Brief project overview/abstract:
The hemolymph of the American horseshoe crab, *Limulus polyphemus*, is harvested to produce Limulus Ameboocyte Lysate, used to detect bacterial contamination in pharmaceuticals, vaccines, and medical devices. As the bleeding process ends with the release of the live animal, the fishery has been classified as “low-impact,” subject to minimal regulation. However, whether the process induces sub-lethal behavioral and physiological changes is unknown. We examined the effects of the bleeding procedure on mortality, behavior, hemocyanin concentration, and heart rate in male and female horseshoe crabs. We monitored these parameters before and after the bleeding process in two laboratory-based groups of males, three laboratory-based groups of females, and one outdoor group of females; these groups were segregated by the data collection system (laboratory- running wheels and Ethovision; outdoor- accelerometers). Activity of females in both the laboratory and outdoor groups significantly decreased during the second week after bleeding. In contrast, activity of males in only one group (Ethovision) decreased during week three after the bleeding process. In both males and females, angular and linear velocity decreased during the week preceding the activity decrease. The percent decrease in hemocyanin concentration in bled females significantly exceeded that of control females in each experimental group. Heart rate of males significantly decreased during post-bleeding weeks 2-3, an effect not observed in the females. These behavioral and physiological changes suggest that the bleeding process confers sub-lethal effects; these effects may impact the fitness of horseshoe crabs in the wild, with potential population-level consequences.
Objectives:
The principle aims of this project were to determine whether the biomedical bleeding process causes sublethal behavioral and physiological changes in female American horseshoe crabs, and to compare the effects on females to effects that we had previously found for male horseshoe crabs. Specifically, we measured activity (percent of time active, total distance moved, and linear and angular velocity), heart rate, and hemocyanin concentration in female horseshoe crabs for two weeks before and four weeks after the biomedical process.

Project findings/progress to date:
We found that female horseshoe crabs exhibited significantly reduced overall activity during the second week after the bleeding process, reduced linear and angular velocity during the first week after the bleeding process, and sustained declines in hemocyanin concentration six weeks after the bleeding process.

Accomplishments:

In this study we documented significant changes in behavior in animals that were exposed to “biomedical bleeding” (activity decreases and orientation changes). These behavioral changes, while apparently temporary, may lead to decreased spawning rates in Limulus and, since the majority of the biomedical harvest occurs during the spawning season, biomedical bleeding could be a factor underlying current decreases in some populations of Limulus. Managers of Limulus populations that are in decline should consider restricting the magnitude of the harvest during the spawning season.

Students Supported (see next page)
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<tr>
<th>Student Name</th>
<th>Where is he/she now?</th>
<th>Institution/Department</th>
<th>Duration of support</th>
<th>Type of support (stipend, travel, supplies, etc.)</th>
<th>Type of degree: Undergrad Master’s PhD</th>
<th>Year degree awarded</th>
<th>Title of thesis if supported by N.H. Sea Grant</th>
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<td>Rebecca Anderson</td>
<td>Plymouth State University</td>
<td>Biological Sciences</td>
<td>Stipend, supplies</td>
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