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Prevalence and distribution of three protozoan symbionts in blue crab (*Callinectes sapidus*) populations across Louisiana, USA

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ABSTRACT: Louisiana has one of the largest blue crab (*Callinectes sapidus*) fisheries in the USA, but little is known about blue crab diseases, parasites, and symbionts in this area. In 2013–2014, large juvenile and adult blue crabs were collected at 4 diverse sites to determine the prevalence of the protozoan symbionts associated with black gill disease (*Lagenophrys callinectes*), buckshot crabs (*Urosporidium crescens*), and bitter crab disease (*Hematodinium perezi*). A high aggregate prevalence of *L. callinectes* (93.2%) was identified across all seasons at all 4 collection sites regardless of salinity. A moderately low aggregate prevalence of *U. crescens* (22.4%) was identified across all seasons and sites. Prevalence of *U. crescens* depended on site salinity, with only 10% of infections detected at sites with <6.3 ppt salinity, and no infections detected at the low salinity site. While *L. callinectes* and *U. crescens* are commensal parasites of blue crabs, infections can result in unmarketable and unappealing meat. In the Louisiana fishery, *H. perezi* has been blamed circumstantially for adult mortalities in the low salinity nearshore fishing grounds. Despite this, *H. perezi* was not detected in any of the large crabs sampled, even from the low salinity sites. The prevalence data reported here for these 3 protozoans are the first to include blue crabs sampled seasonally at multiple locations along the Louisiana coast over the period of a year.

KEY WORDS: Crab symbionts · *Callinectes sapidus* · Protozoans · *Hematodinium* · *Urosporidium* · *Lagenophrys*

INTRODUCTION

The prevalence of diseases, parasites, and symbionts of the blue crab *Callinectes sapidus* Rathbun, 1896, has not been determined in many regions of its natural distribution range including the Gulf of Mexico. While protozoan symbionts such as *Lagenophrys callinectes* (Peritrichida: Lagenophryidae), *Urosporidium crescens* (Haplosporida: Anurosporidiidae), and *Hematodinium perezi* (Peridiniales: Syndiniaceae) are known to occur in Gulf blue crabs, their prevalence has not been examined.

L. callinectes and *U. crescens* are usually innocuous to the blue crab, but can have a negative economic effect when infected crabs appear unappetizing to consumers. *L. callinectes* is a commensal, loricated ciliate that infests blue crab gills and is shed during ecdysis (Couch 1967, Couch & Martin 1982, Shields & Overstreet 2007, Mayen-Estrada & Aguilar-Aguilar 2012). It has been hypothesized that heavy *L. callinectes* infestations can impair respiratory ability and in some circumstances can asphyxiate crabs (Couch 1966, 1967, Couch & Martin 1982, Shields & Overstreet 2007). Infestations with *L. callinectes* can cause brown or black discoloration of the gills (Couch 1967) and result in a syndrome colloquially called black gill disease.

U. crescens hyperparasitizes blue crabs by infecting the trematode *Microphallus basodactylophallus* (Perkins 1971, Couch 1974, Messick & Sindermann

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1992). Uninfected trematodes are lightly colored, approximately one-third of the size of infected trematodes, and more resistant to rupture than infected trematodes (Perkins 1971, Couch 1974, Shields & Overstreet 2007). Infected trematodes are easily recognized as large black spots in the muscle, hepatopancreas, and ventral ganglia of affected crabs that are described colloquially as pepper spot or buckshot crabs (Couch 1974, Messick & Sindermann 1992). *U. crescens* is not pathogenic to the crab, but has the ability to reduce but not eliminate mobility of the infected trematode, with spores released upon their rupture (Perkins 1971, Couch 1974).

H. perezi is a parasitic dinoflagellate found in crab hemolymph and tissues. Infection reduces hemocyte numbers as well as hemocyanin density in hemolymph, thus reducing its oxygen transport ability, and lyses cells in hepatopancreas and muscle tissues (Taylor et al. 1996, Shields & Squyars 2000, Shields et al. 2003). Infected crabs are typically lethargic and possess milky or chalky-white hemolymph with reduced clotting ability (Newman & Johnson 1975, Shields et al. 2003). Lethargy and reduced feeding activities can lead to crab death due to starvation (Taylor et al. 1996, Stentiford et al. 2000, Shields et al. 2003). *H. perezi* has been associated with mass mortality in several crab species as well as with decreased commercial landings of blue crabs along the US East Coast (Messick & Shields 2000, Shields 2003).

The prevalence of *L. callinectes*, *U. crescens*, and *H. perezi* varies based on water temperature. Because *L. callinectes* is lost during molting, prevalence of this ciliate should be highest in adult crabs that molt infrequently and particularly at sites with low water temperatures (Guillory et al. 2001). However, unlike the US East Coast, its prevalence in the Gulf of Mexico has not been highly correlated with water temperature as they tend to stay high year-round (Shields & Overstreet 2007). *U. crescens* hyperparasitization has not been found to vary seasonally in crabs sampled along the US East Coast in autumn and winter (Messick 1998). *H. perezi* epizootics along the US East Coast vary seasonally, peaking in autumn and declining in winter when water temperatures are <9°C (Messick et al. 1999, Lee & Frischer 2004). This seasonality has not been established in crabs inhabiting the Gulf of Mexico.

Parasite prevalence can also vary with salinity. As *L. callinectes* is hypothesized to be the most common commensal parasite of blue crabs (Shields & Overstreet 2007), its prevalence might not vary based on salinity. *U. crescens* hyperparasitization has been documented in crabs inhabiting moderately saline waters (Messick & Sindermann 1992, Messick 1998), but its prevalence in crabs inhabiting low salinity estuaries such as Lake Pontchartrain, Louisiana (LA), USA, have not been investigated. While *H. perezi* has not been recorded in wild crabs inhabiting sites with water salinities <11 ppt, it has been shown experimentally that infections established in higher salinity water where the hypothesized transmissive dinospore is active can be maintained when crabs are moved to low salinity water (Newman & Johnson 1975, Messick & Shields 2000, Coffey et al. 2012). Regardless of the abiotic factors dictating *H. perezi* prevalence, *H. perezi* has been blamed for adult mortalities in the low salinity, nearshore LA fishing grounds.

Little is known about protozoan symbionts of blue crabs inhabiting LA despite it often having the highest commercial catches in the USA, with a dockside value in 2012 of $43.1 million (National Marine Fisheries Service 2013). Research on parasites of blue crabs inhabiting the Gulf of Mexico has been sporadic and geographically limited. Following the Deepwater Horizon oil spill in 2010, this lack of knowledge became evident when processors, fishermen, and shedding facilities reported higher than average die-offs and higher disease and parasite prevalence (J. A. Anderson Lively & J. P. Hawke unpubl. data). Some of the mortalities were attributed tentatively to *H. perezi* and *L. callinectes* even though salinities were too low for *H. perezi*. However, with no baseline data available for the region, fisheries managers and researchers could not determine whether disease or parasite prevalence had increased due to the environmental damage. To determine the consequences of future natural and anthropogenic impacts on parasite prevalence and to more fully understand reasons for declines in commercial landings, our goal was to examine the prevalence of *L. callinectes*, *U. crescens*, and *H. perezi* in blue crabs caught at 4 sites along the LA coast through 2013 to the beginning of 2014.

MATERIALS AND METHODS

Sample collection

Four field sites in Louisiana (LA), USA, were selected for crab collection: Lake Pontchartrain (30° 12′ 55″ N, 89° 45′ 0″ W), Grand Isle (29° 14′ 19″ N, 90° 0′ 11″ W), Cocodrie (29° 21′ 3″ N, 90° 37′ 34″ W), and Rockefeller Wildlife Refuge (29° 41′ 26″ N, 92° 49′ 53″ W) (Fig. 1). These sites vary from low salinity at

Lake Pontchartrain to high salinity at Grand Isle (Table 1).

To assess parasite prevalence, crabs were collected seasonally at each site (Table 1). Sampling occurred in 2013 to 2014 with winter 1 referring to 2013 and winter 2 referring to 2014. As parasite prevalence can vary among crab size classes, only large juveniles and adults with a carapace width (CW) of ≥110 mm were examined. As no single capture method was consistently successful despite constant effort, crabs were collected using various methods including seine, trawl, and dip nets; baited and unbaited traps; and baited lines. Crabs were caught by the authors except for crabs trawled around Cocodrie in summer, autumn, and winter 2. Multiple collection methods were used as one method was not consistently successful despite constant effort. While variation in collection methods might bias parasite prevalence data, this could not be tested for statistically due to crab numbers caught using the various methods being unequal and insufficient for robust testing. A goal of 60 crabs per site per season was established but was not always attained due to abnormally low catch rates in 2013 (Table 1). The winter season was sampled in both 2013 and 2014 to compensate for the low crab catch numbers in winter 1.

After crab capture at each of the 4 sampling sites, salinity was measured using either a YSI 30-10FT or YSI 63-10FT Handheld Salinity, Conductivity and Temperature System. To remove daily and diel variations in water temperatures within a season, data were averaged from water monitoring stations near each site (east to west): (1) Rigolets USGS buoy, (2) Grand Isle NOAA buoy, (3) USGS Houma Navigation Channel buoy in Dulac and Louisiana Universities Marine Consortium Marine Center in Cocodrie, and (4) Freshwater Canal NOAA buoy in Fresh Water Canal Locks. Live crabs were placed on burlap-covered ice for transport to Louisiana State University (LSU), and dissected within 24 h of capture.

Crab dissection

All crabs were processed and dissected alive after being anaesthetized on ice. Prior to dissection, CW was measured to the nearest millimeter, and the crab was sexed and photographed. For hemolymph collection, the joint of the swimming leg and carapace was disinfected with 70% ethanol, and ~1 ml hemolymph was withdrawn using a sterile needle (18 to 25 gauge) fitted to a sterile 3 ml syringe. Hemolymph was preserved in 95% ethanol for *Hematodinium perezi* polymerase chain reaction (PCR) analysis (see below). A sample of gill tissue was frozen at −20°C for *Lagenophrys callinectes* detection (see below).

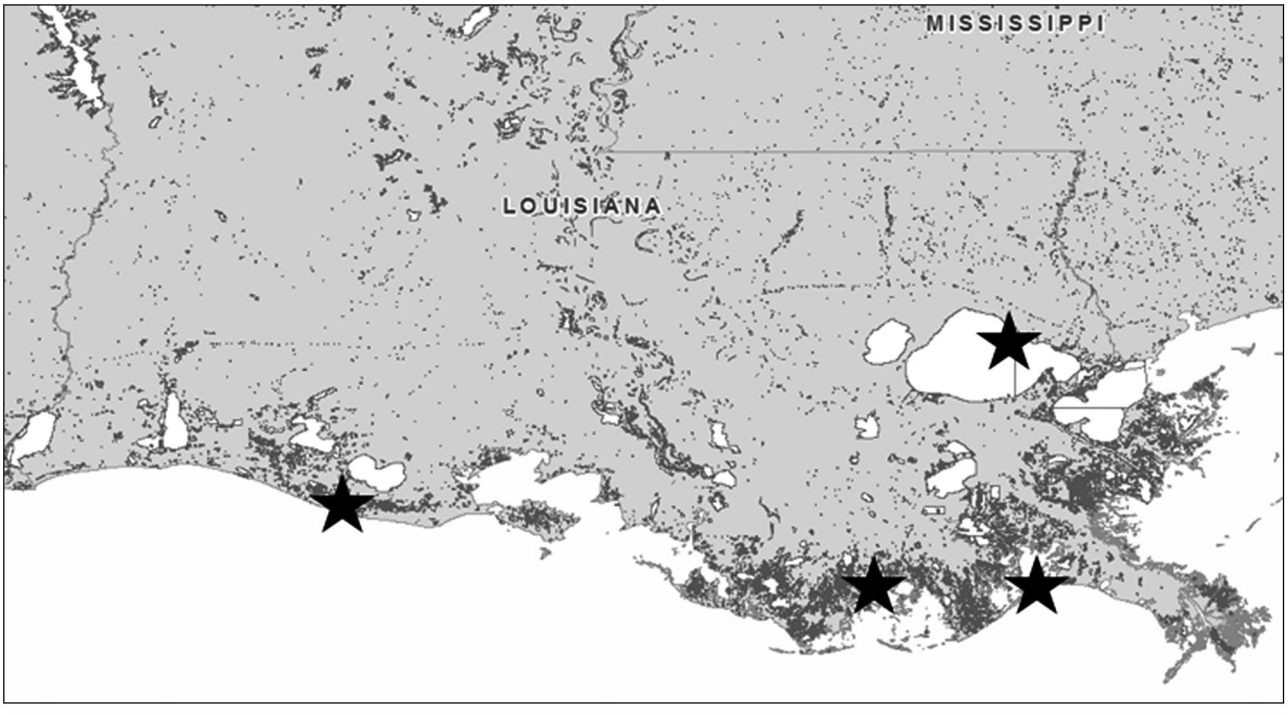

Fig. 1. Coastal Louisiana, USA, with blue crab collection sites marked by stars. From east to west, sites are Lake Pontchartrain, Grand Isle, Cocodrie, and Rockefeller Wildlife Refuge

Table 1. Blue crab numbers collected and water salinities and temperatures at the Lake Pontchartrain, Cocodrie, Rockefeller Wildlife Refuge, and Grand Isle sampling sites between winter 1 (2013) and winter 2 (2014). Salinity and water temperatures expressed as mean ± SD

		Crab number	Salinity (ppt)	Temperature (°C)
Pontchartrain				
2013	Winter 1	0		
	Spring	24	2.08 ± 0.10	15.78 ± 1.46
	Summer	60	2.64 ± 1.72	29.35 ± 0.81
	Autumn	60	6.50 ± 0.74	24.44 ± 2.16
2014	Winter 2	0		
Cocodrie				
2013	Winter 1	0		
	Spring	0		
	Summer	60	5.34 ± 2.30	30.22 ± 0.80
	Autumn	60	11.69 ± 4.12	24.44 ± 2.64
2014	Winter 2	60	11.31 ± 6.45	15.29 ± 4.11
Rockefeller				
2013	Winter 1	17	1.32 ± 1.28	11.70 ± 2.80
	Spring	43	6.43 ± 0.61	18.91 ± 2.93
	Summer	60	12.47 ± 1.95	29.99 ± 0.92
	Autumn	64	11.44 ± 2.10	25.44 ± 2.12
2014	Winter 2	60	10.50 ± 2.42	11.87 ± 2.69
Grand Isle				
2013	Winter 1	7	13.73 ± 0.16	16.89 ± 1.14
	Spring	13	23.95 ± 3.92	17.79 ± 1.59
	Summer	60	14.05 ± 4.35	29.78 ± 1.17
	Autumn	60	23.62 ± 1.93	24.42 ± 2.40
2014	Winter 2	60	23.81 ± 2.42	13.84 ± 2.57

Detection of *Lagenophrys callinectes*

We detected *L. callinectes* on frozen gill sections (<1 cm thick) based on the presence of the circular loricae using a Micromaster Premier light microscope (Fisher Scientific) at a magnification of 10× or higher (Fig. 2; Couch 1967, Couch & Martin 1982, Mayen-Estrada & Aguilar-Aguilar 2012). As the gill area examined was not standardized, infestation intensities were not estimated from the numbers of loricae recorded. When *L. callinectes* was not detected in the first section, 3 sections (minimum) were examined to confirm this.

Detection of *Urosporidium crescens* infected trematodes

During crab dissection, the hepatopancreas and muscle were examined macroscopically for dark, enlarged trematodes infected by *U. crescens* (Fig. 3).

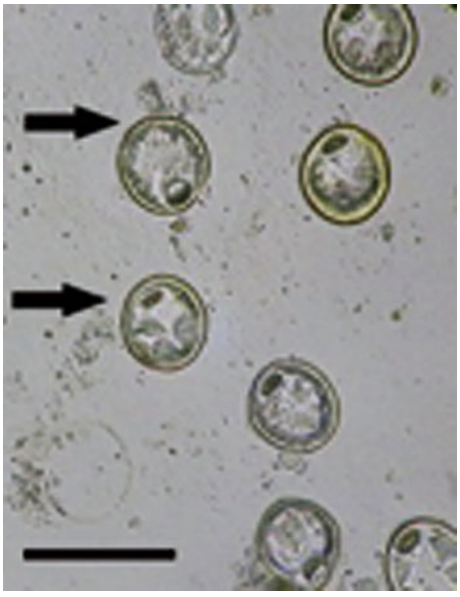

Fig. 2. Light micrograph of unstained *Lagenophrys callinectes* loricae (arrows) present on the gill of a crab collected in winter 1 (2013). Scale bar = 100 µm

Detection of *Hematodinium perezi*

Methods used to extract and detect *H. perezi* DNA by PCR were as described previously (Gruebl et al. 2002, Small et al. 2007, Pagenkopp Lohan et al. 2012). Briefly, 200 µl ethanol-preserved hemolymph was centrifuged to remove excess ethanol. Pelleted hemocytes were lysed overnight and extracted using a DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol. DNA was eluted from columns using 2 × 5 min elution incubations with 100 µl elution buffer. PCR primer pairs used were (1) general metazoan primers nSSU-A and nSSU-B, (2) *Hematodinium* sp. primers HITS1F and HITS1R, and (3) *Hematodinium* spp. primers Hemat-F-1487 and Hemat-R-1654.

Reaction mixtures and thermocycling conditions described previously were modified slightly (Rogers et al. 2015). Briefly, reactions (10 µl) included a negative (no template) control and a positive control of DNA extracted from ethanol-preserved hemolymph of a known infected crab provided kindly by Dr. Jeffrey Shields, Virginia Institute of Marine Science. Positive and negative controls were extracted using the same method used for samples. Amplified DNA products were stained with EZ-Vision DNA Dye (Amresco), separated by 2% agarose gel electrophoresis, and visualized under UV light. If a sample did not amplify using the general metazoan primers

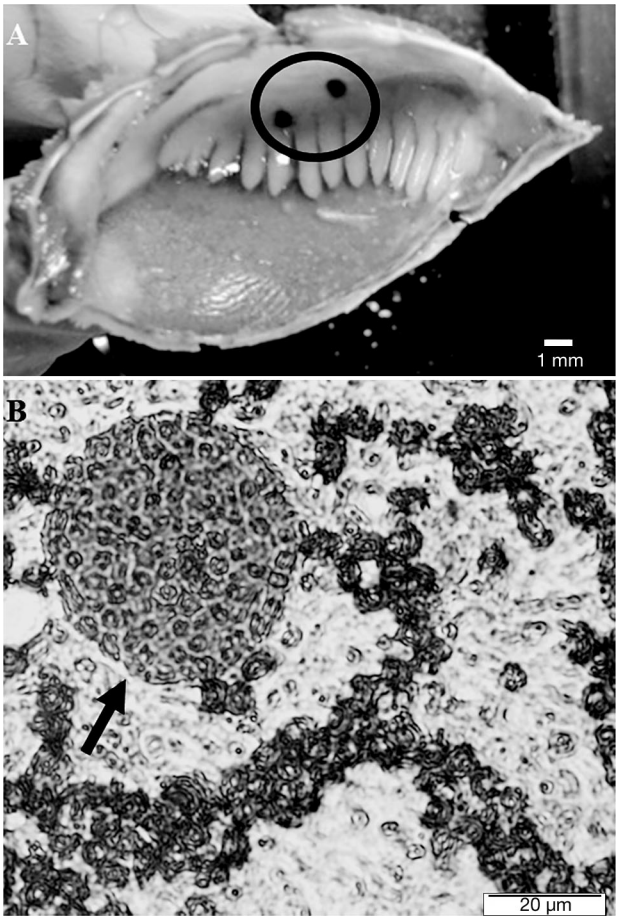

Fig. 3. (A) Crab hepatopancreas with *Urosporidium crescens* infected trematodes appearing as dark conspicuous spots (circle). (B) Transmission electron micrograph of a round *U. crescens* infected trematode (arrow) kindly provided by Yuliya Sokolova, Microscopy Center, Louisiana State University School of Veterinary Medicine, 2013

(~1700 bp DNA band), it was extracted and amplified again. If the second amplification was unsuccessful, that crab was excluded from the *H. perezi* prevalence data.

Statistical analysis

Aggregate prevalence was calculated as the total number of infected individuals divided by the total number of individuals examined. Statistical analyses were performed in RStudio (R Development Core Team 2013). To determine the effect of a predictor variable on the probability of infection by a symbiont, logistic regression analyses were performed (binomial distribution with a logit link) in the R package *stats*. Predictor variables included site, season, site × season interaction, water temperature, salinity, sex, and size. For categorical predictor variables (site, season, sex), a reference category was chosen arbitrarily. The respective reference categories were Grand Isle, autumn, and male.

To evaluate model fit, an analysis of deviance chi-square test was performed in the R *stats* package. When multicollinearity between predictor variables was hypothesized, function *vif* in the R package *car* was used to calculate generalized variance inflation factors (GVIFs). Multicollinearity was present when GVIFs for predictor variables were >4.0. To assess model assumptions of normality of residuals and homogeneity of variance, the residual and normal Q-Q plots of Pearson residuals were examined. The final model was selected from a set of models with individual and combinations of predictor variables based on the Akaike's information criterion (AIC), significant predictor variables, deviance chi-square, and GVIFs. For all statistical tests, alpha was set at 0.05 for statistical significance.

RESULTS

Prevalence of *Lagenophrys callinectes*

The aggregate prevalence of *L. callinectes* in blue crabs (n = 768) collected across all 4 field sites was 93.2% (Fig. 4). Of the sites, Pontchartrain had the highest prevalence (n = 144; 98.6%), and Rockefeller had the lowest (n = 244; 89.8%) (Fig. 4). Prevalence varied by season, water temperature, site × season interaction, and water salinity (Table 2). Multicollinearity was not detected (all GVIFs <3.5) in the full statistical model that contained either water temperature or season, the only correlated predictor variables. The Pearson residuals for the full statistical model deviated slightly from normality.

Prevalence at neither Pontchartrain nor Cocodrie varied significantly by season (Fig. 4, Table 3). At Rockefeller, the summer prevalence (n = 60; 76.7%) was significantly lower than in autumn (n = 64; 90.6%), and at Grand Isle, prevalence during the spring (n = 13; 23.0%) was lower than during autumn (n = 60; 93.3%) (Fig. 4, Table 3). In addition to seasonal variation of *L. callinectes*, there was a significant site and season interaction at Rockefeller during spring (Table 2).

When temperature was used in place of season in the statistical model, water temperature and salinity were significant predictors (Table 2). Slight heterogeneity of variance was associated with salinity. Nei-

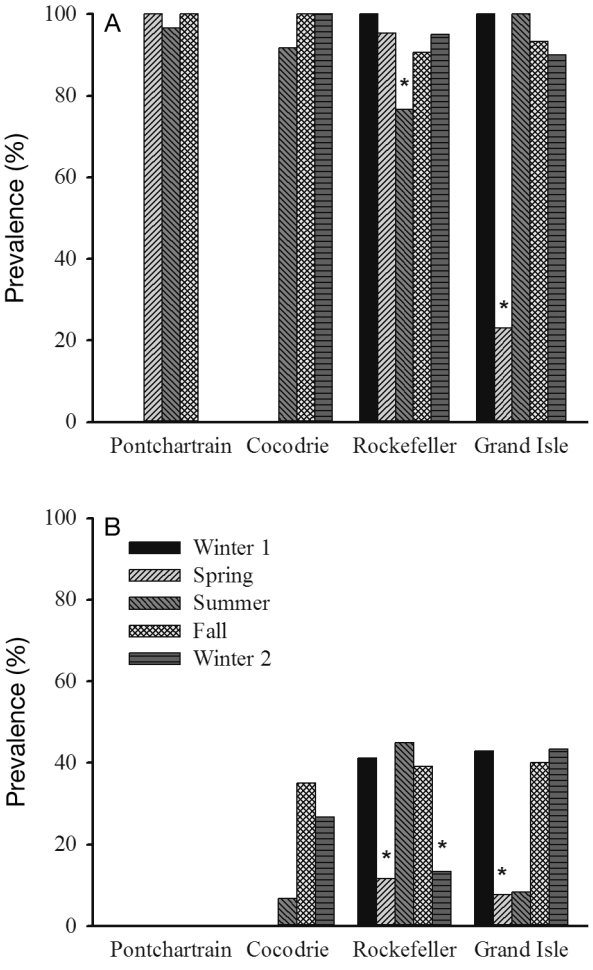

Fig. 4. Prevalence of (A) *Lagenophrys callinectes* and (B) *Urosporidium crescens* in blue crabs collected from Lake Pontchartrain, Cocodrie, Rockefeller Wildlife Refuge, and Grand Isle between winter 1 (2013) and winter 2 (2014). *U. crescens* was not detected at Pontchartrain. Seasons in which prevalence was statistically different to the autumn reference season are indicated (*)

ther crab size (CW) nor sex were significant predictors of *L. callinectes* infestation (Table 2). For size and sex, model fit was equivalent both with and without these predictors (both $p > 0.45$).

Prevalence of *Urosporidium crescens*

The aggregate prevalence of *U. crescens* infected trematodes in blue crabs (n = 768) collected across all 4 field sites was 22.4% (Fig. 4). No infections were detected in crabs from Pontchartrain (n = 144; Fig. 4). Aggregate prevalence was 22.8% at Cocodrie (n = 180) and 29.5% at both Rockefeller (n = 244) and Grand Isle (n = 200; Fig. 4). Overall, the prevalence of *U. crescens* varied by site, season, site × season interaction, and salinity (Table 2). However, there was heterogeneous variance associated with salinity and the distribution of the Pearson residuals of the full statistical model was non-normal. Based on models with individual predictor variables, the full model most closely satisfied the assumption of normality of residuals so all significant variables were retained.

There was no significant seasonal variation at Cocodrie (Table 3), and salinities recorded explained the variation in prevalence across seasons (Fig. 4B; $\beta_{Salinity} = 0.1966$, $p < 0.0001$). Prevalence at Cocodrie was low in summer (6.7%) when low salinities were recorded (5.34 ± 2.30 ppt). In autumn and winter 2, high and low salinities were recorded, and in the sample sets from only the low salinity bayou, 7.1% of autumn crabs (n = 14) and none of the winter 2 crabs (n = 24) were infected.

At Rockefeller, prevalence levels in spring (n = 43; 11.6%) and winter 2 crabs (n = 60; 13.3%) were significantly lower than in autumn crabs (n = 60; 39.1%) (Fig. 4, Table 3). Excluding winter 1 when the crab sample size was low (n = 17), spring (6.43 ± 0.61 ppt) and winter 2 (10.50 ± 2.42 ppt) were the seasons with the lowest average salinities. However, water salinity and temperature were not significant explanatory variables for the seasonal variation at Rockefeller (both $p > 0.3$), which is likely influenced by the winter 1 data set.

At Grand Isle, prevalence during the spring (n = 13; 7.7%) was significantly lower than in autumn (n = 60; 65.0%) (Fig. 4, Table 3). This trend was not explained by water salinity alone as average levels were high in spring (23.95 ± 3.92 ppt). Both season and salinity were significant, uncorrelated explanatory variables ($\beta_{Salinity} = 0.1507$, $p = 0.0202$; GVIFs < 2.5).

In addition to the seasonal variation, there was significant site variation between the Cocodrie and Rockefeller sites and the Grand Isle site (Table 2). Multicollinearity between site and salinity was not detected (GVIFs <3.8). Salinity was a significant predictor for the prevalence of *U. crescens*, where the odds of infection increased by 14.3% for each 1 ppt increase in salinity (Table 2). Of the infections, 90% occurred at salinities >6.3 ppt and 80% occurred at salinities >10.8 ppt. Size and sex were not significant predictors of *U. crescens* prevalence and did not improve the model fit (Table 2; both deviance $p > 0.38$). When water temperature replaced season in the statistical model, it did not have a significant effect on prevalence (Table 2).

Table 2. *Lagenophrys callinectes* and *Urosporidium crescens* prevalence parameter estimates, standard errors, and p-values for predictor variables in logistic regressions using Grand Isle as the reference site category and autumn as the reference season. Significant parameter estimates are highlighted in **bold**. Unlisted interactions that were not significant are identified as not applicable (n/a)

Symbiont	Predictor	Category	Parameter estimate	SE	p-value
Lagenophrys callinectes	Site	Pontchartrain	17.9270	2288.9810	>0.99
		Cocodrie	17.9270	2288.9810	>0.99
		Rockefeller	−0.3704	0.6721	0.5816
	Season	Winter 1	17.9270	6701.4499	>0.99
		Spring	**−3.8430**	**0.8374**	**<0.0001**
		Summer	17.9270	2288.9810	>0.99
		Winter 2	−0.4418	0.6731	0.5115
	Site × Season	**Rockefeller × Spring**	**4.5948**	**1.1872**	**<0.001**
	Water temperature	**n/a**	**0.0893**	**0.0452**	**0.0482**
	Salinity	**n/a**	**−0.1066**	**0.0500**	**0.0322**
	Size	n/a	−0.0040	0.1024	0.9687
	Sex	Male	−0.0431	0.3384	0.8987
Urosporidium crescens	**Site**	Pontchartrain	−15.87	841.2	>0.98
		Cocodrie	**1.339**	**0.5261**	**0.0109**
		Rockefeller	**1.592**	**0.5377**	**<0.01**
	Season	Winter 1	1.446	0.8683	0.0960
		Spring	**−2.213**	**1.081**	**0.0407**
		Summer	−0.8615	0.5935	0.1467
		Winter 2	0.1063	0.3739	0.7762
	Site × Season	**Rockefeller × Winter 2**	**−1.446**	**0.5949**	**0.0151**
	Water temperature	n/a	0.0114	0.0144	0.4290
	Salinity	**n/a**	**0.1336**	**0.0317**	**<0.0001**
	Size	n/a	−0.0535	0.0623	0.3900
	Sex	Male	−0.1185	0.2021	0.5576

Table 3. *Lagenophrys callinectes* and *Urosporidium crescens* prevalence parameter estimates, standard errors, and p-values for seasonal variation at each of the 4 crab collection sites (Lake Pontchartrain, Cocodrie, Rockefeller Wildlife Refuge, Grand Isle) using autumn as the reference season. Significant parameter estimates are highlighted in **bold**

Symbiont	Site	Season	Parameter estimate	SE	p-value
Lagenophrys callinectes	Pontchartrain	Summer	−18.2	3774	>0.99
	Cocodrie	Summer	−19.17	3774	>0.99
	Rockefeller	Winter 1	15.2974	959.5148	0.9873
		Spring	0.7517	0.8416	0.3717
		Summer	**−1.0791**	**0.5264**	**0.0404**
		Winter 2	0.6758	0.7313	0.3555
	Grand Isle	Winter 1	16.9270	4064.6349	>0.99
		Spring	**−3.8430**	**0.8374**	**<0.0001**
		Summer	16.9270	1388.3372	0.99
		Winter 2	−0.4418	0.6731	0.512
Urosporidium crescens	Cocodrie	Summer	−0.7866	0.6711	0.241
		Winter 2	−0.5948	0.4496	0.186
	Rockefeller	Winter 1	0.0880	0.5554	0.8741
		Spring	**−1.5835**	**0.5403**	**<0.01**
		Summer	0.2440	0.3647	0.5034
		Winter 2	**−1.4271**	**0.4581**	**<0.01**
	Grand Isle	Winter 1	1.6159	1.0366	0.1190
		Spring	**−2.2409**	**1.0867**	**0.0392**
		Summer	−0.7394	0.7137	0.3002
		Winter 2	0.1012	0.3751	0.7874

Prevalence of *Hematodinium perezi*

Of the 760 crabs examined from which hemocytes were extracted successfully to generate DNA amplifiable by PCR, none tested positive for *H. perezi*.

DISCUSSION

Regardless of the water salinity at the 4 collection sites in the Gulf of Mexico, a high aggregate prevalence of *Lagenophrys callinectes* was found among blue crabs. This finding was consistent with our expectations as *L. callinectes* has been hypothesized previously to be the most common commensal parasite of blue crabs (Shields & Overstreet 2007). We found potential seasonality of *L. callinectes*

prevalence, with it being lower when molting occurs most commonly. However, this observation might be affected by the lower number of crabs captured in spring. Hypothesized intolerance of *Urosporidium crescens* to low salinity water was supported by it not being found on crabs sampled at the lowest salinity site (Lake Pontchartrain) or in low salinity areas around Cocodrie; however, it was found under novel low salinity conditions at Rockefeller. As expected due to the generally low salinities of the sites sampled in the Gulf of Mexico compared to other regions along the US East Coast (Table 1; Newman & Johnson 1975, Messick & Shields 2000), no *Hematodinium perezi* was detected in any crabs examined.

Prevalence of *Lagenophrys callinectes*

In early studies of blue crabs in Chincoteague Bay, the prevalence of *L. callinectes* was reported to vary seasonally from over 40% in summer and autumn to as low as 0% in winter (Couch & Martin 1982). Among crabs examined from Chesapeake Bay in 1992, its prevalence was similarly found to decrease from 10% in autumn to 3% in winter (Messick 1998). Our overall prevalence of *L. callinectes* identified here among crabs examined in 2013–2014 was over 90%, and thus much higher than in these previous studies. In Mississippi, the prevalence of *L. callinectes* has been found to be correlated primarily with the crab's molt cycle and less so with season or water temperature (Shields & Overstreet 2007). This may be due to water temperatures fluctuating less and molting occurring more frequently in this region compared to the US East Coast, resulting in sporadic periods of low prevalence following ecdysis (Shields & Overstreet 2007). In contrast, among the crabs examined here from LA, water temperature and salinity were identified to be significant predictors of *L. callinectes* prevalence. However, these findings may have been influenced to some extent by the low prevalence detected in the small crab sample set examined from Grand Isle in spring (n = 13) and by the lack of molt stage data to include as an explanatory variable in the statistical analyses.

While molt stage was not determined, the seasonal variation in *L. callinectes* at Grand Isle seems likely to be related to recently molted crabs in our samples (Couch 1967), as April/May are peak blue crab molting months in the Gulf of Mexico (Guillory et al. 2001). The lower prevalence at Rockefeller in summer might also have been related to recently molted crabs in our samples or by the low crab numbers captured during spring (Table 1). More data are needed to substantiate these potential seasonal trends in *L. callinectes* prevalence being lower in summer and spring and unaffected by collection site or salinity. Based on prevalence data reported here for *L. callinectes* and reported previously for blue crabs examined from Chesapeake Bay (Messick 1998), crab size, age or gender are not likely to affect the prevalence of this opportunistic protozoan.

The overall high prevalence of *L. callinectes* detected should not cause alarm for managers of the blue crab fishery in the Gulf of Mexico as it is a commensal symbiont posing a minimal risk to the sustainability of crab populations and as there are no definitive data on it impairing crab respiration and thus health (Couch 1966, 1967, Couch & Martin 1982, Shields & Overstreet 2007). In support of this, heavily infested crabs in our study generally appeared healthy and rarely displayed discolored gills that can be unappealing to consumers. However, as *L. callinectes* is highly prevalent, whether or not high infestation intensities can reach acute levels capable of potential asphyxiation seems worthy of further investigation.

Prevalence of *Urosporidium crescens*

Data on trematodes infected by *U. crescens* are reported only for crabs inhabiting moderate and high salinities (Messick 1998). For example, in Chesapeake Bay, infected crabs were found at salinities that averaged >14.7 ppt (Messick 1998). Similarly, in grass shrimp *Palaemonetes* spp. collected in coastal Georgia, *U. crescens* hyperparasitization of microphallid trematodes by *U. crescens* was only observed in shrimp collected from locations with salinities >22 ppt (Pung et al. 2002). This intolerance of *U. crescens* to low salinity explains the absence of infections in blue crabs examined from low salinity areas such as Lake Pontchartrain. At the Cocodrie and Rockefeller sites, the lowest prevalence levels occurred in the seasons in which crabs were captured at low salinity. Had crabs been captured at the same low salinity sites during all seasons, it is expected that the *U. crescens* prevalence at Cocodrie and Rockefeller would have been uniformly low. However, more consistent crab collections at high and low salinity sites will be needed to resolve whether seasonal trends occur with this parasite.

Salinity was not the sole variable that explained site variation in *U. crescens* prevalence, and fitted statistical values were not always consistent with observed values, especially with regards to salinity.

For example, Rockefeller had the highest prevalence in summer even though salinity was highest at Grand Isle (Table 1, Fig. 4). Therefore, it is possible that a higher crab population density might also have contributed to the higher prevalence at Rockefeller. Crab densities in the Gulf of Mexico are affected by habitat type and commercial fishing pressure, with the Rockefeller site bounded by marsh land and representing a protected wildlife refuge where commercial fishing is prohibited. In contrast, Grand Isle has less dense crab populations that required greater collection efforts and represents an open water habitat subject to intense commercial fishing.

The moderate prevalence of *U. crescens* detected is not alarming ecologically as infection does not appear to harm blue crabs (Perkins 1971). However, a high prevalence of infestation levels can have economic impacts due to affected meat becoming discolored and having a gritty texture that is unappealing (Couch & Martin 1982). In the event of *U. crescens* infestations being prevalent in commercial crab catches, crabbers could be recommended to fish low salinity areas where *U. crescens* infections are less common.

Infestations of *Hematodinium perezi*

Blue crabs with *H. perezi* have been recorded in the Gulf of Mexico, predominantly in Texas, but not in the low salinity areas around LA (Messick & Shields 2000), and only at locations with a salinity of >11 ppt (Newman & Johnson 1975, Messick & Shields 2000, Coffey et al. 2012). *H. perezi* infestations were thus not expected to be found in the large blue crabs examined here. However, it was tested for by PCR due to it being blamed circumstantially in mortality of adult blue crabs captured at low salinity fishing grounds in LA. Additionally, it can proliferate in crabs infected in high salinity water that have migrated to low salinity water, but the hypothesized transmissive state is inactive so odds of transmission in wild crabs inhabiting low salinities are very low (Coffey et al. 2012). Consistent with expectations based on the low average water salinity (11.46 ± 7.28 ppt) across the 4 sampling sites, none of 760 blue crabs examined were identified to be PCR-positive for *H. perezi*. This included crabs collected at Grand Isle, the highest salinity site (20.48 ± 5.53 ppt average water salinity), and although *H. perezi* has been found in up to 29% of crabs sampled from comparable salinity sites in Chesapeake Bay, its prevalence is usually highest in waters with a salinity of >26 ppt (Messick & Shields 2000). In addition, as the prevalence of *H. perezi* is typically highest in smaller juveniles (Messick 1994), the exclusive analysis of adult and large juvenile blue crabs collected from the nearshore fishing grounds might also have contributed to its absence. While this finding is favorable ecologically and economically for the Gulf of Mexico fishery, it might be pertinent to examine smaller sized crabs from higher salinity areas. Additionally, as crabs that died following capture and prior to dissection were not examined, it is possible that handling stress in conjunction with *H. perezi* infection might have been excluded from this study.

Conclusions and future investigations

The data reported here are promising for the sustainability of the blue crab fishery of coastal LA. However, as this was only a 1 yr study at a time when low commercial crab landings were recorded, additional and ongoing surveillance for potentially lethal or harmful protozoan parasites such as *H. perezi*, *Ameson michaelis*, and *Thelohania* sp. as well as reo-like virus should prove valuable in maintaining the long-term health of the fishery.

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