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The Ability of Horseshoe Crabs (Limulus polyphemus) To Detect Changes in Temperature

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Abstract

Previous studies have suggested that horseshoe crabs prefer warm water, suggesting that they may be able to detect changes in water temperature. The overall goal of this study was to test this hypothesis. Our specific objectives were to: 1) find out if horseshoe crabs can detect temperature changes; 2) determine the magnitude of temperature change they can detect, and; 3) determine whether their temperature receptors are located internally or externally. Animals were placed in a light-tight chamber that received a constant flow of cooled seawater. Their heart rates were continuously recorded and a change in heart rate following the addition of warmer water was used as an indicator that they sensed the change in temperature. The results showed that 50% of horseshoe crabs responded to a temperature change of 1°C, while 100% responded to a temperature change of 2.6°C. Over half of the horseshoe crabs also responded to a rate of temperature change of less than 1.5°C. Both of these results indicate that horseshoe crabs can, indeed, sense temperature changes. Also, the horseshoe crabs typically showed a response before their internal temperature changed, indicating that their temperature receptors are most likely located externally.

Introduction

Horseshoe crabs are a valuable species for many reasons. They are used in the fishing industry as bait and in the biomedical industry to make *Limulus* Amebocyte Lysate (LAL), which is used to test for bacterial contamination of biomedical products. In addition to this, they are important members of the marine ecosystem and their eggs provide nutrition for migrating birds (Castro & Myers, 1993; James-Pirri, 2010; Schaller, *et al.*, 2010).

Horseshoe crabs are known to migrate from colder, deeper waters into warmer, shallower waters and beaches for spawning. (Watson & Chabot, 2010; Schaller, *et al.*, 2010; Cheng *et al*., 2015a; Cheng *et al*., 2015b). Preliminary studies indicate that horseshoe crabs prefer warmer waters when given the choice (Cheng, 2014). The previous studies that suggest that horseshoe crabs have the ability to recognize temperature changes motivated us to test this hypothesis.

Our experiment had three main objectives. First, we wanted to determine if horseshoe crabs are able to detect changes in temperature. Second, if they are able to detect changes in temperature, we wanted to determine the threshold, or magnitude, of temperature change that they can detect. And lastly, we wanted to know if their possible temperature sensing receptors are located externally or internally.

Materials and Methods

A group of 4-6 different horseshoe crabs were used for the trials. A total of 17 trials were performed for the study, with 3 trials using female horseshoe crabs and 14 trials using male horseshoe crabs. More male horseshoe crabs were used due to their smaller size that enabled them to fit into the experimental chamber better.

A cardiac assay, similar to the one used by Dufort *et al*. (2001) and Jury and Watson (2000), was used to determine if animals could detect a change in water temperature. The cardiac activity of each *Limulus* was recorded by attaching electrodes bilaterally near the heart (Figure 1) and then connecting them to an impedance converter and an AD Instruments analog to digital converter. All data were recorded using LabChart software.

Internal and external temperature probes were not only used to determine temperature changes in the experimental chamber, but also to try to determine if the horseshoe crabs' temperature receptors are located internally or externally. A response before the internal temperature changed would indicate that the temperature receptors of horseshoe crabs are located externally. On the contrary, if the horseshoe crab responded after its internal temperature changed, it would suggest either that the receptors are located internally or that the response was only due to the physiological response to a higher temperature that has been noted in previous studies (DeFur & Mangum, 1975).

The internal temperature probe was placed inside the body cavity of the horseshoe crab (Figure 1). The external temperature probe was placed in the experimental chamber next to the horseshoe crab. Both temperature probes were connected to a Vernier datalogger and the temperature changes were recorded using PowerLab software.

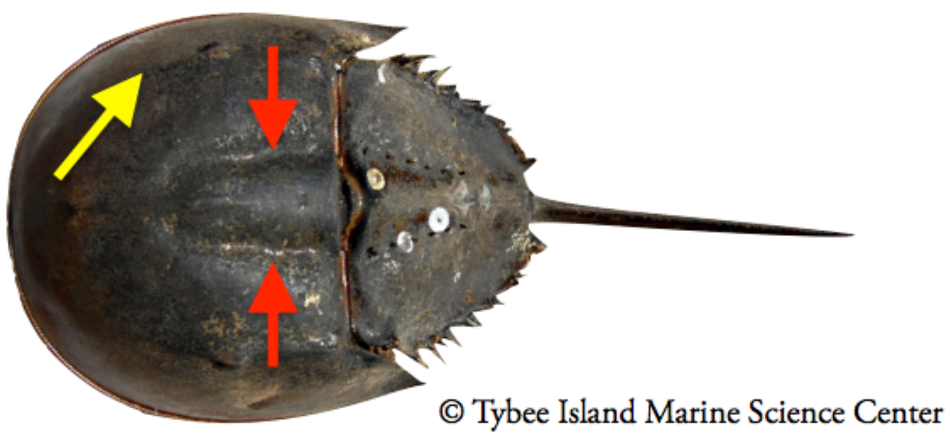

Fig. 1: Location of the electrodes and the internal temperature probe. The two electrodes were located on either side of the heart, indicated by the red arrows. An internal temperature probe was placed within the body cavity, indicated by the yellow arrow.

The trials were conducted in an experimental chamber, where the horseshoe crab was allowed to settle for at least 2 hours before beginning the trial. The experimental chamber was kept dark during the trials to eliminate the horseshoe crab's reaction to light or other external disturbances. To eliminate disturbances in the cardiac rhythm due to the movements of the horseshoe crabs, brick weights were used to immobilize them while they were in the experimental chamber. The horseshoe crabs were allowed at least 40 minutes in between individual trials to recover before any subsequent temperature trial. Overnight the horseshoe crabs were allowed to move freely in a larger tank.

Cold (~12°C) water was continuously pumped from a saltwater aquarium into the experimental chamber, and then allowed to circulate back into the saltwater aquarium through an overflow pipe. To change the water temperature for the trials the pump was moved into a separate container filled with either the same cold seawater (for controls) or a container with hot seawater (Figure 2).

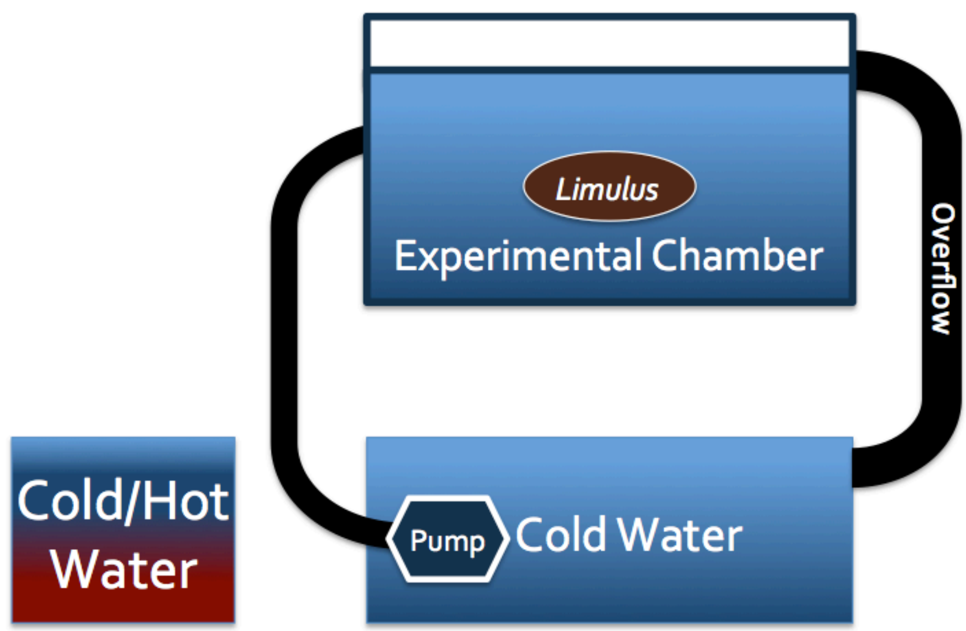

Fig. 2: Experimental setup for determining if horseshoe crabs could detect changes in water temperature. Note that the Experimental Chamber was light-tight so that animals would not react to movements and other external disturbances.

The cardiac assay was used to identify any disruptions in the heart rate of the horseshoe crab during the trials. A prolonged (>35 s) disruption in the heart rate after a stimulus was applied was used as an indication that the subjects detected a change in water temperature.

Results

A typical cardiac response to a change in water temperature is shown in Figure 3. When at rest in the experimental chamber the horseshoe crab heartbeat is very consistent. However, when warm water is added, there was a brief slowing of the heart (bradycardia) followed by an increase in heart rate. This very rapid response indicates that they are capable of sensing the change in temperature.

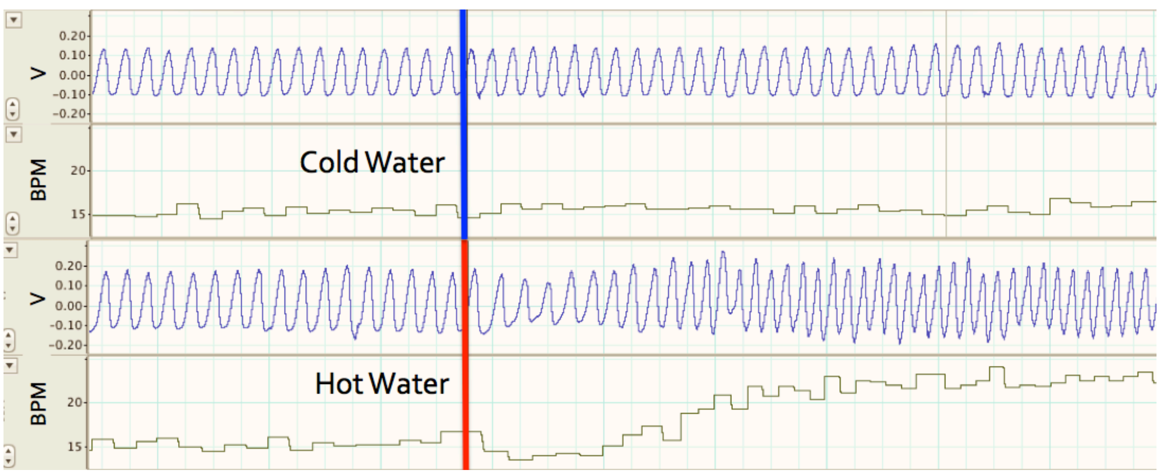

Fig. 3: Sample record showing the cardiac response of a horseshoe crab to a control exposure to water of the same temperature (top), and then to warm water (bottom). The stimuli were applied at the blue or red lines. Note that the heart rate stayed consistent throughout the control. However, as soon as they detected an increase in water temperature, there was an initial decrease in heart rate, followed by a prolonged increase.

Figure 4 below illustrates the response to a trial in accordance with the internal and external temperature changes. Hot water was applied at the time indicated by the red arrow. As the figure shows, the heart rate was fairly consistent before the application of hot water, but significantly increased after the application of hot water. Both the internal and external temperatures increased very slowly in comparison to the heart rate.

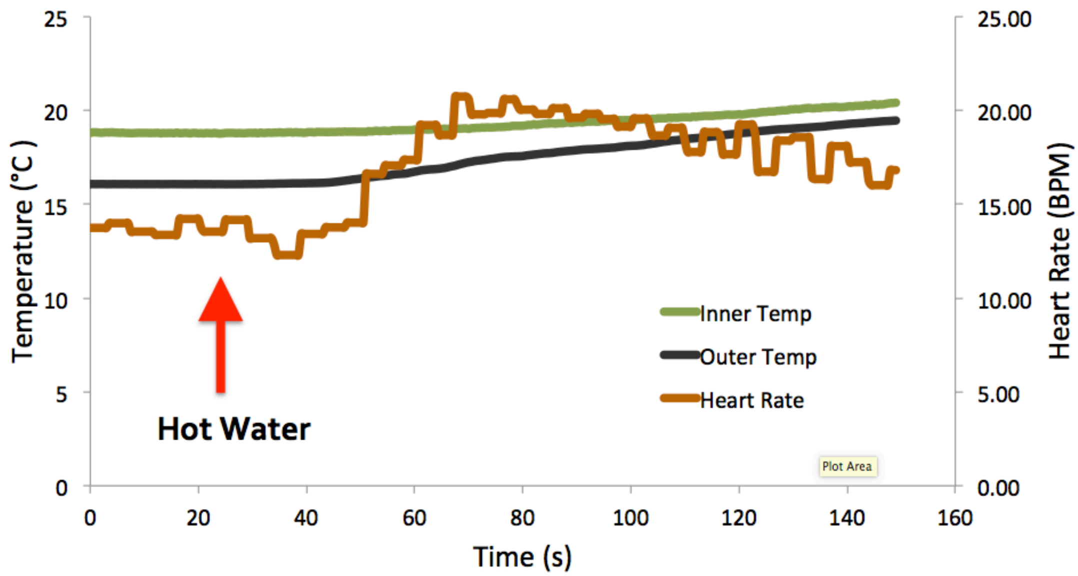

Fig. 4: Changes in heart rate, internal temperature and external temperature in response to application of hot water to the experimental chamber. Note that the horseshoe crab responded before there was a change in internal temperature.

Figure 5 below shows the percentage of horseshoe crabs responding to a certain temperature threshold. The curve representing the percentage of horseshoe crabs responding showed a sudden increase until a threshold of about 0.5°C and then slowly leveled off. All horseshoe crabs responded to a temperature change of 2.6°C, while approximately half of the horseshoe crabs responded to a temperature change of 1°C.

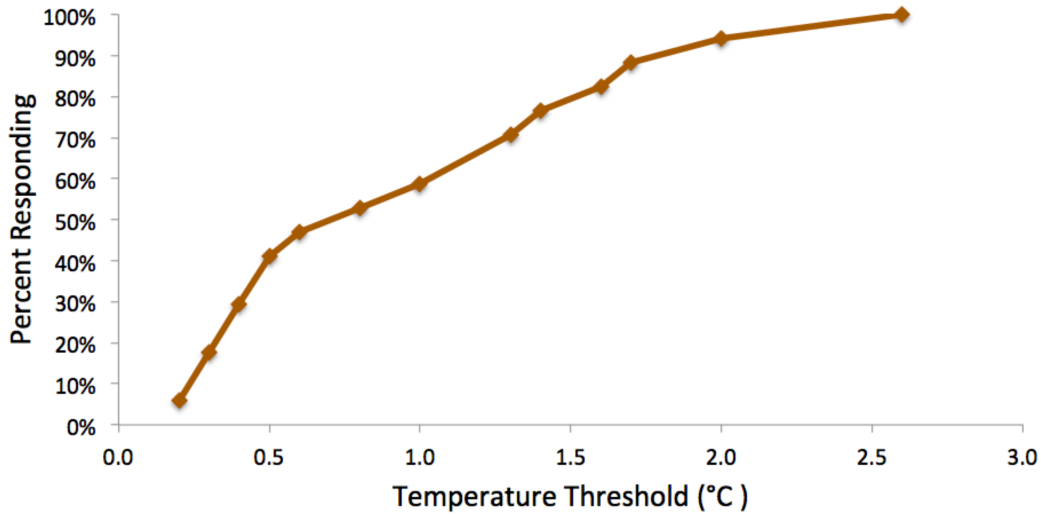

Fig. 5: The percentage of horseshoe crabs responding to different external temperature thresholds.

The response time of each horseshoe crab is illustrated in Figure 6 below. Out of the 17 horseshoe crab trials, 9 horseshoe crabs responded to a rate of temperature change below 1.5°C/min. The remaining 8 horseshoe crabs responded to a rate of change of at least 6°C/min. Overall, the curve showed a decreasing linear trend. A cluster of responses was recorded at a very low rate of temperature change. The remaining responses were scattered over a larger range of rates of temperature change.

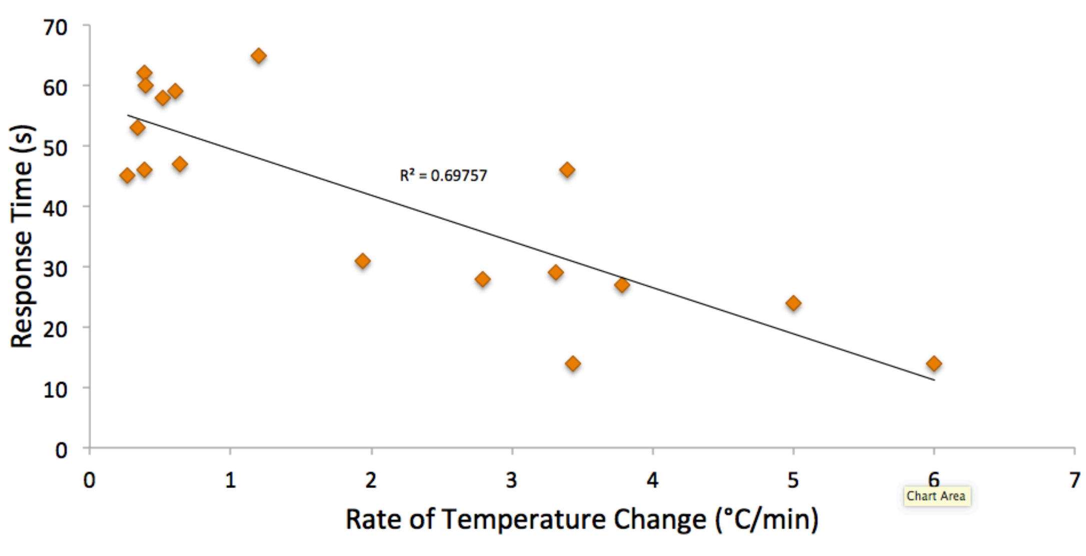

Fig. 6: The relationship between the time it took for animals to respond and the rate of external temperature change. Overall, the response time decreased as the rate of temperature change increased.

Discussion

The results show that horseshoe crabs are not only able to detect temperature change, but they also seem to be very sensitive to it. Figure 5 supports this conclusion, since all of the horseshoe crabs responded to a temperature threshold of only 2.6°C or below, while approximately half responded to a temperature change of only 1°C. This finding is consistent with previous studies performed on other aquatic invertebrates, such as the lobster (*Homarus americanus*), which are estimated to detect temperature changes of 0.15°C and above (Jury & Watson, 2000).

Figure 4 shows a response in the horseshoe crabs before the internal *or* external temperatures change. This could be due to the fact that the input water flowed directly into the horseshoe crabs while the external temperature probe was place further back in the experimental chamber. Even so, it shows that the horseshoe crabs can detect a sudden change in temperature, and suggests that their thermoreceptors are located externally. Sensory receptors of lobsters have been identified to be located on their claws and walking legs (Derby, 1982), so perhaps the same is true for horseshoe crabs. Future studies shall be conducted to determine the exact location of thermoreceptors of horseshoe crabs.

The results also showed that horseshoe crabs' sensitivity to temperature seems to have an inverse relationship with the rate of temperature change. This corresponds with previous studies conducted on the effect of temperature change on survival rates of marine invertebrates (McLeese, 1956). It appears that the higher the rate of temperature change, the more urgently the horseshoe crab will try to adapt to the change.

Conclusions

We concluded in this study that horseshoe crabs can, indeed, detect changes in water temperature. The threshold, or magnitude, of temperature change that we concluded they can detect is ≤ 2.6°C. Due to the fact that the horseshoe crabs responded before a change in internal body temperature had occurred, we concluded that their temperature receptors are most likely located externally.

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