



**Aquatic Animal Drug Approval  
Partnership Program**



# **Histological Determination of Tilapia Gender Following Treatment with 17 $\alpha$ -Methyltestosterone**

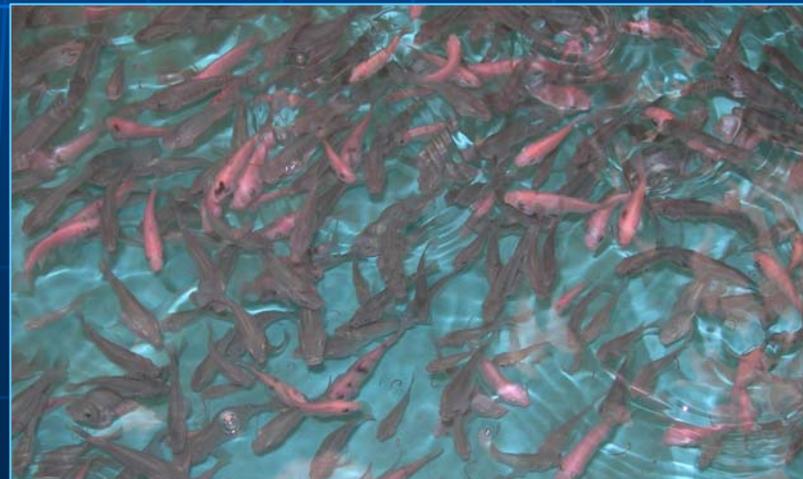
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# Study Objective

- Demonstrate the effectiveness of 17  $\alpha$ -MT-medicated feed administered at a dosage of 9 mg drug/kg fish bw (60 mg drug/kg feed) for 28-d to produce predominantly male populations of tilapia.
  - $H_{a1}: \mu_{\text{treated}} > \mu_{\text{untreated}}$
  - $H_{a2}: \mu_{\text{treated}} > 80\% \text{ males}$



# Background

- 17  $\alpha$ -MT - most commonly used androgen for sex reversal, most effective and economically feasible method for obtaining all male tilapia populations
- Yamamoto began artificially inducing sex reversal in medaka in 1953.
- Eckstein and Spira described effect of sex hormone (MT) on gonad differentiation in *Tilapia aurea* in 1965.
- It has been proven to be effective in a number of different species of tilapia under different conditions at concentrations ranging from 7.5 – 60 mg drug/kg feed.
- Does not alter the genotype, but directs the expression of the phenotype.
- Industry desires males or “non-functional females”

# Study Procedures



- SeaPac of Idaho's Idaho Aquatic tilapia nursery (Buhl, ID)
- Hybrid tilapia (*O. nilotica* x *O. aurea* x *O. mossambique*) fry 6 – 7 d old
- Rangen, Inc. (Buhl, ID) #0 Tilapia Starter (60 mg MT/kg feed)
- Treated (n = 4) and control (n = 4) tanks.
- Fed fish 15% bw for 28-d treatment period (9 mg MT/kg fish bw)

# Assessing Primary Variable

- **Histological evaluation of gonads (testes, ovaries, intersex – (ovatestis))**
  - Collected (without bias) 40 fish from each tank
  - Processed both gonads/fish
  - Examined three 5 um sections/gonad
  - Treatment success = 100% testis in all sections
  - Treatment failure = <100% testis in any one section
    - Lack of information in the literature describing functionality of intersex fish
  - Compared transformed % data (t-test)

# Secondary Variables

- Gender based on gross visual examination of whole gonads
- Mortality
- General fish behavior and feeding behavior during treatment period
- Analytically verified concentration of MT in feed
  - Developed by Marwah and Marwah, Univ. of Wisc. - Madison
  - CanTest Ltd., Burnaby BC Canada (FDA-accepted method)
- Water quality measurements (temp, DO concentration, hardness, alkalinity, pH)

# Study Objective Challenges

- **Demonstrate the effectiveness of 17  $\alpha$ -MT to CVM.**
- **Dealing with:**
  - 6 – 7 d old fish (1 cm; 0.03 g)
  - Strategy to adequately feed small fish but allow enough room to grow to target size
  - Accurately feeding fish at 15% bw
  - Determining desired end-of-study fish size
  - Keeping Murphy's Law at bay for study duration

# Fish Size

Started with really small fish



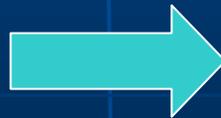
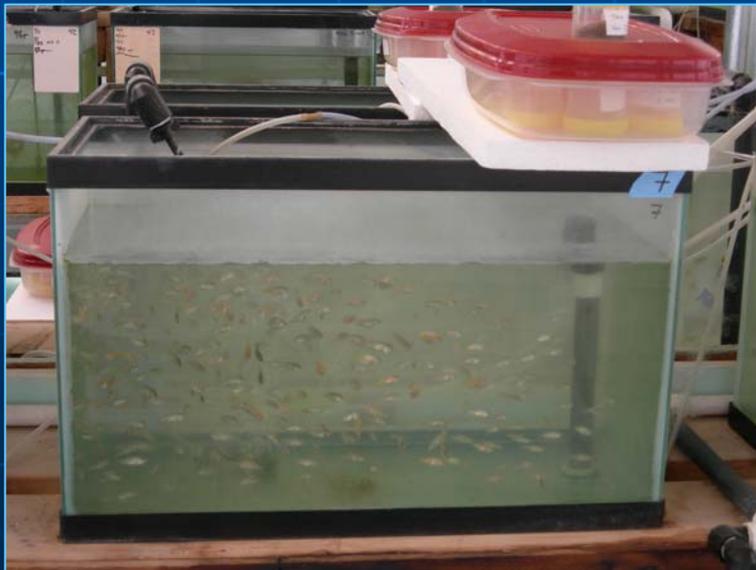
Ended with much larger fish



Used a "tank-pair" system



Reduced numbers as fish grew





## Experimental procedures

- Started with 400 fish/aquaria; ended with 50 fish/tank
- Developed a growth model; verified growth projections periodically – fed fish at 12 – 13% bw
- Target size of fish at end of study – 13 cm
- Study duration 99 d to achieve end-of-study size

# Results

## (Secondary variables)

- Mean cumulative mortality = 4 (range 0 – 6 / tank)
- Behavior and appetite = normal
- Dose verification = 39.5 mg MT/kg feed
- Water temperature, 31°C
- DO concentration, 3.2 mg/L
- Others
  - Hardness, 8 mg/L ( $\text{CaCO}_3$ )
  - Alkalinity, 97 mg/L ( $\text{CaCO}_3$ )
  - pH, 8.4



# Results

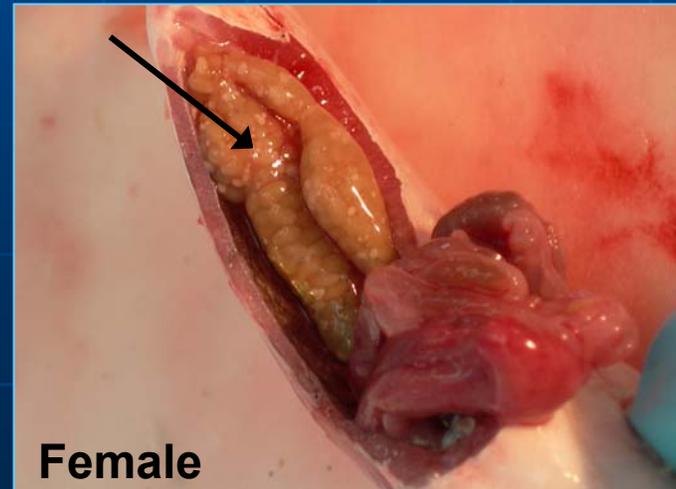
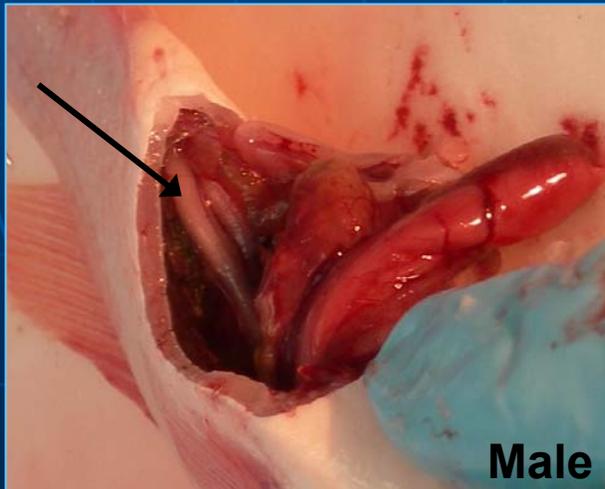
## (Gross visual - Gender)

### ■ Treated

- Male **96.2%**
- Female 3.8%
- Intersex n/a

### ■ Untreated

- Male 41.5%
- Female 58.5%
- Intersex n/a



# Results

## (Histology - Gender)

### ■ Treated

- Males 83.7%
- Females 3.1%
- Intersex 13.2%
- Ratio 1 to 0.2

### ■ Untreated

- Males 38.4%
- Females 60.4%
- Intersex 1.3%
- Ratio 1 to 1.8

**P = <0.001**

# Results

(mean fish length - cm)

## ■ By treatment condition

- Treated 12.4 (9.7 – 15.0)
- Untreated 11.8 (8.9 – 14.4)

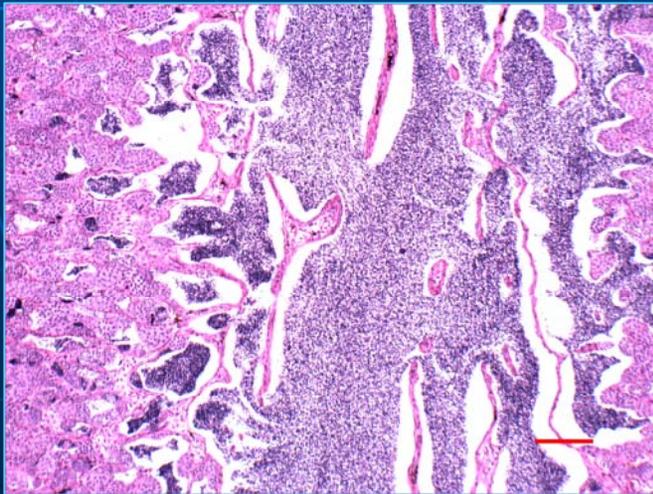
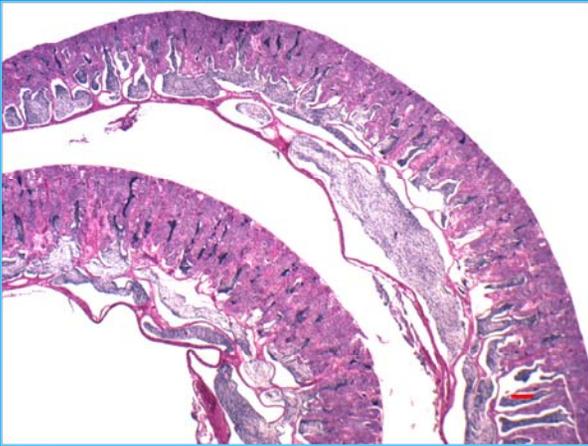
## ■ Regardless of treatment condition

- Male 12.3 (9.5 – 15.0)
- Female 11.3 (9.7 – 13.0)
- Intersex 12.7 (9.9 – 14.5)

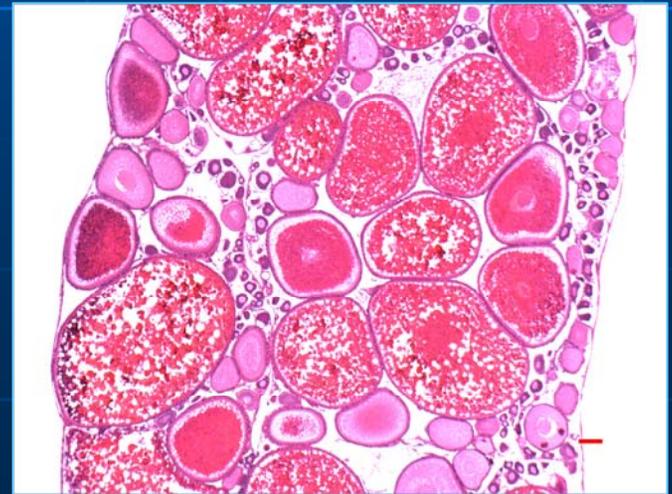
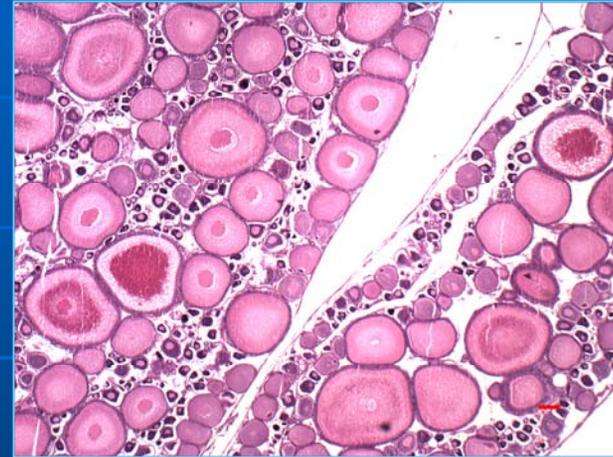


# "Normal" Gonads

- Male (100% testis)

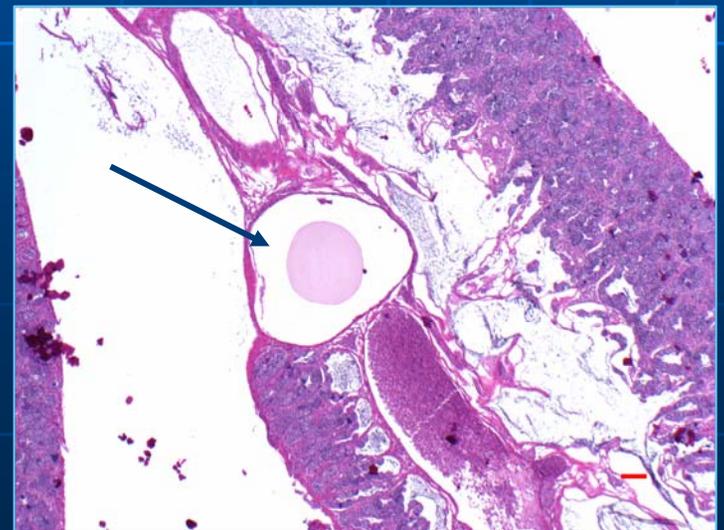
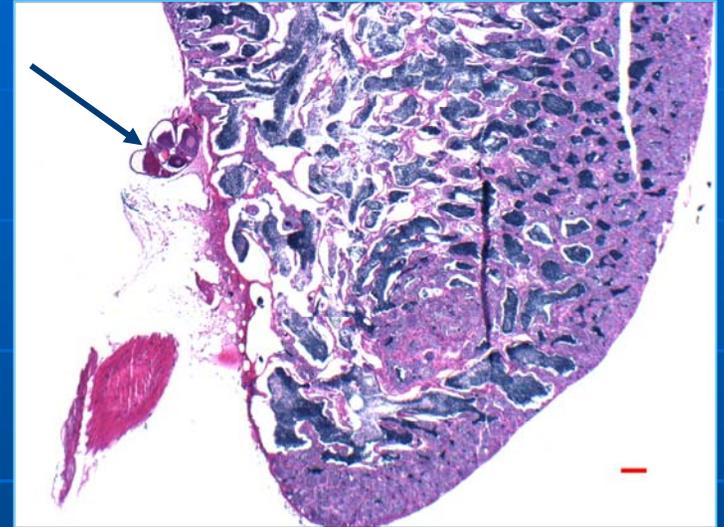
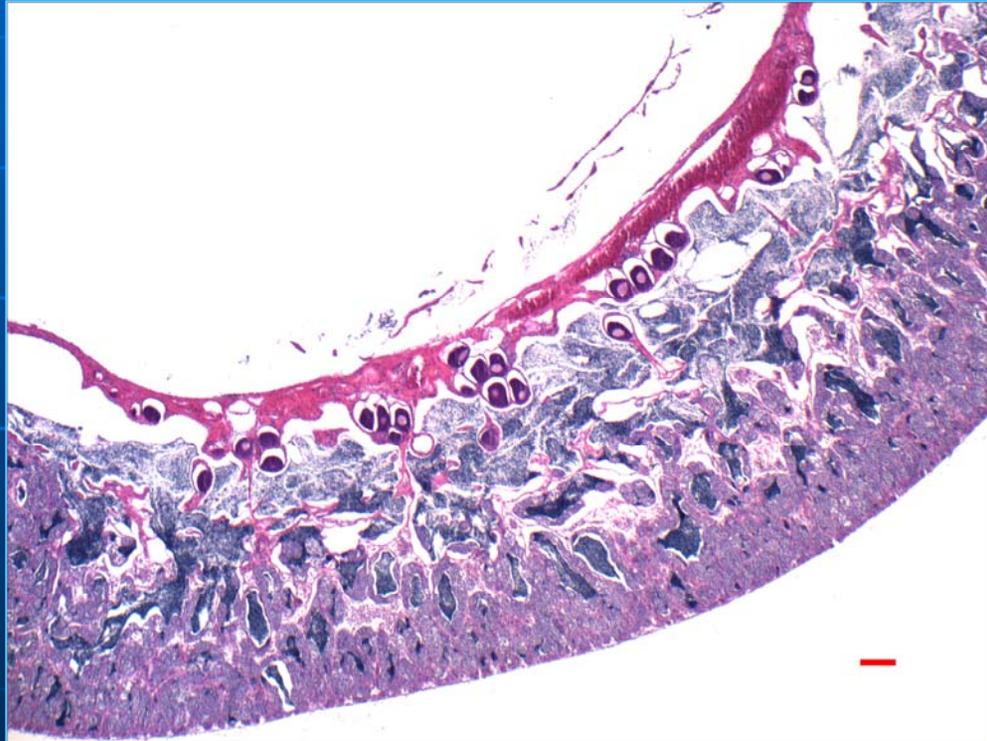


- Female (100% ovary)



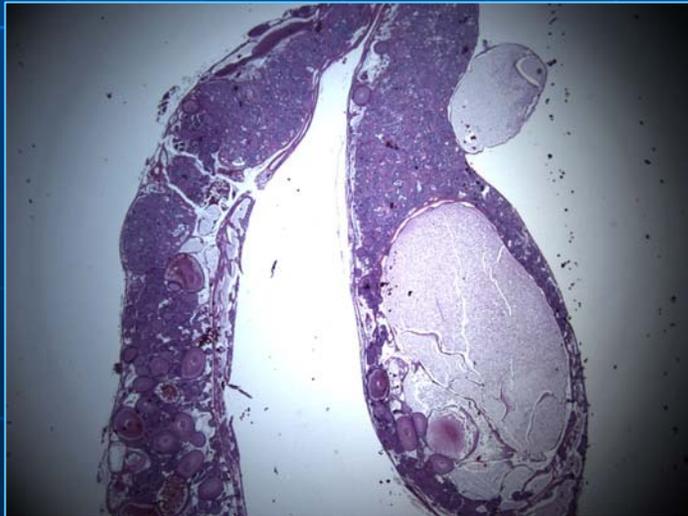
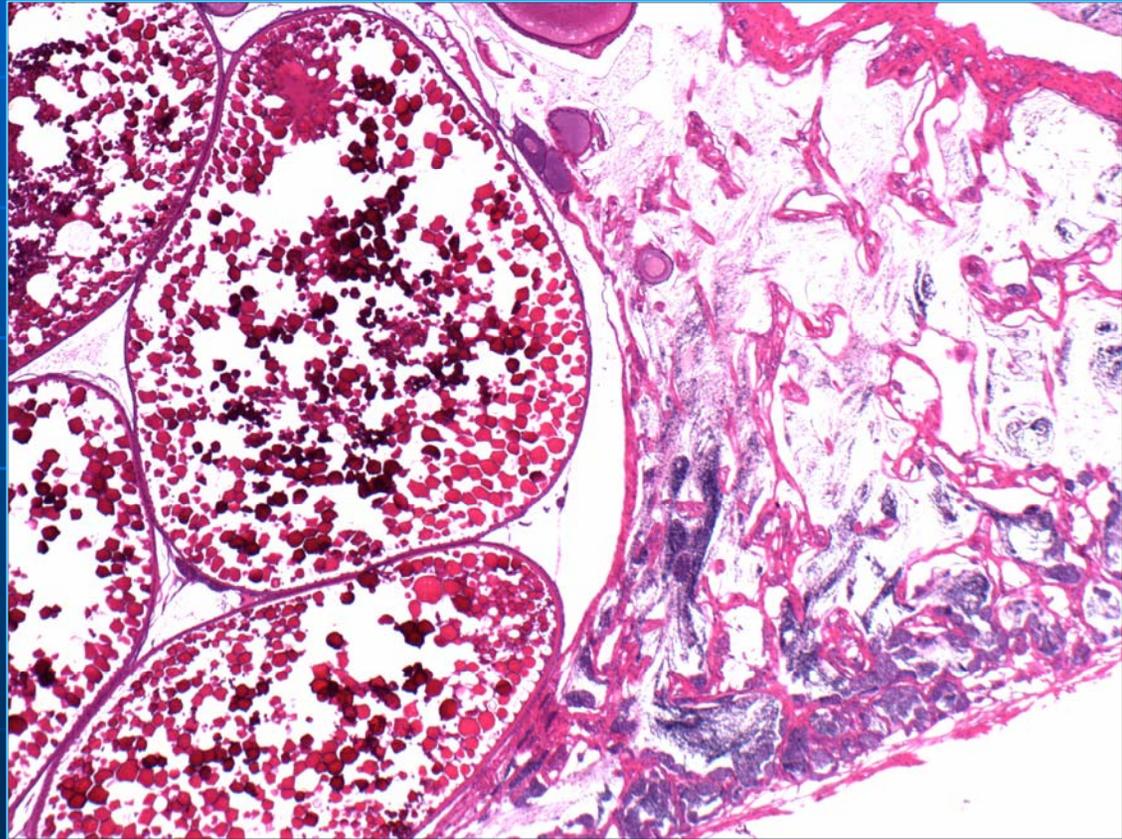
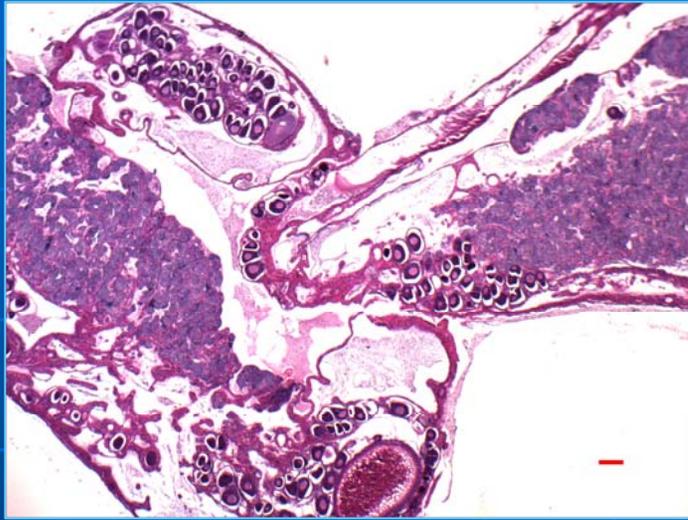
# Mixed Gonads

(reproductively non-functional females)



# Mixed Gonad

(reproductively functional females?)



# Modified Results

## Based on histo results

Designated mixed gonads as either  
treatment success/failure

- Males + reproductively non-functional females

$$84\% + 11\% = 95\%$$

- Reproductively functional females

$$3\% + 2\% = 5\%$$



# Summary and Conclusions

- No evidence of toxicity (mortality, behavior, fish health evaluations).
- Majority of intersex fish - phenotypic males (non-functional females)
- Males and intersex fish were larger than females.
- 17  $\alpha$ -MT fed at a concentration of 40 mg drug/kg feed was effective at producing male populations of hybrid tilapia.



# Acknowledgements

- **SeaPac of Idaho** – Ken Ashley, Ray Gill, and Patty Sheen
- **Rangen, Inc.** – Dave Brock and Doug Ramsey
- **Histopathologist** – Charlie Smith
- **CanTest Ltd.** – Burnaby, BC, Canada
- **Resource** – Phelps and Popma. 2000. Sex reversal of tilapia (Pages 34 – 59 in B. A. Costa-Pierce and J. E. Rokacy, eds. Tilapia Aquaculture in the Americas, Vol. 2)

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