Welcome to the 6th Annual P.U.R.E. Symposium!

Tuesday April 5, 2011
LSF 102

PROGRAM FOR UNDERGRADUATE RESEARCH EXPERIENCE

"Fluorescence Visualization of Cadmium Import in Mitochondria Isolated from Mouse Brain"

Sarah Marie Oliver
Faculty Mentors: Dr. Kirk Dineley and Dr. Latha Malaiyandi

The intracellular accumulation of cadmium and zinc can be detrimental to energy-intensive tissues, such as brain and heart. Although these metal ions disrupt mitochondrial function, the exact mechanism is unclear. Here, we characterize metal effects on mitochondria using a novel paradigm. We isolated mitochondria from mouse brain and adhered individual organelles to microscopy glass. We used the newly developed fluorophore, Leadmium Green to visualize cadmium accumulation in isolated mitochondria. Our data show that the dye responds to cadmium in a concentration-dependent manner. Mitochondrial inhibitors were used to manipulate cadmium import. Both FCCP, a mitochondrial uncoupler, and ruthenium red, an inhibitor of the calcium uniporter, enhanced cadmium uptake in mitochondria in response to cadmium. Our results suggest that cadmium uptake into mitochondria occurs regardless of membrane potential and through a pathway not shared with calcium.

"Prey Size Preference of *Amphiuma means* and Their Predatory Effect on Crayfish Behavior"

Harrison Taylor
Faculty Mentors: Dr. John Ludlam and Dr. Greg Pryor

The two-toed amphiuma, *Amphiuma means*, is a large aquatic salamander and a potential important predator of crayfish. However, little is known about the effects of predation by *A. means* on native crayfish communities. This study was carried out to determine if *A. means* predation on *Procambarus sp.* crayfish is size-dependent. According to optimal foraging theory, medium-sized crayfish should be the main preference of *A. means* because smaller and larger crayfish require more energy expenditure to catch and consume. Seven *A. means* were collected from an agricultural ditch in Effingham, SC and were placed in individual aquatic mesocosms. Six crayfish, two from each of three size categories were also placed in each tank. The percent mortality and location of the crayfish in the tank were checked frequently. There were no significant differences among predation rates; however, there was a slight trend in predation of medium crayfish, as the average number of surviving crayfish fell faster than that of the other two categories over the first few days of the experiment. Small crayfish had the lowest mortality rates. Surviving crayfish were found most often on the edges of the tubs. Predation seems to be highest on medium crayfish possibly due to their weak defenses relative to large crayfish and inability to hide as well as small crayfish. However, crayfish never seem to outgrow predation because even the largest crayfish were preyed upon during the experiment.

"Using PCR to Clone the p53 Promoter From *Callorhinchus milii* (Elephant Shark) and *Danio rerio* (Zebrafish) Genomic DNA"

Megan Thompson
Faculty Mentor: Dr. Erin Eaton

In humans, p53 along with p63 and p73, under normal conditions, activates apoptosis when a cell is beyond repair. It has been discovered in some cases, a mutant p53 is formed and its normal function is either lost, or a malignant form of p53 is produced. The cartilaginous fish, the shark, stands out because this mutation in p53 has not caused the malignant cancers as it has in so many other species. Polymerase Chain Reaction (PCR) was used to amplify the p53 promoter region of the elephant shark DNA, using primers designed from the known sequence of *Danio rerio* (Zebrafish). Zebrafish DNA is used because the entire genome of *Danio rerio* has been sequenced and because the bony fishes are thought to be more evolutionarily related to sharks than human or mice DNA. Using the *Danio rerio* genome as a model, we initiated a parallel project in which we also clone, using similar techniques, the p53 promoter region from this organism. In, conclusion, we are in the process of screening sub-clones which contain the unique 2kb p53 promoter from *Danio rerio*. The sub-clones will be sequenced to confirm their identities.

"Determination of Mercury Levels in Aquatic and Terrestrial Wildlife in the Pee Dee Area"

Ashley Van Laethem
Faculty Mentors: Dr. Jennifer Kelley and Lisa Pike

Mercury poisoning affects hundreds of thousands of people and results in hundred of deaths each year in the United States. The allowed daily intake of mercury as determined by the EPA is 0.4 micrograms per kilogram of body weight, but some South Carolinians are taking in up to three times more than the daily limit in one meal. Mercury poisoning occurs mainly from the eating of contaminated fish. These fish become contaminated through the biomagnification of organic (methyl) mercury in local waterways which spreads up the food chain to top consumers, such as humans. This results in species at higher trophic levels having higher mercury concentrations than species at lower levels. This study is to be used as an educational method to inform the public about levels of mercury contamination in local aquatic and terrestrial wildlife in the Pee Dee area and to develop a method for testing levels of methyl mercury in local wildlife. Methyl mercury concentrations will be tested in local wildlife using cold vapor atomic absorption (CVAA) spectrometry. Samples are prepared as described in EPA Method 245.6. In this method, methyl mercury is released from the samples of fish flesh through acid digestion using sulfuric and nitric acids and then later oxidized with potassium permanganate to Hg^{2+}. Hg^{2+} is then reduced by Sn^{2+} to Hg^{0} (free mercury is the gaseous state). The concentration of free mercury gas can then be determined based on its absorption at a wavelength of 254.7 nanometers. Confirmation of the release of mercury by the analytical method and the ability to detect a known amount of mercury will be determined from analysis of a variety of standard concentrations and compared to a certified sample with a known concentration of mercury. Measured values of methyl mercury in the local fish flesh samples can then be compared to DHEC limits to help inform the public of significant mercury contamination in our area.

The Department of Biology at FMU strongly encourages student participation in research activities. We offer many opportunities for undergraduates to assist in faculty research or develop their own independent research projects. Students can earn academic credit through Special Studies (BIOL 497) and Honors Independent Study.

If you are interested in learning more about P.U.R.E. or available research opportunities, please visit our website at: http://www.fmarion.edu/academic/Biology and click on 'Research' link. You can also contact Dr. Barbeau (tbarbeau@fmarion.edu) or Dr. Pryor (gpryor@fmarion.edu), the coordinators of P.U.R.E. We can answer any questions you might have and get you started on a research project!