The Ecologically Relevant Effects of Trenbolone on *Gambusia holbrooki*

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Abstract

Trenbolone, a testosterone mimic that is often used in the cattle industry, has potential to act as an endocrine disrupting chemical affecting wildlife near cattle feed lots. With three times the bonding affinity as testosterone and a nine month half-life, trenbolone has been found in the runoff and waste of cattle feed lots (Orlando 2004, Bartelt-Hunt 2012). The continued use of trenbolone in the cattle industry could pose a threat to the freshwater environment of the mosquitofish, which are often placed in ponds near cattle as an alternative mosquito control method. Our previous research has show that Trenbolone, at the ecologically relevant levels of 5ng/L and 10ng/L, to have significant effects on the eastern mosquitofish *Gambusia holbrooki* in a laboratory setting. These effects include changes in mosquitofish mating behavior and masculinization of the female reproductive tract. Here we show replicated results of the influence trenbolone has on morphological changes, mating behavior, and female masculinization of the reproductive tract.

Background

Gambusia holbrooki

- Small, hardy fish used as natural mosquito control by farmers with small ponds
- Live bearing fish
- Likely exposed to pollutants in feedlot runoff

What is trenbolone?

- Testosterone mimicking chemical used in implants to supplement beef cattle
- Higher bonding affinity than testosterone, half-life of ¾ a year

Goal of Experiment

- Determine morphological, physiological, behavioral, and histological effects of trenbolone on *Gambusia* holbrooki

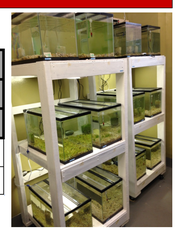
Experimental Setup

- Captive fish lab set up with temperature controls set to 25° C, set photoperiod to simulate mid-summer (prime breeding conditions)
- Male and female fish kept separately for each treatment group: Control, Low dose trenbolone (5ng/L), High dose (10ng/L)
- Doses determined by literature values of trenbolone found in feedlot runoff

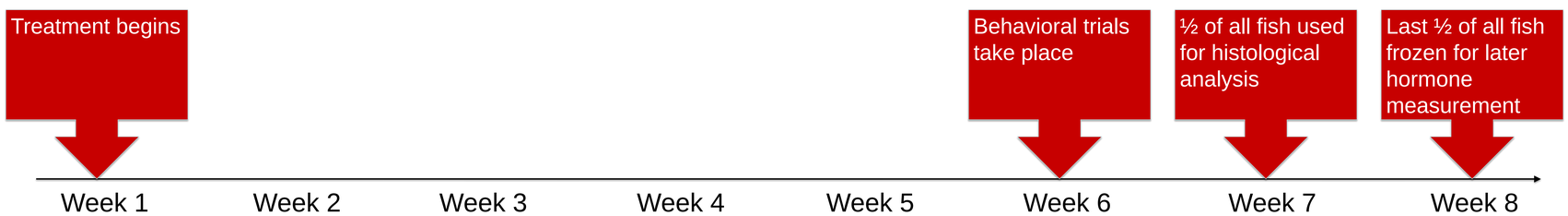
Tanks & Treatments

Table 1

Treatment Group	Control			Low Dose			High Dose		
Dose	EtOH			5ng/L			10ng/L		
Experiment #	1	2	Total	1	2	Total	1	2	Total
# of Males	9	4	13	11	6	17	12	4	16
# of Females	10	16	26	9	4	13	7	18	15



Timeline



Results

Behavioral Trials – Breakdown of behaviors

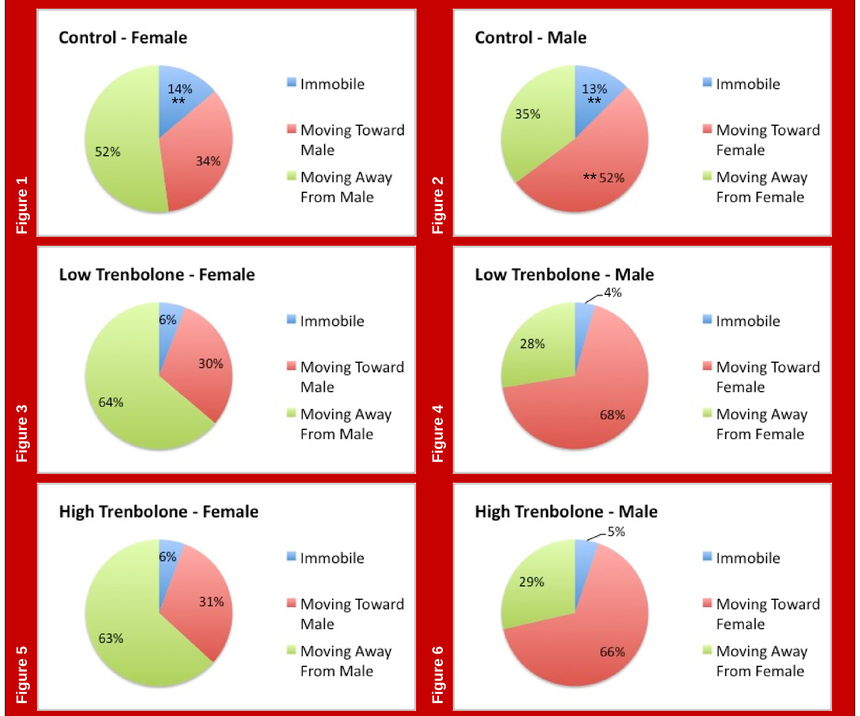
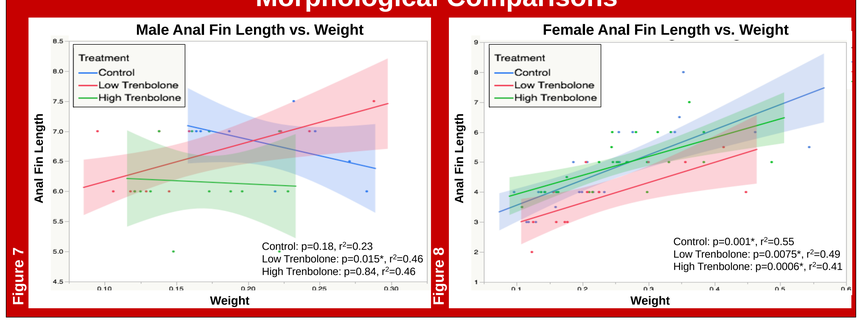


Figure 1-6: Visual breakdown of behaviors during 1 minutes video trials. Using an ANOVA, it was determined the Control group spent significantly less time in mating behavior (**p<0.015).

Behavioral Trials – Velocity and Distance Moved

Table 2	Control	Low Trenbolone	High Trenbolone
Velocity (cm/s)	Female: 5.08**/Male: 4.64**	Female: 2.67/Male: 2.44	Female: 2.82/Male: 2.41
Distance Moved (cm)	Female: 180.23/Male: 156.44	Female: 210.948/Male: 193.37	Female: 230.70/Male: 198.42

Morphological Comparisons



Results

Histological Comparisons

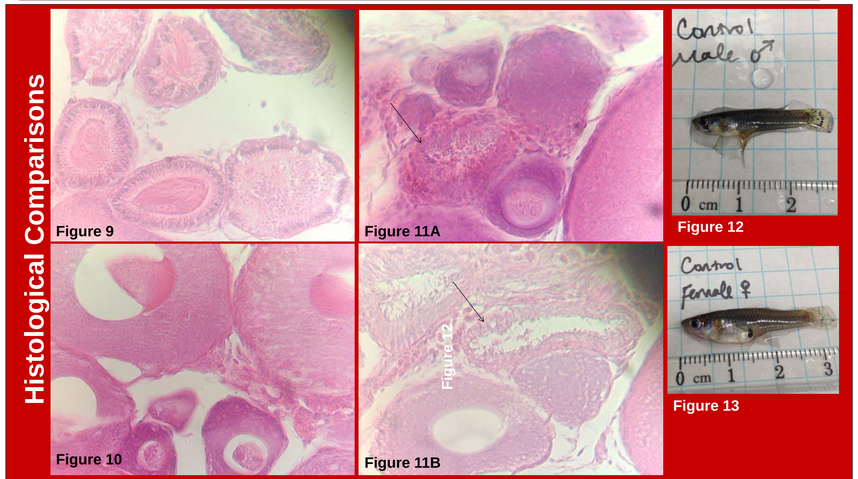


Figure 9-11AB: All slides prepared using H&E stain. Figure 10: Control Female. 40x. Figure 9: Control Male. 40x. Figure11AB: Follicles of High trenbolone dosed female. Possible seminiferous tubules visible (see arrows). Figure 12: Control Male. Note anal fin. Figure 13: Control Female. Note pregnancy spot.

Masculinization of Females

Table X: Shows the number of females with evidence of gonadal masculinization.

Table 3

Treatment	N	% Masculinized
Control	4	0% (0/4)
Low Trenbolone	3	66% (2/3)
High Trenbolone	7	71% (5/7)

Conclusions

- Trenbolone has shown subtle, significant changes of the sexually dimorphic anal fin in males. The low dose male fish showing significantly higher ratios than the high dose and control group.
- Behavioral trials show that the treatment groups are traveling slower and for a longer period of time that the control groups, while still covering the same distance.
- Histological analyzation shows masculinization of female fish at 5ng/L and 10ng/L. An ecologically relevant dosage.
- The effects of trenbolone are subtle but significant in behavioral and morphological effects and may not be immediately apparent in wild-caught fish.

Future Studies

- Multigenerational effects: look at the effects of trenbolone over generations
- Developmental effects: effects of trenbolone on developing young
- Metabolism and duration: how trenbolone effects swim habits
- Behavior: effects of trenbolone on foraging capabilities and same-sex behaviors
- Exposure and Cessation: determine the effects of trenbolone dosage followed by time without trenbolone and explore reversibility of changes
- Continue to explore U-shaped dose response curve

Acknowledgements & Contacts

Special thanks to:

- The Office of Undergraduate Research and Scholarship and the Scholar-Citizen Initiative for funding and travel support
- The Ecophysiology Lab and Michael Barbour
- For additional information please contact Emily Guise at guise.emily@gmail.com or Dr. Sara O'Brien at sobrien3@radford.edu

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