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May, 2022

My initial interest in the Atlantic Horseshoe Crab stemmed from their form [fig.1]. Once becoming fully immersed in learning about horseshoe crabs, it was clear that the Atlantic horseshoe crab's population, as well as the other types of horseshoe crab found over the world are threatened. [1]

The threats facing horseshoe crabs also pose a peril to migratory shorebirds such as the Red Knot, a species that depends on abundant horseshoe crab eggs as a critical food source during its nearly 10,000-mile migration to its Arctic nesting grounds. Other species that are in decline, like weakfish and striped bass, also feed on the eggs. Forage fish, too, flourish by feeding on hatched larvae, thus providing more prey for sportfish high in the food chain. Even loggerhead turtles are an important predator on the crabs, even when they are adults. In addition to the well-documented impacts on fish and shorebirds, these impacts reverberate into local communities and towns that depend on recreational sportfishing, birding and eco-tourism activities as key parts of their economies.[2]

While there are still horseshoe crabs participating in an ecosystem in Delaware Bay, about 200 miles away, in New Haven, Connecticut, it's a different situation. Scientist Dr. Jennifer H. Mattei has been conducting research on the population ecology of the American horseshoe crab for over 20 years and sees the population in a more serious decline. Due to very low population numbers the horseshoe crab is functionally extinct in Long Island Sound:[3]

[1] Smith, David, et al. "IUCN Red List of Threatened Species: Limulus Polyphemus." *IUCN Red List of Threatened Species*, Name, 17 Feb. 2016, www.iucnredlist.org/species/11987/80159830#assessment-information.
[2] *Horseshoe Crab Recovery Coalition – Saving the American Horseshoe Crab*. hscrabrecovery.org/.
[3] Rivard, Nicole. "Friends of Animals." *Friends of Animals*, 21 Mar. 2022, friendsofanimals.org/cts-environment-committee-passes-horseshoe-crab-hand-capture-and-killing-ban/.

> "…their ecological role has been severely diminished. Horseshoe crabs function as a dominant species (not a keystone species) and their abundance is what is of ecological importance. They are no longer a source of food for shorebirds and fish in Long Island Sound. No eggs are washed into the surf. Yes, you do see thousands of horseshoe crabs in some areas of Connecticut but their population density does not allow them to function within the food web as they have in the past. This "functional extinction" has occurred with other species in CT as well including pollinating insects, oysters, Spartina grasses, etc. A management plan that merely keeps a species from going extinct is not good enough. It should keep the Long Island Sound ecosystem functioning sustainably.[4]

After appearing on the planet about 445 million years ago, the stern-looking arthropods have held on through each of the world's five mass extinctions that wiped out nearly all of their contemporaries, including the dinosaurs. They spend most of their time crawling on the bottom of the ocean and are rarely seen above water alive. However, in the Spring and Summer months, after reaching about 10 years old, guided by the moon and tide, the horseshoe crabs rise from the sea floor to lay and fertilize their eggs in huddled masses on the beaches along the Atlantic coast. Horseshoe crab eggs are a food source for numerous birds, reptiles, and fish. Most horseshoe crabs will not even make it to the larval stage before being eaten.[5]

While there are several factors contributing to their decline, overfishing for biomedical use is one of the main reasons. Horseshoe crabs are bled (live) by biomedical companies for a substance in their blood called Limulus amoebocyte lysate, or LAL [fig.2]. LAL is used to detect endotoxins in medicines, equipment and vaccines to protect humans and other domesticated animals. It detects pyrogens. These are gram-negative bacteria such as E. coli, Salmonella, Vibrio cholerae, Shigella, and many others.[6]

In high enough concentrations these bacteria produce and shed endotoxins in a sufficient quantity to cause fever if injected into a human or animal. Thus the Food and Drug

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[4] Mattei, Jennifer. *AN ACT CONCERNING the HAND-HARVESTING of HORSESHOE CRABS in the STATE*. H.B. 5140, 20 Feb. 2022.

[5] Fortey, Richard A. *Horseshoe Crabs and Velvet Worms : The Story of the Animals and Plants That Time Has Left Behind*. Vintage Books, 2012.

[6] Lisa Jean Moore. *Catch and Release: The Enduring yet Vulnerable Horseshoe Crab.* New York University Press, 2018, pp. 109, 110.

Administration (FDA) mandated injectables to be tested for both sterility and pyrogenicity. But there is never enough endotoxin, even in the most poorly maintained systems, to cause severe injury, let alone death, if injected.[7]

Prior to the LAL test, starting in 1944, scientists tested for pyrogens using rabbits, the Rabbit Pyrogen Test, RPT [fig. 3]. This testing method took up to 48 hours and required that three animals per test be injected with a sample batch of a drug intended for intravenous use. If an injected rabbit got a fever, it was taken as indication that endotoxin was present. This test was expensive, requiring resources to keep rabbit colonies alive, clean, and managed in the laboratory. That is, until a cheaper test came along, that didn't require as many rabbit colonies to be bred and maintained. For LAL, adult horseshoe crabs are captured by fishermen using nets that trawl offshore of their known habitats, scooping them out of sea to be bled and then released, or they hand-harvest horseshoe crabs as they come ashore during their annual egg-laying ritual.

I reached out to one of the larger bleeding biomedical facilities, Charles River Laboratories to learn more about the process first hand, however no one got back to me. [8] The closest I can come is to share the experience as recounted by fishery and wildlife scientists who study the bleeding of horseshoe crabs:

> "Throughout the typical biomedical bleeding process, horseshoe crabs are subjected to a variety of potential stressors (i.e., air exposure, increased temperature, handling, blood loss, trauma)... Animals may be held on the deck of a boat or in containers for several hours during collection, transported to the bleeding facility in trucks (that may or may not be air-conditioned), held in the coldroom of a laboratory for several hours at an air temperature of 16-19 degrees C, bled for a period of time, and then held in the coldroom or in a truck until transport back to the dock. Horseshoe crabs that are returned back to the ocean are transferred onto a boat and returned to their approximate point of capture within 72 hours of their collection".[9]
>
> In order to bleed the crab, it is folded up over a bar, and the sinus exposed. The sinus that runs down the back of the carapace is needled. Once inserted, the needle enters the heart of the crab (also known as the *hemocoel*); this procedure is called a *cardiac dorsal puncture*. The blood flows from the needle into a

[7] Lisa Jean Moore. *Catch and Release: The Enduring yet Vulnerable Horseshoe Crab.* New York University Press, 2018, pp. 106, 107.

[8] "Charles River Laboratories | Every Step of the Way." *Www.criver.com*, www.criver.com/.

[9] Hurton, L., and J. Berkson. "Potential Causes of Mortality for Horseshoe Crabs (Limulus Polyphemus) during the Biomedical Bleeding Process." *Undefined*, 2006, p. 294, www.semanticscholar.org/paper/Potential-causes-of-mortality-for-horseshoe-crabs-Hurton-Berkson/202f5266c5b1e65ba7ba2ab5236560bb3258b7e4.

> sterile glass container, filling it with light blue blood. Horseshoe crabs are then marked on their carapace to indicate they have been bled that year so as not to be recaptured and rebled. The blood is then centrifuged, and technicians pour the hemocyanin out and retain the amoebocyte pellet that forms at the bottom of the container. Roughly 3% of the blood is the amoebocyte. Within the laboratory, technicians, in the words of Ron Berzofsky (senior technical advisor at the LAL Division of Wako Chemicals), "bust open the cells to get the lysate. So LAL is actually busted up horseshoe crab blood cells." The pharmaceutical companies then use their own recipes to magnify the chemical sensitivity of the blood's own reaction, making LAL more sensitive than what comes out of the crab. While much is obscured about the collection of horseshoe crab blood, what we do know is that the biomedical bleeding of crabs uses cardiac puncture to collect between 25% and 40% of a crab's blood, and then it is released. This is roughly the "amount of a cup of coffee." By comparison, humans give about 10% of their blood during a voluntary donation.[10]

Due to the amount of stress horseshoe crabs go through, I looked up how many humans have died from endotoxins, but the results are not clear. The horseshoe crab's "life-saving" blood is advertised ubiquitously [fig. 3]. However, the claim of the life-saving capacities of horseshoe crab blood is a bit of a leap. Tom Novitsky, a microbiologist and biochemist who, until 2003, ran Associates of Cape Cod, a leading manufacturer of LAL explains this rationale:

> "All injectables are sterilized to kill living bacteria. Any endotoxin left would hardly be enough to cause septic shock symptoms let alone disease. THERE IS NO PUBLISHED REPORT THAT ENDOTOXIN ALONE IN A MANUFACTURED PHARMACRUTICAL PRODUCT HAS KILLED ANYONE."
>
> "At best, the regulations for pyrogen and LAL testing are another layer of safety for modern pharmaceuticals. So when I see the inference that endotoxins cause "toxic shock" or "diarrhea," the statements have nothing to do with LAL use. In fact, the LAL test has been used in research to follow Gram-negative infections. Endotoxin is probably the direct cause of the symptoms of toxic shock but not the primary cause of the disease. One cannot get toxic shock or diarrhea from a pharmaceutical contaminated with endotoxin (a lot of drugs themselves cause diarrhea if you can believe all the TV ads). I should point out that our intestines have gobs of endotoxin and we are no worse for it. We also consume gobs of endotoxin in our food and drink every day. It is only when endotoxin is injected into our bloodstream or spinal fluid that it becomes a problem. Even then, however, there is no record of any deaths from injected endotoxin from a pharmaceutical except [for] several cases of death caused from blood transfusions, where a small amount of contaminated blood was stored until Gram-negative bacteria grew to such great numbers that the endotoxin they contained killed the recipients in a matter of minutes. It is kind of sad that the FDA never saw a need to do LAL on stored blood prior to transfusions, albeit the incidence of endotoxin toxicity from bacterial blood contamination is extremely rare. As far as saving lives, one can accurately say that the LAL test has saved thousands of rabbits' lives as they were usually put down after they were used for pyrogen tests a few times. Colonies of rabbits were actually bred specifically for pyrogen test use, and now there is far less demand. So it is possible that the horseshoe crab is saving far more rabbit lives than human lives - although those rabbits might not have been bred if they weren't needed for testing."[11]

[10] Lisa Jean Moore. *Catch and Release: The Enduring yet Vulnerable Horseshoe Crab.* New York University Press, 2018, pp. 112.

[11] Lisa Jean Moore. *Catch and Release: The Enduring yet Vulnerable Horseshoe Crab.* New York University Press, 2018, pp. 110, 118.

However, Charles River Laboratories, illustrates the relevance of the rabbit test alongside the bleeding of the horseshoe crabs: **"**The rabbit pyrogen test (RPT) remains a viable mammalian test model when testing for non-endotoxin pyrogens and a variety of products for which LAL is limited."[12]

If endotoxin testing is not as much of a life-saving measure, but rather a quality-control test, is it essential to bleed horseshoe crabs and inject rabbits if the procedures are not necessarily saving any lives? Are there any synthetic alternatives available?

A synthetic version of LAL does exist. It was first invented over 20 years ago in Singapore and already approved in Europe, Japan and China as an equivalent to LAL called recombinant Factor C or rFC for short. However, Dr. Lawrence Niles, a wildlife biologist who co-leads the national Horseshoe Crab Recovery Coalition from New Jersey and has been involved with animal conservation projects for more than 40 years explained, "They [LAL manufacturers] have a lot to lose from switching to rFC." In 2019, Charles Rivers Laboratories hired a lobbyist based in Washington D.C. to pressure the government about its opinions on the topic. "Speedy adoption should not be viewed more favorably than certainty of patient safety," Charles River urged the U.S. Pharmacopeia (USP) in its 2019 letter. Half a year after the USP received Charles River's letter, they announced it was canceling a proposal expected to eventually grant the synthetic equivalent status to LAL in America.[13]

The decision was "based on stakeholder comments and in accordance with USP's public process for standards development." Without the approval for the classification as an industry

[12] "Pyrogenicity Testing | Charles River." *Www.criver.com*, www.criver.com/products-services/biologics-testing-solutions/contamination-and-impurity-testing/pyrogenicity-endotoxin-and-monocyte-activation-testing?region=3601. Accessed 6 May 2022.

[13] "USP Provides Guidelines for Recombinant Factor c (RFC) a Non-Animal-Derived Reagent Critical to Development of Vaccines and Other Sterile Pharmaceutical Products." *Www.usp.org*, www.usp.org/news/rfc-horseshoe-crabs-statement.

standard testing material, U.S. companies will have to overcome the scrutiny of showing that rFC is safe and effective for their desired uses, which may serve as a deterrent for usage of the horseshoe crab blood substitute.[14]

Why is the USP stating that rFC isn't a viable alternative when it's good enough for Europe, Japan and China? Their reason was "based on stakeholder comments" and taken from their website: "USP's quality standards are enforceable in the United States by the Food and Drug Administration and integrated into law in more than 40 countries."[15]

What is the FDA's role? "The federal regulation of food, drugs, cosmetics, biologics, medical products and tobacco is legally mandated by acts of the United States Congress. Since the early-20th century, the laws that establish FDA's regulatory authority have been modified to cover new product areas, expand enforcement powers, provide for new funding sources, modernize surveillance, inspection and investigative methods and enhance public education efforts."[16]

The FDA is legally mandated by acts of the US Congress, so I looked at the relationship between Congress and pharmaceutical companies (such as Charles River). 72 senators and 302 members of the House of Representatives cashed a check from the pharmaceutical industry ahead of the 2020 election — representing more than two-thirds of Congress. Pfizer's political action committee alone contributed to 228 lawmakers. Amgen Inc.'s Political Action Committees or PAC donated to 218, meaning that each company helped to fund the campaigns

[14] "Drugs Standards Group Nixes Plan to Kick Pharma's Crab Blood Habit." Reuters, 30 May 2020, www.reuters.com/article/us lonza-crabs-idUSKBN2360MB.

[15] "USP Provides Guidelines for Recombinant Factor c (RFC) a Non-Animal-Derived Reagent Critical to Development of Vaccines and Other Sterile Pharmaceutical Products." *Www.usp.org*, www.usp.org/news/rfc-horseshoe-crabs-statement.

[16] Commissioner, Office of the. "FDA's Legal Authority." *FDA*, Mar. 2021, www.fda.gov/about-fda/changes-science-law-and-regulatory-authorities/fdas-legal-authority.

of nearly half the lawmakers on Capitol Hill. Overall, the pharmaceutical sector donated $14 million compared to less than $12 million from defense in 2020. [17]

Further, more than 40% of members in Congress, or more than 220 representatives and senators, own individual stocks, collectively holding at least $225 million in stock assets. Pharmaceutical and biotechnology giants are also popular investments for elected officials. Johnson & Johnson and Pfizer, were the most-held pharmaceutical stocks in Congress in 2020, owned by 44 and 37 members, respectively. [18]

Additionally, the FDA has moved from an entirely taxpayer-funded entity to one increasingly funded by user fees paid by the manufacturers that are being regulated. The Prescription Drug User Fee Act was a law passed by the United States Congress in 1992 which allowed the FDA to collect fees [user fees] from drug manufacturers to fund the new drug approval process. The FDA's funding has increasingly come from the industries that it regulates. Of the FDA's total $5.9 billion budget, 45% comes from user fees. These user fee programs must be reauthorized every five years by Congress, and the current agreement remains in effect through September 2022.[19]

Dr. Raeford Brown, a pediatric anesthesia specialist at the UK Kentucky Children's Hospital and chair of the FDA Committee on Analgesics and Anesthetics has spoken out, "The pharmaceutical industry pours millions of dollars into the legislative branch every single year…

[17] Facher, Lev. "Two-Thirds of Congress Cashed a Pharma Campaign Check in 2020." *STAT*, 9 June 2021, www.statnews.com/feature/prescription-politics/federal-full-data-set/.
[18] A 501tax-exempt, The Center for Responsive Politics, et al. "Lobbying Data Summary." *OpenSecrets*, www.opensecrets.org/federal-lobbying/.
[19] White, C. Michael. "Why Is the FDA Funded in Part by the Companies It Regulates?" The Conversation, 13 May 2021, theconversation.com/why-is-the-fda-funded-in-part-by-the-companies-it-regulates-160444.

In 2016, they put $100 million into the elections. That's a ton of money. I'm really much more concerned because Congress is supposed to have oversight for the FDA." [20]

Now, where does the horseshoe crab's blood fit into this increasingly self-regulated and entangled situation? Representatives from Charles River have said that more than 80 million LAL tests are performed annually at a value of over $500 million, and that the company makes the most in the industry. The company itself is worth $13 billion. [21] Additionally, based on the Global Endotoxin and Pyrogen Testing Market Report, the biopharmaceutical industry is "flourishing"[22]. This creates an opportunity for the growth of the market value of endotoxin and pyrogen testing. This type of economic research analysis on the pyrogen testing market is prepared for potential financial backers. Publications such as "Global Endotoxin and Pyrogen Testing Market – Industry Trends and Forecast to 2028" supply individuals with the data they need to figure out investment strategies for hedge funds, individual investors, and retirement products. As such, the data points of pharmaceutical forecasting are narrated for possible shareholders as pyrogen testing as a growth industry. The growth is a potential bonanza for eager investors. The need for LAL consistently expands as new medical devices are innovated and human bodies are continually biomedicalized.[23] More recently, the Covid-19 pandemic led to

[20] McGreal, Chris. "FDA's Opioids Adviser Accuses Agency of Having 'Direct' Link to Crisis." *The Guardian*, 24 Jan. 2019, www.theguardian.com/us-news/2019/jan/24/fda-opioids-big-pharma-prescriptions.

[21] "Charles River Laboratories Net Worth 2010-2022 | CRL." www.macrotrends.net, www.macrotrends.net/stocks/charts/CRL/charles-river-laboratories/net-worth. Accessed 6 May 2022.

[22] Lisa Jean Moore. *Catch and Release: The Enduring yet Vulnerable Horseshoe Crab.* New York University Press, 2018, pp. 120.

[23] "Endotoxin and Pyrogen Testing Market – Global Industry Trends and Forecast to 2028 | Data Bridge Market Research." *Www.databridgemarketresearch.com*, www.databridgemarketresearch.com/reports/global-endotoxin-and-pyrogen-testing-market.

federal vaccination mandates, accelerating research and development putting "additional strain on the American horseshoe crab." [24, 25]

The dwindling horseshoe crab population seems related to the increasing demands of the biomedical industry. To find more precise facts and figures, I looked at the annual report from the Atlantic States Marine Fisheries Commission (the ASMFC is an interstate compact, ratified by the states and approved by the US Congress in 1942). and any state-run entities such as Department of Natural Resources (DNR) [26, 27]. The number of animals collected by Charles River, and the amount known to have died or become unresponsive were blacked out in each of the documents provided by the governmental groups. The rule is set up in order to protect fishermen's identities and the locations they found to be good for extraction. Now multinational corporations are hiding behind that same rule, so no one can see how many crabs they are actually killing. "Why the harvesting of public resources should ever be considered confidential business information is beyond me," said Jason Rylander, a senior counsel at Defenders of Wildlife, a nonprofit focused on animal conservation.[28]

The ASMFC 2019 stock assessment mentioned the lack of transparency about biomedical data across the coast getting in the way of the commission's job. But thanks to an unusual gap in the harvest in South Carolina, there were indications of the company's impact on the local population anyway. Charles River closed all bleeding operations for building renovations in 2016. Before the construction, when Charles River was bleeding horseshoe crabs like it usually

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[24] Reuters. "Factbox: Countries Making COVID-19 Vaccines Mandatory." *Reuters*, 8 Oct. 2021, www.reuters.com/business/healthcare-pharmaceuticals/countries-making-covid-19-vaccines-mandatory-2021-08-16/.

[25] Iovenko, Chris. "The Fight to Save Horseshoe Crabs from the Biomedical Industry." The Verge, 17 Dec. 2021, www.theverge.com/2021/12/17/22840263/horseshoe-crab-blood-medical-industry-controversy.

[26] *Atlantic States Marine Fisheries Commission Compact & Rules and Regulations*. 2016, www.www.asmfc.org/files/pub/CompactRulesRegs_Feb2016.pdf.

[27] *Atlantic States Marine Fisheries Commission*. 2019, www.asmfc.org/files/commissionerManual/ISFMP/HSC%20Stock%20Assessment%20Overview%202019_Final.pdf.

[28] Eisner, Chiara. "Vaccine Testing Is Changing. Why Is This $13B Lab Still Bleeding SC Horseshoe Crabs?" *The State*, 7 Mar. 2022, www.thestate.com/news/local/environment/article248306895.html.

does, the 2015 South Carolina DNR annual report showed that the density numbers for the population in deep water had continued to decline since 2011. However, the next year, when Charles River did not bleed a single horseshoe crab from the state, the records show the density of horseshoe crabs in 2016 was higher than the estimated density in 2015 and was the third highest since 1995, the first year of comparable data. When Charles River returned to harvesting crabs like usual the following year, the density of the animals reverted to a depressed level similar to the one reported before.[29]

Further highlighting the relationship between pharmaceutical companies, industry and government is the lease Charles River has with South Carolina's DNR. In this relationship, Charles River Laboratories, pays the state of South Carolina's DNR near ly $1.5 million a year to lease Morgan Island [infamously referred to as Monkey Island], which was bought with $20.5 million federal tax dollars in 2002. Additionally, Charles River offered a $500,000 in the fall of 2021 for a special "biomedical research license", an offer DNR eventually refused. [30]

Studying the Atlantic Horseshoe Crab and the dwindling population, relationships between industry and government are identified and shown to be linked in self-regulated ways. These relationships are exasperating the demise of an ancient species, as well as mirroring our own situation as humans; highlighting the interconnectivity of all life on Earth.

[29] Eisner, Chiara. "Vaccine Testing Is Changing. Why Is This $13B Lab Still Bleeding SC Horseshoe Crabs?" The State, 7 Mar. 2022, www.thestate.com/news/local/environment/article248306895.html.

[30] postandcourier.com, TONY BARTELME and SHAMIRA MCCRAY. "UNCOVERED: Monkeys and Blood/DNR Gets Millions from Pharma Firm It Regulates." *The Times and Democrat*, 6 Mar. 2022, thetandd.com/news/local/uncovered-monkeys-and-blood-dnr-gets-millions-from-pharma-firm-it-regulates/article_cd93f2ab-0ed5-5d22-bada-6d2f9424b6d8.html.

Figure 1: A dead horseshoe crab on the Connecticut shoreline. Photo by Molly Gambardella, April, 2022.

Figure 2: Taken at the Charles River Laboratory in Charleston, South Carolina. Photo by Timothy Fadek / Corbis via Getty Images, 2014

Figure 3: The Rabbit Pyrogen Test (RPT): https://www.mat-biotech.com/pyrogen-testing

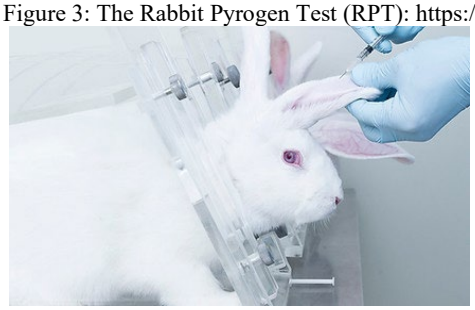
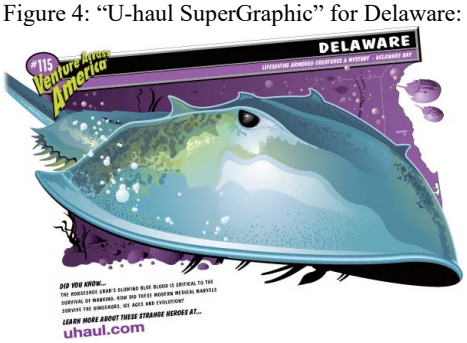


Figure 4: “U-haul SuperGraphic” for Delaware: https://Uhaul.com/Supergraphics/Delaware/Learn-More/Introduction, 2022.



Text on truck: “Did you know the horseshoe crab’s glowing blue blood is critical to the survival of mankind. How did these modern wonders survive the dinosaurs, Ice Ages and evolution?”