

Effects of Blood Extraction on the Mortality of the Horseshoe Crab, *Limulus polyphemus*.

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ABSTRACT

Horseshoe crabs (*Limulus polyphemus*) are bled by biomedical companies for the extraction of Limulus Amoebocyte Lysate (LAL), a clotting agent used in the detection of endotoxins. In 1998, the Atlantic States Marine Fisheries Commission mandated that all biomedical companies collecting horseshoe crabs for the production of LAL study the horseshoe crab mortality rates resulting from the company's blood extraction process. BioWhittaker, a Cambrex Company is one of the largest producers of LAL in the world. During the summer of 1999, bled and unbled horseshoe crabs were transported from BioWhittaker's bleeding facility in Chincoteague, Virginia to the Virginia Seafood Agricultural Research and Extension Center's aquaculture facility in Hampton, Virginia. At the aquaculture facility, they were kept in a tank and their survival was monitored for a period of two weeks. Mortality for bled crabs was 15%, while mortality for unbled crabs was 0%. Because of the importance of horseshoe crabs to a wide variety of interests, proper management requires monitoring and consideration of mortality effects on the population.

INTRODUCTION

Horseshoe crabs (*Limulus polyphemus*) are the target of a large commercial fishery that provides bait for the American eel (*Anguilla rostrata*), whelk (commonly referred to as conch *Busycon* spp.), and, to a lesser extent, catfish fisheries (HCTC, 1998). The horseshoe crab fishery has been increasing rapidly (Loveland et al., 1996; Berkson and Shuster, 1999) and is the subject of much controversy (Berkson and Shuster, 1999).

Biomedical companies also catch horseshoe crabs to produce Limulus Amoebocyte Lysate (LAL), a clotting agent used to detect the presence of endotoxins pathogenic to humans in injectable drugs and all implantable devices (Novitsky, 1984; Mikkelsen, 1988). The LAL test is now a standard used to protect human health around the world, and horseshoe crabs are the only source of LAL.

The Food and Drug Administration (FDA) mandates that biomedical companies release their horseshoe crabs alive after they have been caught and bled. Because mortality rates in the capture and bleeding processes are poorly understood, the Atlantic States Marine Fisheries Commission mandated in 1998 that all biomedical companies actively bleeding horseshoe crabs estimate mortality rates resulting from their bleeding process. Proper management of the resource requires information on all human induced sources of mortality.

BioWhittaker, a Cambrex company, is the largest producer of LAL in the world. In response to the ASMFC mandate, BioWhittaker requested Virginia Tech to conduct

the mortality study. The objective of the study was to evaluate the mortality rates between bled and unbled crabs in a controlled holding facility.

METHODS

To examine the impact of blood extraction on the horseshoe crabs used by BioWhittaker, mortality rates were compared between crabs that underwent the bleeding process ("bled") and crabs that were suitable to undergo the bleeding process but did not ("unbled"). On 8 July 1999 and 22 July 1999, horseshoe crabs were captured using BioWhittaker's standard operating procedure of trawling in the Atlantic Ocean off the coasts of Chincoteague, Virginia and Ocean City, Maryland. After capture, horseshoe crabs were brought to BioWhittaker's bleeding facility in Chincoteague. The procedure was the same for each of the two sample and monitoring periods. Ten newly-matured, male crabs (identified by pristine shell condition and boxing-glove lower claws) were randomly selected from all of the crabs obtained in that day's trawls. These ten were not put through the bleeding process and served as a control in the experiment. They were packed in coolers labeled "unbled," and set aside. Ten additional newly-matured, male crabs were then randomly selected from the remaining crabs and underwent BioWhittaker's normal bleeding process. Upon completion of the bleeding process, the crabs were packed in coolers labeled "bled."

All coolers containing horseshoe crabs, both "bled" and "unbled," were immediately packed in an air-conditioned vehicle, and transported to the Virginia Seafood Agricultural Research and Extension Center's aquaculture facility in Hampton, Virginia. Here, the "unbled" crabs were marked so as to distinguish them from the "bled" crabs, and all crabs were placed into a re-circulating marine aquaculture system. The horseshoe crabs remained under observation in Hampton for a period of two weeks. They were maintained in appropriate conditions (Brown and Clapper, 1981), fed a diet of squid, and monitored daily.

At the conclusion of each two-week period, the status of each crab (dead or alive) was recorded. The results from the two samples were combined and the overall percentage mortality was calculated for the bled and the unbled groups. All surviving horseshoe crabs were removed from the tank, placed in the coolers, packed in an air-conditioned vehicle, and returned to BioWhittaker's bleeding facility in Chincoteague, Virginia. They were then returned to the Atlantic Ocean in accordance with BioWhittaker's standard operating procedures.

RESULTS

The results for each of the two-week periods on mortality differences between crabs bled by BioWhittaker and crabs suitable for bleeding, but not undergoing the bleeding process, are summarized in Table 1. Bled crabs had an overall mortality rate of 15% compared to the 0% mortality rate of the unbled crabs.

DISCUSSION:

The results obtained in this study show that there is a level of mortality resulting from the blood extraction process. This is consistent with results obtained in previous studies (Rudloe, 1983; Thompson, 1998). Based on a tagging study in Florida, Rudloe (1983) found that bleeding increased mortality by 10% during the first year after bleeding, and 11% during the second year. Thompson's (1998) study on horseshoe

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TABLE 1. Comparison of mortality rates between bled and unbled groups of horseshoe crabs.

Dates	Unbled Horseshoe Crabs			Bled Horseshoe Crabs		
	# of crabs monitored	# of crabs that died	% dead at study end	# of crabs monitored	# of crabs that died	% dead at study end
07/08/99 – 07/22/99	10	0	0%	10	0	0%
07/22/99 – 08/05/99	10	0	0%	10	3	30%
Totals:	20	0	0%	20	3	15%

crabs in South Carolina estimated mortality rates associated with LAL processing to be 15%.

Each LAL producer has a unique operation in processing horseshoe crabs. They each have unique bleeding methods, method of capture, distance and method of travel to bleeding lab, holding time and conditions, and methods of return most appropriate to their own setting and situation. The results found in this study reflect those of BioWhittaker and may not be reflective of other companies' procedures.

Additional examination of the effect of blood extraction on mortality of horseshoe crabs would provide more detailed information. Further studies examining this effect are planned and will involve larger sample sizes. The sample sizes used in this study were limited by the size of the holding tank at the aquaculture facility.

This study examined the survival of the crabs in a controlled environment (a re-circulating marine system), as opposed to their natural environment. While this may not be reflective of the mortality rate of crabs returned to the wild, it may be thought of as a maximum mortality that these crabs could experience. Transfer and holding may induce stress on the crabs. Thus, the survival of the bled crabs could be compromised in translocation and confinement in the tank.

Further, this study looked only at newly matured, male crabs in an attempt to minimize variation of external influences. In each set of monitored crabs, the only difference between the two groups was whether or not they underwent the blood extraction process. Capture, transport, sample size and holding period were consistent in all groups of crabs. Additional research should also look at differences in mortality in other age and gender classes.

The Food and Drug Administration estimates that 260,000 horseshoe crabs were caught, bled, and returned to the ocean by biomedical companies in 1997 (HCTC, 1998). The commercial fishery reported landings of 1,885,883 pounds in 1997 with a 100% mortality rate, down from the 5,153,630 reported in 1996 (HCTC, 1998). These numbers are known to be incomplete, with actual landings likely to be substantially higher (HCTC, 1998). When comparing the commercial fishery to BioWhittaker's bleeding process, in terms of the numbers of crabs caught and their associated mortality rates, it is evident that the bleeding process has a substantially smaller impact than the commercial fishery on the horseshoe crab population.

CONCLUSIONS

In conclusion, it is evident that the blood extraction process performed by biomedical companies results in some mortality of horseshoe crabs. This study reports the impact of BioWhittaker's bleeding process to be 15%. Because horseshoe crabs are a valuable resource to numerous interests, any impact on their mortality rates should be carefully monitored. With this information, managers can regulate the resource ensuring the needs for horseshoe crabs can be met on an ongoing basis.

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