

**Sophia Gambale**  
**Class of 2022**  
**Forensic Science - Biology Concentration**  
**The Effects of 17 $\alpha$ -Ethinyl Estradiol on Crayfish Aggression**  
**Mentor: Dr. Christopher O'Brien**  
**Department of Forensic Science**

The compound, 17 $\alpha$ -Ethinyl estradiol (EE2) is a synthetic estrogenic compound and one of the primary components of birth control; the metabolites of EE2 are readily found in sewage as a result of its widespread inclusion in many birth control pills (Halling-Sorensen et al. 1998; Daughton et al. 1999). As wastewater treatment plants are not equipped to remove pharmaceuticals and personal care products (PPCPs) their final repository is the aquatic environment, primarily rivers and streams (Breton and Boxall 2003; Daigle, 2010). Accumulation of EE2 in water and sediment as a result of densely populated regions with birth control users can have serious consequences on wildlife and implications on the natural behavior of various organisms (Barreiros et al., 2016). Crayfish fill an ecological niche within aquatic freshwater ecosystems acting as omnivorous scavengers (Reynolds, Souty-Grosset, and Richardson, 2013). Exposure to EE2 has been proven to have implications for crustaceans in both marine and fresh water; however, the research on its effects in regard to crayfish is still in its infancy (Daigle, 2010; Shirley, 2021). It has been proven that the species *Procambarus clarkii* is a naturally aggressive, dominant species and in turn has been increasing in number and location making crayfish an ideal candidate for research on their behavior (Gamradt et al., 1997).

This study was conducted on the premises of 20 Atwood Place and utilized female Red Swamp Crayfish (*Procambarus clarkii*) which were shipped from Louisiana. Research objectives for this project included maintaining a viable colony of crayfish, determining the natural behavior of the crayfish in colony, and analyzing the behavior of the crayfish upon exposure of EE2. Dosages of EE2 consisting of 0 ppm, 0.5 ppm, and 1.0 ppm were added to each tank and left to incorporate; two female crayfish were put into each tank as well. Crayfish were left to interact with one another for a duration of 7 days wherein they were monitored via video and physical observation to document, categorize, and tally their behaviors. Review of the footage and firsthand observation showed pseudocopulation between pairs of female crayfish in the 1.0 ppm tank of EE2 in trials 2, 3, and 4. Additionally, postcopulatory grooming was seen by the dominant female crayfish, which is a common behavior among crayfish when copulating (Mason, 1970). Due to necessary alterations in the experimental set-up that interfered with the trial period, trial 1 was not included in the observations. Pseudocopulation has been documented in male crayfish and is said to occur to reduce anger, subordinates in pseudocopulating pairs have been proven to have a greater chance of survival and both individuals are more likely to face a lower risk of mortality from fighting (Riddihough, 2006). Although pseudocopulation was not witnessed in all the trials in this study, it is recommended that further research be conducted wherein there are longer trial periods to see if an accumulation of the compound overtime can result in pseudocopulation or other altered behaviors in tanks with lower dosages. Additionally, further research into how much EE2 crayfish bioaccumulate at these dosages both in their carapace and muscle would be beneficial as an indicator of potential long-term effects that may come from continuous exposure to EE2.

## References

- Barreiros, L., Queiroz, J.F., Magalhães, L.M., Silva, A., & Segundo, M. Analysis of 17- $\beta$ -estradiol and 17- $\alpha$ -ethinylestradiol in biological and environmental matrices — A review, *Microchemical Journal*, Volume 126, 2016, Pages 243-262.
- Breton, R. & Boxall, A. 2003. Pharmaceuticals and personal care products in the environment: regulatory drivers and research needs. *QSAR and Combinatorial Science* 22: 399-409.
- Daigle, J. "Acute responses of freshwater and marine species to ethinyl estradiol and fluoxetine" (2010). LSU Master's Theses. 4005.  
[https://digitalcommons.lsu.edu/gradschool\\_theses/4005](https://digitalcommons.lsu.edu/gradschool_theses/4005)
- Daughton, C. & Ternes, T. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* 107 (6): 907-938.
- Gamradt, S.C., Katz, L.B., & Anzalone, C.B. 1997. Aggression by non-native crayfish deters breeding in California newts. *Conservation Biology*, 11: 793-796.
- Halling-Sørensen, B., S. Nors Nielsen, P. Lanzky, F. Ingerslev, H. Holten Lützhøft and S. Jørgensen. 1998. Occurrence, fate, and effects of pharmaceutical substances in the environment - a review. *Chemosphere* 36 (2): 357-393.
- Mason, J. C. (1970). Copulatory behavior of the crayfish, *Pacifastacus trowbridgii* (Stimpson). *Canadian Journal of Zoology*, 48(5), 969-976.
- Reynolds, J. & Souty-Grosset, C. & Richardson, A. (2013). Ecological Roles of Crayfish in Freshwater and Terrestrial Habitats. *Freshwater Crayfish*. 19. 197-218.
- Riddihough G. (2006). Submit or Perish. *Science*, 314(5805), 1516.
- Shirley, M. "Effects of Synthetic Estrogen (17 $\alpha$ -Ethinyl Estradiol) on Male Fiddler Crab Aggression" (2021). Honors College Theses. 643.