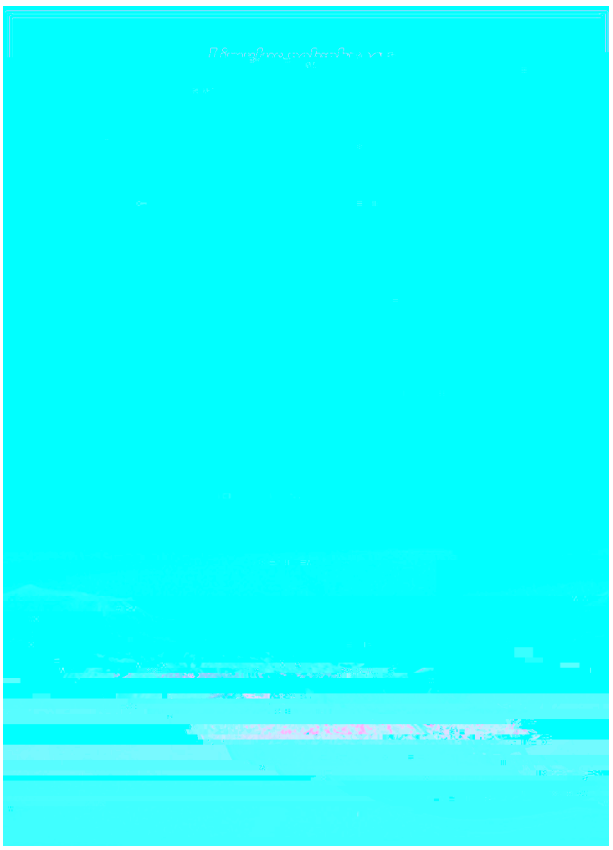

FIGURE 1 | Basic anatomy of the horseshoe crab (*L. polyphemus*) **(Top)**; and photograph of a mature female **(Bottom)**.

This ancient aquatic arthropod, more closely related to scorpions and spiders than to crabs (Størmer, 1952), belongs to its own distinct class, Merostomata (Woodward, 1866), literally meaning "legs attached to mouth." The name "horseshoe crab" is derived from the Limulus polyphemus' most recognizable features, its extended prosoma (or cephalothorax), a large shell that resembles a horseshoe (Figure 1). Commonly referred to as a "living fossil," the horseshoe crab has been able to survive nearly unchanged for an estimated 200 million years (Walls et al., 2002; Kin and Blazejowski, 2014)–prior to recent population

FIGURE 2 | Diagram of horseshoe crab circulatory system (Diagram modified from Patten, 1912; copyright permission, reuse or modifications of **Figure 2** are not required for this image as it is in the Public Domain and holds no copyright).

However, as the increasing biomedical industry requirements of horseshoe crab blood and the species link to migratory shorebirds viability were realized, new oversight agencies were established to mediate the risks from over-harvesting, and restrictions were placed on the number of horseshoe crabs collected for bait in order to regulate populations. These agencies further generated programs for stock management, developed state quota regulations, and established best practices for biomedical harvesting. In 2015, 583,208 horseshoe crabs were harvested as bait for eel and whelk (Atlantic States Marine Fisheries Commission, 2016), a significant reduction from the millions that were once harvested (Atlantic States Marine Fisheries Commission, 2013).

IMPACT OF AMEBOCYTE HARVESTING ON HORSESHOE CRAB BEHAVIOR AND PHYSIOLOGY

The Atlantic States Marine Fisheries Commission (ASMFC) reported that in 2015, 559,903 horseshoe crabs were transported to biomedical facilities for the production of LAL (Atlantic States Marine Fisheries Commission, 2016). The raw materials for the preferred LAL test require careful extraction of blood from horseshoe crabs. Established methods entail introduction of a hypodermic needle placed directly into the exposed pericardial membrane of the horseshoe crab to draw from 50 to 400 mL of blood, depending on the sex and maturity of the horseshoe crab (Figure 2). The plasma is centrifuged, and LPS-free reagents, such as Na_2EDTA or 3% NaCl, are added to help prevent clotting after extraction; this can occur as a result of the unintended introduction of endotoxins or other external factors, including undue stress during extraction and exposure to extreme temperatures. Cxr05(t)

prospects for overall horseshoe crab population growth (Atlantic States Marine Fisheries Commission, 2013). Later addenda to the Horseshoe Crab FMP specified annual state-by-state landing quotas across the east coast and contributed to the establishment of the Carl N. Schuster Jr. Horseshoe Crab Reserve, a 1,500 square mile harvest-free zone (Atlantic States Marine Fisheries Commission, 2000). Historically, the most comprehensive data on horseshoe crab abundance has been based on the Benthic Trawl Survey conducted by Virginia Polytechnic Institute (VPI), but the survey faces inconsistent funding circumstances. Other studies including the Delaware Trawl Survey, the New Jersey Delaware Bay Trawl Survey, and the New Jersey Ocean Trawl Survey have been established and helped intermittent funding limitations (Smith et al., 2016). Funding for future years of the studies is undergoing evaluation.

Addendum IV of the FMP delayed harvest in Maryland and Virginia and restricted bait harvest in Delaware and New Jersey to 100,000 male-only crabs. It was approved in May 2006; and the addendum was extended through October 2013 (

febrile reactions and thus requires no horseshoe crab byproducts, in contrast to the LAL and rFC assays (Stang et al., 2014). The MAT has been used reliably to resolve discrepancies between LAL test results; however, it has been shown to be ine□ective in the presence of cytotoxic agents (Dobrovolskaia et al., 2014; Stang et al., 2014). The standard MAT procedure also lacks the sensitivity to detect the required amount of pyrogens on medical surfaces (which is also a limitation of the LAL assay). While the MAT has been optimized to detect such pyrogens, including the ability to ensure sensitivity by incubating test materials in the MAT, the modified version can take up to 20 h and is therefore too time-consuming for practical application in most settings (Stang et al., 2014).

Although the rFC and MAT methods produce results comparable to the LAL test (Alwis and Milton, 2006; Thorne et al., 2010; Hermanns et al., 2011) while conserving the horseshoe crab and surrounding ecosystems, the widespread adoption of these alternative tests may prove to be extremely challenging. The industry has been reluctant to transition to newer methods due to the complex validation procedure and subsequent redesign of the manufacturing processes that would necessarily accompany the change to procedures that have been established and followed for approximately 40 years (Cohen, 1979; U. S. Department of Health and Human Services, 2012).

In fact, revising the current system to improve e□ciencies in horseshoe crab use may be more viable in the near term. Rather than adopting alternative tests, some biomedical companies have opted to make existing tests more sustainable. For example, LAL assays with specially designed cartridges have been developed to reliably screen for endotoxins, while also using one-twentieth of the raw horseshoe crab material required by conventional LAL tests (Wainwright, 2013).

Another alternative would be the use of a line of amebocytes that could be cultured in vitro. Research in this arena has yielded promising but inconsistent results (Joshi et al., 2002; Hurton et al., 2005); whereas, mounting pressures on the harvest of horseshoe crabs may yet help justify continued e□orts and investment into this approach.

ALTERNATIVES TO CURRENT HORSESHOE CRAB HARVESTING PRACTICES

As more may be learned from further study, ranching of horseshoe crabs could be considered to help replenish populations. An instructive 56-day study of horseshoe crabs in captivity revealed decreases in body weight and deteriorating health, as reflected in various biological markers, including hemocyanin and amebocyte concentrations, which declined significantly (Coates et al., 2012). Although these changes occurred at all temperatures over time, horseshoe crabs held in higher temperatures (23°C) experienced the most significant decreases in these key metrics. To achieve the lowest horseshoe crab mortality and highest blood quality during biomedical bleeding, a more systematic understand

this approach has not been conducted on a large scale, and any objections to egg collection that might interfere with shorebird feeding would need to be addressed before advancing this notion to a broader initiative (Mishra, 2009; Schreibman and Zarnoch, 2009).

Finally, various steps could be employed to reduce fishing industry demands on wild horseshoe crab populations, such as alternative and/or synthetic baits for whelk and eel, which could be used in lieu of horseshoe crabs. Such alternatives utilizing reduced quantities of horseshoe crab have been researched and field-tested with encouraging results (Ferrari and Targett, 2003; Fisher and Fisher, 2006).

As horseshoe crabs harvested for bait have outnumbered biomedical counts in recent years (Atlantic States Marine Fisheries Commission, 2016), a reduction in bait harvest is vital for conservation of horseshoe crab populations. Yet, because biomedical harvesting does not typically result in immediate mortality, the full impact might be underestimated and unaccounted for once fatigued, traumatized, and sometimes

the theory and performed primary research reviews. AD, KD, CK, and TB verified the materials and methods. TB encouraged AD, JK-G, KD, and WA to investigate the biomedical medial industry impact on horseshoe crabs. AD supervised the findings of this work. All authors prepared analysis of the primary research and contributed to the final review manuscript.

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