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Closing report: faculty summer research award 2004.

### The role of serotonin in pH regulation by larval mosquitoes

The primary goals of the proposed research were to quantify changes in serotonin levels in larval mosquito in response to environmental changes in pH, and to determine whether alaproclate increases serotonin levels in vivo. A further goal of the proposed research was to identify the organs involved in pH regulation in larval mosquitoes by screening for increases in mitochondrial numbers or densities, using fluorescent probes, in animals acclimated to waters of different pH values.

#### Accomplishments:

I made substantial progress on each of my two main goals, but did not complete either project. However, I also made some important progress in other areas that were not explicitly discussed in my proposal. A significant amount of my research effort this summer was directed at guiding research projects of two undergraduates who expressed interest in working with me this summer. I estimate that I spent about 20 hours per week supporting and guiding their research projects. These projects were completed successfully, and are expected to contribute to future research publications with these students as coauthors. I should point out, though, that support of student research is not cost-effective if your only goal is to obtain data. It is generally both easier and quicker to do the work yourself. However, support of undergraduate research is an important component of the educational experiences of the next generation of scientists.

The first goal of the proposed research was to quantify changes in serotonin levels in larval mosquito in response to environmental changes in pH, and to determine whether alaproclate (and other serotonergic agents) increases serotonin levels in vivo. Samples of hemolymph from animals reared under different conditions of salinity and pH, and of animals exposed to the serotonergic agents alaproclate, quipazine, and methiothepin have been collected and processed, and stored in the -70 freezer. However, the summer ended before these samples could be analyzed. They should keep until such time as I can complete the analysis of these samples.

The second goal was to localize the organs involved in pH regulation in larval mosquitoes by screening for increases in mitochondrial numbers or densities, using fluorescent probes, in animals acclimated to waters of different pH values. This project, though not yet completed, has borne significant fruit. We determined that changes in mitochondrial densities and distributions occur in response to changes in pH, in several regions of the alimentary canal and excretory system. These changes are consistent with upregulation of acid excretion by the Malpighian tubules in response to acid loading. Base excretion proved more difficult to interpret. Specimens of larvae raised at different pH have

been fixed and stored for further analysis using cryosectioning and transmission electron microscopy, to provide greater resolution and address changes in other tissues that were not accessible in the original study.

Working with undergraduate Ritchie Samandu, we determined that serotonergic agents that we have found to cause pH-dependent mortality, also influence rates of acid/base excretion. Methiothepin caused acid excretion rates to decline from  $70.2 \pm 3.70$   $\mu\text{mol/g/hr}$  to  $61.6 \pm 2.4$   $\mu\text{mol/g/hr}$ , while quipazine caused acid excretion rates to increase to  $79.1 \pm 5.01$   $\mu\text{mol/g/hr}$ . These changes were statistically significant. In contrast, alaproclate did not cause any detectable change in acid excretion rates.

Marcus Vieira and I performed a series of experiments using pH indicators in vivo. Larvae ingest the indicators, which are visible through their cuticles, showing the pH of different gut regions in living, unrestrained larvae. Using a series of indicators, we determined that the rectal fluid is always acidic, even in larvae living in water of pH of 11. This was very surprising, as our initial hypothesis, based on information in the primary literature, was that the rectal fluid would be highly alkaline because either the alimentary or excretory systems were involved in base elimination. Because the rectum is acidic, however, base excretion must occur elsewhere. The most likely location would be the anal papillae. We also found that a pH indicator will accumulate in the anal papillae of larvae in acidic water. When these larvae were then transferred to alkaline water, the indicator remains in the anal papillae but very rapidly changes color, demonstrating that the pH within the anal papillae changes very rapidly in response to changing environmental pH. In contrast, the hemolymph is known to remain relatively constant (Clark et al. 2004). Although these data are difficult to interpret, they are consistent with stimulation of base excretion by anal papillae.

In addition to these accomplishments, I also initiated studies in collaboration with Dr. Jim McLister to determine the metabolic costs of pH regulation. These studies, still underway, have established the techniques to measure oxygen consumption of larvae in waters of different pH, and show that pH regulation does have a metabolic cost. These studies, when complete, should complement very nicely the changes in mitochondrial densities and localization within various tissues, described above.

Finally, I worked with Dr. Robert Pope to develop techniques to assay carbonic anhydrase activity in situ. This enzyme is expected to be involved in pH regulation. We are interested in determining whether activities of this enzyme change in a pattern similar to changes in mitochondrial densities. We established the techniques, but we have not yet collected the data.

A handwritten signature in black ink that reads "Thomas M. Clark". The signature is written in a cursive style with a vertical red line to its right.