



# *Trojan Sex Chromosomes*

*Can Biased Sex Ratios Drive A  
Population To Extinction?*

*Chris Jeszke – Ashton Hatchery*



# *Trojan Sex Chromosomes*

## *Collaborative Fisheries Bureau Investigation:*

- Schill, Frew, Horton, Grunder, Heindel – IDFG Fisheries Bureau*
- Matt Campbell – IDFG Eagle Genetics Lab*
- Phil Mamer – IDFG Eagle Health Lab*
- Meyer, Koenig, Dillon – IDFG Nampa Fisheries Research, Region 3*
- Doug Engemann, Chris Jeszke, Paul Martin – IDFG Ashton / Springfield Fish Hatchery*



# *Trojan Sex Chromosomes*

## *Exotic Introductions Problematic*

- Relatively few *practical and effective* alternatives exist to deal with non-natives
  - *Chemical*
  - *Removal/depletion*
  - *Construction of barriers*

*Logistically difficult, can be costly, may have collateral damage to non-target organisms*



# *Trojan Sex Chromosomes*

**Multiple Interpretations in Literature:**

Gutierrez and Teem

Cotton and Wedekind

*Daughterless Technology*



# Daughterless Technology

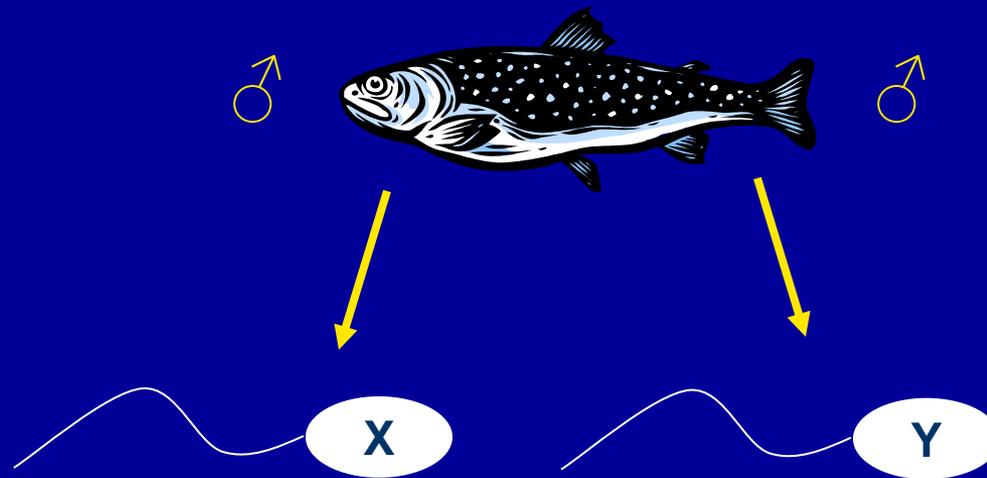
Salmonids share a common trait with most mammals in that they are

MALE HETEROGAMETIC

Male individual of a given species produces two different “kinds” of gametes with respect to sex chromosomes

# Daughterless Technology

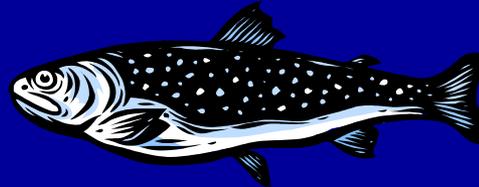
## MALE HETEROGAMETIC



**Either X or Y sex chromosomes**

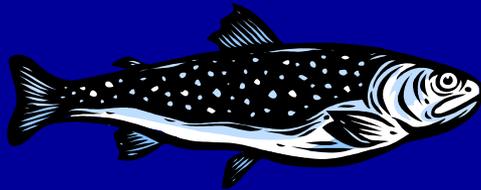
# Daughterless Technology

♀

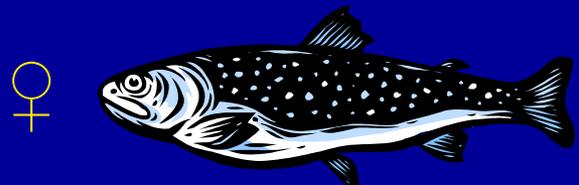


	X	X
X	XX	XX
Y	XY	XY

♂

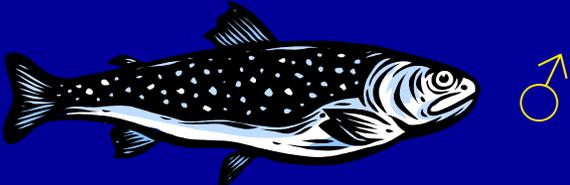


# Daughterless Technology

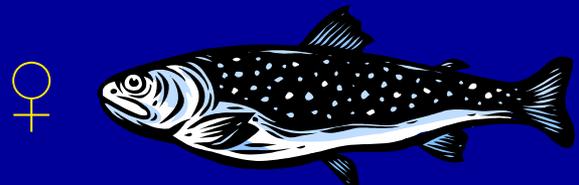


	X	X
X	XX	XX
Y	XY	XY

50% XX



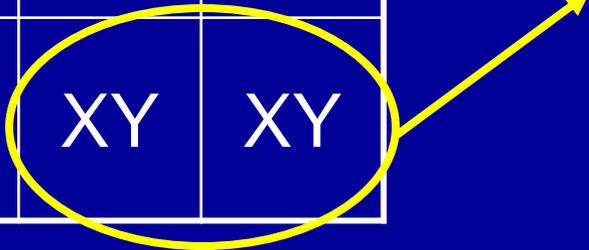
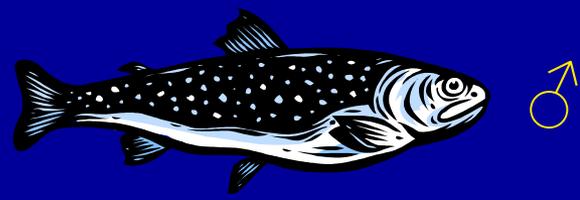
# Daughterless Technology



	X	X
X	XX	XX
Y	XY	XY

50% XX

50% XY



# Daughterless Technology



**Fxx = phenotypic female (F), genotypic female (xx)**

**Mxy = phenotypic male (M), genotypic male (xy)**

# Daughterless Technology



Fxx

+ TESTOSTERONE = Mxx



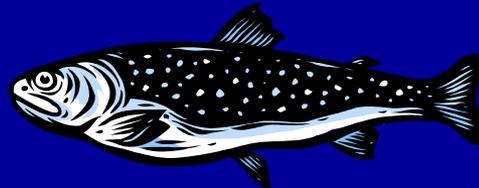
Mxy

+ ESTROGEN = Fxy

# Daughterless Technology

Why? = trout aquaculture

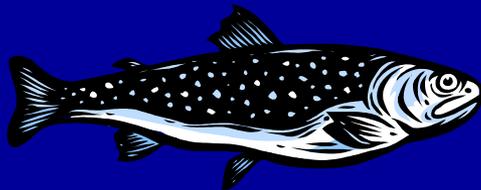
Fxx



	X	X
X	XX	XX
X	XX	XX

100% XX

Mxx



# Daughterless Technology

Why? = tilapia aquaculture

Fxy



**Generation #1**



Mxy

	X	Y
X	XX	XY
Y	XY	YY

**25% XX**

**50% XY**

**25% YY**

# Daughterless Technology

Why? = tilapia aquaculture

F<sub>xy</sub>



**Generation #1**



M<sub>xy</sub>

	X	Y
X	XX	XY
Y	XY	YY

25% XX

50% XY

25% YY

# Daughterless Technology

Why?

Fyy



Generation #2



Myy

	Y	Y
Y	YY	YY
Y	YY	YY

100% YY

“supermales”



# Daughterless Technology

- Gutierrez and Teem Model:

Repeated introductions of individuals that are phenotypically sex reversed from that of their genotype will provide disproportionate influx of one sex chromosome into subsequent generations, biasing sex ratio, and leading to potential population extinction



# Daughterless Technology

- Assumptions:
  - Altered stocks are identical to wild with regards to mating advantage (no size, performance, selection differences - current research is lacking)



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- Offspring viability identical to wild  
(current research is lacking)



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- Altered stocks are identical to wild with regards to mating advantage (no size, performance, selection differences - current research is lacking)
- Offspring viability identical to wild

– Model assumes iteroparity  
(unknown ?)



# Daughterless Technology

- Assumptions:
  - Altered stocks are identical to wild with regards to mating advantage (no size, performance, selection differences - current research is lacking)
  - Offspring viability identical to wild
  - Model assumes iteroparity (unknown ?)
  - All individuals have same life span and mortality rates (unknown)



# Daughterless Technology

- Assumptions:

- Altered stocks are identical to wild with regards to mating advantage (no size, performance, selection differences - current research is lacking)
- Offspring viability identical to wild
- Model assumes iteroparity (unknown ?)
- All individuals have same life span and mortality rates
- No elements in system that can cause spontaneous masculinization or feminization (steroids, temperature, social environment, stocking density)



# Daughterless Technology

- Rate at which  $F_{xx}$  eliminated relies on:
  - Rate at which  $F_{xx}$  eliminated through mortality



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  - Rate at which  $F_{xx}$  eliminated through mortality
  - Rate at which  $F_{xx}$  are generated in matings



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- Rate at which  $F_{xx}$  eliminated relies on:
  - Rate at which  $F_{xx}$  eliminated through mortality
  - Rate at which  $F_{xx}$  are generated in matings
- Relative proportion of  $F_{xx}$  to  $F_{yy}$  in population over progressive rounds of mating



# Daughterless Technology

- Rate at which  $F_{xx}$  eliminated relies on:
  - Rate at which  $F_{xx}$  eliminated through mortality
  - Rate at which  $F_{xx}$  are generated in matings
  - Relative proportion of  $F_{xx}$  to  $F_{yy}$  in population over progressive rounds of mating
- Number of  $F_{yy}$  introduced and for how long (model used 3.2% introduction of  $F_{yy}$  in a population of several hundred individuals = extinction after a few decades)



# Daughterless Technology

- Reduction in population size dependent on magnitude and frequency of  $F_{yy}$  = can have *mild management* of a system to *complete eradication*
- If possible, this technology is non-permanent = sex ratio returns to unity if  $F_{yy}$  ceases before  $F_{xx}$  extinct (example - unexpected and/or unwanted effects detected)



# *Daughterless Technology*

- No (apparent) collateral ecological damage
- Only target species impacted (potential issues with hybridization?)

# Daughterless Brook Trout?

Fall 2008

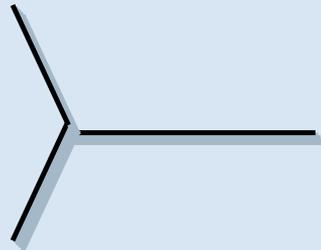


XX Female (F<sub>xx</sub>)



XY Male (M<sub>xy</sub>)

#1



~50% XX, 50% XY



17 beta-estradiol  
20 mg/kg diet, 60 days

# Daughterless Brook Trout?

#2



XX Females (Fxx)



XY Neo-Females (Fxy)



Develop Sex Markers?



XX Females (Fxx)



XY Neo-Females (Fxy)



Retain  
XY Neo-Females  
(Fxy)

# Daughterless Brook Trout?

Fall 2010

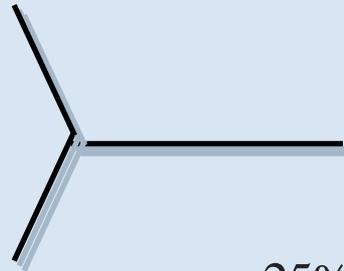


XY Neo-Female (Fxy)



XY Standard Male (Mxy)

#3



~25% XX, 50% XY, 25%YY



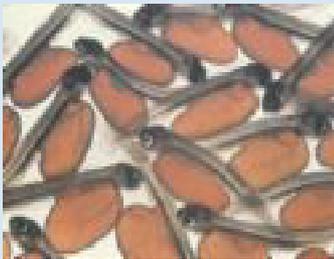
Need Sex Markers

# Daughterless Brook Trout?

Fall 2010



~25% XX, 50% XY



~25% YY (Myy)

~ 1/2 of production



17 beta-estradiol  
20 mg/kg diet, 60 days



YY Neo-Females? (Fyy)

# Daughterless Brook Trout?

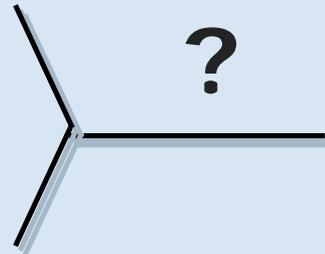
Fall 2012



#5 YY Neo-Female (Fyy)



YY "Super male" (Myy)



~100%YY (Myy)



**Broodstock  
Production**

# What's Happening Now?

- Need reliable genetic sex markers for Brook trout
- Ability to produce salmonid Fyy with functional gametes is unknown
- Field evaluations (triploidy's big sister?)

# What's Happening Now?

- 2011 – progeny ponded in one raceway at Ashton:

**208 controls + 208 treatments; 4 unique (un-related) family types that can be crossed in 2012 – 2013 release if success.**

**Assume ~ 25% xx, 50% xy, 25% yy in general population**

- 2012 – Sex markers have been obtained, can select Fyy's from population and determine whether or not oocytes are viable
- Spawning took place in ~ Nov 2012
- ~ 25 Myy and ~ 25 Fyy were spawned.

# What's Happening Now?

- If developing oocytes, are they viable? Spawning took place in ~ Nov 2012
- Spawning produced ~ 25 Myy and ~ 25 Fyy that were spawned.
  - $25 * 500$  fecundity = 12,500 green eggs
  - 12,500 green eggs @ 70% survival to eye = 8,750 eyed
  - ~ 75% eyed to sub-yearling survival = 6,563 fish
  - ~ 6,563 sub-yearlings available for release in 2013
  - Other crosses were made with know gender control fish to confirm genetic markers



# What's Happening Now?

- Eggs are currently in incubation
- Eggs from first spawning have eyed up
- Gender ratios will be confirmed within the month
- If all offspring are Myy, sex markers are accurate, can move towards production
- Wait and see....update in Boise in 2013