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Variations in prevalence of viral, bacterial, and rhizocephalan diseases and parasites of the blue crab (*Callinectes sapidus*)

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ABSTRACT

Prevalence of blue crab diseases and parasites has not been consistently monitored in the Gulf of Mexico. To establish current prevalence levels and to more fully understand population dynamics, commercial landing trends, and effects of future natural and anthropogenic disasters on animal health, we measured the prevalence of white spot syndrome virus (WSSV), *Loxothylacus texanus*, shell disease, and *Vibrio* spp. in blue crabs collected from Louisiana in 2013 and the beginning of 2014. We used PCR to detect WSSV and *L. texanus* infections, visual gross diagnosis for *L. texanus* externae and shell disease, and standard microbiological culture techniques and biochemical testing for *Vibrio* spp. We found no crabs infected with WSSV or *L. texanus*. Absence of *L. texanus* parasitization was expected based on the sampled salinities and the sampling focus on large crabs. Shell disease was present at a level of 54.8% and was most prevalent in the winter and summer and least prevalent in the spring. *Vibrio* spp. were found in the hemolymph of 22.3% of the crabs and prevalence varied by site, season, and sex. Additionally, three of 39 crabs tested were infected with reo-like virus.

1. Introduction

Louisiana (LA) often has the largest blue crab (*Callinectes sapidus* Rathbun, 1896) fishery in the USA. The LA fishery was valued at $43.1 million in 2012 (National Marine Fisheries Service, 2013). The profitability and sustainability of this fishery depend on healthy populations and appropriate management. However, in the Gulf of Mexico little is known about population health, and population health has not been thoroughly considered in the fishery management plan. Specifically, data on prevalence of diseases and parasites in LA blue crabs are limited, and therefore, managers and researchers have not been able to determine the effects of recent anthropogenic (e.g. oil spills) and natural (e.g. floods, hurricanes) disasters on the incidence of diseases and parasites.

Infections and parasitism in blue crabs range from asymptomatic to fatal. For example, the blue crab is an asymptomatic carrier of *Whispovirus* sp. or white spot syndrome virus (WSSV), the virus that causes white spot syndrome in shrimp and crayfish (Chang et al., 2001; Shields and Overstreet, 2007). White spot syndrome virus has caused mass shrimp mortalities globally (Shields and Overstreet, 2007). The determination of WSSV prevalence in wild blue crab populations is ecologically important because of possible transmission of the virus from crabs to more susceptible hosts such as shrimp (Shields and Overstreet, 2007).

Conversely, parasitization by *Loxothylacus texanus* or reo-like virus (RLV) is harmful to the infected crab (Shields and Overstreet, 2007). *L. texanus* causes sterilization and suppression of host ecdysis, both of which decrease population numbers and the number of legal sized crabs available to be commercially landed (Ragan and Matherne, 1974; Shields and Overstreet, 2007). *L. texanus* larvae are only viable at salinities above 12, and parasitization by this barnacle typically peaks during the late summer and fall when waters are warm (Adkins, 1972; Shields and Overstreet, 2007). While *L. texanus* infections are not fatal, RLV infections are. Symptoms of RLV include tremors, lethargy, and eventual paralysis (Johnson, 1977; Shields and Overstreet, 2007). Reo-like virus is hypothesized to cause mass mortalities in commercial shedding facilities on the East Coast (Bowers et al., 2011).

Bacteria such as *Vibrio* spp., *Pseudomonas* spp., and *Aeromonas* spp. can also be harmful to infected crabs by causing shell disease and intense hemolymph infections that can be fatal (Noga et al., 1994; Shields and Overstreet, 2007). Infectious bacteria are ubiquitous across a wide salinity range but peak in abundance when waters are warmest (Huq et al., 1984; Welsh and Sizemore, 1985).

Shell disease is a common result of bacterial shell infections following shell damage. It can be detrimental to infected crabs when necrotic lesions form and expose internal organs and tissues to

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pathogens in the ambient water (Millikin and Williams, 1984). Shell disease prevalence is dependent on water temperature as temperature affects crab ecdysis. Shell disease is typically most prevalent when crabs molt infrequently and when they burrow frequently (Sandifer and Eldridge, 1974; Shields and Overstreet, 2007).

To more fully understand population dynamics, commercial landing trends, and effects of future natural and anthropogenic disasters on aquatic animal health, we determined the prevalence of diseases and parasites in blue crabs collected along the LA coast in 2013 and the beginning of 2014. In this paper we focus on our findings for WSSV, *L. texanus*, bacterial infections, shell disease, and RLV. Results for protozoan symbionts (*Lagenophrys callinectes*, *Urosporidium crescens*, and *Hematodinium perezi*) have been reported previously (Rogers et al., in press). This was the first prevalence study conducted on several diseases and parasites over the span of a year at multiple locations along LA's coast.

2. Materials and methods

2.1. Sample sites

The four sites for collection of crabs were (East to West): Lake Pontchartrain, Grand Isle, Cocodrie, and Rockefeller Wildlife Refuge. These sites comprise a salinity gradient from low salinity at Lake Pontchartrain and to high salinity at Grand Isle (Table 1). Both Rockefeller and Cocodrie have moderate salinities (Table 1).

2.2. Sample collection

Crabs were collected seasonally (Table 1) with seines, trawls, baited traps, unbaited traps, dip nets, and baited lines. Multiple collection methods were used because one method was not consistently successful. Sixty crabs per site per season was our target sample size, but this was not always possible due to abnormally low catch rates (Table 1). The winter season was sampled twice (January–February 2013 and December–February 2014) due to low sample size in winter 1. We primarily collected large juvenile and adult blue crabs that had a carapace width (CW) of 11 cm or larger because prevalence can vary across life stages due to molt frequency. However, a subset of crabs that were less than 10 cm CW were collected for *L. texanus* assessment (Table 1) because infection by this parasite typically stunts juvenile crab growth (Adkins, 1972; Overstreet et al., 1983). Live crabs were placed on burlap-covered ice in coolers for transport to Louisiana State University.

At the completion of each crab collection, salinity was measured with a YSI 30-10FT or YSI 63-10FT (Yellow Springs, OH; Table 1). To limit daily and diel water temperature variation within a season, water temperature data were collected from nearby monitoring stations (East to West): (1) Rigolets USGS buoy and Lake Pontchartrain Basin Foundation weekly water quality data; (2) Grand Isle NOAA buoy; (3) USGS Houma Navigation Channel buoy in Dulac and LA Universities Marine Consortium Marine Center in Cocodrie; and (4) Freshwater Canal NOAA buoy at Fresh Water Canal Locks. Water temperatures were averaged across the collection season for each monitoring station.

2.3. Hemolymph extraction and Vibrio spp. detection

Hemolymph and bacterial infection methodologies were outlined by Rogers et al. (2015). Briefly, with a sterile needle and syringe, approximately 0.5 mL hemolymph was drawn from an

Table 1
Sampling parameters (sample sizes, n; average salinity; average water temperature) in 2013 and the beginning of 2014 at Lake Pontchartrain, Grand Isle, Cocodrie, and Rockefeller Wildlife Refuge. Salinities and water temperatures reported as mean ± standard deviation.

Site	Parameter	Winter 1	Spring	Summer	Fall	Winter 2
Pontchartrain	n^1	0	24	60	60	0
	n^2	0	24	45	45	0
	n^3	0	0	32	0	0
	n^4	0	23	0	0	0
	n^5	0	24	0	10	0
	Avg. Salinity	–	2.08 ± 0.10	2.64 ± 1.72	6.50 ± 0.74	–
	Avg. Temp. (°C)	–	15.78 ± 1.46	29.35 ± 0.81	24.44 ± 2.16	–
Grand Isle	n^1	7	13	60	60	60
	n^2	7	13	45	45	41
	n^3	8	0	53	0	0
	n^4	7	12	36	32	49
	n^5	7	13	23	47	39
	Avg. Salinity	13.73 ± 0.16	23.95 ± 3.92	14.05 ± 4.35	23.62 ± 1.93	23.81 ± 2.42
	Avg. Temp. (°C)	16.89 ± 1.14	17.79 ± 1.59	29.78 ± 1.17	24.42 ± 2.40	13.84 ± 2.57
Cocodrie	n^1	0	0	60	60	60
	n^2	0	0	45	45	42
	n^3	0	0	11	0	0
	n^4	0	0	33	20	10
	n^5	0	0	55	15	29
	Avg. Salinity	–	–	5.34 ± 2.30	11.69 ± 4.12	11.31 ± 6.45
	Avg. Temp. (°C)	–	–	30.22 ± 0.80	24.44 ± 2.64	15.29 ± 4.11
Rockefeller	n^1	17	43	60	64	60
	n^2	17	42	44	44	43
	n^3	5	0	47	0	0
	n^4	17	39	33	26	6
	n^5	17	36	0	1	12
	Avg. Salinity	1.32 ± 1.28	6.43 ± 0.61	12.47 ± 1.95	11.44 ± 2.10	10.50 ± 2.42
	Avg. Temp. (°C)	11.7 ± 2.80	18.91 ± 2.93	29.99 ± 0.92	25.44 ± 2.12	11.87 ± 2.69

– indicates no sample collection.
n^1 = number of large juvenile and adult (⩾11 cm carapace width) blue crabs collected and analyzed for shell disease.
n^2 = number of large juveniles and adults analyzed for white spot syndrome virus.
n^3 = number of small juvenile (<10 cm carapace width) blue crabs collected for *Loxothylacus texanus* detection.
n^4 = number of large juveniles and adults analyzed for *Loxothylacus texanus* by PCR.
n^5 = number of uncontaminated, hemolymph samples tested for *Vibrio* spp.