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Will Recombinant Lysate Save The Horseshoe Crabs?

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Your Endotoxin & Glucan Experts

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There have been a number of recent articles stating that a synthetic alternative to a reagent manufactured using blood extracted from horseshoe crabs could save the species[i,ii]. This suggestion incorporates some erroneous assumptions and ignores some important facts. Consequently, the simple answer to the question posed in the title is, no; there is little truth to the idea. This article examines the assertion that the synthetic reagent could save the horseshoe crab and explains the fallacies that it is based upon.

Unpacking the Assertion

In order to address the assertion it is necessary to identify the various components and implications involved. These include:

1. The nature of the reagent and its synthetic counterpart.
2. The status of the horseshoe crab population.
3. The nature of the threats to horseshoe crabs.
4. The potential for synthetic reagent to positively impact the horseshoe crab population.

These four areas are addressed in turn, followed by correction of a few general misconceptions about horseshoe crabs and some fact-based conclusions.

1. The Reagents – Natural and Synthetic

The natural reagent in question is *Limulus* amebocyte lysate (LAL), which is prepared from the white blood cells (amebocytes) of the horseshoe crab, *Limulus polyphemus*. LAL is exquisitely sensitive to a potent bacterial toxin (endotoxin) and is used commercially in a test to ensure the safety of injectable drugs, intravenous solutions and many medical devices. A related LAL test is also used to aid diagnosis of invasive fungal infection, a condition to which people with impaired immune systems are particularly susceptible. Although many do not know it, almost everyone in the developed world has benefited from the LAL test.

Current alternatives to naturally sourced LAL are based on a genetically engineered, synthetic version of Factor C or rFC. Factor C is the first protein in the enzyme cascade of horseshoe crab blood, which recognizes endotoxin. The synthesized protein is specific to endotoxin; it does not detect the marker for invasive fungal infection, nor does it benefit from working in concert with other factors in the naturally sourced product.

2. Horseshoe Crab Populations

The assertion that the synthetic reagent could save the horseshoe crab suggests that the population is in decline and perhaps in danger of extinction, as has been stated in some articles[iii, iv]. The International Union for Conservation of Nature (IUCN), has determined that the American horseshoe crab is vulnerable[v]. Is this the case?

There is no evidence of an overall decline in the total number of horseshoe crabs over the time that reasonably reliable data have been available, which is roughly the last 20 years. However, just because overall numbers appear stable does not mean that some local subpopulations are not declining. Horseshoe crabs exist as multiple populations along the east coast of the United States of America and some can be healthy and growing while others wane. Consequently, a more complete answer requires different regions be considered individually.

The Atlantic States Marine Fisheries Commission (ASMFC) have concluded that population trends in the Florida region are variable with some declines and at least one increase. Numbers of horseshoe crabs are either stable or increasing in the coastal waters of New Jersey, Maryland, Virginia, North Carolina, South Carolina and in the Delaware Bay[vi]. These areas comprise the largest populations in the United States, leading to the overall conclusion given above, that the total number of horseshoe crabs appears to be stable or even increasing. Both organizations expressed declining population levels in New York and New England waters, including New Hampshire and Maine, which have small populations. (Maine is at the northern end of the geographic range of the American horseshoe crab.) The most recent Compliance Report by the Massachusetts Division of Marine Fisheries (MADMF) for the ASMFC[vii] states that in 2016 trawl surveys, horseshoe crab abundance exceeded median levels, continuing an upward trend reported the previous year. In the southern New England region of Massachusetts waters, more horseshoe crabs are reported than any other point in survey history, which goes back over 30 years. This upward trend continues today and is largely believed to be the result of management actions in 2010 that prohibited harvest during peak spawning periods, cut daily possession limits and other measures that helped preserve the resource.

In conclusion, while there are concerns about some Florida and some Northeastern regional populations, the total number of horseshoe crabs appears to be at least stable and could be increasing at present. Contrary to the more sensational headlines, the horseshoe crab is not in danger of extinction.

3. Threats to Horseshoe Crab Populations

Several articles have stated that the biomedical fishery is a major contributor to horseshoe crab mortality. Is this correct? The answer is clearly "No." The principal, non-natural source of mortality is the bait fishery. During the years 2010-2015, (a period over which numbers are readily available for both bait and biomedical fisheries[viii]), the average number of horseshoe crabs caught for bait is 45% greater than that taken by the biomedical fishery. In addition, the mortality for the bait fishery is 100% while the great majority of biomedical horseshoe crabs survive when returned to the water. The ASMFC estimates a mortality rate of 15% for biomedical horseshoe crabs. Thus the bait harvest accounts for 90% of the fishery mortality and clearly has a much greater potential to impact horseshoe crab populations. To claim that the biomedical fishery is substantially affecting horseshoe crab populations is to ignore the elephant in the room, which is the bait fishery. The effect of the biomedical fishery is relatively insignificant.

Also, there is no biomedical fishery in the areas where local declines are reported. The majority of biomedical horseshoe crabs are taken from (and returned to) Atlantic coastal waters between South Carolina and New Jersey, including the Delaware Bay, and from Massachusetts waters. Populations in these areas are reportedly stable or increasing.

Other threats to horseshoe crabs include habitat degradation due to shoreline development and beach reinforcement, which result in loss of suitable spawning habitat.

4. Can the Synthetic Reagent "Save the Horseshoe Crab"?

Even a complete switch to a synthetic reagent would have minimal effect on horseshoe crab populations because the biomedical industry is a relatively small contributor to fishery mortality.

Cost and Risk - Regulatory considerations of rFC

The FDA enforces the standards established by the US Pharmacopeia which require all parenteral drugs, injectable products and certain devices to pass an endotoxin test before the product is released for sale or use. Life science manufacturers are bound by the harmonized pharmacopeial standard Chapter <85> Bacterial Endotoxins Test. Regarding the reagent for the test, the BET chapter specifies "This reagent refers only to a product manufactured in accordance with the regulations of the competent authority." In the United States the competent authority is the FDA, who has rules on the licensure of BET reagents. Recombinant lysate cannot receive an FDA license and Recombinant lysate is not included in the harmonized US/EU/JP pharmacopeia. Unlicensed reagents can be validated and used according to USP provisions for alternative methods, but manufacturers often prefer a lower risk path using FDA licensed reagents with which they and FDA inspectors have decades of knowledge and experience. When pharmaceutical and medical device manufacturers submit their drug, biological product or device applications to the FDA, they include a description of the endotoxin test method to be employed. Almost 100% of the issued FDA approved products currently on the market that include BET cite naturally sourced lysate.

Adoption of a synthetic reagent in place of natural LAL is not likely to be rapid. The rFC reagent has been commercially available for about 15 years but it has found little acceptance in the market place. To quote Burgenson of Lonza, the company that developed the test, "We built it and they didn't come". This is for a number of reasons, including the refusal of FDA to license recombinant reagent (as it does naturally sourced LAL), and the lack of inclusion of rFC in the major Pharmacopeia, which set standards for testing healthcare products and specifies that the reagent used in endotoxin tests be "… manufactured in accordance with the regulations of the competent authority." Consequently, anyone wishing to use rFC in place of LAL must demonstrate to the satisfaction of regulatory authorities its equivalence to LAL. As a result, Lonza is still dependent on horseshoe crabs for the great majority of endotoxin test kits that they sell. Finally, the rFC test is inherently less sensitive the unaltered naturally sourced product because it only uses the first step from the natural reaction. In the past the rFC kits were priced higher than naturally sourced product and rFC assays necessitate the use of more expensive, specialized instrumentation to read the test, factors which may have contributed to its slow adoption.

Other Misrepresentations and Misconceptions

Some articles cite mortality rates of biomedical horseshoe crabs as high as almost 30%. In some experimental studies of effects of the biomedical use of horseshoe crabs[ix, x], the conditions used exceed established industry best practices for crab handling (sometimes greatly), resulting in reports of high mortality and sub-lethal effects. In one study[x], no mortality occurred for crabs handled under conditions of low stress, whereas up to 29% mortality occurred in the highly stressed group. Like all animals, if horseshoe crabs are not treated well, they do not fare well. Proper handling of horseshoe crabs by the biomedical industry is important to maximize the survival of horseshoe crabs returned to the water. This is recognized by the biomedical companies and the ASMFC who have together formulated a code of best management practices[xi] to assure that horseshoe crabs are handled humanely and promptly returned to the water. In Massachusetts adherence to the ASMFC best management practices is a condition of authorization to receive biomedical horseshoe crabs and the Division of Marine Fisheries monitor the practices used. As previously noted, the ASMFC uses an estimated mortality rate of 15% for biomedical horseshoe crabs.

Conclusion

This article makes it clear that adoption of the rFC test would have minimal impact on horseshoe crab populations because the manufacture of LAL is a rather minor contributor to horseshoe crab mortality. Perhaps this is fortunate because it would likely take time for widespread adoption of the rFC test given the current regulatory climate and the current lower sensitivity of rFC.

Consequently, adoption of rFC could not “save the horseshoe crab.” Perhaps the best news is that despite some regional declines, the overall horseshoe crab population appears to be stable and the largest populations may be increasing. This is not to suggest that complacency is warranted. The ASMFC is closely monitoring populations and the bait and biomedical fisheries. Manufacturers of LAL and the users of many healthcare products will continue to depend on these healthy populations for some time.

References

i. Kramer, D. “Inside the Biomedical Revolution to Save Horseshoe Crabs and the Shorebirds That Need Them, ” Audubon Magazine, May 11, 2018. https://www.audubon.org/news/inside-biomedical-revolution-save-horseshoe-crabs-and-shorebirds-need-them. Accessed August 20, 2018.

ii. “Alternative to horseshoe crab blood for medical testing may save species,” The Press of Atlantic City, May 12, 2018. https://www.pressofatlanticcity.com/news/breaking/alternative-to-horseshoe-crab-blood-for-medical-testing-may-save/article_53d54309-84bf-5e6f-8aa7-5d19c5a14c88.html. Accessed August 20, 2018.

iii. Could the multi-million dollar industry that bleeds 500,000 horseshoe crabs a YEAR for medical research drive them to extinction? Dailymail.com. 13 April 2017. Updated: 14 April 2017. http://www.dailymail.co.uk/news/article-4410928/Horseshoe-crab-extinct-overharvesting.htm. Accessed August 20, 2018.

iv. Ancient Horseshoe Crab Endangered Over Blue Blood Profits. Stephen Seifert. The Alternative Daily. https://www.thealternativedaily.com/horseshoe-crab-endangered-over-profits/ Accessed August 20, 2018.

v. Limulus Polyphemus. The IUCN Red List of Threatened Species. http://www.iucnredlist.org/details/11987/0. Accessed August 20, 2018.

vi. ASMFC Horseshoe Crab and Delaware Bay Ecosystem Technical Committees Meeting, October 5, 2016. http://www.asmfc.org/files/Meetings/2016AnnualMeeting/HorseshoeCrabBoardSupplemental.pdf. Accessed August 20, 2018.

vii. Massachusetts 2016 Compliance Report to the Atlantic States Marine Fisheries Commission – Horseshoe Crab. https://www.mass.gov/files/documents/2017/09/19/compliance%20report%202016%20public_0.pdf. Accessed August 20, 2018.

viii. 2016 Review of the Atlantic States Marine Fisheries Commission Fishery Management Plan for Horseshoe Crab (*Limulus polyphemus*). http://www.asmfc.org/uploads/file/58b70d1eHSC_FMPReview_2016.pdf. Accessed August 20, 2018.

ix. Anderson RL, Watson WH 3rd, Chabot CC. Sublethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, Limulus polyphemus. Biol Bull. 2013; 225(3):137-51. https://www.ncbi.nlm.nih.gov/pubmed/24445440. Accessed August 20, 2018.

x. Hurton L, Berkson J. Potential causers of mortality for horseshoe crabs (Limulus polyphemus) during the biomedical bleeding process. Fish. Bull. 2006; 104:293–298. https://vtechworks.lib.vt.edu/bitstream/handle/10919/48012/hurton.pdf?sequence=1&isAllowed=y. Accessed August 20, 2018.

xi. Biomedical Best Management Practices, Horseshoe Crab Biomedical Ad-Hoc Working Group Report, October 3, 2011 http://www.asmfc.org/uploads/file/biomedAdHocWGReport_Oct2011.pdf. Accessed August 20, 2018.