

Office of Undergraduate Research & Scholarship
2014 Summer Undergraduate Research Fellowship Application

Student applicant's name: Emily Guise

Email address: eguisse@radford.edu

Faculty mentor: Dr. Sara O'Brien

Major/department: Biology

Title of project: Effects of Ecologically Relevant Exposure to Trenbolone

Will your project involve human subjects or animals? Yes No

Have you applied for IRB or IACUC approval? Yes No

Note: Although prior IRB/IACUC approval is not needed for the application process, it will be **required before April 14th** in order to qualify for an award. Surveys require IRB approval.

Students are highly encouraged to make an appointment or visit the Office of Undergraduate Research and Scholarship on Tuesdays 9am-12pm and Fridays 12-3pm in Reed 354 for feedback on their drafted proposal.

Use 12-point, single-spaced font throughout the application. The description is not to exceed two pages (excluding references) under any circumstances and should address the points below.

Project Narrative:

- Provide a brief description of the purpose of your proposed study. State your research question or new knowledge created and the innovative nature of the project. Why is this important to study? How does it contribute to the field?
- Describe the methodology/creative process of your study. Describe your qualifications for conducting this study. What is your experience with the procedures and instrumentation to be used in this study?
- Appropriate references should be cited throughout, using the style most appropriate to your discipline (the reference list does not count toward the one page limit)

Anticipated Outcomes and Learning Objectives:

- What products and/or outcomes are expected from this project? Outcomes include: academic papers, creative work such as paintings or musical compositions, conference papers, journal articles, and performances.
- What is the contribution to the scholarly and arts community?
- What is the contribution to society?
- What do you anticipate learning from this research?
- At this end of this project what personal development is expected to take place?
- What are your long-term goals and how will this project help you realize them?

Supporting References:

A list of appropriate references should be included in the professional style standard for the discipline.

Project Narrative

INTRODUCTION & BACKGROUND:

Endocrine Disruptor Chemicals (EDCs) are environmental pollutants that interfere with an organism's natural endocrine system function at a variety of exposure levels. EDCs are known to accumulate in ecosystems affecting their inhabitants with effects that are often less known (1,2). One example of an EDC is trenbolone. Trenbolone is a relatively new EDC with very little research exploring its environmental effects thus far, despite being commonly used in many countries, including the US. As trenbolone is a synthetic androgen, with three times more bonding affinity than testosterone, trenbolone is an ideal compound commonly used in the beef cattle industry to promote faster and significantly more growth-around 50 extra pounds at time of slaughter (2,3,4). Despite trenbolone implementation method, as a food additive in other countries, or dispersed through an implant, trenbolone is still known to be excreted by the cattle through urine and manure, and has been detected in feedlot runoff in levels highly relevant to freshwater species (5,6). One of the more disturbing characteristics of trenbolone is its molecular stability, with a half-life of $\frac{3}{4}$ of a year (5), suggesting it will be waiting around to be used for non-intended purposes for over a year. Trenbolone's widespread use and consistent appearance in runoff are directly relevant to the health of our environment, and the goal of my research is to explore the freshwater effects of trenbolone.

As an androgen, trenbolone is known to reduce typical female behavior in adult female fish, affecting their reproductive behaviors and resulting in more masculine physical features at nominal doses (2). However, to my knowledge there haven't been any studies testing the long-term effects of trenbolone on freshwater fish. If trenbolone causes a slight reduction of female fish's reproductive behavior (when exposed to trenbolone only as adults), then how will the fish's offspring be affected as it has been exposed to this androgen during its development? In this experiment, I would like to test the effects of long-term trenbolone exposure on the freshwater fish's multigenerational reproductive efforts in the controlled environment of a lab.

METHODOLOGY:

For my experiment, I will collect multiple samples of cow manure from several local beef cattle feedlots that use implants distributing trenbolone, and first test them for the presence and level of trenbolone in the manure/runoff using a trenbolone ELISA kit. I have experience running an ELISA to measure levels of cortisone while participating in a BIOL 491 and I am excited to use it again for future work, as it is a great skill to have as a growing biologist. Throughout the experiment, Dr. Sara O'Brien has graciously offered to help me learn the correct procedures and to offer her guidance during the experiment. The samples that exhibit the highest presence of trenbolone will be used as "runoff" to expose the experiment group of fish.

Initially, there will be five fish tanks: two tanks will be female (one trenbolone treated and one untreated), two will be male (one treated and one untreated), and the fifth tank will be used for breeding and behavior analysis, and will be untreated. The treated fish will be exposed to trenbolone for two weeks, after which a female fish will be placed in the fifth breeding tank with a male fish to breed while their behavior is analyzed. The same breeding procedure will be used for the control group at a separate time. The fish will be returned to their respective tanks during gestation. The species of fish used will be live-bearing fish, meaning that during gestation, the eggs remain inside the mother as a source of care. This means that the females

who are pregnant and are being exposed to trenbolone, are also exposing their young, resulting in exposure during the young's physical development, pre-birth. Once hatched, the young will be checked for any changes in their typically sexual dimorphic features. Then, as they mature, we will evaluate their ability to reproduce and the characteristics of their young.

PROJETERED SCIENTIFIC OUTCOMES:

Ultimately this study should elucidate the effects of trenbolone on at least two entire generations of fish and to measure the changes in reproductive behavior and their ability to produce viable young compared to the control. Additionally behavioral and morphological differences between exposed and unexposed fish will be explored at many life history states (juvenile, adult, breeding, etc.).

I plan to analyze the reproductive behavior using Noldus software, which can use video recordings of the tank to analyze the social behavior of the fish. For example, the software can measure the amount of time the male fish spends chasing the female and the amount of time the female fish spends swimming away from the male (8). The data that Noldus provides will allow me to statistically compare the behavioral differences between the trenbolone exposed fish, and the unexposed fish.

For almost a year now, I have been actively participating in the Ecophysiology Lab here at RU, which partly involves weekly scientific paper discussion. These papers have primed me for asking scientific questions and ways of finding out those answers. This is my first experiment I have designed and am very excited to get to actually complete it this summer. I am currently IACUC certified and will seek approval for this experiment in the coming months.

Anticipated Outcomes and Learning Objectives

OUTCOMES:

My intent with the experiment is to present the conclusion of my research at national conferences which the RU Ecophysiology Lab has participated in the past. Additionally I will seek publication in a scientific journal if warranted. At the very least, this study will provide preliminary data to develop additional experiments and seek additional funding for research. Overall, this information is relevant to the health of the natural world as we are finding information on the effects of trenbolone on development that is currently unknown. My goals in this project are to become comfortable with experimental design, fieldwork, project management, data management, statistical analysis, technical analysis, animal husbandry, and oral and written dissemination of scientific results.

LEARNING OBJECTIVES:

As a student interested in attending medical school or doing research in graduate school, I feel that it is very important to have experience planning experiments, as there are always questions to be asked in all places, and I want to be someone asking (and seeking answers to) those questions. I feel that this experiment will help me decide where I want to end up, be it in graduate school or medical school. Either way, I believe that the experiences I have within this project will follow me wherever I go.

References

1. Li, Y., Luh, C.J., Burns, K.A., Arao, Y., Jiang, Z., Teng, C.T., Tice, R.R., Korach, K.S. (2013). Endocrine-disrupting chemicals (EDCs): In vitro mechanism of estrogenic activation and differential effects on ER target genes. *Environmental Health Perspectives*, 121 (4), 459-466.
2. Saaristo, M., Tomkins, P., Allinson, M., Allinson, G., Wong, B. B. M. (2013). An androgenic agricultural contaminant impairs female reproductive behaviour in a freshwater fish. *Plos One*, 8 (5), 1-7. doi: e62782
3. Whittie, W. D., (2011, May 4). *Implant Calves This Year?*. Retrieved February 2, 2014 from Virginia Cooperative Extension: http://pubs.ext.vt.edu/news/livestock/2011/05/LU_05-04-11-2.html
4. Schiffer, B., Daxenberger, A., Meyer, K., Meyer, H. H. D. (2001). The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: Environmental studies. *Environmental Health Perspective*, 109 (11). 1145-1151.
5. Ankley, G. T., Jensen, K.M., Makynen, E. A., Kahl, M. D., Korte, J. J., Hornung, M.W. (2003). Effects of the androgenic growth promoter 17- β -trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environmental Toxicology and Chemistry*, 22 (No. 6), 1350-1360.
6. Bartlet-Hunt, S. L., Snow, D. D., Kranz, W. L., Mader, T. L., Shaprio, C. A. (2012). Effect of growth promotants on the occurrence of endogenous and synthetic steroid hormones on feedlot soils and in runoff from beef cattle feeding operations. *Environmental Science & Technology*, 46, 1352-1360. doi:10.1021/es202680q
7. Yarrow, J. F., McCoy, S. C., Borst, S. E. (2010). Tissue selectivity and potential clinical applications of trenbolone (17- β -hydroxyestra-4,9,11-trien-3-one): A potent anabolic steroid with reduced androgenic and estrogenic activity. *Elsevier*, 377-389.
8. Green, J., Collins, C., Kyzar, E. J., Pham, M., Roth, A., Gaikwad, S. (2012). Automated high-throughput neurophenotyping of zebrafish social behavior. *Journal of Neuroscience Methods* (210). 266-271. doi:10.1016

Emily Taylor Guise

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EDUCATION:

North Carolina State University, 2010-2012
Radford University, 2012-2015 (expected)
Bachelor of Science in Biology

Raleigh, NC
Radford, VA

MAJOR INDEPENDENT RESEARCH TOPIC:

Radford University, Ecophysiology Lab - Mentored by Dr. Sara O'Brien
Guise, E. "Trouble with trenbolone? Examining the influence of common run-off pollutant on *Gambusia holbrooki* development and behavior" Spring 2013 - Current

GRANTS AND AWARDS:

Summer Undergraduate Research Fellowship, Radford University, Spring 2014. Provided by RU Office of Undergraduate Research. \$2500
Highland in Action Grant, Radford University, Spring 2014. Provided by Scholar Citizen Initiative Office. \$2000
Biology Research Award, Radford University; Spring 2014. Provided by RU Biology Department. \$1000

PRESENTATIONS & PUBLICATIONS:

Scholarly

Guise, E., O'Brien, S. 2014. Effects of Ecologically Relevant Exposure to Trenbolone. 2014 RU Summer Research Celebration.
Guise, E. 2014. Submitted for review. *Microtus richardsoni*: natural history, distribution, and classification of the water vole. Animal Diversity Web.
Guise, E. 2014 Eportfolio: [Link]
Guise, E. 2014. My Eportfolio. 2014 Highlander in Action Scholar-Citizen Initiative Showcase.

Outreach

Guise, E. 2014. Women in Science and Undergraduate Research. Invited Presenter/Panelist. RU Summer Bridge Program: encouraging high school woman to major in STEM fields.
Guise, E., Self, K., O'Brien, S. 2014. Establishing a Toxin-free Home: decreasing your exposure to endocrine disrupting chemicals. Outreach presentation at the 2014 Christiansburg Minorities Health fair.
Guise, E. 2014 The Truth About Your Food. Radford University Whim online magazine.
Dudley, E., Guise, E. 2014. Roachzilla: Bugs, hormones, and beyond. Outreach presentation at the 2014 Blacksburg and Roanoke Virginia Science Festivals.

RELEVANT COURSE WORK AND TECHNIQUES:

Coursework

Endocrinology. Radford University. Fall 2014.
Biochemistry. Radford University. Fall 2014.
Pathophysiology. Radford University. Summer 2013.
Microbiology. Radford University. Spring 2013.
Women in Science and Engineering. NC State University. 2010-2012. Completed 4 semester program, learned what it means to be a woman in science and the difficulties women face.

Lab/field Techniques Mastered:

- ELISA/EIA test kits
- Water sample collection
- Animal care including avian, reptilian, and aquatic animals
- General dissection
- Dilutions and reagent preparation
- Behavioral monitoring with Noldus Ethovision software
- Accurate Pipetting technique
- Bacterial culture
- Titrations, distillations, etc.
- H¹-NMR, IR, and Mass Spectrometry

TEACHING EXPERIENCE:**Teaching Assistant:**

Human Anatomy and Physiology for Nurses, Fall 2014-Current – Lead TA– Radford University

Retention Course for Students Interesting in Pursuing Research, Fall 2014-Current – Radford University

Advanced Human Anatomy and Physiology for Majors, Fall 2013-Spring 2014 – Radford University

EMPLOYMENT:

Radford University, New Student Programs, Retention Program. Fall 2014
Peer Instructor. Co-teach UNIV 100 to incoming freshman interested in Research.

Radford University, Learning Assistance Resource Center. Fall 2014
Tutor. Tutor students in Anatomy and Physiology.

Radford University, Anatomy and Physiology Lab. Spring 2013 - Current
Lab Assistant. Assist with dissection, and general cadaver maintenance.

Radford University, Admissions. Fall 2013 - Current
Student Admissions Representative. Give campus tours, attend admissions events.

Radford University, Waldron College Dean's Office. Summer 2013-Fall 2013
Office Assistant. Worked front desk, office work, assisted Dean and advisors.

NC State, The National Society of Leadership and Success. Spring 2012
Intern. Started chapter of NSLS on campus.

OUTREACH & SERVICE:

IACUC Student Representative. Radford University. Fall 2014. Serve as student representative on IACUC board, review all incoming protocols involving institutional animal research, ensure recommendations are met and compliance is achieved.

Greenhouse Tour Guide Coordinator. Radford University. Fall 2014. Recruit, train, and schedule student tour guides for Science Day at RU.

Vivarium SPF Search Committee Student Representative. Radford University. Spring 2014. Participated in phone and on campus candidate interviews as student representative. Arranged student meeting with candidate.

Wakemed Cary Hospital Volunteer. Cary, NC. Spring 2012. Restocked rooms, assisted nurses and patients, cleaned rooms. 2 hours weekly.

Knitting Instructor Volunteer. Raleigh Rescue Mission, Raleigh, NC. Spring 2012. Taught knitting classes weekly to homeless women and children. Arranged collection of donated materials.