Lisa Rania Ganser

University of Miami. Coral Gables, Florida

2004 EPA Star Graduate Fellowship Conference

Next Generation Scientists—Next Opportunities

The Effects of the Herbicide Atrazine on the Sexual Differentiation of the Brain of *Bufo marinus*

Environmental Issue

The endocrine system serves as one of the main control systems of the body. During the amphibian larval period, hormones help to organize bipotential reproductive tissues in the brain and the gonads into their male or female forms. It is during this critical period of organization that certain man-made chemicals may disrupt the normal endocrine cascade that governs the function and form of reproductive tissues. These endocrine disruptors, often found in pesticides and industrial wastes, have been known to affect the morphology of the gonads as well as the mating behaviors of exposed animals.

I will collect cane toads, *Bufo marinus*, from various sites in South Florida and breed them in the laboratory. The timing of sexual differentiation will be determined with histological methods. Immunohistochemistry will be used to determine any sexual differences in brain morphology by assessing any variation in arginine vasotocin (AVT) receptor populations in the areas of the brain responsible for mate calling, call phonotaxis, and orientation to breeding sites.

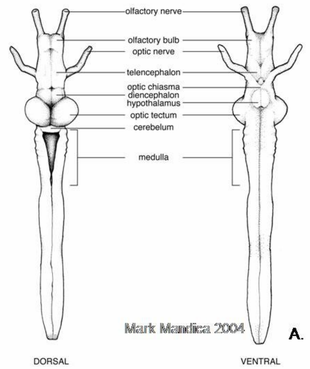
After the timing of sexual differentiation of the gonads and any sexual differences in brain structure are described, new cane toad specimens will be treated with the putative endocrine disrupting herbicide, atrazine. A previous study (Hayes et al. 2002) concluded that atrazine feminized the gonads of male clawed frogs (*Xenopus*) I will then quantify any effects of atrazine on the sexual differentiation of the brain and gonads of the cane toad. In this preliminary study, I have described neuroanatomical structures relevant to mating behavior in *Bufo marinus*. My next goal is to determine whether or not there are sex specific differences in these regions. These data are necessary in order to determine if there are effects of atrazine on the development of neuroanatomical structures pertinent to reproduction.

Scientific Approach

- **Develop cane toads as models for atrazine-induced endocrine disruption.**
  - -Treat larval and adult Cane toads with varying concentrations of atrazine (following Hayes et al. 2002).
  - -Will the presence of a Bidder's organ make toads more susceptible to endocrine disruption?
  - -Determine the timing of sexual differentiation of the gonads of *Bufo marinus*.
  - -Determine any effects of atrazine on the development of the gonads of *B. marinus*.
- **Augment the study by Hayes et al. (2002) to include the effects of atrazine on the brain of the cane toad.**
  - -Identify neuroanatomical structures of the cane toad with especial attention to areas in charge of mating behaviors
  - -Use immunocytochemistry to detect and quantify sex differences in the brain.
  - -Treat larval and adult cane toads with varying concentrations of atrazine (following Hayes et al. 2002).
  - -Determine any effects of atrazine on areas of the brain of *B. marinus* that are pertinent to reproductive behaviors.
- **Preliminary work:**
  - -I have used histology to identify neuroanatomical structures in the brain of *Bufo marinus*.
  - -The next step will be to utilize immunocytochemistry to identify areas of the brain that are governed by sex hormones.

Preliminary Results

Figure 1. Gonads of *Bufo marinus*. A. Male. B. Female
Testes and ovaries were dissected from adult, field-collected *Bufo marinus* for subsequent histological examination in the laboratory.



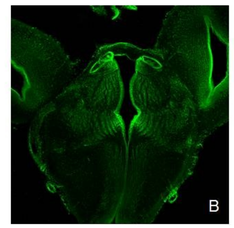


Figure 2. Brain of *Bufo marinus*. A. Drawing of Cane toad brain with regions and landmarks. B. Confocal micrograph of the diencephalon region of the brain.

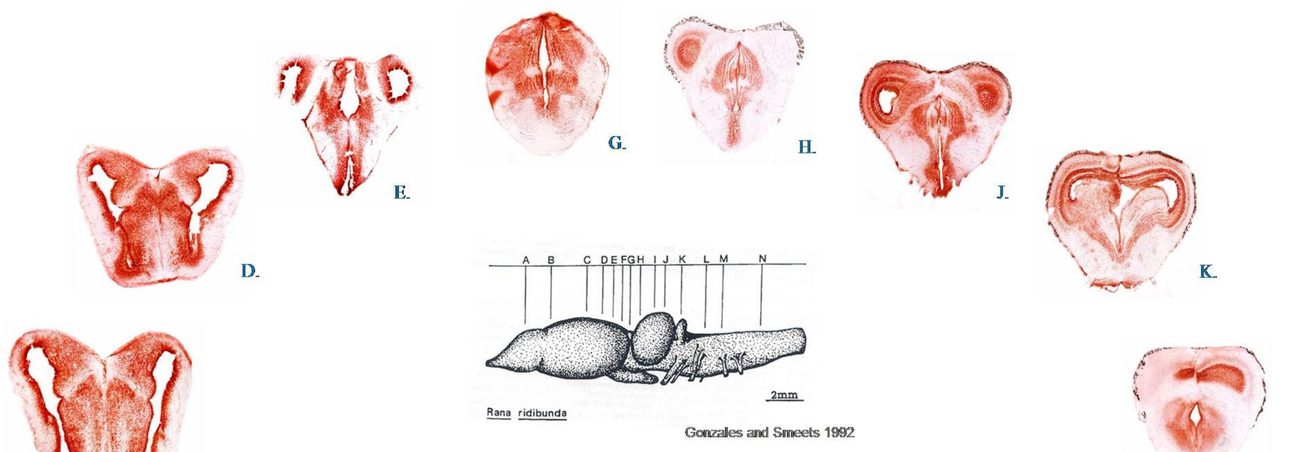
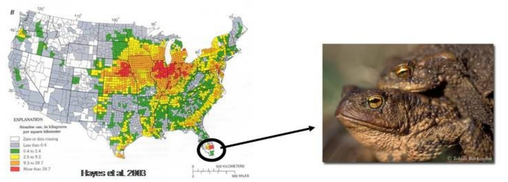


Figure 3. Safranin O stained sections through the brain of *Bufo marinus*. Letters represent regions of the brain outlined in the figure of the brain of *Rana ridibunda* from Gonzalez and Smeets (1992).

Impact

My study will provide insight into the potential endocrine disrupting effects of atrazine. Treatment with atrazine during the larval period may affect the organization of reproductive tissues including the gonads and areas of the brain influencing mating behaviors. Exposure to atrazine during adulthood may disrupt the normal activation of these tissues.

Because atrazine is quite prevalent in the environment, the possible impact of endocrine disruption on the reproductive capabilities of this species and other wildlife in Florida may threaten the survival of affected populations.



Literature Cited

Gonzalez, A and WJAJ Smeets. 1992. Comparative analysis of the vasotocinergic and mesotocinergic cells and fibers in the brain of two amphibians, the Anuran *Rana ridibunda* and the Urodele *Pleurodeles waltlii*. J. Comp. Neurol. 315: 53-73.

Hayes, T.B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A.A. Stuart. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at ecologically relavant doses. Proc. Natl. Acad. Sci. 99: 9900-9904.

Hayes, T.B., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ. Health Pers. 111(4): 568-575.

Acknowledgements

-Dr. Zhongmin Lu
-Seth Tomchick
-Mark Mandica
-Cynthia Jeyapaul
- The U.S. EPA
- O'Lab
- Dr. Jim O'Reilly

Email: ganser@bio.miami.edu
http://www.bio.miami.edu/zlu
http://www.bio.miami.edu/oreilly/ganser.html

This fellow is sponsored by EPA's STAR or Greater Research Opportunities (GRO) Program.