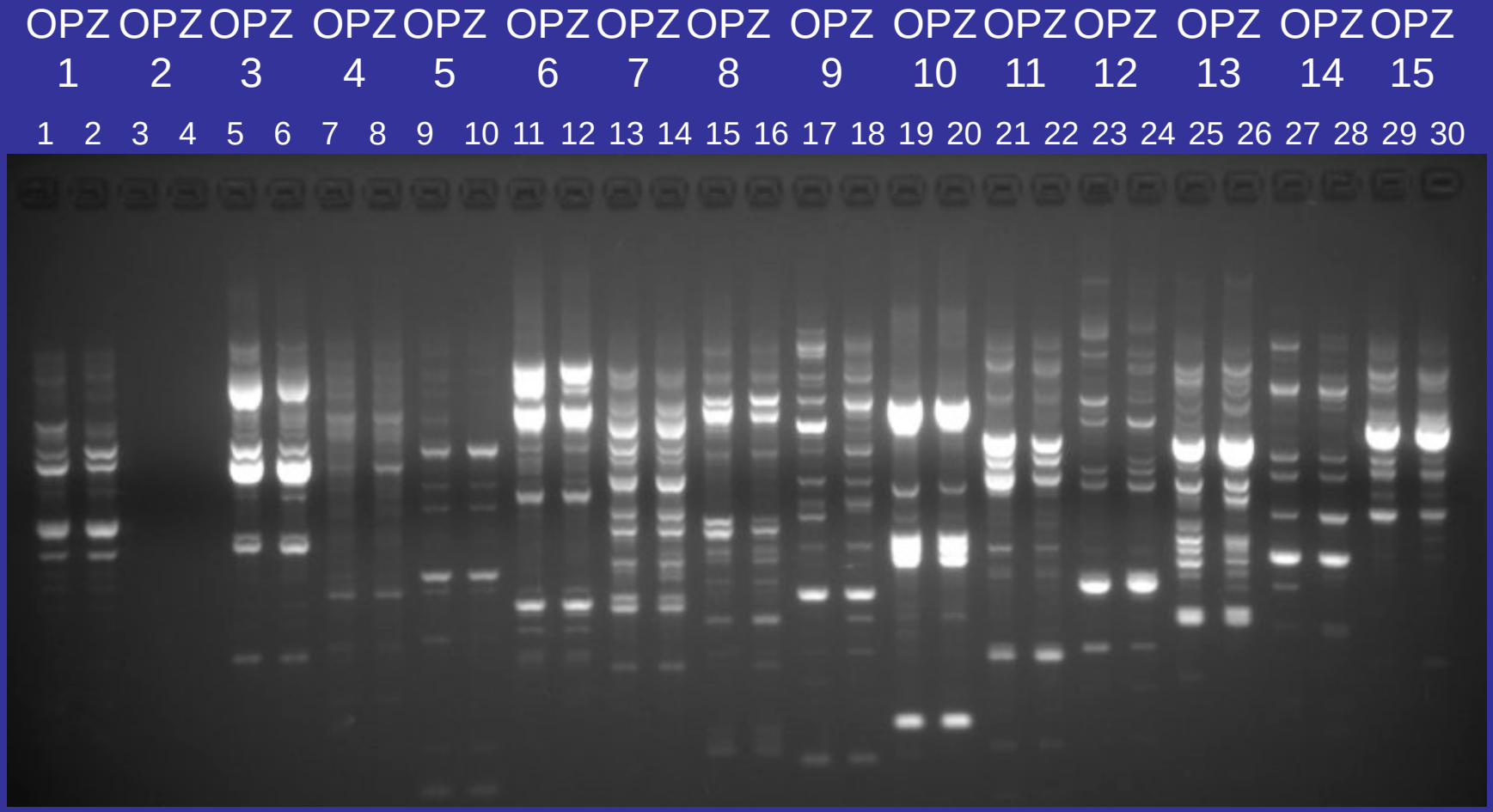


Male-specific DNA band

(Chen et al., 2009)

Could this same DNA marker be used to identify males in silver carp, tilapia or African jewelfish?

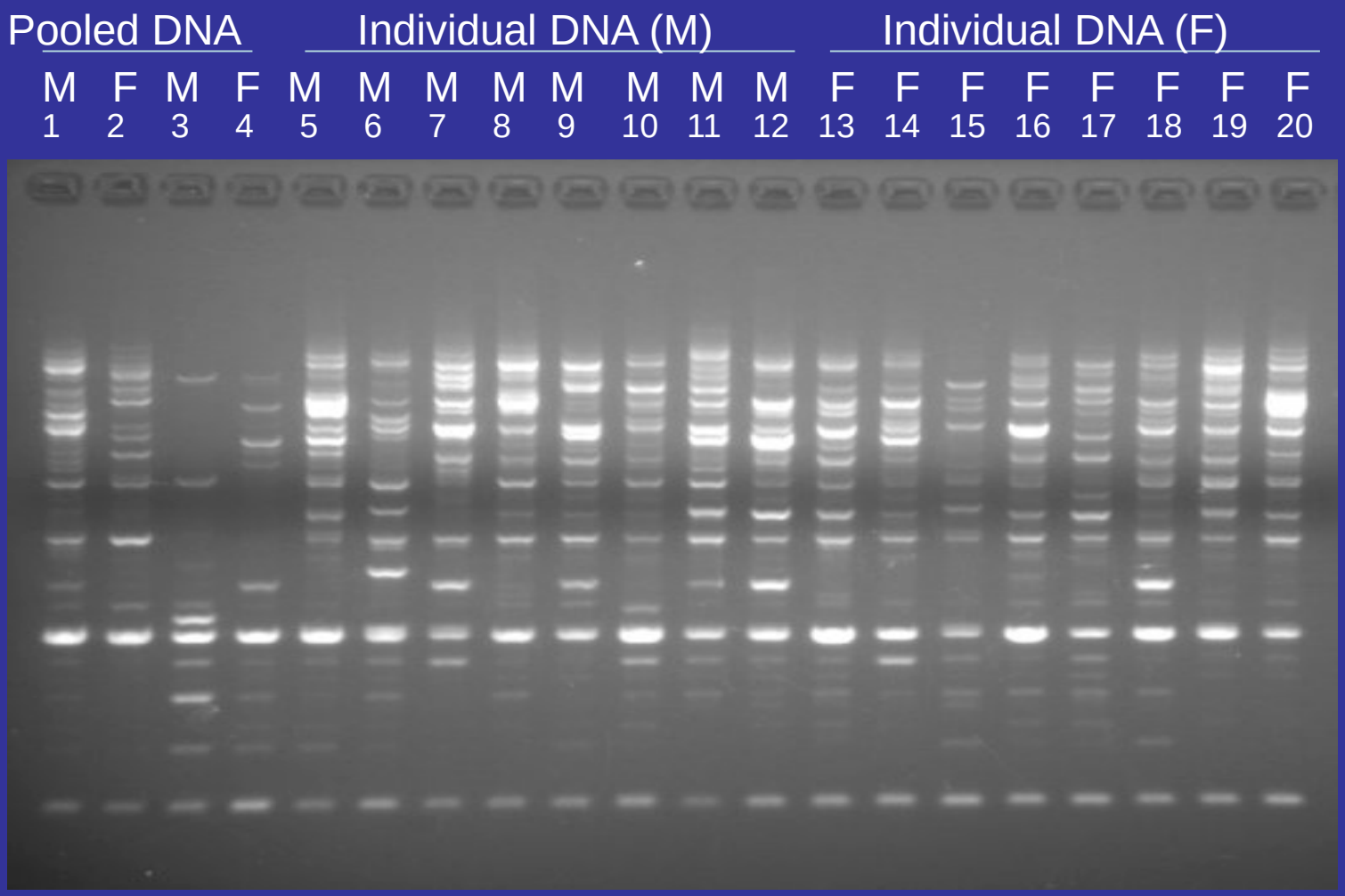
PCR Screening for Sex-Specific DNA Markers in African Jewelfish



Odd # lanes = pooled male-specific DNA from African Jewelfish
Even # lanes = pooled female-specific DNA from African Jewelfish

DNA fragments from PCR reactions using RAPD primers OPZ1–OPZ15 are separated on a 1.5% agarose gel.

No Primers Were Identified that Demonstrate Sex-Linkage in Individual Fish DNA



Primer OPZ6 applied to individual males and females (no sex –linkage)

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 YY broodstock are being developed for this species by USGS.

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

USGS will sequence the African Jewelfish genome to aid in the identification of sex-specific markers.

Problems with the Trojan Y Chromosome Genetic Biocontrol Strategy

1. **Requires continuous addition of an autocidal Trojan fish over a long period of time.**

2. **Adding too few autocidal Trojan fish will not cause extinction.**

3. **Requires decades to achieve eradication.**

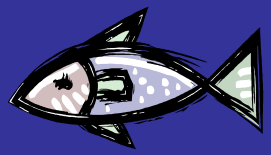
Could We Develop Better Genetic Biocontrol Strategies by Modeling Them on Natural Systems?

Amazon Trojan (AT)
– based upon the Amazon Molly

Trojan Female Technique (TFT)
– based upon mitochondrial defects causing male sterility in insects (Gemmell 2013).

Amazon Mollies Produce Only Amazon Mollies as Progeny

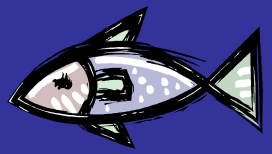
♂



P. mexicana

Mxy

♀

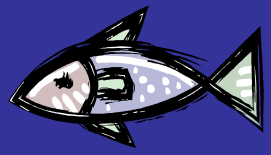


P. mexicana

Fxx

	X	Y
X	XX	XY
X	XX	XY

♀



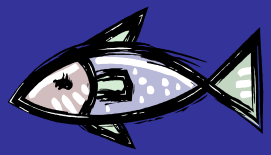
P. formosa

Fxx

XX	XX	

P. Formosa (*Amazon Molly*) Causes Local Extinction of *P. mexicana*

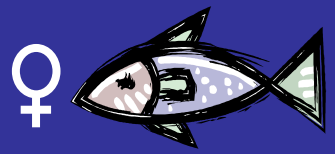
♂



P. mexicana

Mxy

♀

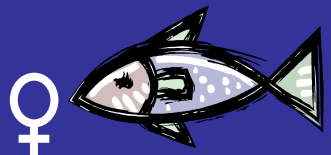


P. mexicana

Fxx

	X	Y
X	XX	XY
X	XX	XY

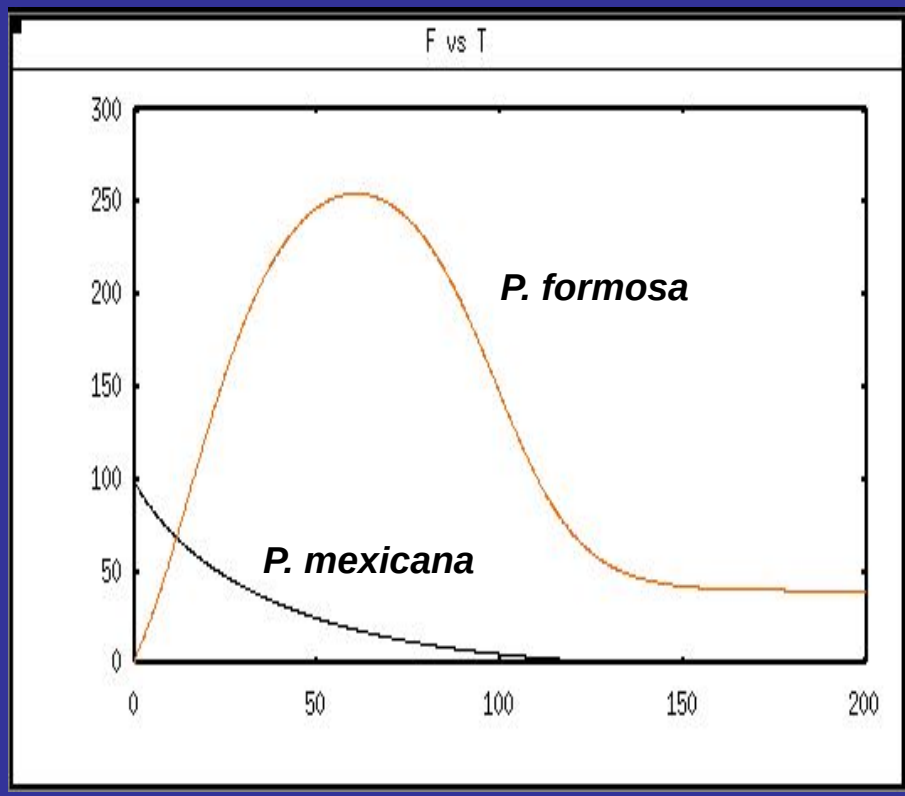
♀



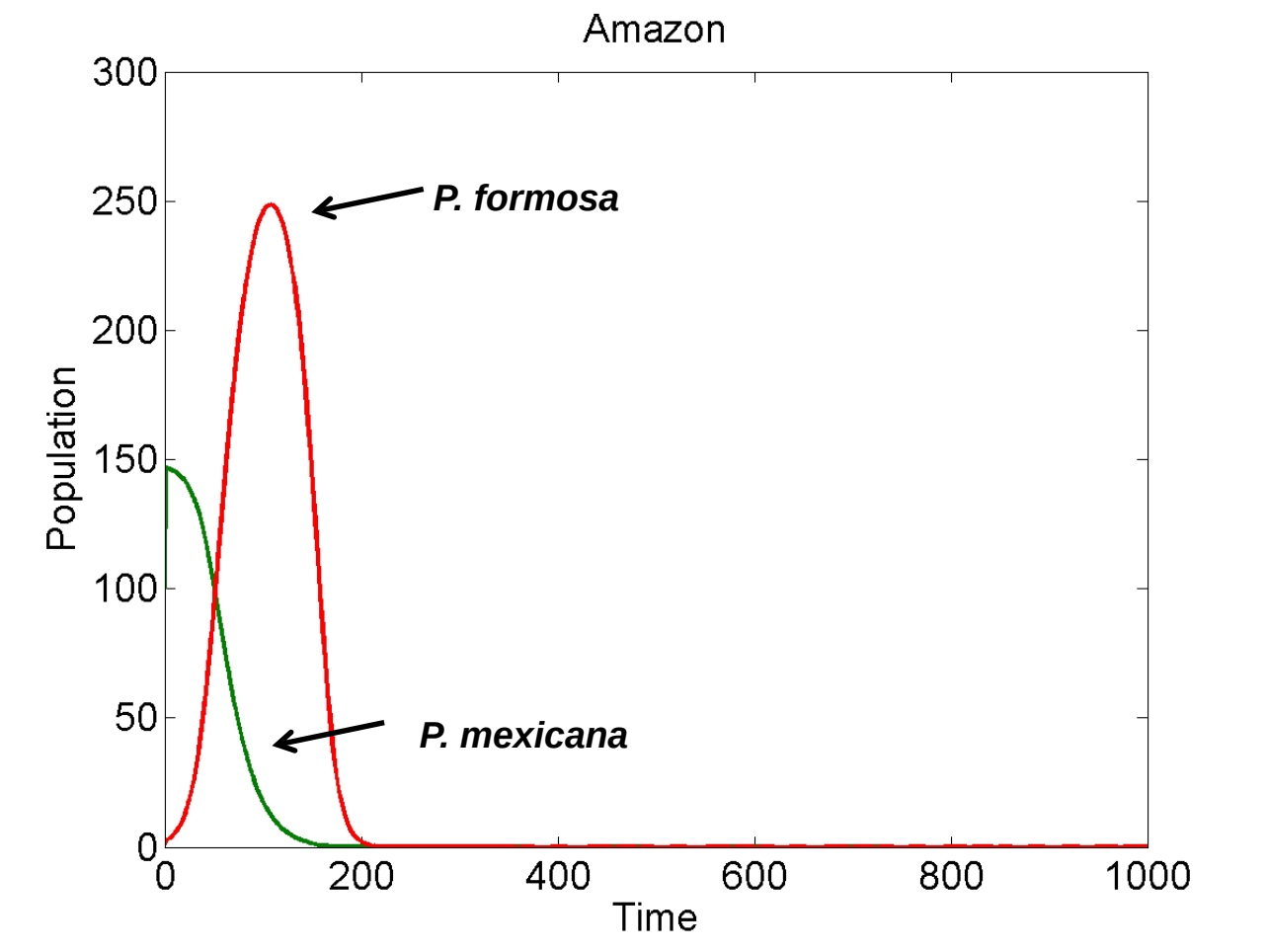
P. formosa

Fxx

XX	XX	



Extinction of *P. mexicana* Will Occur With the Addition of Just a Single Amazon Molly



How could an Amazon Trojan be Developed?

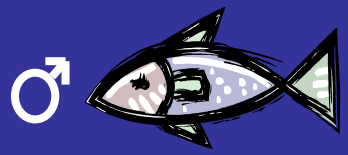
– requires knowledge of the unique reproductive features of the Amazon Molly

1. How do Amazon Mollies produce diploid eggs?

2. How does the Amazon Molly egg nucleus avoid fusion with the sperm nucleus?

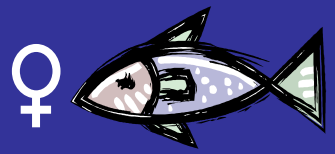
Can the reproductive differences be linked to specific genes that can be genetically modified in an invasive fish species?

Trojan Female Technique
A non-GMO Alternative to the Amazon Trojan?



Lionfish
Mxy

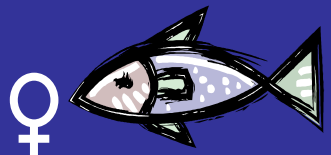
m=mitochondrial defect



Lionfish
Fxx

	X	Y
X	XX	XY
X	XX	XY

Motile Sperm (fertile)

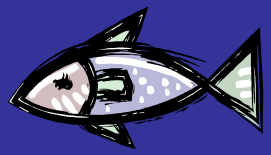


TRT Lionfish

	X	Y
Xm	XXm	XYm
Xm	XXm	XYm

Nonmotile Sperm (sterile)

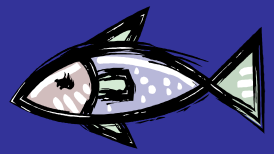
Trojan Female Technique
A Non-GMO Alternative to The Amazon Trojan?

♂ 

Lionfish

Mxy

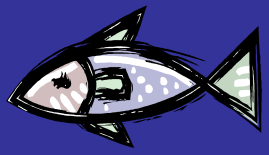
m=mitochondrial defect

♀ 

Lionfish

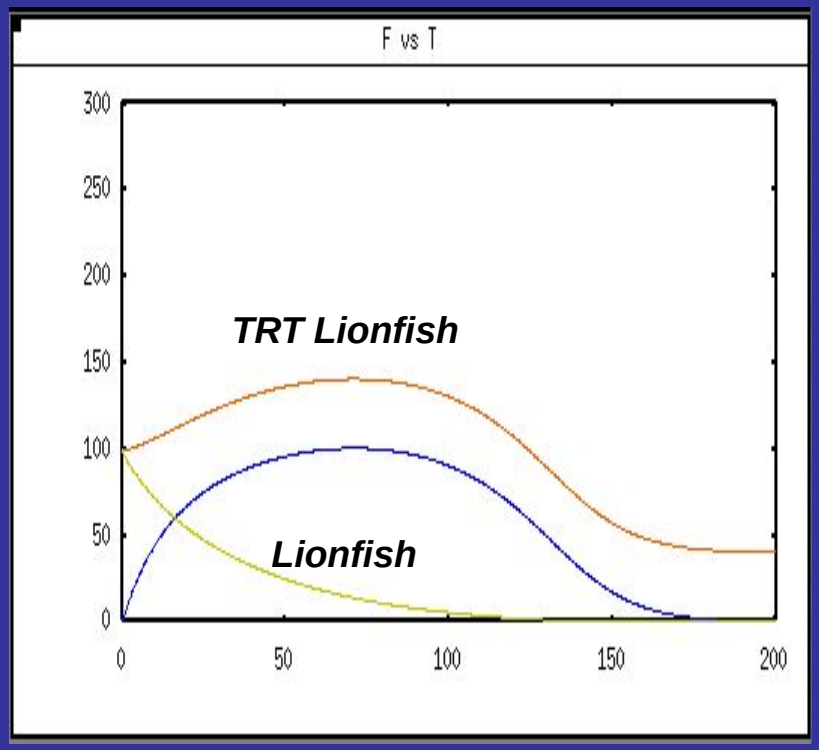
Fxx

	X	Y
X	XX	XY
X	XX	XY

♀ 

TRT Lionfish

	X	Y
Xm	XXm	XYm
Xm	XXm	XYm



Trojan Female Technique

Requires that a mitochondrial mutation is isolated that causes male sterility in the invasive species .

TRT females must be added continuously to achieve extinction, but in smaller numbers compared to the Trojan Y Chromosome strategy.

Conclusions

Other genetic biocontrol strategies that result in propagation of an autocidal fish within the system should be considered (Amazon Trojan, Trojan Female Technique).

The Amazon Trojan would be the most effective, requiring only a single autocidal Trojan fish to achieve eradication.

The TFT strategy might offer a non-GMO alternative to the Amazon Trojan.

$60,000