

HORSESHOE CRAB (*Limulus polyphemus*) HEMOLYMPH BIOCHEMICAL AND IMMUNOLOGICAL PARAMETERS

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ABSTRACT

The horseshoe crab (*Limulus polyphemus*) is one of four extant species remaining worldwide, and can be found along the western Atlantic coast from Maine to the Gulf of Mexico.⁶ Demand for this species of horseshoe crab continues to grow. Horseshoe crab eggs are an important protein source to the successful spring migration of at least eleven species of migratory shorebirds, a component of the horseshoe crab's hemolymph (Limulus Amoebocyte Lysate or LAL) is used worldwide to detect the presence of endotoxins in injectable drugs and implantable medical devices^{4,5}, and horseshoe crabs are also used as bait for a growing eel and conch fisheries along the east coast of the United States.⁷ Thus, horseshoe crabs are an essential component of a healthy coastal ecosystem, an essential contributor to human health, and an integral part of the coastal economies of the eastern United States. Unfortunately, the status of wild horseshoe crab populations may be in jeopardy due to overharvesting, environmental degradation and habitat destruction^{1,2,3}.

While the horseshoe crab has existed in its present form for over 350 million years, very little is known about the basic biology of this organism. While the characteristics of the crab's "blue blood" and the composition of LAL have been studied in detail, basic information on the horseshoe crab's life cycle, dietary requirements, and physiological parameters are completely lacking. To better evaluate the health of wild populations of horseshoe crabs, biochemical parameters of the horseshoe crab's hemolymph were determined from a collection of fifty wild adults (29 male and 21 female). The horseshoe crab values seemed to parallel many other aquatic marine species that fluctuate their osmolality and other hemolymph parameters according to their external environment, developmental stage, and reproductive cycle. However, several biochemical parameters were also notably different from ambient seawater. Results of the biochemistry parameters (mean values) for the hemolymph of the horseshoe crab were: total protein (8.15 g/dl), glucose (58.5 mg/dl), creatinine (0.7 mg/dl), cholesterol (0.8 mg/dl), sodium (389.5 mEq/l), potassium (12.5 mEq/l), chloride (445.1 mEq/l), calcium (39.0 mg/dl), magnesium (96.1 mg/dl), phosphorus (3.4 mg/dl), triglycerides (5.3 mg/dl), amylase (9.3 U/l), lipase (32.7 U/l), alkaline phosphatase (12.1 U/l), aspartate aminotransferase (5.4 U/l) and gamma glutamyl transferase (0.92 U/l).

In addition, the relative position of horseshoe crabs in the Phylum Arthropoda has been the subject of much debate, specifically their systematic proximity to crustaceans and arachnids. Using SDS-PAGE, Western blotting and agarose gel immunoprecipitation techniques, hemolymph proteins were compared to evaluate the hypothesis that horseshoe crabs are more closely related to representatives of arachnids than crustaceans. Hemolymph proteins were compared between a horseshoe crab (*L. polyphemus*); representatives of crustaceans: the American lobster (*Homarus americanus*) and blue crab (*Callinectes sapidus*); insects: the Madagascar hissing cockroach (*Gromphadorina portentosa*); and arachnids: the emperor scorpion (*Pandinus imperator*) and the Mexican red-legged tarantula (*Bracypelma emilia*). Our immunological results supports the conclusion that present-day horseshoe crabs are more closely related to arachnids than crustaceans and insects.

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