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Assessing the Current State of Louisiana's Crawfish Fishery: Trends and Challenges in Wild Capture and Aquaculture

Mahala G. Gambill

ASSESSING THE CURRENT STATE OF LOUISIANA’S CRAWFISH FISHERY: TRENDS AND CHALLENGES IN WILD CAPTURE AND AQUACULTURE

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Mahala Grace Gambill
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Abstract

Crawfish are found throughout the southern U.S.; however, Louisiana is by far the largest producer. The state's industry includes harvesting wild crawfish from natural habitats such as bayous, swamps, and marshes, and crawfish reared in outdoor ponds. Although commercial harvest records are available, characterization of Louisiana's wild capture crawfish fishery in recent years is undocumented, and analysis of environmental variables that could be correlated with annual harvest totals have not yet been explored. Using trip ticket data from the Louisiana Department of Wildlife and Fisheries, I characterized monthly and annual trends within Louisiana basins to assess changes in crawfish harvest over the past 20 years. To understand the wild harvest fishery through changes in hydrological conditions within the Atchafalaya Basin, where more than 80% of wild harvest occurs, I used a linear regression to look at the effects of Atchafalaya River discharge and monthly precipitation accumulation on monthly crawfish harvest from 1999–2020 during months of peak harvest.

Louisiana's crawfish industry is comprised of two unique yet similar components: wild harvest and pond production. Research on crawfish biology and improved methods for pond production has been ongoing since the early 1960's. Challenges within the crawfish aquaculture industry are documented but of most recent concern is the emergence of white spot syndrome virus (WSSV). WSSV possesses the ability to create mass mortality outbreaks with little notice to farmers. Outbreaks often present in multiple conditions and can be hard to quantify. While white spot syndrome virus was first documented in Louisiana crawfish ponds in 2007, recorded laboratory testing that provides spatial and temporal outbreak data are available beginning in 2020. To better understand WSSV and the challenges it presents within Louisiana's crawfish industry, I used data from the Louisiana Animal Disease Diagnostic Lab on positive testing

ponds. In addition, I recorded pond temperature to assess spatial and temporal trends to better understand the effect of environmental variables on a viral outbreak

Chapter 1. Wild harvest of crawfish in Louisiana

1.1. Introduction

Louisiana is well known for its wild capture crawfish fishery, where recreational and commercial fishermen have harvested crawfish from the lower Mississippi River floodplain since at least the late 1800s. Additionally, some evidence suggests that commercial trade in crawfish existed during the early 1800s, with the first commercial recorded harvest occurring in 1880 and consisting of 23,400 pounds worth $2,140. (McClain and Romaire, 2004). Record keeping before the late 1950s is limited; however, since 1988 commercial harvests have averaged 17 million pounds per year, with a high of 50 million pounds in 1993 and a low of 392 thousand pounds in 2000 (Isaacs and Lavergne, 2010).

1.1.1 Commercial crawfish harvest

To harvest wild crawfish legally for commercial purposes, an individual must hold two licenses: a commercial fishing license and a commercial crawfish trap license (Louisiana Commercial and For-Hire Fisheries Rules and Regulations, 2021). Crawfish are typically caught in baited wire mesh traps, soaked in ponds, swamps, or slow-moving rivers. There are no limits on season, size, or possession for commercial crawfish harvests except when specified within wildlife management areas where commercial crawfishing may be allowed. The Louisiana Department of Wildlife and Fisheries (LDWF) is responsible for monitoring and managing wild crawfish through gear, licensing, and reporting requirements. Harvest controls are not necessary to protect the crawfish resource as their populations are resilient and influenced by environmental conditions rather than fishing mortality (McClain et al. 2007). While biologists conduct studies on wild crawfish, they do not sample, monitor, or survey the populations as with other fishery resources. Currently, only one program is set to collect commercial fishery dependent data on the inland fisheries in Louisiana. This data includes commercial crawfish harvest. Under Louisiana law, when a licensed commercial fisherman sells or transfers his catch to a wholesale or retail seafood dealer, he must present his license to the dealer for license verification and provide the dealer with information necessary to complete a commercial trip ticket (Isaacs 2020, LDWF 2022). The Trip Ticket Program has officially been collecting commercial landings data in Louisiana since its implementation under Louisiana Department of Wildlife and Fisheries (LDWF) in January 1999. As landings are sold to wholesale or retail seafood dealers, the dealers must collect specific information about the fisher, vessel, and dealer as well as the area fished, species landed, quantity, and dockside value. Each month dealers will send the data back to LDWF who maintain a database of trip data. Commercial fishermen who sell their catch directly to consumers are also required to complete and submit trip tickets.

Louisiana is home to over 39 different species of freshwater crawfishes (Walls 2009), but the red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus zonangulus*) make up nearly the entire annual commercial crawfish harvest (McClain and Romaire, 2004). Furthermore, wild crawfish is the most economically important freshwater species recorded through the trip ticket program. In 2016, crawfish landings alone accounted for $12.6 million of the freshwater fisheries landings, which is about 70% of the total value for freshwater fisheries landings for that year (Bonatakis, 2019).

1.1.2 Crawfish Biology and Life History

The genus *Procambarus* includes crawfish belonging to the family *Cambaridae* and currently includes 161 species (Longshaw et al. 2016). However, the characterization of Louisiana's crawfish fisheries is encompassed by the two previously identified *Procambarid* species common to Louisiana. The red swamp crawfish is native to the Southern Mississippi Valley and Northern Mexico although it has been introduced in other areas of North America, Europe, Africa, Asia and South America and commercial harvests have been recorded from Europe, Asia, and Africa (Huner and Barr, 1991). The white river crawfish naturally occurs in the southern states along the Gulf of Mexico and northward up the Mississippi River drainage, possibly as far as the confluence of the Mississippi and Ohio Rivers (McClain and Romaire, 2007). Red swamp and white river crawfishes have similar habitat requirements that include riparian zones that experience natural cycles of flooding and drying, conditions that are common to much of Louisiana.

In spring, mating occurs in the open waters. As the water levels decrease in subsequent seasons and the temperatures increase, females retreat to burrows to continue the reproduction process. Crawfish of all ages, sizes, sexes, and maturity stages will dig or retreat to burrows to survive periods of dewatering. Crawfish burrows are usually dug by a single individual, with burrow diameter determined by the size of the crawfish and usually containing a single female, or sometimes a male and female together (Correia et al. 1995, Longshaw et al. 2016). Successful survival and reproduction within the burrows depend on many factors, such as the severity and length of the dry period, characteristics of the burrow, and health of the animal, but it is evident that Louisiana crawfish have evolved over millions of years to reproduce within the protection of their burrows (Duffy and Thiel, 2007). When water levels increase, all crawfish begin to emerge from burrows and hatchlings are released from the tails of females. Juveniles mature during winter and spring while water levels are high. Wild crawfish harvest is driven by varying patterns of seasonal hydrology from the Mississippi River and precipitation patterns in the upper and middle Mississippi River Valley watershed and thus aligns with the life history of these crawfish species (McClain et al. 2004).

The seasonality of wild crawfish harvest relies on many environmental variables that allow for spawning and growth of individuals (McClain et al. 2007). Many of Louisiana's coastal basins contain flooded, low-lying areas, areas that flood periodically with drier periods, and upland forests. Species indigenous to these basins have adapted to this cycle of flooding and drying, as have the animals that feed on crawfish. Although crawfish are steadily abundant some years because of increased water levels in the basins and other natural wetland areas, crawfish landings notably fluctuate in other years. The variable annual abundance of these crawfish may be influenced by changing water levels and other environmental factors.

It is documented that flood regime plays an important role in fisheries production in Louisiana River basins across multiple species (Alford et al. 2013). An increase in flood duration and magnitude should benefit populations of organisms that utilize floodplain habitats by enhancing their growth and reproductive output. That is, density-dependent effects caused by mating and nursery habitat availability increases will lead to greater biomass and growth of the organism. Also, density-independent effects may lead to greater forage production because of high nutrient loads diverting from the Mississippi River during each flood. I predicted a positive correlation between flood duration, magnitude, and crawfish abundance. Another environmental variable, precipitation, is of interest as river discharge and precipitation totals are not entirely correlated. That is, discharge can be high due to precipitation elsewhere in the state or country when local precipitation totals are low. The inverse could also be true: local precipitation accumulation can be high when river discharge levels are low.

Landings data were analyzed from the trip ticket program (1999–2020) to answer three questions. These questions are:

1) How have wild crawfish landings changed over the past 20 years,

2) Does the magnitude of Atchafalaya River flow predict wild crawfish landings on a monthly and annual basis, and

3) Does the presence of drought in the Atchafalaya River basin predict crawfish landings on a monthly and annual basis.

1.2. Materials and Methods

1.2.1 Data Sources

Data on wild crawfish landings was provided by the Louisiana Department of Wildlife and Fisheries Trip Ticket Program and contained wild crawfish harvest data from 1999–2020. The trip ticket information is completely confidential and protected under state and federal law. As such, any information leading to fishermen identification is withheld. Additionally, any data manipulation that leads to three or less individual sale records being reported could lead to fishermen or dealer identification (LA Rev Stat § 56:301.4). Due to these confidentiality regulations, data was accessed for the geographic area at the river-basin level. When delimited to parish, sometimes there are less than three records in that area. Data recorded on individual trip tickets includes the common name of the species (both prominent crawfish species are recorded as "wild crawfish"), month and year of harvest, quantity of crawfish landed (in pounds), the value of total landing ($), and river basin of crawfish harvest. Data from trip tickets measures crawfish harvest, which is usually quantified by unit effort. However, effort is not measured in this fishery as with other fisheries, but recorded landings serve here as a proxy for crawfish production as recorded landings crawfish increase as crawfish abundance increases due to favorable environmental conditions.

Data on river flow was acquired from The U.S. Geological Survey's (USGS) National Water Information System (NWIS), an application that supports the acquisition, processing, and long-term storage of water data. Water Data for the Nation serves as the publicly available portal to much of the water data maintained within NWIS. Monthly summaries of flow data from 1999–2020 were accessed from the Simmesport observation site (site number: 61608).

Monthly precipitation accumulation totals (mm) are from the Norm91m dataset compiled using a parameter-elevation regression on independent slopes model (PRISM) (PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, data created 4 Feb 2014). PRISM is a high-resolution model for climate data estimations and was developed by Oregon State University (Jeong et al. 2018). Monthly and annual averages for multiple climate elements are available at a grid resolution of 4km. Values were clipped to fit the Atchafalaya basin shapefile and totaled monthly (Figure 1.1).

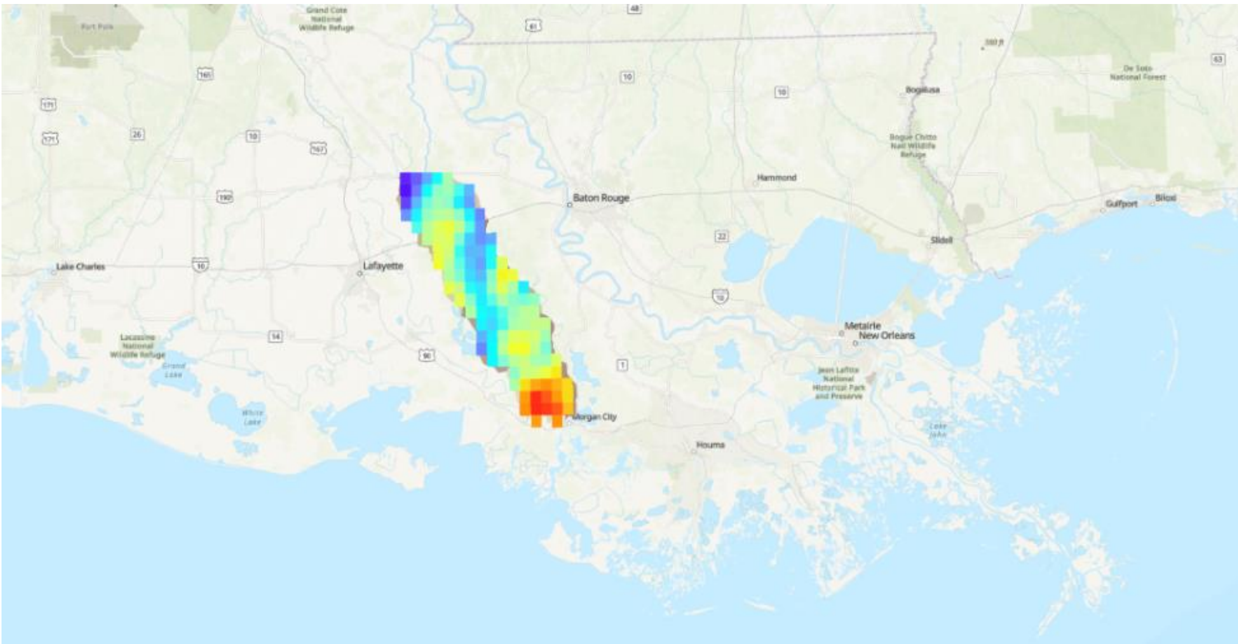

Figure 1.1. Highlighted area denotes the Atchafalaya River Basin in which 4km PRISM grid cells were superimposed on this shapefile in ArcGIS to compile monthly precipitation accumulation throughout the basin.

1.2.2 Analysis

Over the past 20 years, commercial landings data were analyzed for trends in annual landings and value. To summarize Louisiana wild crawfish harvest, measures of variability were assessed for each basin, and across all years and months containing recorded landings. Regarding trends in annual harvest due to seasonality, a simple linear regression was used to evaluate annual landings as a response to the time of harvest (month and year). After state-wide descriptive analyses were complete, I focused on analyzing only the Atchafalaya River Basin (ARB), because it accounted for more than 85% of landings recorded in all basins and thus represented the most robust basin for in-depth analyses of environmental factors over time.

Before modeling the association between crawfish production and hydrological and metrological variables in the ARB, a beta regression was used to determine which years included landings that were statistically different that the annual average across the dataset. A beta regression is commonly used for modeling proportions as the beta distribution is for interval data contained between 0–1. Initial model selection focused on my intent to model the cumulative proportion of annual catch by month where each month per year would contain a value between 0–1 as landings increase annually. *Year* was treated as a fixed effect so that multiple comparisons could be run to compare years. Years with large slope estimates indicated landings taking place earlier in the year as most of the annual catch was reported over a shorter time frame, whereas years with lower slope estimates indicated a more gradual increase in crawfish landings throughout the year. For this analysis, beta regression was used with the R package "betareg" using the *logit* link function (Cribari-Neto and Zeileis 2010).

To investigate the impacts of river discharge on landings within the ARB, a simple linear regression was implemented. Annual landings was the response of lagged discharge across the months of peak harvest from 1999–2020. Monthly discharge values were lagged one month to compare previous month discharge with subsequent monthly landings as I assume a temporal disconnect present between monthly discharge and recorded landings and that river discharge may affect growth or survival prior to landings; in other words, previous months' river conditions may influence landings. Similarly, a simple linear regression was used to examine annual crawfish landings as a response of lagged monthly precipitation accumulation throughout the ARB for months of peak harvest. All statistical analyses were performed with R programming (R Core Team 2020).

1.3. Results

Since the trip ticket program began in 1999, a total of 237,940,688 pounds of wild crawfish landings have been recorded across multiple basins. This equates to a value of $209,302,298. 89% of these landings occurred within the Atchafalaya River Basin (211,705,463 pounds and $186,482,417; Figure 1.2). Because most of the total landings are contained within the ARB, further analysis exclusively focuses on landings from this basin. Annual landings in the ARB ranged from 384,168–16,920,744 pounds across all years. Similarly, the lowest value in one year was $671,292 while the highest was $14,365,839. Monthly landings ranged from 1,232 –6,270,530 pounds with across all years. This variability is expected due to the annual cycles of when crawfish are harvested. The highest monthly landings of wild crawfish were recorded in May of 2013, in the ARB. Monthly landings of wild crawfish were mostly contained within the months of February to June (Figure 1.3).

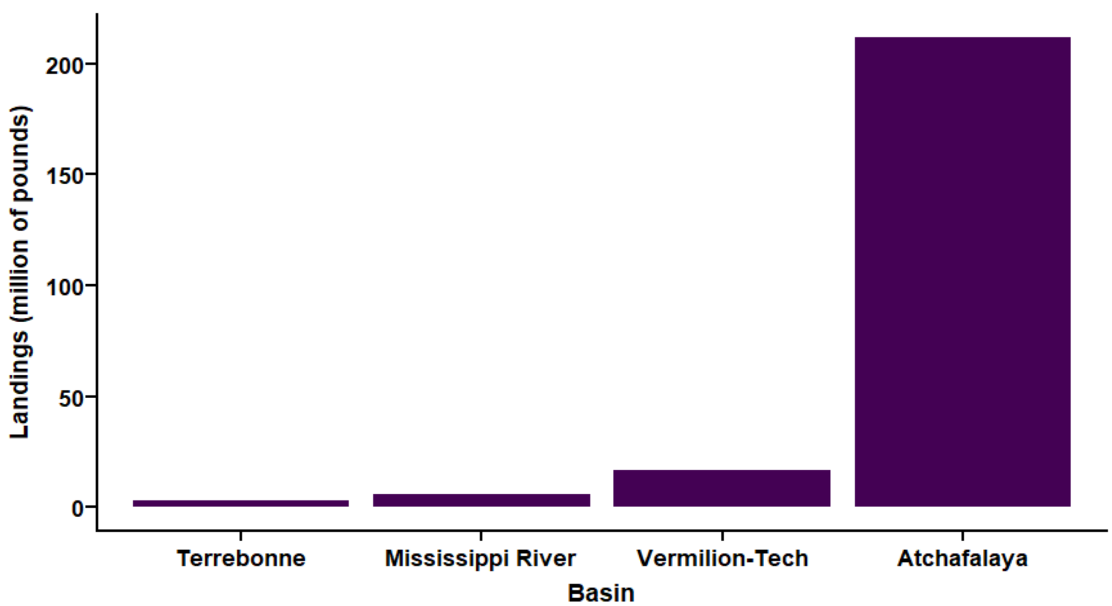

Figure 1.2. Bar plot of total crawfish landings in millions of pounds from 1999–2020 in four major river basins in Louisiana. The Atchafalaya River basin contains most crawfish landings (89%) over the entire period of study.

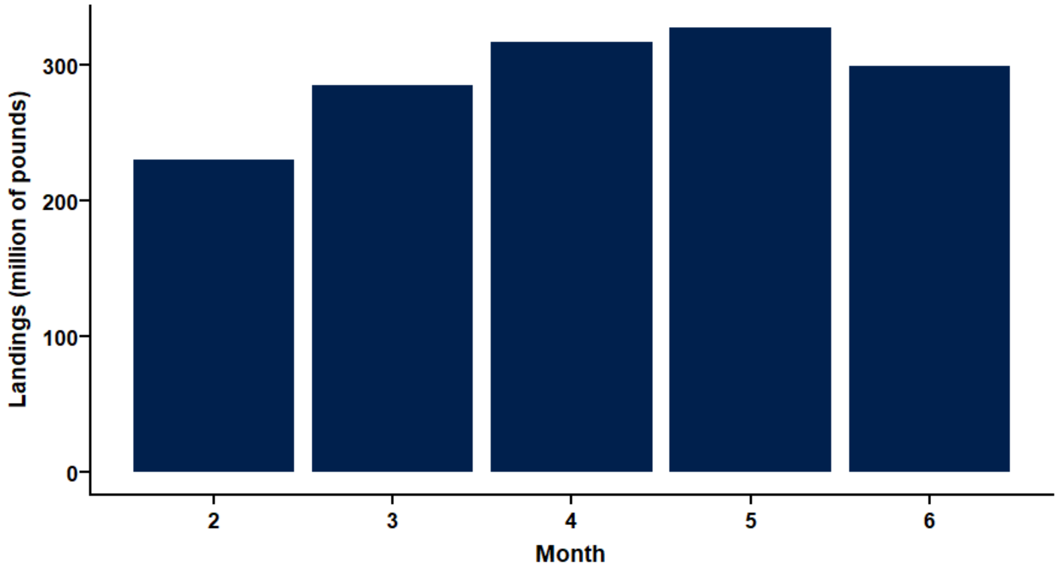

Figure 1.3. Bar plot of total crawfish landings in millions of pounds from 1999–2020 by month in the Atchafalaya River basin.

The beta regression estimated that annual landings proportions in 2000, 2006, 2011 and 2012 and were statistically different from all other annual landings (Figure 1.4). A simple linear regression of lagged monthly discharge in the ARB predicting monthly wild crawfish landings was only significant in the months of February (p <0.001) and March (p < 0.05) (Figure1.5). A simple linear regression of lagged monthly precipitation accumulations in the ARB were not significant in predicting the landings of any month during peak wild crawfish harvest (Figure 1.6).

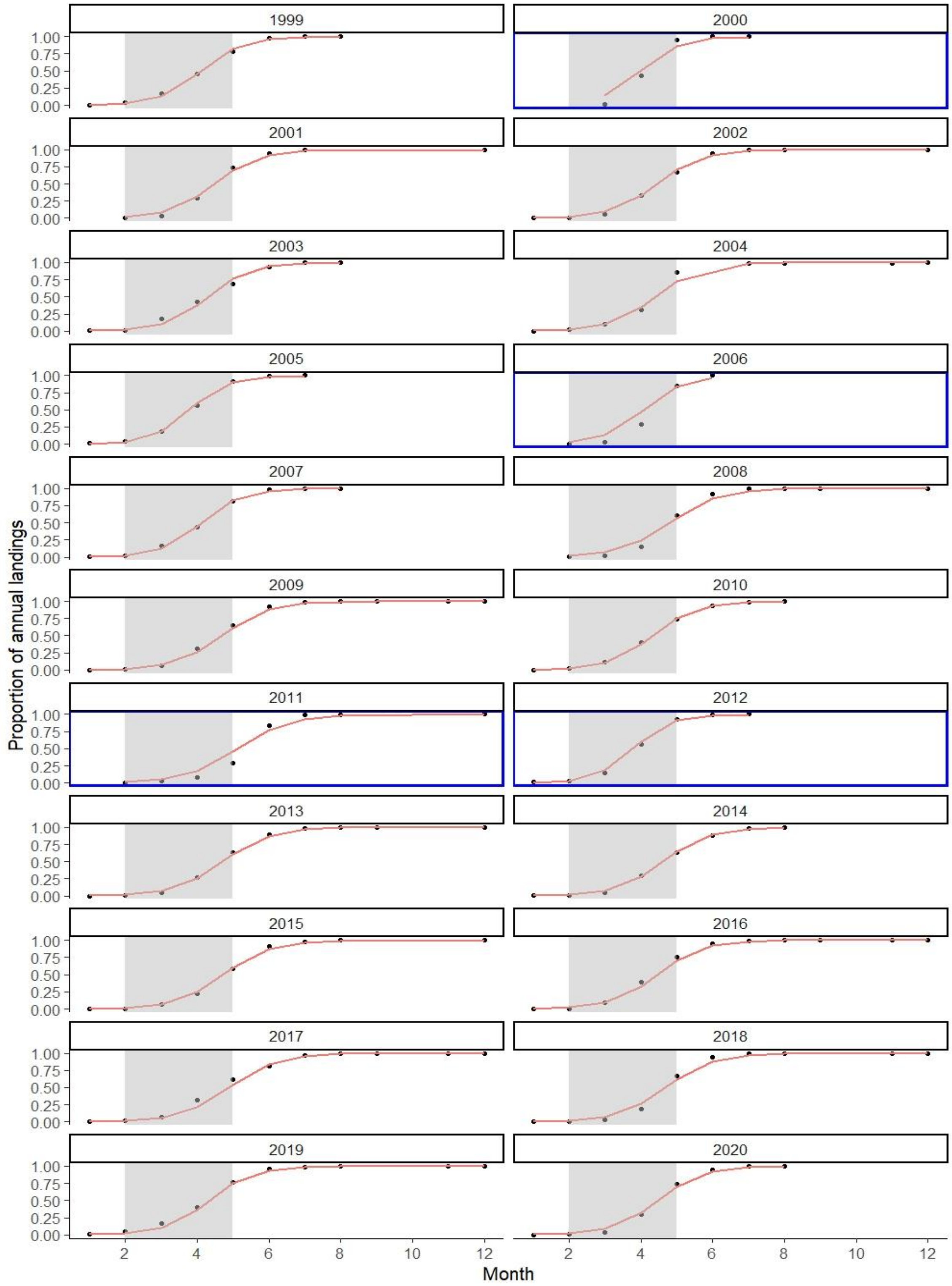

Figure 1.4. Proportions of monthly landings throughout each year. A beta regression showed significant years that are highlighted with a blue border. Each point represents the annual proportion of monthly landings accumulated in that month and previous months of the same year. Months of peak harvest, February-May, are highlighted in grey.

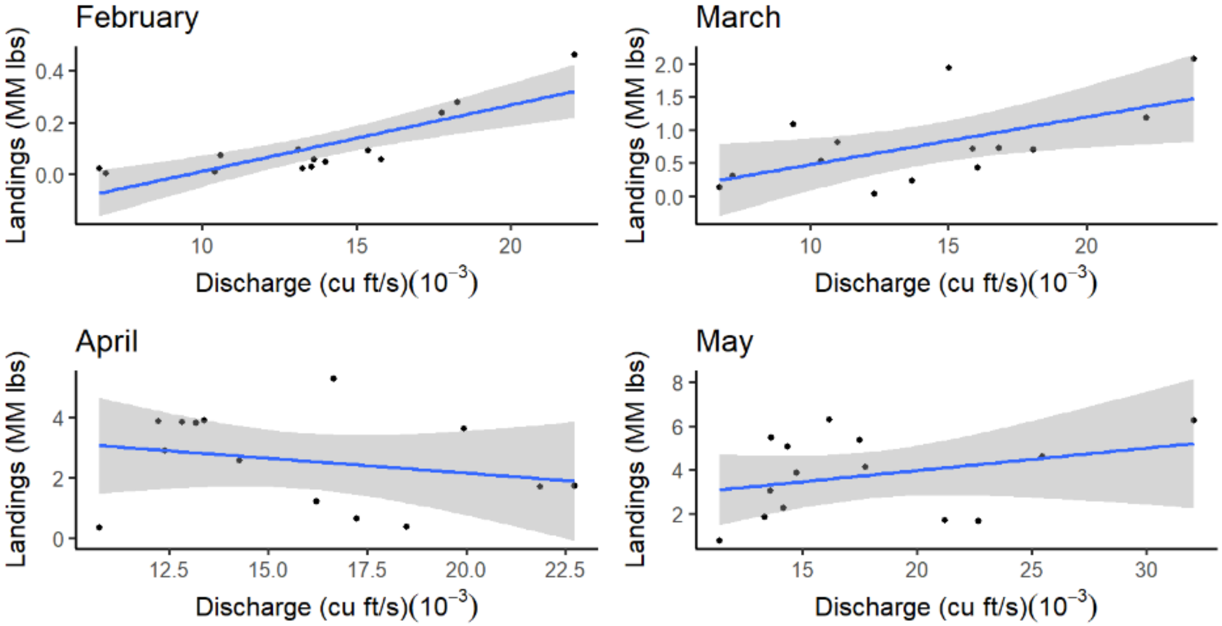

Figure 1.5. A linear regression for monthly landings in peak harvest months (February–May) as a response of monthly discharge of the previous month.

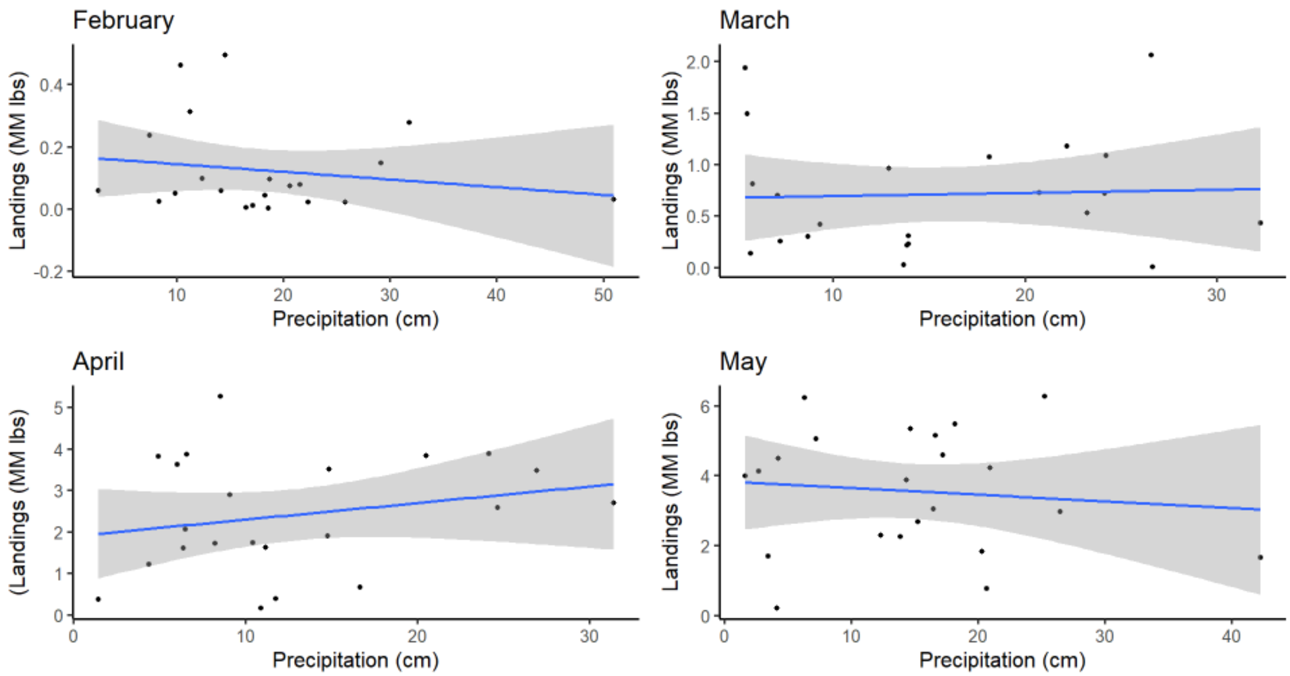

Figure 1.6. A linear regression for monthly landings in peak harvest months (February-May) as a response of monthly precipitation accumulation of the previous month.

1.4. Discussion

Assessing differences in crawfish harvests on an annual basis over the past 20 years may provide insight into the effects of localized and statewide weather events as well as reporting discrepancies across the implementation period of the trip ticket program. In 2000, not all months recorded harvest and the proportion of landings as time progressed through spring months of the year shows a positive correlation as only five months of the year had reported landings. This could be due to a lack of reporting of crawfish harvest within the Atchafalaya basin or could show the effects of implementation of the trip ticket program as the program was only enacted one year prior. 2005 was marked by a weather event well known to most Gulf Coast state residents. Hurricane Katrina made landfall in Louisiana on August 29, 2005, flooding all parishes around Lake Pontchartrain and widespread flooding developed due to storm sure over southeastern Louisiana. 2011 was characterized by a massive flooding even during the early Spring and the Bonnet Carre spillway was partially opened on May 9, 2011. While proportioned landings in both 2011 and 2012 were significantly different from other years, landings in 2012 could be a more direct reflection of hydrological variables regarding increased crawfish abundance due to increased flooded habitat availability. While crawfish landings in this analysis came from the Atchafalaya River Basin, further analysis coupling parameters from the Mississippi River over different time intervals could reveal additional correlation between extreme weather events and increased harvest in fisheries in following years.

In addition to localized extreme weather events that may lead to greater production of forage because of high nutrient loads being diverted from the Mississippi River during each flood, analysis of monthly discharge seems to be most influential in the early Spring. Localized precipitation is not as influential in predicting crawfish abundance, which is not entirely correlated to river discharge because discharge measurements can be high due to precipitation elsewhere in the state or country when local precipitation totals are low. To what extent this reduction in wild harvest might reflect long-term trends in water management, climate, and habitat alteration remains uncertain. On a global scale, climate change is expected to increase the frequency, intensity, and impacts of some types of extreme weather events. For example, sea level rise increases the impacts of coastal storms and warming can place more stress on water supplies during droughts (Smith et al. 2010, Portmann et al. 2008). As research develops on the effects of climate change, specifically on hurricane and drought events, fisheries data analysis could provide insight into updated management measures.

Historically, the harvest of wild crawfish has occurred in Louisiana. In recent years, however, many of the traditional areas of wild harvest do not reflect the abundance of crawfish present to harvest. Since 2000 less than 20% of Louisiana's crawfish harvests on average have come from the wild fishery (LDWF, Freshwater Fishery Report 2019), which clearly demonstrates either environmental changes resulting in low catch, the dominance of the crawfish aquaculture industry or a combination of both. Also of importance is effort within the wild crawfish industry, however, the number of wild harvesters in operation is not documented within the data used. Additional analysis of fisherman effort since 2000 could further address the relationships between crawfish landing reporting and crawfish production.

Also contributing to the decline observed in wild crawfish caught is the non-sustainable nature of wild harvest. The harvest of wild crawfish is decreasing while the demand grows, a trend leading to a fishery that cannot be sustained through wild harvest alone. Similarly, the price of crawfish is constantly increasing because of the rising demand and lower supply. Furthermore, seasonality of wild crawfish harvest relies on many environmental variables that allow for spawning and growth of individuals to reach a size desirable for market (McClain et al. 2007). In southern Louisiana, the Atchafalaya River Basin consists of a network of diverse flood plain habitats that are hydrologically connected at varying degrees during winter-spring floods. These seasonal floods bring nutrients and unpredictable harvests which coupled with increasing consumer demand, provided much of the motivation for development of crawfish aquaculture in the late 1940s (Avery and Lorio 1996). During this time, rice farmers developed a method to farm crawfish which provided an opening for expansion of the farm-raised crawfish industry.

Today, it is generally thought that farm-raised and wild-caught crawfish crops complement each other—farm-raised crawfish are available later in the season as during low flow periods (late summer–fall), natural habitats lose much of their connectivity and the circulation of flow throughout the network of the ARB is severed (Alford et al. 2013). Although the annual timing, amplitude, and duration of the flood pulse varies, typically, peak water levels, and ARB floodplain inundation tend to occur in the late spring with the drawdown period from late summer through early fall (Fontenot et al. 2001, Bonvillain et al. 2008, Piazza 2014). As such, the effect of time on wild crawfish landings is likely related to the growth of crawfish aquaculture within the industry.

Louisiana is well known for its wild capture crawfish fishery, where recreational and commercial fishermen have harvested crawfish from the lower Mississippi River floodplain since at least the late 1800s. Annual landings from the past 20 years are greatest within the Atchafalaya basin. However, since 1999, annual landings have decreased within this basin alone. Providing those who still participate in the wild crawfish fishery with more information on how their catch and livelihood may fluctuate due to environmental conditions can help make them more resilient in the future. It is known that environmental conditions influence crawfish harvests (Huner et al. 1988, Romaire and Lutz 1989), but no other study has aimed to directly quantify the influence of changing environmental variables on the harvest of wild crawfish in Louisiana.

Chapter 2. White spot syndrome virus in farmed crawfish: spatio-temporal trends and environmental factors

2.1. Introduction

Although crawfish have social, economic, and ecological significance in several regions around the world, Louisiana dominates the crawfish industry of North America in both aquaculture and wild capture fisheries. Wild crawfish have been recreationally and commercially harvested in Louisiana for years, but harvest is often limited to four months out of the year. Unpredictable harvests and increasing consumer demand provided much of the motivation for development of crawfish aquaculture. In the late 1940s, rice farmers developed a method to farm crawfish. Today, farm-raised, and wild-caught crawfish yields generally complement each other—farm-raised crawfish are available during late fall through mid-spring, and, if conditions are favorable, wild-caught crawfish dominate the market from mid-spring to early summer. While crawfish are cultivated for food in states neighboring Louisiana, the impact of crawfish cultivation within Louisiana is substantial. Crawfish aquaculture is the most profitable aquaculture endeavor in Louisiana, representing roughly 54% of the total gross farm value generated across all commercial aquaculture enterprises in 2019. Recent field estimates indicated farm-raised crawfish production occupied 247,753 acres, equating to a gross farm value of $201 million (Louisiana Summary Agriculture and Natural Resources 2019).

2.1.1. Crawfish Aquaculture

The creation of crawfish aquaculture as a successful commercial endeavor can be partially attributed to the fact that no highly technical cultivation practices are required to ensure success

(Louisiana Summary Agriculture and Natural Resources 2019). Unlike the rearing of many aquatic species that require hatcheries and formulated feeds, current crawfish farming practices are based on annual hydrological cycles and conditions that reflect the natural environment. Crawfish are notably fecund organisms and can provide sufficient recruitment under natural conditions, removing the need for hatcheries that support other fisheries. Although many areas have naturally occurring crawfish, normally, crawfish brood stock are introduced into new ponds or ponds that have been out of production for a year or more. Stocking of brood stock usually occurs from late spring to early summer prior to the summer draining of ponds. Permanent ponds (ponds only used for crawfish production) usually require no further stocking as the populations are self-sustaining. Ponds are typically drained annually to mimic the natural and seasonal water level fluctuations (McClain et al. 2007).

Crop rotational systems that include crawfish often involve a rice harvesting component. In one rotational approach, crawfish and rice are rotated in the same physical location within a year, and this practice is conducted for several consecutive years. In another rotational strategy, the rice crop is planted in alternate locations annually and crawfish aquaculture then occurs in different fields each year to conform to typical field rotations of the agronomic crops. Ponds are drained during summer to establish a forage crop that serves as the foundation of a detritus-based food web when the pond is reflooded before crawfish harvest begins (McClain et al. 2007). Ponds are reflooded in autumn and water depths are typically maintained at 20–60 cm (Louisiana Summary Agriculture and Natural Resources 2019). The advantages of this system over crop rotation within the same field are that each crop can be better managed, and the crawfish production season can be extended.

The red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus zonangulus)* are the species of greatest commercial importance in Louisiana. Thirty-nine species and sub-species of crawfish have been identified in Louisiana (Walls 2009); however, red swamp crawfish comprises 70–80% of annual crawfish catch in Louisiana (McClain et al. 2007) and represents the most important aquaculture species. The red swamp crawfish is native to the states bordering the Gulf of Mexico from Texas to Alabama, northward up the Mississippi River drainage into Tennessee and Illinois, and southward into eastern Mexico (Louisiana Summary Agriculture and Natural Resources 2019).

2.1.2. White Spot Syndrome Virus

One threat to the success of the crawfish aquaculture industry is the annual recurrence and spread of white spot syndrome virus (WSSV) (Sánchez-Paz 2010). WSSV is highly pathogenic and often induces mass mortality in crustacean aquaculture operations worldwide (Lightner 1996, Flegel & Fegan 2002). Furthermore, the virus has a broad host range and can infect > 90 aquatic crustacean species (Escobedo-Bonilla et al. 2008), including crawfish (Jiravanichpaisal et al. 2001). First described in penaeid shrimp in 1992 in Taiwan (Chen 1995), WSSV continues to plague Taiwan and has contributed to an overall economic loss of billions of dollars in commercial shrimp farms (Lightner 2011). The virus reached the United States in 1995 (Lightner 1996) with the first diagnosed case documented in a South Texas shrimp farm, and it was suggested that the most probable route for its introduction was through an Asian imported frozen-bait shrimp commodity (Hasson et al. 2006). Between late 1995 through early 1997, multiple WSSV cases were documented among feeder crayfish populations at the National Zoo in Washington, DC (Richman et al. 1997). WSSV has also been reported in wild shrimp stocks in the Atlantic off South Carolina and Georgia and was responsible for severe losses of farmed Whiteleg shrimp in South Carolina in 1997 (Prior et al. 2001). WSSV was first identified in natural and farmed crawfish populations in Louisiana in 2007. In farmed shrimp mortality is rapid—typically 3–10 days after infection—and cumulative mortality with individual ponds is generally between 90% and 100%. In Louisiana, farmers can experience greater than 90% mortality in traps when infections occur (Baumgartner et al. 2009).

Although WSSV pathogenesis has been extensively studied, there is currently no effective treatment to cure white spot disease (WSD) (Hernández-Pérez et al. 2020). Perhaps the most crucial stage in the dynamics of virus infections is its mode of transmission. In general, transmission of viruses can occur through two pathways: horizontally (transmitted among individuals of the same generation by direct contact, or indirectly, by ingestion of infected organisms), and vertically (virus is passed from an infected female parent to her F1 progeny). However, some modes of transmission are more effective than others: transmission by ingestion of infected tissue is over an order of magnitude higher than cohabitation transmission (Lo et al. 1996, Lotz et al. 2002). Similarly, studies completed on red swamp crawfish in Louisiana support this theory that when a crawfish dies from WSSV, large crawfish eat it and become infected, while small crawfish avoid interacting with larger ones and so remain uninfected. However, results indicated no difference in mortality patterns among size groups (Lutz, 2022).

Additionally, some studies have shown that WSSV can remain latent in organisms without causing mortality, thereby preserving the virus in the environment or host until conditions are suitable for an outbreak (Tsai et al. 1999; Sanchez-Martinez et al. 2007). As such, crawfish aquaculture systems face three potential disease states regarding WSSV: (1) uninfected with white spot syndrome virus, (2) infected with virus but not showing signs of infection, or (3) infected with virus and presenting with signs of infection. Signs of disease outbreak are usually characterized by lethargic crawfish that do not move much once they are collected from the trap and floating crawfish at the edges of ponds or on the banks of pond levees (Romaire and Lutz 1989). White calcified spots appearing on the exoskeleton are diagnostic of WSD in some but not all host species (Chou et al. 1995). Because these spots are not always present, and because similar spots can be produced by bacteria, high alkalinity, and stress, they are not considered a reliable sign for preliminary diagnosis of this disease (Verbruggen et al. 2016).

2.1.3. Environmental stressors

Crustaceans, including shrimp and crawfish, are poikilothermic animals with open hemolymph systems; meaning that their body temperature depends mainly on the water temperature (Hopkin et al. 2006). While shrimp species are affected by biological and non-biological factors in the surrounding water, water temperature plays an important role in the innate immunity of shrimp to WSSV infection (Chen et al. 2019). Similarly, crawfish reside in ponds that vary in temperature according to the surrounding environment and are therefore subject to the prevailing pond water temperature. Although responses to biological factors are often considered part of organismal immunity, these responses can also be encompassed in a broader term of environmental adaptations. Therefore, it is not surprising that environmental stress and immune responses are closely related in crawfish and other aquatic animals (Chen et al. 2019). While it has been shown that variables such as water pH, temperature, and other physical and chemical factors weaken the immune system of these organisms, some studies have shown that varying intensities of environmental stress may induce immune-like effects, enacting a form of protection.

The effects of temperature on the outcome of WSSV infections are already documented. In Whiteleg shrimp (*L. vannamei)*, heat stress (determined as 33 °C) during the early stages of WSSV infection effectively increased WSSV resistance and reduced cumulative shrimp mortality (Rahman et al. 2006). In tropical countries such as Ecuador and Thailand, the prevalence of WSSV in grow-out ponds and hatcheries is reduced in the warm season (Rodriguez et al., 2003, Withyachumnarnkul et al., 2003). Further, experimentally WSSV-infected shrimp kept at high (> 32 °C) (*L. vannamei* or *Marsupenaeus japonicus*) or at low (12–15 °C) (*M. japonicus* or crayfish *Astacus astacus* and/or *Pacifastacus leniusculus*) water temperatures showed reduced and delayed mortality (Chen et al. 2019). Despite these results, the mechanism by which high water temperature induces a reduction in mortality of WSSV-infected organisms is unknown. Temperatures above 16 °C and below 32 °C allow WSSV replication in susceptible hosts such as shrimp, crabs, and crayfish (Corbel et al. 2001, Jiravanichpaisal et al. 2006). In this context, water temperature is considered one of the most important environmental factors for crustaceans because it influences metabolism, oxygen consumption, growth, molting, and survival (Coman et al. 2002).

2.1.4. White spot syndrome virus testing

Three metrics are often used detect WSSV in crawfish ponds: Polymerase Chain Reaction (PCR) test results, C_t values, and farmer identification. Real time quantitative PCR, also commonly referred to as qPCR, methods are commonly used to detect WSSV in DNA extracted from gill tissue (Pace et al 2016). Provided with the result of the qPCR test is a C_t value. C_t (threshold cycle) is a relative measure of the concentration of target in the PCR reaction. Many factors impact the absolute value of C_t besides the concentration of the target; however, in isolation, C_t

values provide a relative measure of viral quantity in the specimen, but do not provide the actual quantity. The C_t value is associated with the amount of PCR product in the reaction. The lower the C_t value, the more PCR product that is present. This is because it takes fewer PCR cycles for that product to be detected over the background signal. When conducting qPCR testing, a range of C_t scores indicates a strong, moderate, or weak positive result. When using the qPCR method for WSSV testing, C_t scores from 10–20 indicate a strong positive, 21–30 a moderate positive and 31–40 a weak positive (personal communication, John Hawke, LADDL).

2.1.5. Objectives

Several studies have demonstrated differences in WSSV strain virulence in crustaceans (Chou et al. 1995, You et al. 2010, Gao et al. 2014); however, WSSV in Louisiana aquaculture crawfish has not been exclusively quantified or described. Furthermore, effects of environmental stressors on the Louisiana strain of WSSV in producing crawfish ponds is yet to be explored but presents growing concern. The pond culture systems used in crawfish aquaculture provide an opportunity for the virus to spread rapidly and potentially decimate harvest yields. Annual reinfections of both permanent and rice rotational ponds are well documented within multiple parishes but the proportionality of this is yet to be quantified (personal communication, Mark Shirley, LSU AgCenter). Additional challenges are presented in virus identification and mortality event determination (e.g., farmer assessed outbreak vs. scientific testing). Spatial data on the spread of WSSV can be hard to quantify and verify within Louisiana in a pond setting because currently all testing is voluntary and has an associated cost (personal communication, John Hawke, LADDL). However, voluntary submission shows that dozens of farmers are demonstrating concern of the spread of WSSV. As with most viruses in outdoor systems, environmental variables may affect outbreak. It is well documented that drastic changes in water temperature are correlated with rapid viral replication and these changes may be indicative of mass mortality events (You et al. 2010). As such, these environmental variables may hold information that helps describe WSSV outbreaks and inform strategies enacted to mitigate effects. This research aims to increase understanding of the interactions between red swamp crawfish and WSSV by defining the scope of the virus and its annual reoccurrence in Louisiana, assessing methods of detection, and quantifying the effects of environmental variables as triggers for disease outbreak. With his overarching goal in mind, multiple data sources will be used to ask four specific questions. These questions are:

1) What is the spatial extent of white syndrome virus in Louisiana,

2) Within the identified spatial context of WSSV, what is the proportion of consecutive year infectivity,

3) What are the relationships between known WSSV diagnostic measures, and

4) Are changes in pond water temperature related to WSSV infectivity and induced mortality events.

2.2. Materials and Methods

2.2.1. Data Collection

The Louisiana Animal Disease Diagnostic Lab (LADDL), housed at Louisiana State University (LSU) School of Veterinary Medicine, provides specific PCR identification of pathogens, and confirmed the presence of WSSV in Louisiana crawfish in 2007 (LSU AgCenter 2007).

Submissions of crawfish are sent to the LADDL annually by farmers and county agriculture agents. Some samples of crawfish were submitted from 2008–2015, however, in 2016 WSSV showed up as a major problem in some indoor soft-shell operations and became of larger concern in 2017 in pond raised crawfish (Pace et al 2016). Therefore, PCR results from 2020–2022 were accessed to describe and understand the spatial occurrence of the virus during these three years. All test submissions were voluntary in 2020. PCR results in 2022 were part of a 12-week study in which weekly testing allowed for monitoring of the weekly status of infected ponds to include more robust testing than previous opportunistically sampling.

In 2021, collaboration with Louisiana Sea Grant marine extension agents lead to established relationships with farmers were interested in sharing historic information about their experience with WSSV and were comfortable with a temperature logging device being deployed in a crawfish pond currently in production for the 2021 and 2022 harvest seasons. HOBO TidbiT MX Temperature 400' Data Logger – MX2003 were placed in 23 crawfish ponds across Vermillion, Acadia, Jefferson Davis, and St. Landry parishes at the beginning of the crawfish harvesting season (February 1) in 2021. HOBO loggers recorded hourly temperature (C°) until May 1, 2021. In 2022, it was determined that because WSSV could be present in a pond without any symptomatic indicators, weekly PCR could be beneficial in allowing us to examine the effects of environmental variables on WSSV presence and disease outbreak. In 2022, HOBO loggers were deployed in 11 crawfish ponds across Vermillion, Acadia, Jefferson Davis and St. Landry parishes at the start of the harvest season and recorded hourly temperature (C°) until May 19, 2022. All ponds were identified by unique latitude and longitude and a unique farm identifier.

2.2.2. Data Analysis

Pond coordinates of positive PCR test results from 2020–2022 were compiled and summarized to quantify the persistence of WSSV across multiple years. Maps showing pond coordinates with positive PCR test results were created to quantify infectivity in multiple parishes within Southern Louisiana visually. WSSV PCR results from submitted tests were compared over three years: 2020, 2021, and 2022 to investigate annual infectivity or ponds that exhibited biannual infectivity. A logistic regression with a random effect for each pond was used to examine the correlation between farmer assessed outbreak and reported C_t score value in ponds testing positive for WSSV in 2021 and 2022. Similarly, a logistic regression with the same random effect for each pond assessed the correlation between reported PCR testing and C_t score value. A mixed effects model, with a fixed intercept and random slope was used to assess weekly average temperature, vegetation type and vegetation type on reported C_t score values. This type of mixed effects model accounts and allows for differing C_t values among ponds tested but establishes that all ponds start with a negative test for WSSV after the first week of testing. Ponds with a negative PCR test do not have an associated C_t score as WSSV was not detected. As previously mentioned, a range of C_t scores was established with an upper limit of 40 equating to the least presence of WSSV. For this analysis, all negative PCR tests were given an associated C_t score of 40. All statistical analyses were performed with R programming (R Core Team 2020).

2.3. Results

In 2020, 72 samples were submitted from different ponds across the state. Of these 72 total samples, 47 were positive for WSSV and 25 were negative. However, unique pond coordinates of submitted samples were only available for 29 of these 72 submissions, all of which tested positive. In 2021, 42 pond samples were tested, 22 positive and 20 negative. Identifying coordinates were available for 34 samples (19 positive, 15 negative). In 2022, 132 total samples were tested from 22 different ponds. Locations were available for 11 of the 22 tested ponds (11 positive, 5 negative). Heat maps over the three years show the persistence of WSSV and positivity rate in southern Louisiana parishes although a reporting bias is present (Table 1.1, 1.2, Figure 2.1).

Table 2.1. Positive PCR test results in each year showing month of test. Totals are the sum of all positive tests recorded in that year

	2020	2021	2022
March	2	0	0
April	24	23	9
May	2	8	5
June	0	3	0
Total	28	34	14

Table 2.2. Positive PCR test results in each year showing parish of pond location. Totals are the sum of all positive tests recorded in that year.

	2020	2021	2022
Acadia	2	2	1
Evangeline	1	6	0
Jefferson Davis	7	3	2
Lafayette	0	0	0
St. Landry	1	6	2
St. Martin	1	0	0
Vermillion	16	17	9
Total	29	34	16

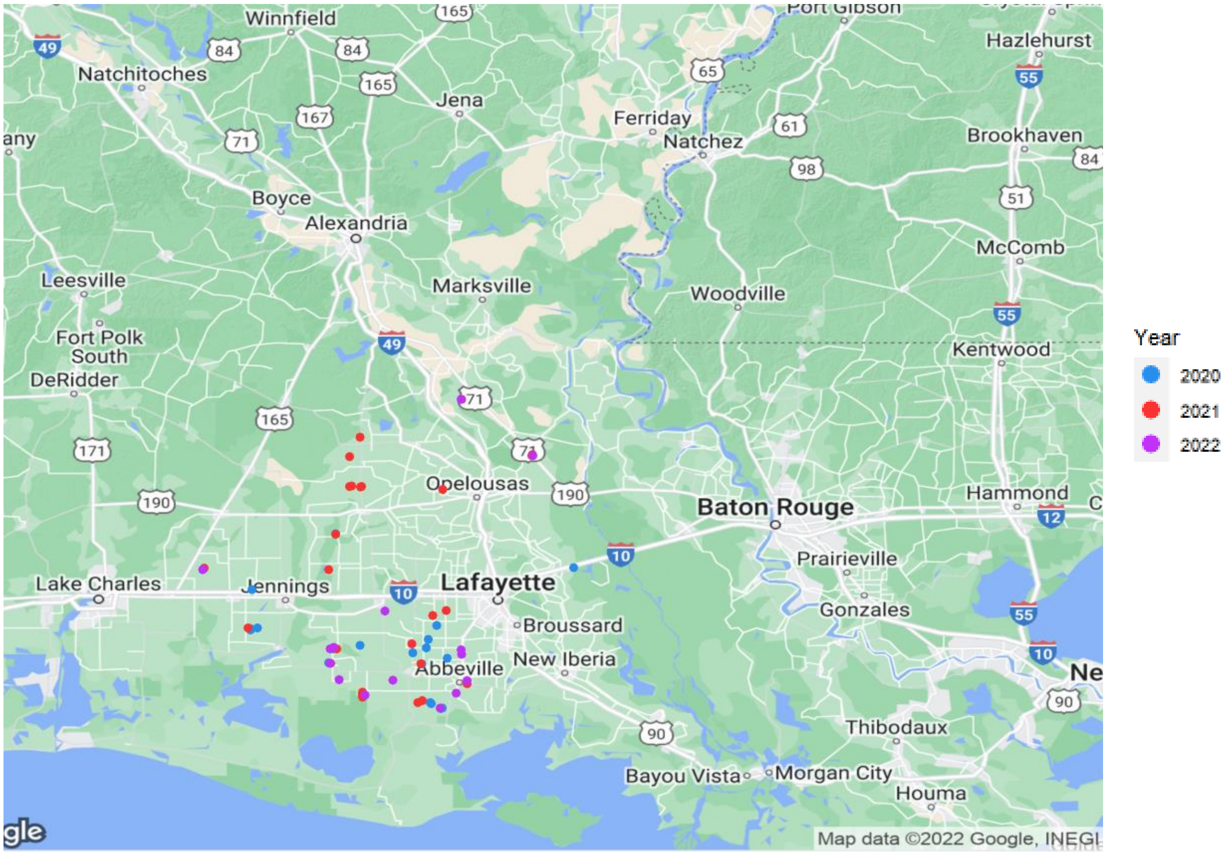

Figure 2.1. Locations of WSSV detections through PCR testing annually from 2020 to 2022.

Across the three-year study period, 14 ponds were tested in each year. As previously mentioned, these samples are from both opportunistic and weekly sampling completed through farmer submission and weekly testing. Five of these annually monitored ponds were infected in both 2021 and 2022. One pond tested posted for WSSV in both 2021 and 2022. Additionally, 2 ponds presented with biannual infectivity, with WSSV being detected in both 2020 and 2022. Zero ponds tested were infected across all three years of study (Table 1.3)

Table 1.13. Parish locations and annual WSSV infectivity of 14 pond locations that were tested annually from 2020 to 2022.

Pond ID	***2020 Infection***	***2021 Infection***	***2022 Infection***	***Parish***
1.	x	x		Acadia
2.	x	x		Lafayette
3.	x	x		Jefferson Davis
4.	x	x		Vermillion
5.	x			Evangeline
6.		x	x	Vermillion
7.	x			Jefferson Davis
8.	x			Vermillion
9.		x		Vermillion
10.		x		St. Landry
11.			x	Vermillion
12.	x		x	St. Landry
13.	x		x	Vermillion
14.	x		x	Vermillion

C_t scores < 40 indicate ponds having a positive PCR test result. C_t score is correlated with PCR quantity, such that lower C_t scores indicate greater viral quantity in the PCR test. (Figure 1.5). Farmer-assessed outbreak also follows a decreasing trend as lower C_t scores are correlated with farmer identification of viral presence (Figure 1.6). Mean weekly temperature is significant in predicting the associated C_t score of a positive PCR test while vegetation type and percent vegetation remaining each week do not influence C_t score (Figure 1.7).

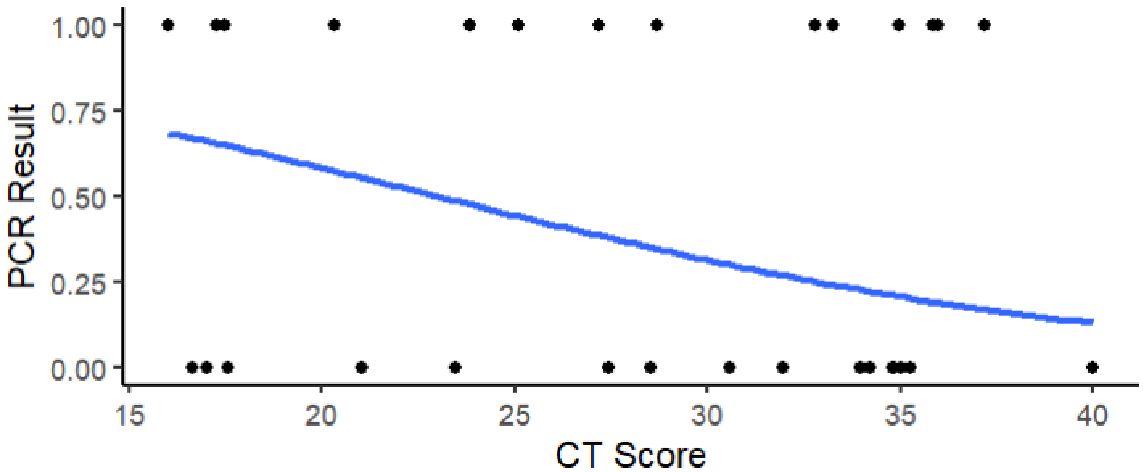

Figure 2.2. PCR positivity (1) or negativity (0) regressed against Ct score. Black dots indicate observed test results and associated Ct scores, and the blue line represents the relationship between the two metrics.

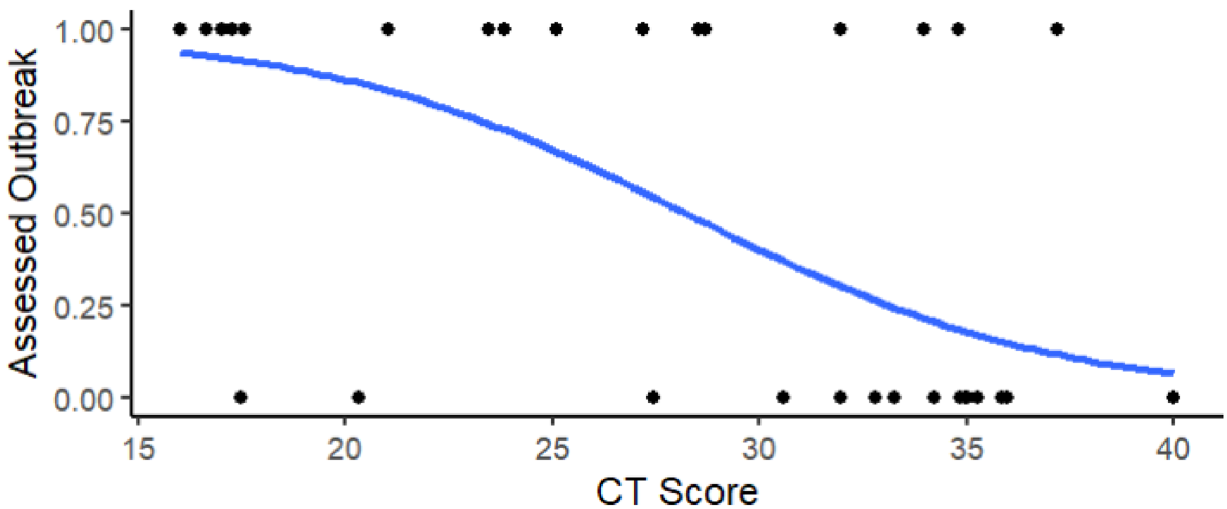

Figure 2.3. An assessed outbreak of 1 indicates positive detection of WSSV by a farmer while an outbreak of 0 indicates no virus detected. Ct score is regressed against farmer assessed outbreak of WSSV. Black dots indicated farmer assessed outbreak, and the blue line is the relationship between the two metrics

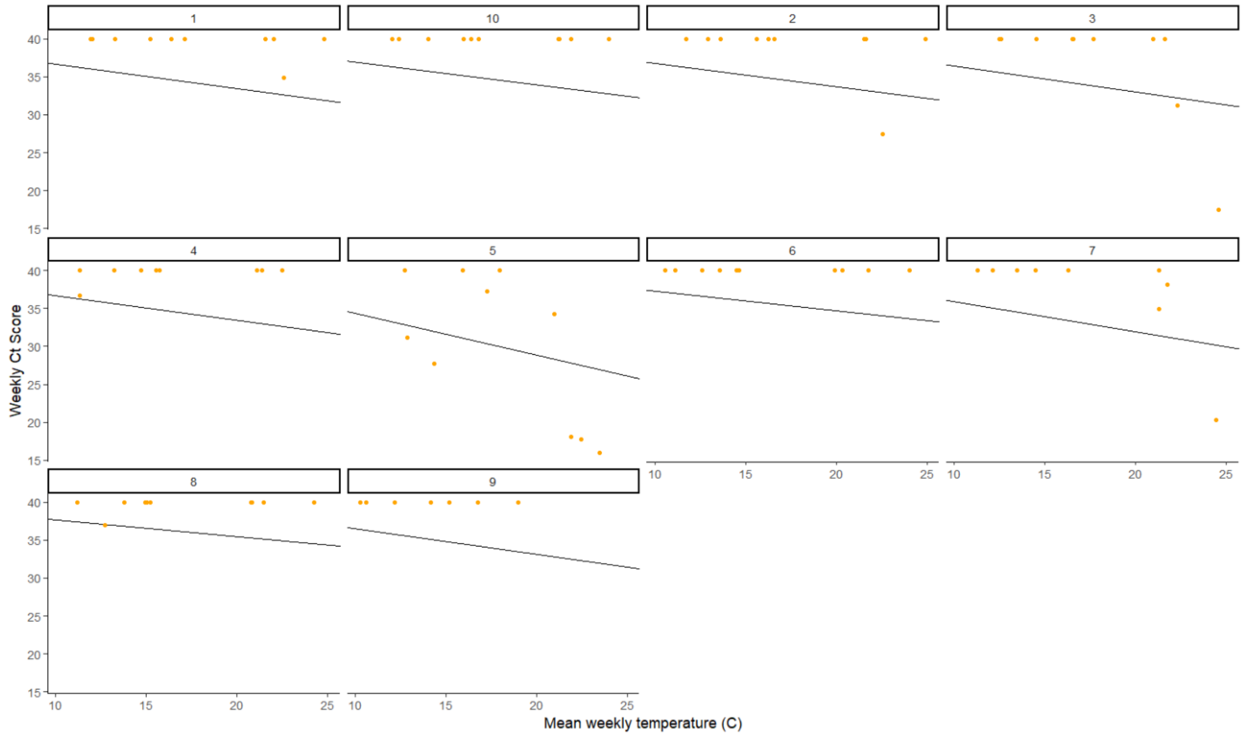

Figure 2.4. A mixed effects model, with a fixed intercept and random slope assess weekly average temperature type on reported C_t score values for each pond location.

2.4. Discussion

Although testing in 2020 and 2021 was opportunistic and samples were taken mainly from ponds already showing signs of WSSV, the positive detections of the virus across multiple parishes and months of the harvest season show the persistence of WSSV in southern Louisiana. Most positive detections occurred in April. When samples were submitted by farmers, most would notice an outbreak in late March and early April as crawfish harvest starts to pick up and water temperatures rise. Samples in 2020 and 2021 were submitted to the LADDL for testing were collected with the assumption of white spot presence. Therefore, a reporting bias must be accounted for if only focusing on PCR testing from these years. Additionally, because I wanted to look at persistence of the virus in individual ponds through consecutive years, ponds that had previously been tested or where suspected of having an outbreak of WSD were included in study data collected in 2022.

Farmer assessed outbreak is important in detecting WSSV and the effects of disease outbreaks in different parishes across southern Louisiana. However, this assessment is subjective and varies depending on how often a pond is harvested and how attentive farmers are to the crop. Crawfish operations vary in size, some being more for personal consumption while others are more focused on commercial production. Larger commercial operations often have multiple workers, some of which might not be as likely to look for characteristic signs of WSSV outbreak. Additionally, some signs of WSSV infection could be equated to problems with dissolved oxygen and additional lab testing is not conducted.

Studies show infected crawfish die more quickly at intermediate temperature (Jiravanichpaisal et al. 2006). Intermediate temperatures most likely occur in the late Spring in Southern Louisiana. Of course, farmed crawfish are frequently exposed variable environmental conditions including temperature fluctuations, low dissolved oxygen levels, and more recently, saltwater intrusion (Green et al. 2011, Reid et al. 2019). Disease occurrence is affected by the interplay between environmental conditions, host, and the pathogen; yet specific temperature thresholds that may contribute most are hard to pinpoint. The temperature change within a day usually affects the fluctuation of water temperature, and this condition is known to induce WSD outbreaks with a low level of WSSV infection in cultured shrimp (Kautsky et al. 2000).

On both a global and national scale, environmental changes attributes to the effects of climate change are well documented. Future years are projected to be characterized by increases in daily high and low temperatures. Because crawfish ponds are of shallow depths, warmer atmospheric temperatures will also affect water temperatures. If warmer springs and summers are observed, the subjection of pond reared crawfish to intermediate temperatures that increase mortality associated with WSSV are likely to be observed. Additionally, warmer temperatures early in the year could lead to ranges of weekly temperature much more variable than are currently seen. Such abrupt changes in temperature also add increase potential for mortality of crawfish within these outdoor pond systems. Additionally, an earlier study suggested that heavy rainfall events induced WSSV outbreak due to the change in water temperature and salinity (Peinado-Guevara & López-Meyer 2006). Extreme precipitation events have also become more prevalent across the United States as warmer air temperatures can hold additional water vapor. Increases in heavy precipitation may not always lead to increases in total precipitation over the crawfish season but localized extreme weather events have more potential for WSSV mortality events in individual ponds. These are additional factors that could be linked to an effect of temperature or other environmental variables that affect WSSV infectivity and disease outbreak. Understanding additional environmental effects could also provide additional insight into how WSSV outbreaks fluctuate from year to year.

While the effects of WSSV on local commercial crawfish operations are still being investigated, the virus is relatively new to this region, and the long-term effects are unknown. Crawfish harvests constitute the bulk of the commercial aquaculture profits in Louisiana, and the presence of WSSV poses a threat to the success of the industry. Individual farmers have voiced concern about transmission, identification, and long-term effects of WSSV since it was identified in Louisiana. Continued partnership and collaboration with stakeholders in this industry will allow for the identification and clarity of multiple additional variables at play surrounding this complex challenge. Although red swamp crawfish are generally hardy organisms, the pond design of crawfish aquaculture provides an opportunity for the virus to spread rapidly and potentially decimate harvest yields. Knowledge concerning the virulence and infectivity of the Louisiana strain of WSSV in native crawfish and other crustacean species will be useful for investigating disease prevention and potential management strategies. Future work in understanding how environmental variables such as temperature, dissolved oxygen and rainfall affect crawfish susceptibility and viral spread throughout a time course of WSSV infection could help explain differences in WSSV outbreaks and positivity rates annually.

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Vita

Mahala "Haley" Gambill was born and raised in Crumpler, North Carolina. She completed her Bachelor of Science in Fisheries, Wildlife and Conservation Biology from North Carolina State University in 2020. In August of 2020, she moved to Baton Rouge, Louisiana to attend Louisiana State University for her Master of Oceanography and Coastal Sciences. Haley anticipates completion of her master's degree in December of 2022. In August of 2022, she will begin a full time position with LSU AgCenter and Louisiana Sea Grant working as a Fisheries Extension Agent for Terrebonne and Lafourche parishes. She looks forward to continued involvement in addressing coastal issues and working with stakeholders within important Louisiana fisheries including the crawfish industry.