# Mosquito Fish Ethinylestradiol and Testosterone Exposure Experiment

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# Goals

- Learn the effects of Estrogen and Testosterone
- Replicate accidental spills that can occur in the environment
- Pave the way for future studies
- Give back to mother nature

# Introduction

- Scientific name Gambusia Affinis
- Mosquitofish are viviparous
- Live in freshwater systems
- Diets are very diverse but mainly include Mosquito larva
- Behavior is aggressive towards Medaka (Japanese fish)
- Morphological changes

**Figure 1** Female mosquitofish top left, and male mosquitofish bottom right

# Procedure-3 Day Behavioral

- Control, 50 ng/L T2, 50 ng/L EE2
- Fed 0.5 mL brine shrimp daily
- 1 female mosquitofish per replicate
- All pregnant
- Controlled light conditions
- Measure weights & standard length of body
- Euthanized with MS22

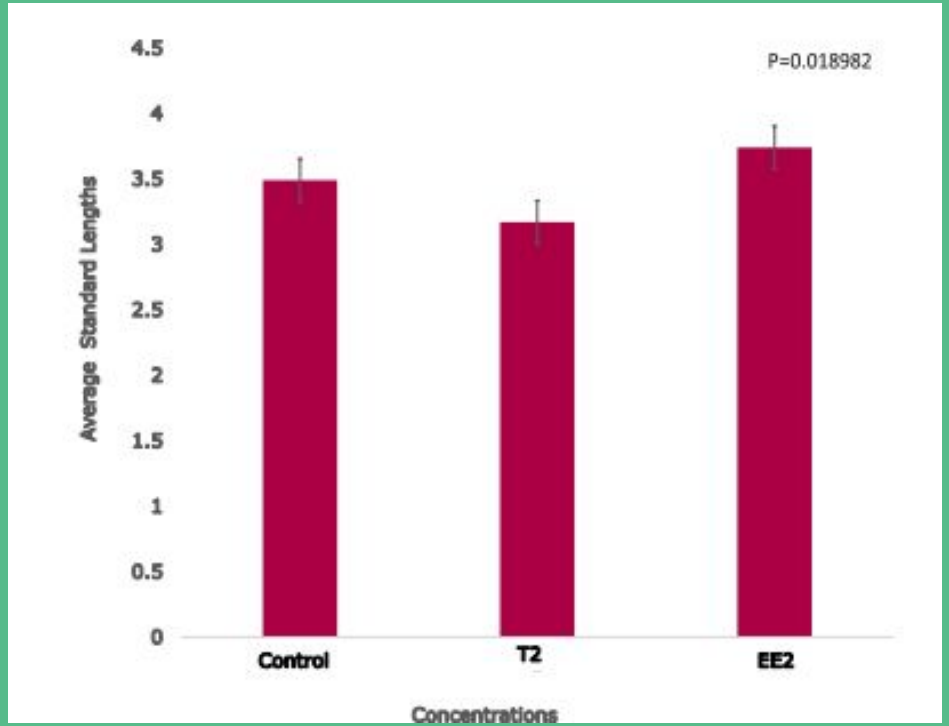

**Figure 2:** Standard length measured from tip of mouth to just before the tail fin

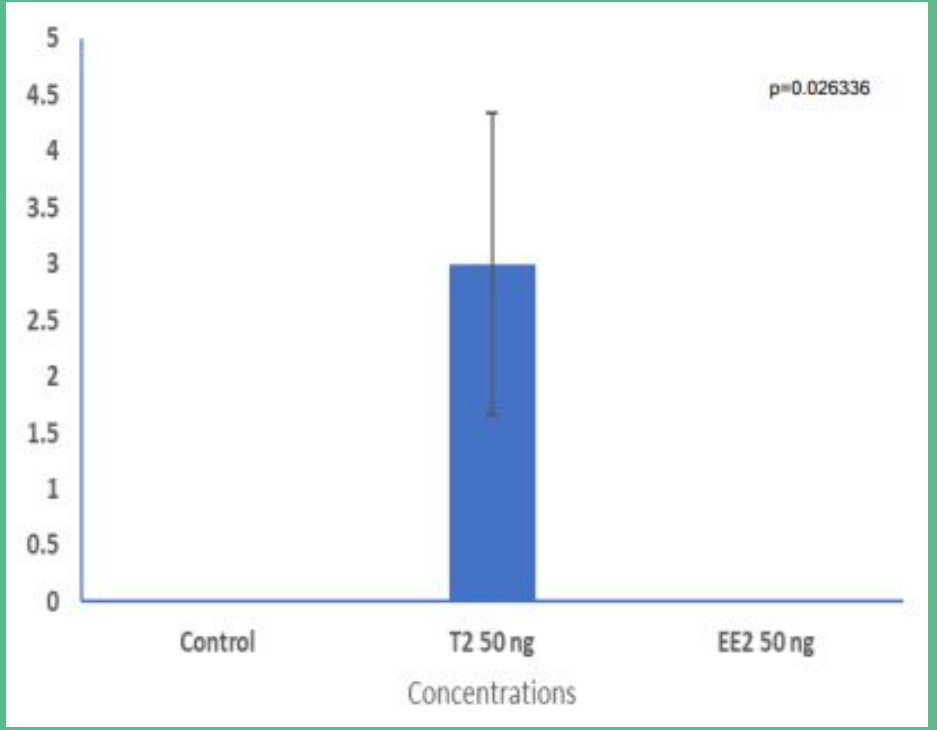
**Figure 3**: Housing Station

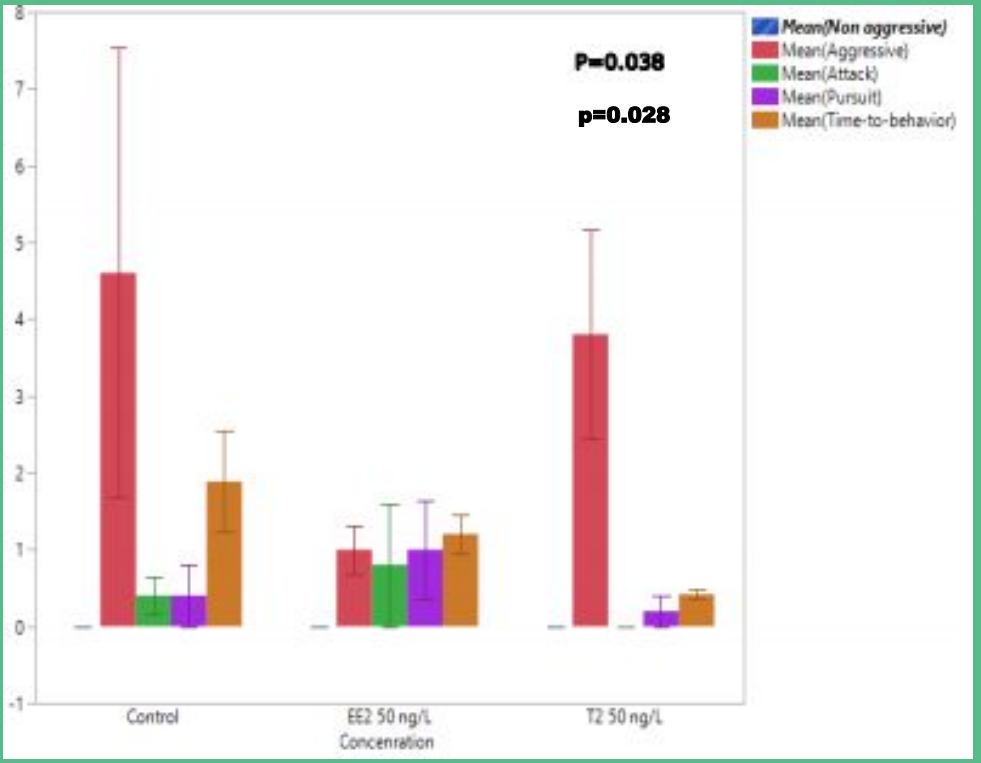
# Behavior Recording

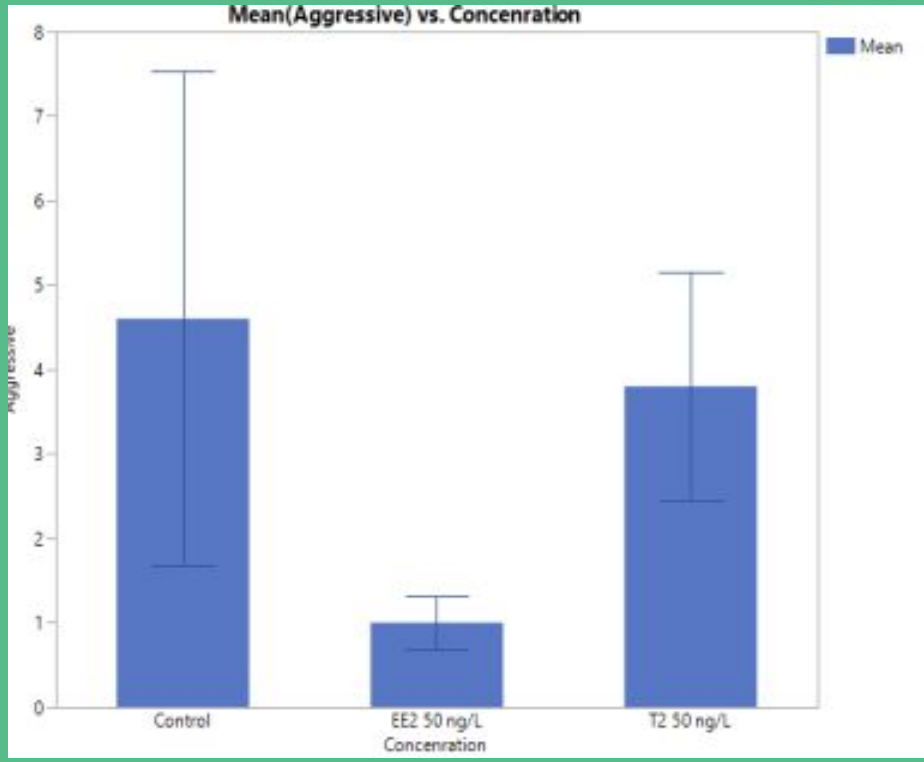
- Timed interaction for 5 minutes each
- Female least killifish

# Results


P=0.018982
Average Standard Lengths
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
T2
EE2
Concentrations


p=0.026336
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
T2 50 ng
EE2 50 ng
Concentrations

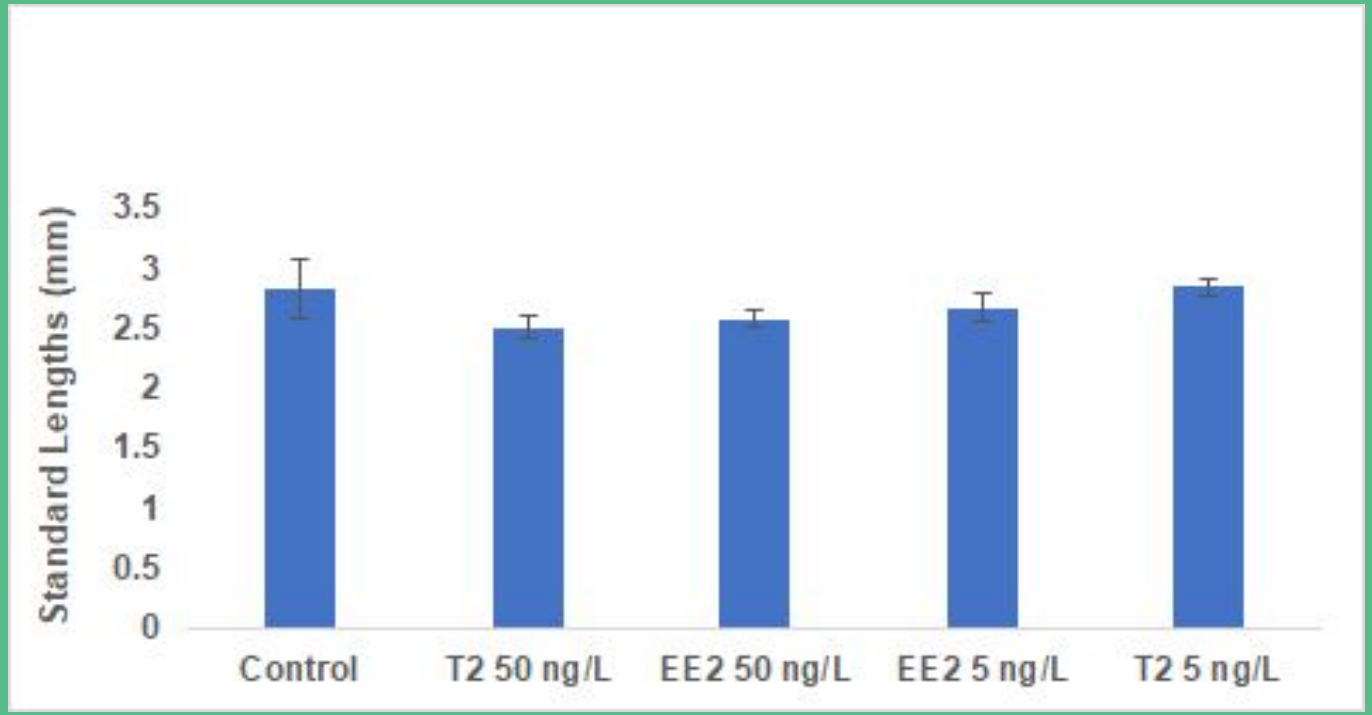

Mean(Non aggressive)
Mean(Aggressive)
Mean(Attack)
Mean(Pursuit)
Mean(Time-to-behavior)
P=0.038
p=0.028
8
7
6
5
4
3
2
1
0
-1
Control
EE2 50 ng/L
T2 50 ng/L
Concenration


Mean(Aggressive) vs. Concenration
Mean
8
7
6
5
4
3
2
1
0
Control
EE2 50 ng/L
T2 50 ng/L
Concenration

# Procedure-7 Day Morphological

- Control, 5 ng/L T2 & EE2, 50 ng/L T2 and EE2
- Fed 0.5 mL brine shrimp daily
- 1 female mosquitofish per replicate
- Some pregnant, some not pregnant
- Controlled light conditions
- Measure length of anal fins, weights, & standard length

# Results


Standard Lengths (mm)
3.5
3
2.5
2
1.5
1
0.5
0
Control
T2 50 ng/L
EE2 50 ng/L
EE2 5 ng/L
T2 5 ng/L

# Discussion

| Behavioral | Morphological |
| --- | --- |
| ● Decrease in standard lengths<br>● Exposure to T2 at 50 ng/L induced birth<br>● Estrogen exposure caused increased aggression | ● Decrease in standard lengths |

# Conclusion

- EE2 and T2 may have devastating effects on mosquitofish populations
- As EE2 and T2 increase, the body length and anal fin size decrease
- High levels of T2 induce early birth in pregnant females

**Future Research**

- Effects of T2 on pregnant females
- Longer studies for behavior
- Histology
- Formulate models to determine how organism level effects translate into population level impacts

# Citations

Hieu M. Dang, Yoshihiko Inagaki, Yuka Yamauchi, Takaski Kurihara, Cong H., Yutaka Sakakibara. Acute Exposure to 17α-Ethinylestradiol Alters Aggressive Behavior of Mosquitofish (Gambusia affinis) Toward Japanese Medaka (Oryzias latipes) pp. 643-748. Published 2017 Feb 3. https://link.springer.com/article/10.1007/s00128-016-2016-z

Xeugeng Wang, Diamond Hill, Donald E. Tillitt, Ramji K. Bhandari. Bisphenol A and 17α-ethinylestradiol-induced transgenerational differences in expression of osmoregulatory genes in the gill of medaka (Oryzias latipes). Pp. 227-234. Published 2019 June. https://www.sciencedirect.com/science/article/pii/S0166445X19301948

Emmanuelle Vulliet, Marine Falletta, Pedro Marote, Thierry Lomberget, Jean-Olivier Païssé, Marie-Florence Grenier-Loustalot. Light induced degradation of testosterone in waters. Pp. 3554-3559. Published 2020. https://www.sciencedirect.com/science/article/pii/S0048969710004584