

**SUB-LETHAL BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF THE
BIOMEDICAL BLEEDING PROCESS ON FEMALE AMERICAN HORSESHOE
CRABS, *LIMULUS POLYPHEMUS***

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The hemolymph of the American horseshoe crab, *Limulus polyphemus*, is harvested to produce Limulus Amebocyte Lysate (LAL), a reagent used to detect bacterial contamination in pharmaceuticals, vaccines, and medical devices. As the harvest process entails the release of live animals, the biomedical fishery has been classified as low-impact and is under minimal regulation, with a continuous increase in harvest magnitude since 2004. Females constitute over 80% of this harvest, and population trends from heavily-harvested regions suggest that the process may negatively affect female behavior and vitality. Though mortality rates (10-30%) in response to the hemolymph extraction process have been examined, whether the process imparts sub-lethal behavioral and physiological alterations in female *L. polyphemus* is unknown. To address this question, we examined the effects of the harvest procedure on mortality, behavior, and hemocyanin concentration in three laboratory-based groups and one outdoor group of female horseshoe crabs. There were several significant effects of this procedure. During the first week after the bleeding process, linear velocity decreased, and, during the second week after the bleeding process, overall activity decreased; bled animals also reduced expression of circatidal behavioral rhythms during the second week post-bleeding. Six weeks after the bleeding process, the percent of original hemocyanin concentration remaining in bled animals was significantly less than that of control animals in three of the four groups. These behavioral and physiological changes suggest that the bleeding process confers sub-lethal effects; these effects may impact the fitness of female horseshoe crabs in the wild, with potential population-level consequences.