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

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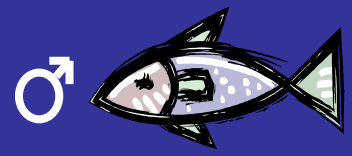
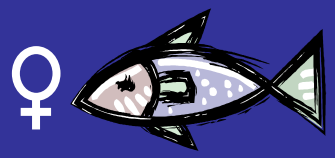
XY Sex-Determination



XY

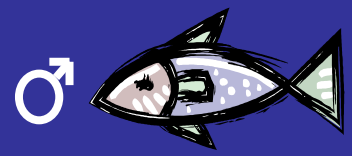


XX

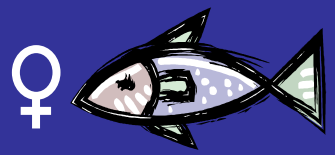
	X	Y
X	XX	XY
X	XX	XY

Males/Females
Ratio 1:1

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

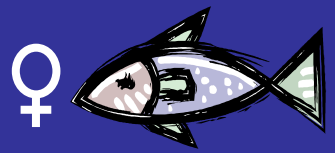


Mxy



Fxx

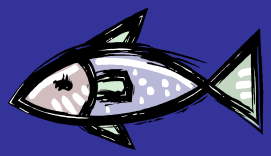
	X	Y
X	XX	XY
X	XX	XY



Fyy

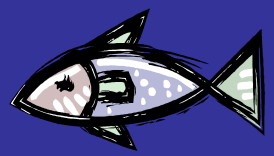
	X	Y
Y	XY	YY
Y	XY	YY

Myy males are viable and produce only male offspring

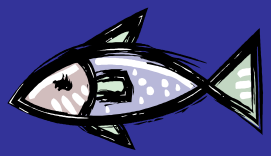
♂ 

Mxy

	X	Y
X	XX	XY
X	XX	XY

♀ 

Fxx

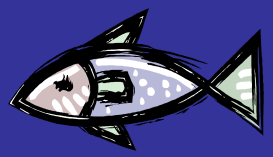
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

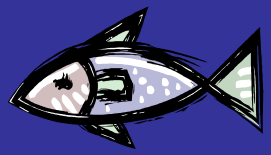
Males/Females Ratio 1:0

	X	Y
Y	XY	YY
Y	XY	YY

♀ 

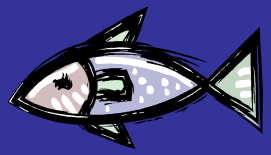
Fyy

Four different matings are possible, leading to increased male production

♂ 

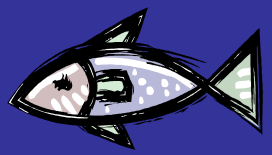
Mxy

	X	Y
X	XX	XY
X	XX	XY

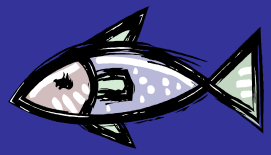
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

♀ 

Fxx

♀ 

Fyy

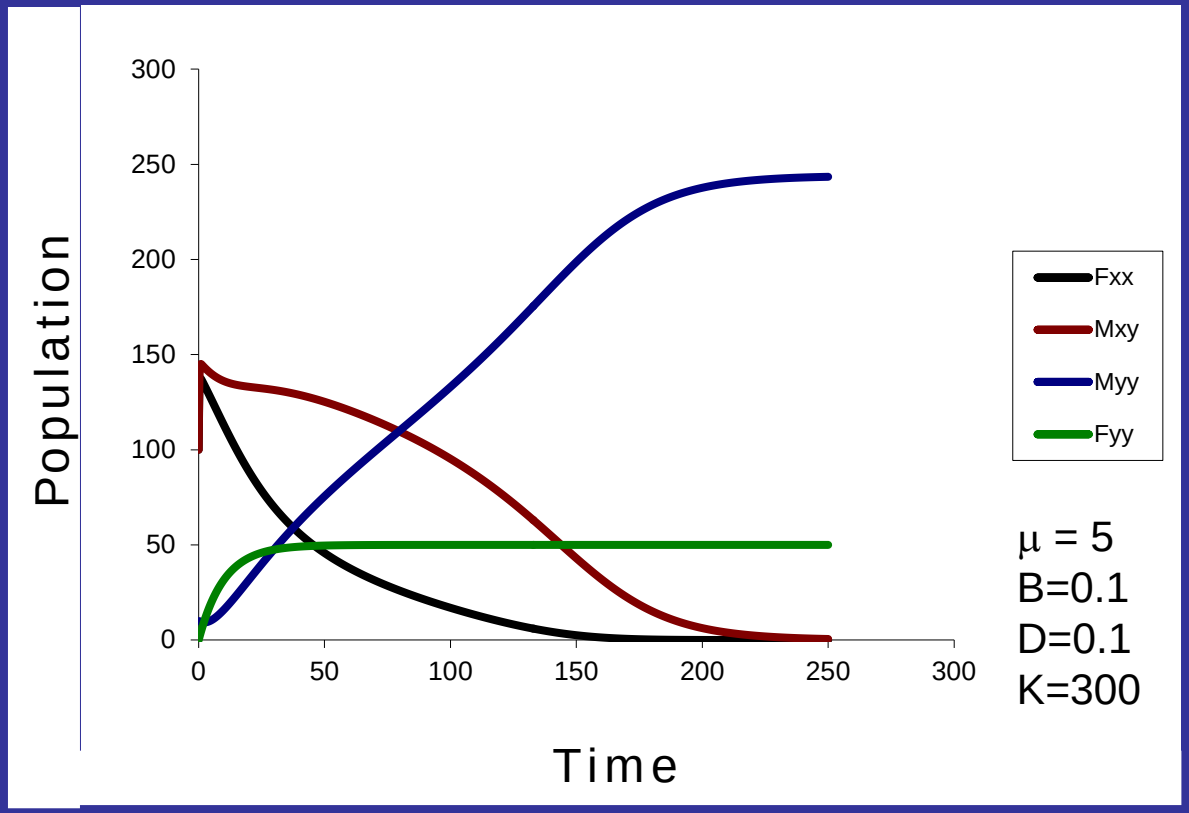
	X	Y
Y	XY	YY
Y	XY	YY

	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).

Why a Trojan Y Chromosome strategy might be an appropriate technique for controlling invasive species

- Species specific
- Requires no new technology development
- Involves standard aquaculture techniques, no recombinant DNA
- Trojan Y chromosome fish have been already been produced in one species (*Oreochromis niloticus*)
- Reversible

TYC Species Requirements

1. The target fish must have a XY sex-determination system

2. The target fish must be amenable to hormone-induced sex reversal

3. A female fish with two Y chromosomes (Fyy) must be viable and mate at the same efficiency as wildtype

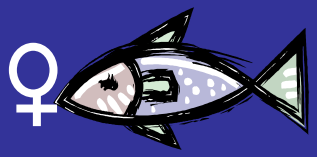
4. The target fish must be amenable to propagation via aquaculture

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.



Fyy

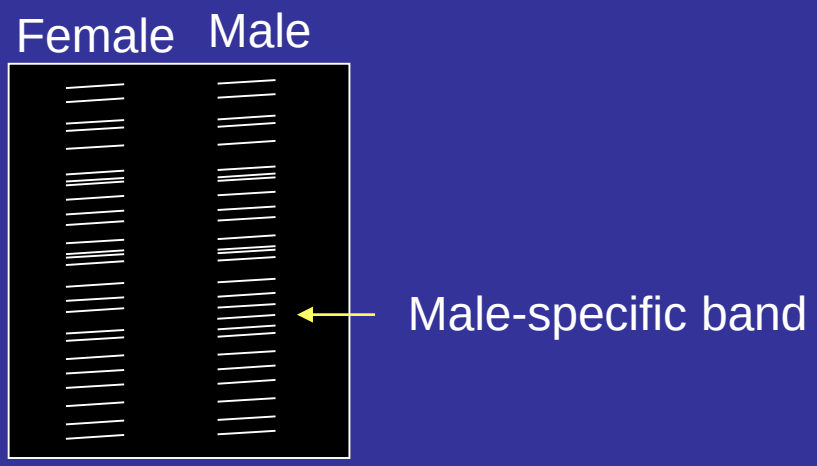
RAPD PCR

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

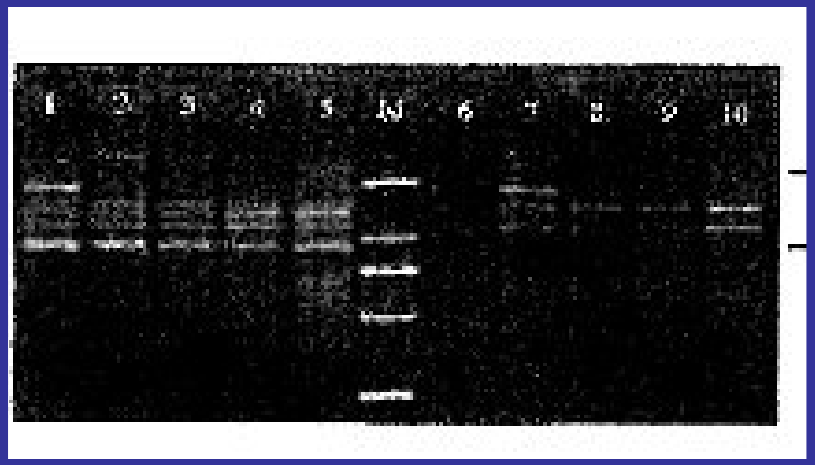
Silver Carp

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?

A Male-specific Carp Marker Can be Used to Design 10-mer RAPD PCR Primers

GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
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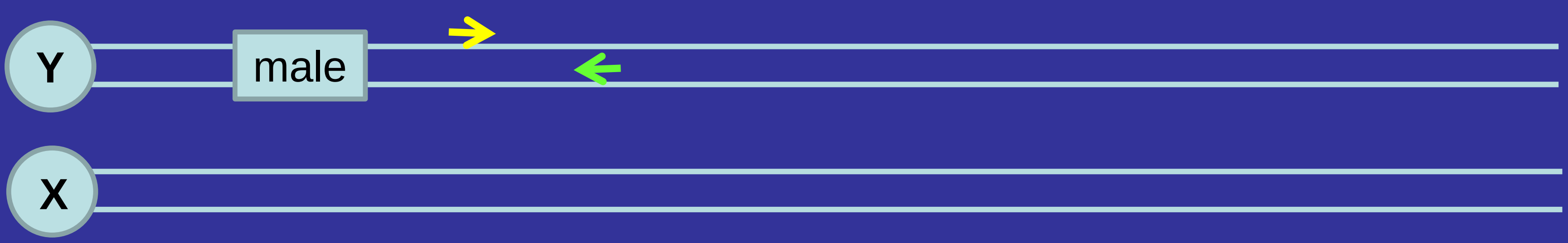
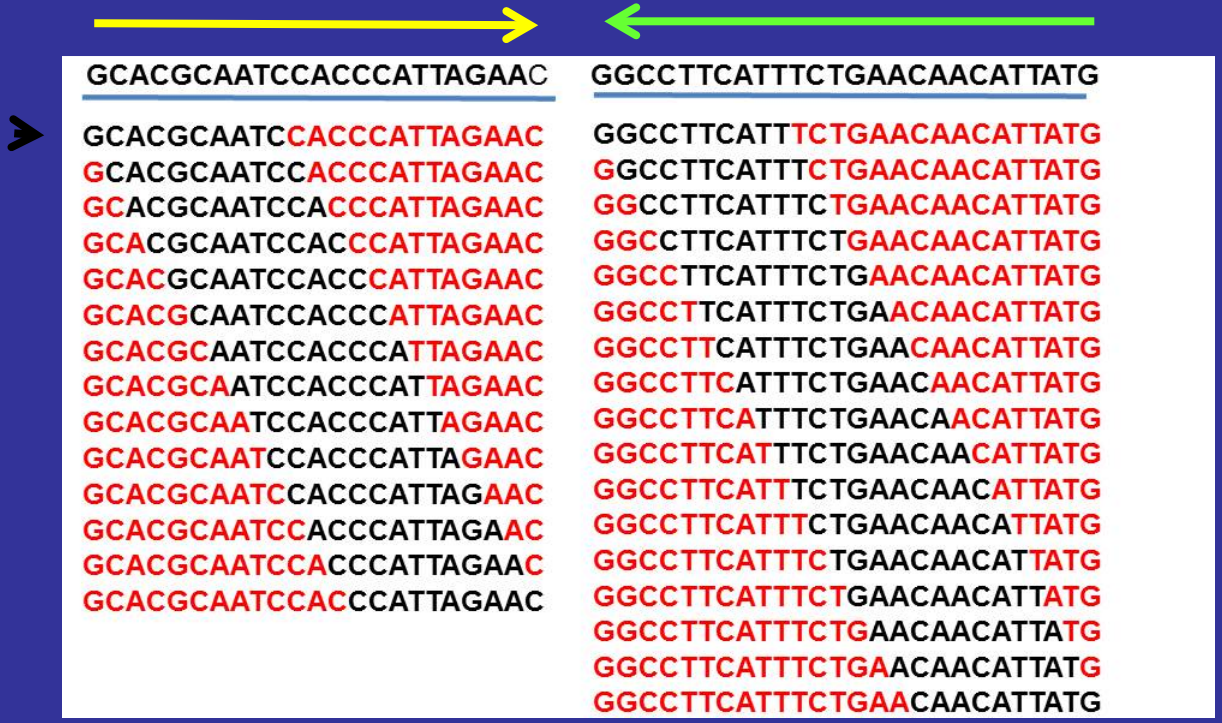
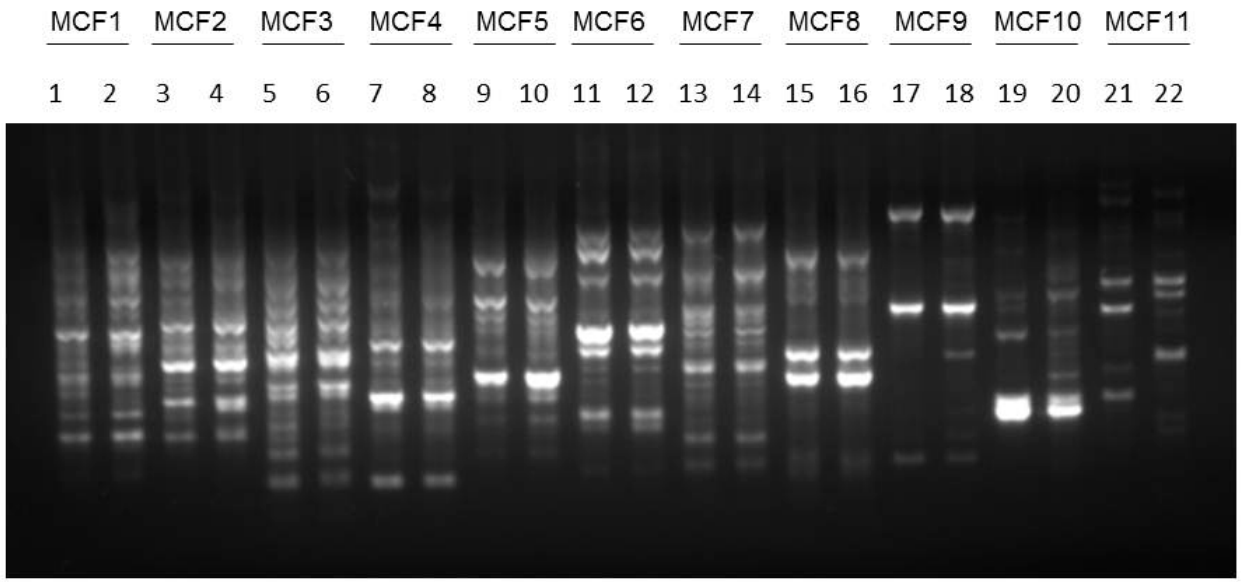


A Male-specific Carp Marker Can be Used to Design 10-mer RAPD PCR Primers



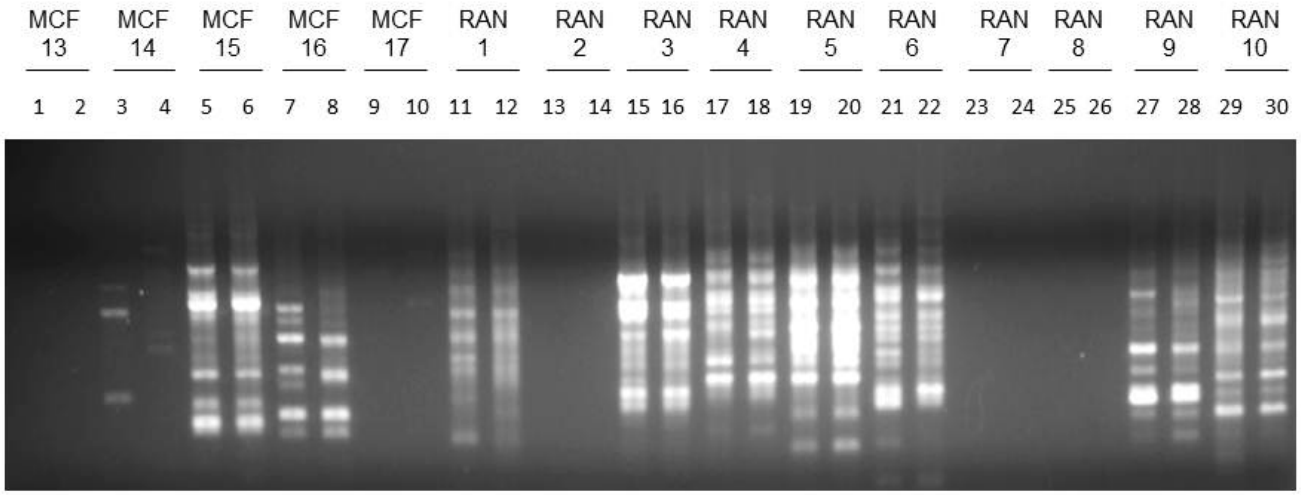
PCR Screening for Sex-specific DNA Markers in African Jewelfish



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.

PCR Screening for Sex-specific DNA Markers in African Jewelfish

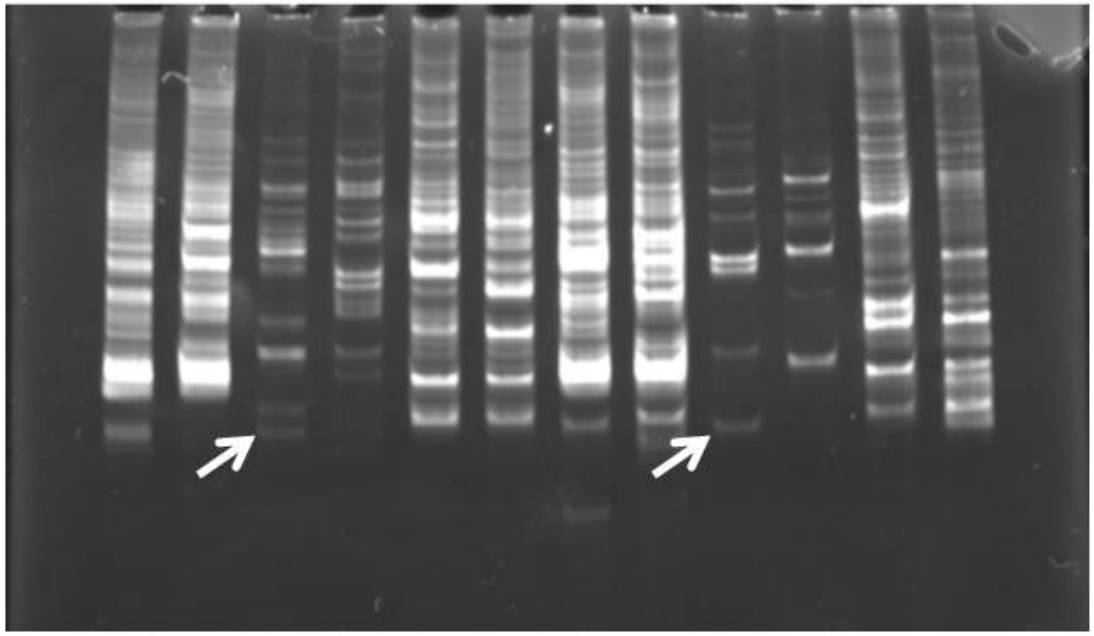


Odd # lanes = male-specific African jewelfish DNA pool 1 (M2-M9)
Even # lanes = female-specific African jewelfish DNA pool 1 (F2-F9)

DNA fragments from PCR reactions using RAPD primers MCF13-MCF17 and RAN1-10 are separated on a 1.5% agarose gel.

For some primers used, no DNA fragments were produced in the PCR reactions (lanes 1,2,9,10,23-26).

PCR Screening for Sex-specific DNA Markers in African Jewelfish



Lanes 1, 3, 5 male-specific African jewelfish DNA pool 1 (M2-M9)
Lanes 2, 4, 6 female-specific African jewelfish DNA pool 1 (F2-F9)
Lanes 7, 9, 11 male-specific African jewelfish DNA pool 2 (M10-M15)
Lanes 8, 10, 12 female-specific African jewelfish DNA pool 2 (F10-F15)
Samples separated on 6% acrylamide gel stained with ethidium bromide.
The white arrow indicates a possible male-specific DNA band for MCF14.

Conclusions

No sex-specific markers isolated as yet for African Jewelfish, silver carp or tilapia.

Larger numbers of fish will be included in pooled male-specific and female-specific DNA pools.

Screening will continue for all three invasive fish, with help from USGS on African Jewelfish.