

**Abstract**

Horseshoe crabs have existed for 445 million years, yet in recent years, the survival of *L. polyphemus*, or the Atlantic horseshoe crab, has been threatened by overharvesting, bleeding by biomedical companies, and stranding. To better understand their decline, 116 dead specimens found on Wallops Island were examined to find trends in physical characteristics and potential causes of mortality. The data collected was compared to data from beaches in Delaware and Georgia. The results suggest that the Virginia and Delaware populations are genetically distinct, there may be a higher mortality rate in female horseshoe crabs, and telson breakage is not the major cause of death in this population. Mass horseshoe crab death events and their causes should continue to be investigated.

**Background**

Horseshoe crabs have survived five mass extinctions, and their impeccable ability to survive stems from their hardy anatomy and high adaptability. They have been known to be able to survive in extremely low levels of dissolved oxygen and can tolerate wide variance in salinity. Horseshoe crabs also have unique blood that coagulates upon contact with bacteria and builds an effective barrier for wounds. Besides their remarkableness as being essentially living fossils, horseshoe crabs are vital to the ecosystem because their eggs serve as food for migratory birds, and the abundance of these eggs affects site selection. The fact that these horseshoe crabs have survived for so long, but have now reached a vulnerable conservation status demonstrates the detrimental effects of human impact.

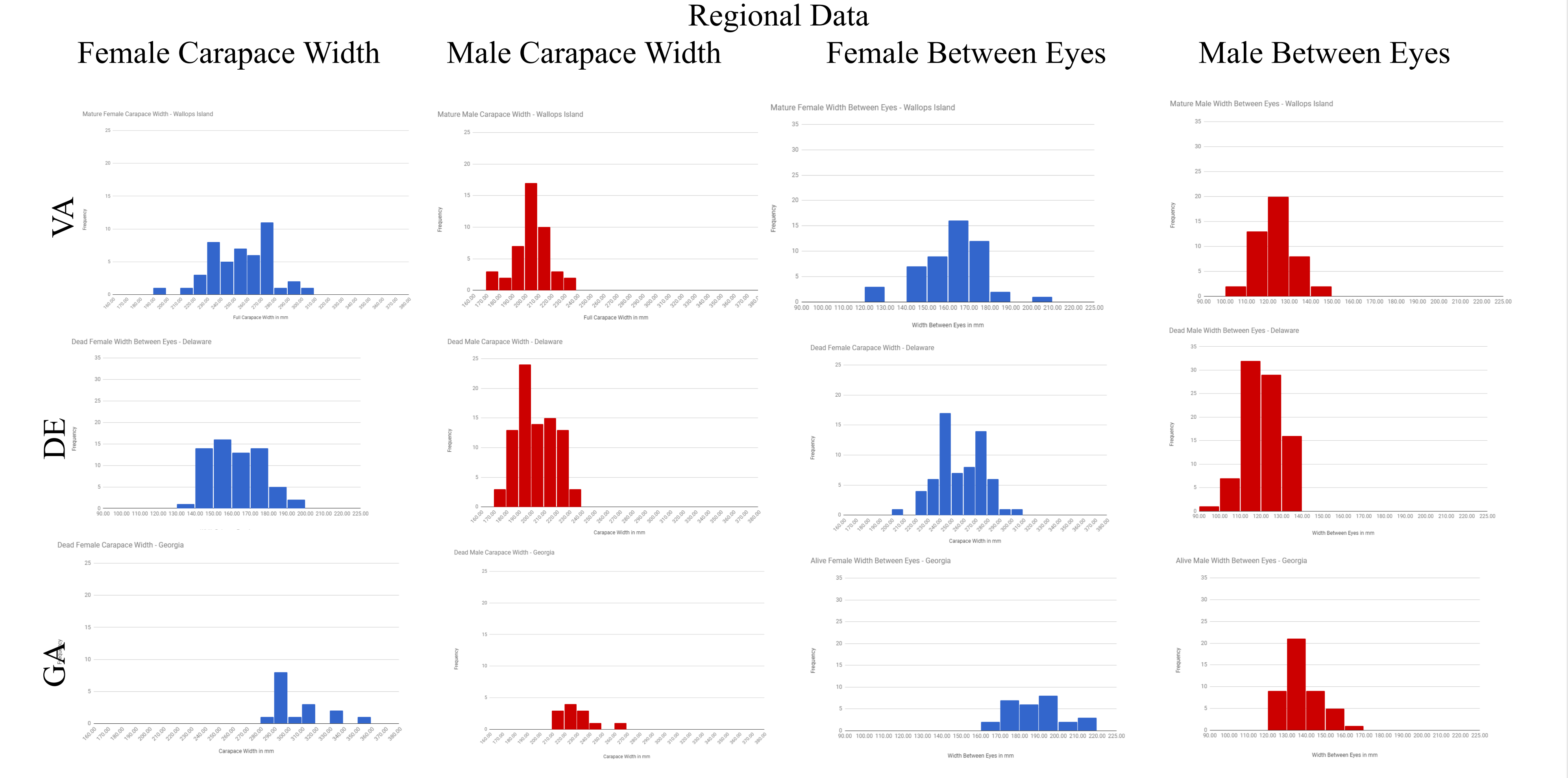
Harvesting horseshoe crabs to use as eel and whelk bait is their major cause of decline, but quotas implemented over the past 15 years have helped limit this damage. Horseshoe crabs are collected and bled by five biomedical companies in the U.S. in order to obtain limulus amoebocyte lysate, a valuable product needed to detect bacterial endotoxins for products to receive FDA approval. The bleeding procedure is supposed to be nonlethal, and the crabs are returned to the wild, but studies suggest dangerous side effects including disorientation or debilitation for two weeks, difficulty spawning afterwards, lower hemocyanin levels, and even mortality rates ranging from 8% to 29%. The final known major cause of mortality in these creatures is stranding. Horseshoe crabs come onto shore to spawn and evade predators. They can survive on land if their gills are kept moist, but if they are flipped over and exposed to sun and seagulls, they will die. Their long telsons help to flip themselves back over in these situations. However, this body part is very fragile, so broken or shortened telsons may increase the likelihood of death in this way.

# Analysis of Deceased *Limulus polyphemus* to Find Causes of Mortality on Wallops Island

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**Results**



Gender Ratios				Telson Data	
	VA	DE	GA	Virginia	Delaware
Total	--	1.68:1	1.33:1	Average mature female: 204.222	Average mature female: 207.974
Dead	0.96:1	1.29:1	0.75:1	Average mature male: 169.929	Average mature male: 175.104
				Percent broken: 12.121%	Percent broken: 17.296%
				Total broken: 8/66	Total broken: 87/503

Types of Telson Condition



Broken telson that started to heal. We can tell it was broken before death.





Broken telson that had a clean break. Potentially broken post mortem.



What a full telson should look like, intact to the tip.

**Methods and Materials**

For each specimen, measurements were taken of the distance between the compound eyes, the prosoma width, the telson length, and the full carapace length. Presence of an amplexus scar, nature of the pedipalps, and curvature of the carapace were observed to approximate sex and maturity. The presence of mollusks or crustaceans on the carapace as well as unique scars were also noted.



**Conclusion**

The ratios of males to females among the Georgia and Delaware populations were both higher than that of Georgia. The Virginia data set was also unique by having more female than male specimens. The observed sex ratio of 0.964:1 was very close to 1:1, suggesting a higher reproductive capacity if one assumes the demographics of the live population match that of the dead. However, when looking at the two past data sets with the live specimens excluded, the gender ratios are lower. While it is possible that there are more females in the Wallops Island population than is typical, this trend suggests a higher mortality rate for the females.

The difference in sizing between the specimens from Virginia and Delaware and those from Georgia suggest a genetic difference. With such a miniscule difference between Virginia and Delaware, we can assume they are part of the same geographical region, but the genetic difference from the Georgia species could show a potential divergence in the Atlantic horseshoe crab.

Out of our measured crabs, over 10% of them were sexually immature. If we assume that mortality is caused by spawning, then no sexually immature crabs would be found, and under this assumption, there is less than 0.001% chance that the immature crabs were due to variation. Because of this, we can conclude that there is another major factor killing *Limulus polyphemus* besides coming up to spawn.

**Future Plans**

Future research could be done on finding changes in organic matter, vehicular use, water parameters for prey, ideal parameters for spawning, and gill cementation.