Effects of blood extraction on horseshoe crabs (*Limulus polyphemus*)

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Horseshoe crabs (*Limulus polyphemus*) are caught by commercial fishermen for use as bait in eel and whelk fisheries (Berkson and Shuster, 1999)—fisheries with an annual economic value of $13 to $17 million (Manion et al.[1]). Horseshoe crabs are ecologically important, as well (Walls et al., 2002). Migratory shorebirds rely on horseshoe crab eggs for food as they journey from South American wintering grounds to Arctic breeding grounds (Clark, 1996). Horseshoe crabs are also essential for public health (Berkson and Shuster, 1999). Biomedical companies bleed horseshoe crabs to extract a chemical used to detect the presence of endotoxins pathogenic to humans in injectable and implantable medical devices (Novitsky, 1984; Mikkelsen, 1988). Bled horseshoe crabs are returned to the wild, subject to the possibility of postbleeding mortality. Recent concerns of overharvesting have led to conflicts among commercial fishermen, environmentalists acting on behalf of the shorebirds, and biomedical companies (Berkson and Shuster, 1999; Walls et al., 2002).

In order to create an effective, sustainable management policy for the horseshoe crab resource, the completion of a stock assessment that incorporates human-induced mortalities is necessary. A stock assessment is not currently available because of a lack of critical information on the horseshoe crab population (Berkson and Shuster, 1999). One critical piece of information needed is an estimate of the mortalities involved in the biomedical bleeding process. With an estimated 260,000 horseshoe crabs bled in 1997 (HCTC[2]), the last year with data available, mortalities may not be negligible.

Five biomedical companies on the Atlantic coast of the United States bleed horseshoe crabs in the laboratory for the production of Limulus Ameobocyte Lysate (LAL). The horseshoe crabs are caught by fishermen under contract to biomedical companies, bled, then returned to their point of capture.

The LAL test used to detect endotoxins in humans is derived from the blue, copper-based blood of the horseshoe crab. Although alternate tests exist for the detection of endotoxin, the LAL test is the most effective because it is capable of detecting as little as one millionth of a billionth of a gram of endotoxin (Mikkelsen, 1988). The LAL test is now a standard test used to protect human health around the world, and horseshoe crabs are the sole source of LAL.

Each biomedical company maintains its own procedures for harvesting horseshoe crabs, extracting the horseshoe crabs' blood, releasing the bled horseshoe crabs, and developing the LAL substance. In 1998, the Atlantic States Marine Fisheries Commission (ASMFC), the Commission responsible for horseshoe crab management in the United States, mandated that all biomedical companies actively bleeding horseshoe crabs estimate mortality rates resulting from their bleeding process (Schrading et al.[3]). Because of the unique methods of the different biomedical companies, each company was required to quantify its own rate of mortality.

BioWhittaker, a CAMBREX company, is the largest producer of LAL. In response to the ASMFC mandate, BioWhittaker requested that Virginia Tech conduct the mortality study for their company. Our objective was to determine horseshoe crab mortality for a two-week period following the bleeding process.

Methods

We compared mortality rates between horseshoe crabs that underwent the bleeding process (bled) and horseshoe crabs that were suitable to undergo the bleeding process but were not bled (unbled). Throughout the 1999, 2000, and 2001 bleeding seasons (June through August), BioWhittaker obtained horseshoe crabs by trawling in the Atlantic Ocean off the coasts of Chincoteague, Virginia, or Ocean City, Maryland (or off both coasts). After capture, the horseshoe crabs were brought to BioWhittaker's bleeding

[1] Manion, M. M., R. A. West, and R. E. Unsworth. 2000. Economic assessment of the Atlantic coast horseshoe crab fishery, 71 p. Division of Economics, U.S. Fish and Wildlife Service, Arlington, VA.

[2] HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast, 9 p + figures and tables. Horseshoe Crab Technical Committee, Atlantic States Marine Fisheries Commission. Washington, D.C.

[3] Schrading, E., T. O'Connell, S. Michels, and P. Perra. 1998. Interstate management plan for horseshoe crab, 59 p. Atlantic States Marine Fisheries Commission, Washington D.C.

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Table 1

Comparison of mortality rates between bled and unbled groups of horseshoe crabs captured near Chincoteague, Virginia, and Ocean City, Maryland, 1999–2001.

	Unbled horseshoe crabs			Bled horseshoe crabs		
Dates monitored	No. of crabs monitored	No. of crabs that died	% dead at study end	No. of crabs monitored	No. of crabs that died	% dead at study end
8–22 Jul 99	10	0	0%	10	0	0%
22 Jul 99–5 Aug 99	10	0	0%	10	3	30%
19 Jun 00–3 Jul 00	30	0	0%	30	0	0%
7–21 Jul 00	30	0	0%	30	0	0%
1–15 Aug 00	30	1	3.3%	30	6	20%
6–20 Jun 01	30	0	0%	30	0	0%
20 Jun 01–04 Jul 01	30	0	0%	30	2	6.7%
15–29 Aug 01	30	0	0%	30	5	16.7%
Total	200	1	0.5%	200	16	8%

facility in Chincoteague, Virginia. At the bleeding facility, we randomly selected a predetermined number (10 in 1999, 30 in 2000 and 2001) of newly matured male horseshoe crabs (identified by pristine shell condition and the presence of boxing-glove lower claws [Shuster[4]]) from all of the horseshoe crabs obtained in that day's trawls. We selected newly matured male horseshoe crabs to minimize covariance in our study. These horseshoe crabs were not bled and served as a control in the experiment. They were packed in coolers labeled "unbled," and set aside. The same number of newly matured male horseshoe crabs were then randomly selected from the remaining horseshoe crabs and underwent BioWhittaker's normal bleeding process. Upon completion of the bleeding process, the horseshoe 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 in Hampton, Virginia. The horseshoe crabs were removed from the coolers and the unbled horseshoe crabs were marked with external tags to distinguish them from the bled horseshoe crabs. These markings were unobtrusive and did not cause any undue stress to the unbled horseshoe crabs. All of the horseshoe crabs were placed in four replicated, flow-through holding tanks, and equal numbers of bled and unbled horseshoe crabs were held in each tank. The horseshoe crabs remained in the tank system at Hampton for two weeks. Horseshoe crabs were maintained in appropriate conditions (Brown and Clapper, 1981), and monitored daily. Horseshoe crabs that died during the two-week period were removed and returned to the ocean at the time of their death.

At the conclusion of each two-week period, the status of each horseshoe crab (dead or alive) was recorded. All surviving horseshoe crabs were removed from the tank, placed in coolers, packed in an air-conditioned vehicle, returned to BioWhittaker's bleeding facility in Chincoteague, Virginia, and returned to the Atlantic Ocean in accordance with BioWhittaker's standard operating procedures. This procedure was repeated eight times during summers 1999, 2000, and 2001. The results from each of the replicates were combined, and the overall percentage mortality was calculated for the bled and unbled groups.

Using Fisher's exact test, we evaluated statistical significance of differences in mortality between the bled and unbled horseshoe crabs (Mehta and Patel, 1999). We then calculated a 95% confidence interval for average differential mortality using the common odds ratio in the statistical program StatXact (Mehta and Patel, 1999).

Results

A Fisher's exact test for statistical significance showed differences between mortality rates in bled and unbled horseshoe crabs (P=2.085E-04). Bled horseshoe crabs (n=200) had an overall mortality rate of 8% compared to the 0.5% mortality rate of the unbled horseshoe crabs (n=200; P<0.001) (Table 1). Thus, this study estimates average differential mortality between bled and unbled horseshoe crabs to be 7.5%. The 95% confidence interval for this average differential mortality ranges from 0.14% to 38.1% as calculated with the common odds ratio (Mehta and Patel, 1999).

Discussion

Our results indicate that horseshoe crab mortality due to bleeding is relatively low. Two small-scale studies had previously estimated postbleeding mortality. Rudloe (1983), observing bled and unbled horseshoe crabs in a penned cove in Florida, found that bleeding increased mortality by

[4] Shuster, C. N., Jr. 1999. Managing the horseshoe crab resource: it's the adult age that counts, 32 p. Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA.

10% during the first year after bleeding, and 11% during the second year. Thompson (1998) estimated that mortality associated with LAL processing was 15% during the first week following blood extraction by observing bled and unbled horseshoe crabs in tanks in South Carolina.

Each LAL producer has a unique bleeding method, method of capture, distance and method of travel to the bleeding laboratory, a different holding time and conditions, and method of return of the bled crab that is most appropriate to that company's setting and situation. The results found in this study reflect those of BioWhittaker and may not be reflective of other companies' procedures.

We examined the survival of the horseshoe crabs in a controlled environment (tank), as opposed to their natural environment. Our survival rate for horseshoe crabs may not reflect the survival rate of horseshoe crabs returned to the wild. Transfer and holding processes induce stress on the horseshoe crabs. Thus, the survival of the bled horseshoe crabs could be compromised by translocation and confinement in tanks. However, the tank environment may provide protection for horseshoe crabs when they are in a weakened state and are more susceptible to predation following blood-extraction.

Further, this study looked only at newly matured male horseshoe crabs in an attempt to minimize variation of external influences, so that the only difference between the two groups was whether or not they underwent the blood extraction process. Additional studies should examine differences in mortality in other age and sex classes.

The Food and Drug Administration estimates that 260,000 horseshoe crabs were caught, bled, and returned by biomedical companies when last reported in 1997 (HCTC[5]). Assuming the 7.5% mortality rate found in our study is applicable to each biomedical company, and assuming that the number harvested for the biomedical companies has stayed relatively constant, we estimate that approximately 18,750 horseshoe crabs die yearly as a result of the biomedical procedure. In comparison, the commercial fishery reported landings of 5,543,000 pounds in 1999 and 3,756,000 pounds reported in 2000, all with a 100% mortality rate (NMFS, 2002). In the overall picture of the magnitude of horseshoe crabs caught and the associated mortality rates, it is evident that the bleeding process has a substantially smaller impact than the commercial fishery on the horseshoe crab population. However, information on both biomedical and commercial fishery-induced mortality are necessary to determine the total harvest mortality of horseshoe crabs.

The information presented in this study provides an estimate of the postbleeding mortality rate, an element of human-induced mortality on horseshoe crabs. This is one critical piece of information required to conduct a stock assessment and to develop an effective management strategy.

[5] HCTC (Horseshoe Crab Technical Committee). 1998. Status of the horseshoe crab (*Limulus polyphemus*) population of the Atlantic coast, 9 p. + figures and tables. Horseshoe Crab Technical Committee, Atlantic States Marine Fisheries Commission. Washington, D.C.

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