

Editorial

Horseshoe crab behavior: Patterns and processes

Christopher C. Chabot, Guest Editor, Plymouth State University, Plymouth, NH, USA, chrisc@plymouth.edu
Winsor H. Watson III, Guest Editor, University of New Hampshire, Durham, NH, USA, win@unh.edu

1 Introduction

The American horseshoe crab *Limulus polyphemus* has long served as a source of delight and inspiration to a broad cross-section of scientists - from naturalists to neuroscientists. Periodically, for the last hundred years or so, new discoveries have been made about this ancient creature that both enlighten scientists and reinforce the importance of conserving this ancient species.

Largely as the result of their annual migration to spawn each spring and early summer, their mating behavior has long been the subject of many publications, ranging from children's books to articles in scientific journals. Then, in the middle of the twentieth century, as scientists were beginning to turn to animal model systems to study fundamental processes underlying animal behavior, H. Keffer Hartline and his colleagues took advantage of the unique design of the *Limulus* compound eye to elucidate many key principles of visual processing (earning him the Nobel Prize in 1967). Subsequently, Bob Barlow (to whom this volume is dedicated) and his colleagues demonstrated that the brain, via efferent nerves that were driven by a circadian clock, modulated the sensitivity of these same compound eyes at night. But horseshoe crabs were not just contributing new information to neuroscientists. Also at the Marine Biological Laboratory, Drs. Bang and Levin were investigating another unique and interesting aspect of horseshoe crabs, their blue blood, which contains molecules that are exquisitely sensitive to endotoxins that occasionally contaminate stored human blood. This work demonstrated the value of this species as a source of natural products for the biomedical industry. Most recently, it has become apparent that horseshoe crabs are part of an important food web, with their eggs serving as a key source of nutrition for migrating birds. This finding has renewed interest in the behavior and ecology of this species and has mobilized efforts to preserve this vital natural resource.

2 Key Questions about Horseshoe Crab Behavior

Despite the long history of investigations into the behavior of horseshoe crabs many important questions remain. In general, these questions fall into two categories: First, we still know little about their normal behavior in their natural habitat, because, with the exception of the mating season, they are difficult to observe underwater. Second, there is much to learn about the neural mechanisms underlying these behaviors.

2.1 Horseshoe crab behavior in their natural habitat

Horseshoe crab mating behavior is dramatic and simple to observe. As a result, much has been written on this subject. Nevertheless, many important questions continue to be pursued and many of these are addressed in this special volume. 1) What mechanisms give rise to the male skewed sex ratio documented on many spawning beaches? Is it a function of the natural sex ratio, or an emergent property resulting from behavioral differences between males and females? 2) How do males locate females and which males are most successful? Is vision sufficient or could some type of chemical communication also be involved? 3) How do the horseshoe crabs that always mate at high tide in the spring and early summer know that the tide is high? 4) Do horseshoe crabs, like some turtles, return to the same beach to mate year after year and, if so, how do they locate the beach?

After the mating season horseshoe crabs return to deeper water where they are difficult to observe, and thus a number of very fundamental questions continue to exist about their activities during most of the year. 1) Do they remain resident within bays and estuaries or do they move offshore into deeper water in the fall and winter? 2) What is the relationship between areas where they breed and locations where they spend the rest of the year? 3) What do they eat and what is their typical foraging behavior? 4) Do they continue to express a tidal rhythm of locomotion during the remainder of the

year, like they do during the mating season?

2.2 Neural mechanisms underlying horseshoe crab behavior

While much has been learned about the physiological basis of behavior in *Limulus*, there are, of course, many unanswered questions, including several that are raised by the authors in this Special Issue: 1) Where are the biological clocks that control eye sensitivity and locomotion and are the molecular mechanisms used by these clocks similar to those in other species? 2) What hormones or neurohormones are involved in linking the output of their biological clocks to changes in visual sensitivity and locomotion? 3) How are the rhythmic movements of the gills, legs, and heart driven and coordinated during walking, eating, breathing and swimming? 4) What environmental cues do horseshoe crabs use to modulate their behavior on a seasonal basis? 5) If horseshoe crabs can synchronize their activity to the tides, how do they sense the tides?

These, and other questions, are all addressed in this volume, even though answers to all of them have not been attained.

3 Contributions to the Special Issue

The twelve papers in this volume present significant new data along with thorough reviews of our current understanding of a broad cross-section of *Limulus* behaviors. First, there are several papers that address issues of migratory movements during breeding and non-breeding seasons. Smith et al., using radio transmitters affixed to freely moving crabs in a very large “open” estuary (Delaware Bay), found that crabs rarely return to previously visited beaches to spawn in subsequent years and that stranding mortality is correlated with age and epibiont growth. James-Pirri, and Schaller et al., using underwater acoustic tags in smaller enclosed bays, found that horseshoe crabs in New England bays express seasonal movements of ~ 2–6 km/year and rarely, if ever, move out of the bays. Animals in the Schaller et al. study appeared to generally move up into the bay and into shallower water in the spring and summer and down-estuary towards the ocean and into deeper water, during the winter; findings that were consistent with the James-Pirri study. These results were corroborated and extended by Watson and Chabot who carried out a higher resolution analysis of the patterns of activity exhibited by horseshoe crabs. This study showed that during their high activity months (April–October) animals were more active during high tides

than low and during the day versus the night. Further, both males and females approached mating beaches during only 33% of the high tides they experienced. Lee provides some complementary data showing that horseshoe crabs appear to be actively foraging and digging pits during high tides on submerged tidal flats during much of the summer and fall. Thus, the tidal rhythms of activity documented by Watson and Chabot are likely to be the result of these foraging activities.

Several papers in this issue address questions related to horseshoe crab mating behavior and the behavior of juveniles and larvae. For example, the issue of how horseshoe crabs locate potential mates is addressed by Saunders et al., who show that male horseshoe crabs use both chemical and visual information to find potential mates. The use of chemical and visual cues in juvenile and larval horseshoe crabs is also assessed by Medina and Tankersley. They show that visual orientation of larval and juvenile horseshoe crabs changes upon exposure to habitat and predator cues and that the direction of the response undergoes an ontogenetic shift following metamorphosis. Mattei et al. show that reproductive tactics are influenced by population density and that single males may be at a selective disadvantage in populations with low densities and highly variable operational sex ratios. Further, in at least in some populations with a low density, horseshoe crabs may have difficulty locating mates.

The underlying physiological mechanisms controlling horseshoe crab behavior, especially behavioral rhythms, are also explored in depth in this issue. Chabot and Watson summarize data showing that horseshoe crabs exhibit clear circatidal rhythms of locomotion that can be entrained by tidal cues. They also provide new evidence that these tidal rhythms may be driven by two endogenous clocks, each with a period of 24.8 h (termed circalunidian). When combined with field data that are presented in multiple papers in this issue, it is clear that these endogenous clocks help to synchronize breeding behavior during the breeding season as well as foraging behavior before and after this time of year. Horseshoe crabs also express daily rhythms of behavior and a circadian rhythm of visual sensitivity. Dalal and Battelle provide new evidence about the potential mechanisms underlying this change in visual sensitivity. They show that octopamine regulates gene expression in *Limulus* lateral eyes by activating a cAMP cascade. Octopamine also modulates the expression of certain rhythmic behaviors expressed by horseshoe

crabs, such as feeding. Wyse summarizes these data as well as the neural control of ventilation, gill cleaning, and locomotion.

Another important thread that connects many of the papers in this issue, including most of those cited above, is the effect of external factors on behavior and physiology. Botton et al. shows that development in horseshoe crabs is highly plastic and is influenced by several factors, including temperature, salinity, dissolved oxygen, and the presence of pollutants. The high tolerances of horseshoe crab embryos and larvae to changes in these environmental factors may help to explain the current geographic distribution and their evolutionary persistence. Botton et al. also review the early developmental stages of horseshoe crabs as well the ecological importance of their eggs. Lastly, they describe the behavior and dispersal of the larvae and note that they exhibit circatidal rhythms of swimming that likely enable them to emerge from their nests.

4 This Special Issue is Dedicated to Dr. Robert Barlow

The atmosphere in Woods Hole, Massachusetts in the 1970's was an intoxicating mix of brilliant neuroscientists eagerly squeezing every bit of data out of their marine species of choice, hardworking "townies" living life to the fullest, and relaxed people from all over the world passing through on their way to Martha's Vineyard or Nantucket for a vacation. For one of us (WHW), and many other young scientists, there was no better place on the planet. Each day we mixed experiments, stimulating lectures, and occasional dips in the ocean. Each day we witnessed, first hand, the depth and breadth of neuroscience and behavior, ranging from cutting edge studies of voltage sensitive channels to the link between synaptic plasticity and slug learning. Somehow, I wanted to make all of this part of my own career. But, unfortunately, I just could not see how it was possible; until I met Bob Barlow.

Bob had it all. He was a brilliant scientist, he had a wonderful family that was integrated into the fabric of life in Woods Hole, he was an active swimmer and SCUBA diver, and he had an aura and charisma that was special. Most importantly, for me, he found a way to combine his interest in the basic neural mechanisms underlying vision, with his desire to understand the role that vision played in the normal behavior of horseshoe

crabs in their natural habitat. This period of time was the pinnacle of the growth of the field of Neuroscience and, while everyone talked about the importance of linking neural mechanisms to behavior, few people, on their own, were able to make this leap. Bob Barlow was one of these special people.

One of the great things about Woods Hole was that, no matter how famous people were, it seemed like we were all one big family. So, it was not uncommon to have a casual chat with a Nobel Prize winner at the Fish Monger or the Captain Kidd or the Woods Hole Beach. I can still remember being at a small gathering in a house right on Eel Pond in the center of town, when I started up a conversation with Bob. It kept building in intensity because my enthusiasm to pick his brain was equaled by his excitement about his recent discovery of efferent axons emanating from the brain of horseshoe crabs that modulated the sensitivity of their eyes at night. Finally, much to my surprise, he asked me and a few other graduate students, if we wanted to see one of his experiments live! Needless to say, we jumped at the opportunity and the next thing we knew we were listening to the sound of spikes on the loudspeaker while we watched electroretinograms drift past us on the oscilloscope. How could you not want to have a career in Neuroscience after an experience like that?

Over the next few summers I became increasingly impressed with Bob and his efforts to extend his work into the lives of horseshoe crabs in their natural habitat. I watched him SCUBA dive to observe animals at night, I worked with one of his colleagues, Len Ireland, on some early acoustic telemetry experiments, and I walked the beach with his students at Mashnee Dike, observing mating horseshoe crabs by the light of the full moon. These were just the types of things I wanted to do with my own scientific career and Bob was demonstrating to me, as a perfect role model, that they were all possible and that this multidisciplinary approach to Animal Behavior was the most rewarding path. I'm sure many of the authors represented in this special issue on *Limulus* Behavior have also been inspired, directly or indirectly, by Bob Barlow and his research. He demonstrated first-hand the benefits of searching for an understanding of both the behaviors expressed by animals in their natural habitat, and the mechanisms underlying those behaviors. We will all miss Bob, but his impact on most of us will live on for many years to come.