Article

The potential functions of mechanoreceptors found on trilobite larva of *Limulus polyphemus* (Linnaeus, 1758)

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Abstract

In this study trilobite larvae and early juvenile stages of *Limulus polyphemus* (Linnaeus, 1758) are examined using light microscopy and scanning electron microscopy to examine the mechanoreceptors present on the cuticle. Three forms of mechanoreceptors were described on the trilobite larvae of L. polyphemus, (1) tactile sensilla, (2) peg sensilla and (3) helical sensilla. Tactile sensilla are adapted to detect touch. Peg sensilla are adapted to detect water currents and near field sound. The helical sensilla are new to science and their function is not known. The genus Limulus Müller 1785 has a fossil record from the Jurassic era, 148 million years. Before the Common Era (BCE) to the present. Having survived through the mass extinction at the end of the Mesozoic era, it is referred to as a "Stabilomorph." As a Stabilomorph the morphology of mechanoreceptors, specifically sensilla capable of sensing particle motions, found on trilobite larva of L. polyphemus may shed some light on the mechanisms of near field sound or particle motion detection in Jurassic seas. To detect particle motion, near-field sound energy, the sensory organ must extend through the boundary layer. The thickness of the boundary layer (δ) for particle motion in water is equal to [kinematic viscosity/sound wave frequency]^{1/2}, less than 200 μ m in the range of human hearing. The value of δ in air is 2.54 [kinematic viscosity/sound wave frequency]^{1/2}, about 1 to 2 millimeters in the range of human hearing. Therefore, the mechanoreceptors active in the detection of particle motion are typically shorter in aquatic animals compared to terrestrial animals. Near field sound energy is the dominant sound energy at a distance less than or equal to the wavelength.

Keywords horseshoe crabs; marine chelicerates; stabilomorphs; sensilla; Limulus polyphemus.

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1 Introduction

The horseshoe crab *Limulus polyphemus* (Linnaeus, 1758) is often referred to as a "living fossil." The genus *Limulus* Müller 1785 has been present in the fossil record for 142 million years with no notable change in its external morphology (Dunlop, 2012; Kin and Błażejowski, 2014; Błażejowski, 2015). The term

"Stabilomorph" has been used for taxa that have survived relatively unchanged morphologically through one or more mass extinction events, for which *L. polyphemus* qualifies (Kin and Błażejowski, 2014). The species represents a potential window into life in the distant past. This study focuses on the mechanoreceptor organs in the trilobite larva of *L. polyphemus*.

Horseshoe crabs lay their eggs in the beach sand near the spring high tide level. The developing animal undergoes four molts before hatching from the egg. When the eggs hatch, at a subsequent spring tide, the trilobite larva (Fig. 1) crawl to the surface and move down the beach to the ocean. In some cases, the larva overwinters in the interstitial fluid on the beach and enters the ocean in the spring of the following year (Botton, et al., 1992; Botton, 2010). The first instar after hatching is called the trilobite larva because of its resemblance to the members of the Class Trilobita, which became extinct at the end of the Paleozoic Era.

Three forms of mechanoreceptors were described on the trilobite larvae of *L. polyphemus*, (1) tactile sensilla, (2) peg sensilla and (3) helical sensilla (Fornshell, 2021). Tactile sensilla were found along the frontal and lateral margins of the carapace of the prosoma (Fig. 2). The tactile sensilla were 80 μ m long and a maximum of 5 μ m in diameter at their base. Peg sensilla 30 μ m to 100 μ m long with a diameter of 1 μ m to 2 μ m arising from a socket of about 5 μ m in diameter (Fig. 3) were found on the dorsal surface of the carapace near the lateral ridge and compound eye.



Fig. 1 Ventral and dorsal views of the trilobite larvae of *Limulus polyphemus*.

Peg sensilla like those found on the dorsal carapace of the trilobite larva are adapted to sense water currents and near-field sound energy in the form of particle movements (Dohrn, 1881; Helfer and Schlottke, 1935; Fornshell and Harlow, 2018; Fornshell, 2019, 2021). Such mechanoreceptors have been shown to be capable of functioning in near-field sound communication between animals (Santer and Hebets, 2008). Kaplan et al. (1976) established the presence of mechanoreceptors on the dorsal carapace of *Limulus* with a morphology and innervation pattern comparable to those found on crustaceans. Sensilla with a helical form were present on the pedipalps, second and third walking legs. and pusher on the ventral side of the prosoma of trilobite larva. Some of these helical sensilla form a double helix structure by twisting around adjacent sensilla (Fig. 4). The helical

sensilla arose from a socket about 10 μ m in diameter and were 123 μ m to 170 μ m long and 5 μ m in dimeter at their base. The length of a single helix varied from 17 μ m to 55 μ m (Fornshell, 2021).

2 Materials and Methods

Fifty trilobite larvae of *L. polyphemus* archived in the collections of the Natural History Museum originally fixed in 5% formaldehyde at the time of collection and stored in 70% ethyl alcohol were progressively dehydrated in 75%, 90%, and 100% ethyl alcohol solutions (Fornshell, 2021). After being critical-point dried, the larvae were coated with gold palladium alloy (Fornshell, 2021). The images were produced using a Zeiss EVO MA15 Electron Microscope (Zeiss, Tokyo, Japan). All measurements were made from the electron micrographs (Fornshell, 2021).



Fig. 2 The ventral margin of the carapace of the prosoma showing the tactile sensilla.

3 Results and Discussion

Kin and Błażejowski (2014) Described *Limulus darwini* from well-preserved three-dimensional fossils found in deposits in the coastal sediments of the Kcynia Formation (Upper Jurassic @ 148 million years ago) in central Poland. While they did not have a single complete specimen, they did have several fragments from which a complete animal could be reconstructed. There are no identifiable morphological differences in *L. darwini* and *L. polyphemus*. Both animals are members of the near shore benthos. Their physical environment has not altered greatly, but due to the mass extinction at the end of the Mesozoic, their biological environment must be significantly different. The morphology of the mechanoreceptors may also have undergone little or no changes as well. Because they are separated in time by at least 148 million years, however, it is generally believed that their DNA would have changed enough to make them distinct species (Lidgard and Love, 2018).



Fig. 3 The carapace of the prosoma in the region of the right compound eye showing the sensilla adapted for detecting particle motion and water currents.

The helical sensilla are present on all specimens, the double helix form is not present in all specimens. Also, it is distinctly different from the peg sensilla which lack directionality. The functioning of mechanoreceptors found on trilobite larvae need to be investigated in laboratory experiments to establish the actual function of these sensilla. The helical sensilla are found in the trilobite larval stage but are absent in juvenile horseshoe crabs as small as 15 mm body length. In the juvenile the typical "Peg sensilla" are found (Fig. 7). This loss of morphological features found in the larval stages during the transition to juvenile stages is also observed in sea spiders, the only other extant marine chelicerate. The loss during larval development of plesiomorphic structures such as the palps and ovigerous appendages is found in several families of the Pycnogonida (Fornshell, 2014). The helical sensilla found in the trilobite larva of *L. polyphemus* may also be interpreted as plesiomorphic in the Limulidae. As such the helical sensilla are potentially vestigial mechanoreceptor organs.

We calculate a boundary layer thickness in water of 223μ m for sound energy at 0.02 kHz, 141 μ m at 0.05kHz, 100 μ m at 0.1 kHz, 44.7 μ m at 0.5kHz, 36.6 μ m at 1 kHz and 22.4 μ m at 2 kHz. In air the boundary layer thicknesses are many times thicker (See Figures. 5 and 6). The peg sensilla then, can detect sound energy from a source within a few meters and at frequencies above 0.5 kHz.

The sound energy emitted by a source has two components, Kinetic Energy (KE) in the form of particle motion and Potential Energy (PE) in the form of pressure waves (Popper and Hawkins, 2018). Near field sound energy, particle motion or KE, is the dominant sound energy at a distance less than or equal to the wavelength. At distances greater than the wavelength the energy in the pressure waves, PE, is dominant (Popper and Hawkins, 2018). To detect the KE of particle motion, near-field sound energy, the sensory organ must extend through the boundary layer. In air, the boundary layer is defined as $2.54 \times [kinematic viscosity/sound wave frequency]^{1/2}$ in air (Barth, 2004). The boundary layer thickness in water is decreased to a value of about [kinematic viscosity/sound wave frequency]^{1/2} (Fletcher, 1992; Devarakonda et al., 1996; Barth, 2004).



Fig. 4 The helical and double helix sensilla found on the walking legs two and three of the trilobite larva. The two helical sensilla descending from the top of the image are attached to walking leg two.

The peg sensilla and helical sensilla (Fig. 3, 4). on the trilobite larva of *L. polyphemus* are potentially active in detecting particle motions in the fluid environment of the animal. The physics of their interactions with the fluid environment are dominated by the principles of Stokes Flow, that is, the viscus forces are dominant (Fletcher, 1992). To detect particle motions, the sensory organ must extend through the boundary layer.

In water, then a sensilla could be expected to detect a sound source at 200 Hz at ca. 7 m from the source. sound source at 2 kHz would be detected ca. 75 cm from the source and a 10 kHz source would be detected ca. 15 cm from the source. The ability of these sense organs to detect a sound source is then relatively short in absolute terms, but large in terms of the size of the animal.

There is no evidence at this time as to what the helical sensilla are used for. The sensilla with a double helix form would permit the detection of particle motion and or water currents in a single plane perpendicular to the axis formed by their sockets. As such they would have a distinct directivity in their response to stimuli. This is distinctly different from the peg sensilla which have no directionality in their sensitivity to sound energy.



Frequency in Herts

Fig. 5 Showing the boundary layer δ in microns verses the frequency in Hertz in water.



Fig. 6 This graph shows the boundary layer δ in microns vs the frequency in Hertz in air.



Fig. 7 Showing the peg sensilla on the pusher or fourth walking leg of juvenile horseshoe crabs.

The peg sensilla and tactile sensilla are found in other major taxa in the phylum Arthropoda and are examples of primitive or pleiomorphic morphological traits. Given the antiquity of the genus *Limulus*, this indicates that in many ways the soundscape in coastal seas of the Jurassic era was not hugely different from the present-day conditions. The length of the double helix sensilla indicates that they can detect near field sounds at low frequencies, ≤ 0.05 kHz. Whereas the peg sensilla on the walking legs of juvenile horseshoe crabs can sense frequencies ≤ 0.02 kHz. The double helix and helix sensilla, if interpreted as vestigial organs, may also indicate and additional component in the sound scape in the Jurassic seas.

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