Local populations of eastern oyster from Louisiana differ in low salinity tolerance

Suggested running head: low-salinity tolerance of Louisiana oyster populations

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U.S. Geological Survey, Louisiana Fish and Wildlife Cooperative Research Unit, School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA E-mail: mlapeyre@agcenter.lsu.edu Tel: (225) 578-4180 [A]Abstract

Eastern oysters support a critical commercial industry and provide many ecosystem services to coastal estuaries yet are currently threatened by changing estuarine conditions. A changing climate and the effects of river and coastal management are altering freshwater inflows into productive oyster areas, causing more frequent and extreme salinity exposure. Although oysters are tolerant to a wide range of salinity means and variation, more frequent and extreme exposure to low salinity (5) impacts oyster populations and aquaculture operations. This study assessed four Louisiana, USA oyster stocks to explore population-specific responses to low salinity exposure. Hatchery-produced progeny $(10 - 25$ mm) were deployed in baskets kept offbottom on longline systems in a low salinity (mean ± 1 SEM daily salinity of 8.7 \pm 0.2, ranging $1.2 - 19.0$) and a moderate salinity (mean ± 1 SEM daily salinity of 16.8 ± 0.3 , ranging 4.8 – 30.0) environment for one year, beginning in December 2019, with growth and mortality determined monthly. Significant differences in cumulative mortality between stocks at the end of the study were found at the low salinity site, with the greatest increase in cumulative mortality occurring mid-July to mid-August. Mortality differences between stocks suggest that some oyster populations (i.e., stocks) may be better suited to low salinity or low salinity events than others. This difference may be attributed to similarity between site of origin and grow out site conditions and/or to greater salinity variability and therefore higher phenotypic plasticity in some oyster populations compared to others. The identification of oyster stocks able to survive under extreme low salinity conditions may facilitate the development of "low salinity tolerant" broodstock to support aquaculture in areas experiencing and predicted to experience low salinity events.

[A]Introduction

The eastern oyster, *Crassostrea virginica* (hereafter, oyster), is a keystone species that provides critical ecosystem services and supports a productive commercial fishery in northern Gulf of Mexico (nGoM) estuaries (Coen et al. 2007, La Peyre et al. 2019a). Estuaries across nGoM face increasing environmental variability from climate change and management activities that impact freshwater inflow (Das et al. 2012, Powell and Keim 2015, CPRA 2017). These changes in freshwater inflow across previously productive areas, along with overharvesting and habitat destruction, are causing oyster population declines (Beck et al. 2011, Beseres Pollack et al. 2012). To balance high market demand with declining abundance, aquaculture production has become increasingly popular (Maxwell et al. 2008, Walton et al. 2013, Wadsworth et al. 2019), but its success may depend on identification of broodstock tolerant of predicted and changing water conditions.

Estuarine oyster aquaculture systems are dependent on the suitability of local water conditions (i.e., salinity, temperature, dissolved oxygen concentration, food availability, turbidity, water movement) for production (Shumway 1996, Bayne 2017). Of these conditions, salinity has a major influence on oyster growth and mortality in nGOM estuaries. Oysters are tolerant to a wide range of salinities, but their populations generally grow and survive best in mid-range salinities (Lowe et al. 2017). In Louisiana in particular, which has supported over 64% of nGoM oyster production in the past 10 years (NOAA 2020), many oyster producing areas already experience low annual mean salinities below 10 or frequent exposure to extreme low-salinity events (≤ 5) , sometimes for extended periods. The existence of oyster populations in areas exposed to lower salinity regimes, and continued oyster production in these areas suggests potential for local adaptation.

Increasing field and laboratory evidence from nGoM oyster populations indicate population specific responses to water quality conditions, including salinity (Leonhardt et al. 2017, Miller et al. 2017, Casas et al. 2017, Marshall et al. 2021a, 2021b). Marshall et al. (2021a) compared the performance of hatchery-produced progeny of two Texas and two Louisiana oyster populations at low (\sim 6 annual mean) and moderate (\sim 16 annual mean) salinity sites in Alabama and in salinities ranging from $2 - 44$ in a laboratory setting. Results indicated clear population responses with one Texas population surviving better at high salinity but worse at low salinity than either Louisiana populations (Marshall et al. 2021a), supporting earlier work indicating population specific adaptations (Barber et al. 1991, Dittman et al. 1998, Burford et al. 2014). Across a narrower geographic range, significant differences in the performance of oysters produced from broodstocks collected from three public oyster grounds in Louisiana and deployed along a salinity gradient were also reported by Leonhardt et al. (2017). Whether more locally adapted oyster populations could exist across Louisiana estuaries with low annual mean salinities or frequent and extended exposure to low salinity events needs to be investigated further (Leonhardt et al. 2017).

This study compares the performance of hatchery-produced progeny oysters from four oyster populations that exist in areas of Louisiana estuaries suspected to frequently have lower than optimal salinity conditions by determining their growth, mortality, condition index, and *Perkinsus marinus* infection intensity at a low and a moderate salinity site. This study is timely considering that predicted increases in precipitation and runoff in the southeastern USA alongside more frequent extreme rain events are increasing oyster exposure to both extended and acute low salinity (Powell and Keim 2015, Carter et al. 2018). Moreover, Louisiana estuaries face additional low salinity events from land loss management involving large-scale river

diversions into estuaries (Das et al. 2012, CPRA 2017), which may result in decreased salinity across oyster production locations and, consequently, increased oyster mortality (Soniat et al. 2013, Lavaud et al. 2021). Identifying oyster populations adapted to low salinity regimes and more tolerant of extreme low salinity provides a path to support development of broodstock for aquaculture, particularly given recent research suggesting heritability of low salinity tolerance (McCarty et al. 2020).

[A]Methods

[B]Broodstock collection sites

Oyster broodstocks were collected in early 2019 from four sites along the Louisiana coast: Sabine Lake (29°47'6.00"N, 93°55'5.02"W), Calcasieu Lake (29°51'2.34"N, 93°16'59.81"W), Point Au Fer (29°18'54.05"N, 91°21'49.30"W), and Pass a Loutre (29°11'32.24"N, 89°2'43.58"W) (Figure 1). Natural reefs exist across these locations, which represent a wide range of environmental conditions and riverine influence (Table 1).

Sabine Lake is an estuarine lake located at the southern end of the Sabine River Basin that experiences freshwater inflow from the Neches and Sabine rivers, consists of 22,280 ha of water bottom with oyster reefs in the southernmost portion of the lake, and has been closed to oyster harvest since the early 1960s (Louisiana Department of Wildlife and Fisheries (LDWF) 2019). Salinity data (bottom) for this stock collection site were obtained from the LDWF longterm sampling station (#3014; LDWF 2018) located at the site of broodstock collection (Figure 1, Table 1).

Calcasieu Lake is an estuarine lake located at the southern end of the Calcasieu River Basin that experiences freshwater inflow from the Calcasieu River, consists of 23,580 ha water bottom with oyster reefs, and supports oyster production (LDWF 2019). Salinity data (bottom) for this stock collection site was obtained from the LDWF long-term sampling station (#3018; LDWF 2018) located in close proximity (0.14 km southwest) to the broodstock collection site (Figure 1, Table 1).

Point Au Fer is a primarily open water brackish system that experiences freshwater inflow from the Atchafalaya and Vermilion rivers. The collection site supports a historic natural reef that is not open to harvest, but is proximal to public oyster seed grounds that experience extensive oyster mortalities except in years with reduced freshwater from the Atchafalaya River, therefore supporting intermittent oyster harvests (LDWF 2019). Salinity data for Point Au Fer was obtained from the Coastwide Reference Monitoring System (CRMS) data recorder CRMS6304-H01 (CPRA 2021) 14.3 km northeast of the broodstock collection site and from LDWF long term sampling station (#2101; LDWF 2018) located 25.6 km southeast to the broodstock collection site (Figure 1, Table 1).

Pass a Loutre is located on the eastern side of the Mississippi River Delta where the Mississippi River enters the Gulf of Mexico. The collection site in this location is not sampled for oysters regularly, and no other oyster reefs are known to exist in this area. The site receives extensive freshwater inflow from the Bohemia Spillway, Caernarvon and Bayou Lamoque freshwater diversion structures, and main-stem Mississippi River distributaries. Surface and bottom salinity data was obtained from LDWF (LDWF 2018) at a site 2.2 km west of the broodstock collection site (Figure 1, Table 1).

[B]Spawning and spat grow-out

One day after collection, broodstocks were transported to the Louisiana Sea Grant Oyster Research Farm (LASGRF) located off Grand Isle, LA (Figure 1). Oysters were placed in baskets (75 cm x 22 cm x 20 cm, 12 mm mesh size) suspended on an adjustable long-line system nearshore (ALS, BST Oyster Co., Cowell, South Australia) until they were spawned at the Michael C. Voisin Oyster Hatchery adjacent to LASGRF.

In summer 2019, individual oysters from each broodstock were naturally induced to spawn by increasing water temperature in a controlled setting (Wallace et al. 2008). Gametes were collected from spawning individuals and the eggs from each female were fertilized by sperm from each male and pooled (Table 2). Larvae were raised to the pediveliger stage and were then set on microcultch to produce single oyster spat using standard hatchery procedure (Wallace et al. 2008). The spat were grown in upwelling nursery systems until they grew past 6 mm in shell height, at which time they were transferred to baskets (6 mm mesh) on the long-line system at LASGRF until their use in this study. Once oysters grew past 12 mm shell height, they were transferred into 12 mm mesh baskets to improve water flow.

[B]Experimental design

The hatchery-produced progeny of each of the four broodstocks were deployed in December 2019 in baskets suspended on ALS at two grow-out sites, which differed in their salinity regimes. The moderate salinity site was at LASGRF and the low salinity site was located off the Louisiana Universities Marine Consortium facilities (LUMCON) in Cocodrie, LA. Four baskets of 100 oysters each were deployed at each grow-out site for the four stocks (4 stocks x 4 baskets x 2 sites x 100 oysters). Baskets were placed on the long line in a randomized block

design to account for unmeasured variation in the growing environment. Oyster mortality and growth were monitored monthly through November 2020. At each sampling, the numbers of live and dead oysters in each bag were recorded and dead oysters were discarded. The shell height (mm), the distance from shell umbo to distal edge, was then measured for a random subset of 25 oysters per bag. Mean shell height of each stock at the time of deployment is summarized in Table 2.

In October 2020, near the completion of this study, twenty oysters from each stock (five per basket) were haphazardly collected to determine *Perkinsus marinus* infection intensity (parasites per g wet tissue), infection prevalence (number of infected oysters / total number of oysters sampled * 100), and body condition index (100 x dry tissue weight / (whole weight shell weight)) as described by La Peyre et al. (2003, 2019b). *P. marinus* infection intensity was categorized as either no infection (0 parasite per g wet tissue), light infection $(1 - 10^4)$ parasites per g wet tissue), moderate infection $(10^4 - 5 \times 10^5)$ parasites per g wet tissue), or heavy infection $($ >5 x 10⁵ parasites per g wet tissue) (Casas et al. 2017). Condition index was used to indicate how well an oyster uses its shell cavity for tissue growth reflecting overall health and reproductive status, and estimates meat quality (Haven 1960, Lawrence and Scott 1982, Mann 1992).

[B]Water quality

Daily mean salinity and temperature data for LASGRF was obtained from the USGS 073802516 recorder Barataria Pass at Grand Isle, LA (USGS 2021), with missing data filled in using the closest USGS recorder (291929089562600 Barataria Bay near Grand Terre Island, LA Data Recorder; $R^2 = 0.8798$; USGS 2021). The primary recorder is located 6.6 km northeast of

the deployment site. Daily mean salinity and temperature data for LUMCON was obtained from the Marine Center Environmental Monitoring Station (LUMCON 2021). The recorder is located 0.4 km west of the deployment site.

[B]Data analyses and statistics

All statistical analyses were conducted using R v.3.6.3 (R Core Team 2020). A *P* < 0.05 was used to determine significance for all tests. Unless indicated otherwise, mean ± 1 SEM are presented. Mean daily salinity and temperature at both grow-out sites were compared using a paired t-test. Interval and cumulative mortality were calculated following the method of Ragone Calvo and Burreson (2003) and cumulative mortalities at the end of the study were compared using a two-factor (stock, site) Analysis of Variance (ANOVA) for each grow-out site followed by a Tukey post-hoc test. Mean growth rates ($mm \, \text{mo}^{-1}$) of each stock at both grow-out sites over the study duration were calculated by subtracting mean shell height from each basket $(n=4)$ at the start of the study from the mean shell height of the same basket at the end of the study, standardized to a 30-day month. Mean growth rates $(nm \, \text{mo}^{-1})$ were compared using a twofactor (stock, site) ANOVA followed by a Tukey post-hoc test. *P. marinus* infection intensity and condition index of oysters sampled in October 2020 were compared using a two-factor (site, stock) ANOVA followed by a Tukey post-hoc test.

[A]Results

[B]Water quality

Over the study period, mean daily salinity at LUMCON was significantly lower than at LASGRF (paired t-test, $t = 31.5$, $df = 343$, $P < 0.001$). The LASGRF grow-out site generally experienced moderate salinity throughout the study duration with a mean of 16.8 ± 0.3 , ranging 4.8 – 30.0 (USGS 2021; Figure 2). The LUMCON grow-out site generally experienced low salinity throughout the study duration with a mean salinity of 8.7 ± 0.2 ranging $1.2 - 19.0$ (LUMCON 2021; Figure 2). A period of extended, extreme low salinity (2.6 ± 0.2) was observed at LUMCON from mid-June through mid-July (Figure 2, Table S.1). At both sites, salinity trended downward from December through March, plateaued from April through July, and trended upward from August through December (Figure 2).

Over the study period, daily temperature followed expected seasonal trends and was within expected ranges for this region (paired t-test, $t = -3.514$, $df = 343$, $P \le 0.001$; Figure 2). Although significant differences in temperature were found between grow out sites, they likely did not impact oyster growth and mortality as trends were consistent and largely similar over time, and statistical differences are likely due to large sample size. At the LASGRF grow-out site, temperature ranged $10.7 - 32.5$ °C with a mean of 23.7 ± 0.3 °C throughout the study duration (USGS 2021; Figure 2). At the LUMCON grow-out site, temperature ranged 9.9 – 32.6°C with a mean of 23.9 ± 0.3 °C (LUMCON 2021; Figure 2). At both sites, temperature plateaued from December through February, increased from March through June, plateaued from July through August, and decreased from September through December (Figure 2).

[B]Mortality

At the study's completion, there was a significant site by stock interaction on cumulative mortality $(F_{3,31} = 9.835, P \le 0.001)$. This interaction is due to no significant difference between

the Sabine Lake stock (25.1%) at LUMCON and all stocks at LASGRF (<14%), but significant differences between the cumulative mortality of all stocks at LASGRF and the Calcasieu Lake (42.8%), Pass A Loutre (53.1%), and Point Au Fer stocks (70.2%) at LUMCON (Figure 3). The greatest increase in cumulative mortality at LUMCON was observed between mid-July and mid-August, a period of high mean temperatures $(30.5 \pm 0.3^{\circ}\text{C})$ and low mean salinity (7.2 ± 0.5) , and notably following a period (mid-June through mid-July) of very low mean salinity $(2.6 \pm$ 0.2) (Figure 2, Figure 3).

[B]Growth Rate

At the end of the experiment, oysters at LASGRF (mean shell height of 83.1 ± 0.5 mm) were larger than oysters at LUMCON (mean shell height of 47.4 ± 0.4 mm) (Figure 4). There was a significant site by stock interaction for overall mean growth rate over the study duration $(F_{3,31} = 6.403, P = 0.002)$. Overall mean growth rate was significantly higher at LASGRF than at LUMCON for all stocks (Table 3; Tukey HSD, *P* < 0.001). At LASGRF, only the Point Au Fer stock had a significantly higher overall mean growth rate than the Sabine Lake (Tukey HSD, $P =$ 0.03) and Calcasieu Lake (Tukey HSD, *P* = 0.007) stocks (Table 3). There were no differences in overall mean growth rates between stocks at LUMCON (Table 3).

[B]Condition index

When oyster condition indices were measured in October, a significant site by stock interaction was found $(F_{3,155} = 13.80, P \le 0.001)$. No significant differences could be shown between stocks at LASGRF, but the Calcasieu Lake stock at LUMCON had a significantly lower condition index than the stocks from Sabine Lake, Pass a Loutre, and Point Au Fer (Tukey HSD, $P < 0.001$; Table 3).

[B]*Perkinsus marinus* infection intensity

When determined in October, there was no significant interaction or effect of stock, but there was a significant effect of site with *P. marinus* infection intensities at LUMCON significantly lower than at LASGRF, with some moderate and heavy infection intensities only occurring at the LASGRF site $(F_{1,155} = 30.709, P \le 0.001$; Table 3). Prevalence of infection was higher at LASGRF ($>90\%$) than at LUMCON ($\leq 35\%$) for all stocks (Table 3).

[A]Discussion

F1 progeny of four Louisiana oyster populations suspected to be frequently exposed to low salinity (< 5) were assessed for differences in tolerance to low salinity. The broodstocks were collected at low to moderate salinity sites in estuarine lakes (i.e., Sabine, Calcasieu) or at the mouth of large rivers with high freshwater inflow (i.e., Point Au Fer at the mouth of the Atchafalaya River, Pass a Loutre at the mouth of the Mississippi River). Overall, oysters at the low salinity grow-out site had higher cumulative mortality compared to oysters at the moderate salinity site, with most mortality occurring between mid-July and mid-August concurrent with high temperatures. However, the most noteworthy differences were in cumulative mortalities between stocks at the low salinity site with the Sabine Lake stock having the lowest cumulative mortality (25.1%) and the Point Au Fer stock having the highest cumulative mortality (70.2%). The Sabine Lake stock also tended to have the highest growth rate at the low salinity site while the Point Au Fer stock had a greater growth rate than the Sabine Lake stock at the highest

salinity grow out site. Mortality differences between stocks suggest that within Louisiana, discrete populations of oysters may be better suited to frequent low salinity or low salinity events than others.

The overall higher performance of the Sabine Lake stock, in terms of lowest mortality and highest growth rates, at the low salinity grow out site could be because, based on best available data, mean salinity at the Sabine Lake site of origin was most similar to the mean salinity at the low salinity grow-out site (Sabine Lake: 13.2; LUMCON: 8.9) suggesting that Sabine Lake stock may be better adapted to these low salinity conditions. In contrast, the mean salinity at the Point Au Fer site of origin (16.4) was much higher than the mean salinity at the low salinity grow-out site, and this lack of previous exposure of this oyster population to low salinity may partially explain why the Point Au Fer oysters died faster than the Sabine Lake oysters (Marshall et al. 2021a). However, these site salinities are estimations based on data available in proximity to the collection sites and it is not clear if this hypothesis is fully supported due to infrequent data collection in proximity to sites and low continuous recorder coverage within the water column. While phenotypic differences in performance between stocks are evident at the low salinity site, the specific contribution of their genomes versus their epigenomes in low salinity tolerance will need to be determined in their subsequent (F2) oyster generations.

The observed difference in low salinity tolerance between stocks could be related to the relative condition of the various oyster stocks (e.g., level of genetic diversity, harvest pressure). Specifically, the oysters with the lowest mortality (Sabine Lake) are the progeny of broodstock taken from an area that has been closed to harvest for over 50 years and may contain an oyster population that has not experienced severe bottlenecks caused by high harvest pressure. As a

result, this population may have higher levels of genetic diversity and be better able to survive highly variable estuarine environmental conditions including extreme salinity (Hilbish and Koehn 1987, Hawkins and Day 1999, Reed 2005, Guo et al. 2018). Interestingly, the mean monthly salinity over a ten-year period (2009-2019; Supplementary Table 1) at the Sabine Lake site is more variable than at the other study sites which could have contributed to an increase in plasticity of the Sabine Lake oysters. More amplitude fluctuations in environmental conditions have been predicted to lead to increased phenotypic plasticity (de Jong 1995, Via et al 1995, Kassen 2002, Bitter et al 2021). Higher plasticity in salinity tolerance was also observed in the progeny of oysters collected in Aransas Bay, Texas compared to the progeny of oysters collected from three other nGoM estuaries that experience less salinity variation than Aransas Bay from year to year (Marshall et al. 2021a). Plastic divergence may also be generated by changes in epigenome or through other mechanisms (e.g., maternal investment) and be inherited without changes in DNA sequences across generations (Johnson and Kelly 2020; Griffiths et al. 2021).

The progeny of two broodstocks collected at outflow areas of a large river (Pass a Loutre and Point Au Fer populations) exhibited high mortality at the low salinity grow-out site despite the hypothesized exposure to frequent and extended low salinity at their sites of origin. One explanation for these unexpectedly high mortalities could be the presence of water stratification at the sites of origin, supported by monthly data at the Pass a Loutre site with 2009 – 2019 data showing bottom mean salinity 10.1 units higher than surface mean salinity and significantly higher than the mean daily salinity (2.1 ± 0.1) ; CRMS0161-H01) reported on the adjacent marsh (LDWF 2018), CPRA 2021). While Louisiana estuaries are generally described as shallow and well-mixed, it is possible that high river inflow, such as at Pass a Loutre and Point Au Fer, results in localized stratification, with nearby marshes flooded with freshwater while bottom

waters remain more influenced by marine waters (Laevastu and Hela 1970), but support of this hypothesis is limited by the availability of water quality data. Alternatively, it is possible that these poorly studied oyster populations are periodically exposed to acute salinity changes causing frequent die-off with concomitant loss of genetic diversity. Oysters from Calcasieu Lake had the second lowest mortality at the low salinity grow out site. This result was somewhat unexpected as this oyster population has historically been grown in intermediate to high salinity waters (Leonhardt et al. 2017). However, it is important to note a reduction of mean monthly salinity in Calcasieu Lake by about 4.3 (i.e., station 3003) in recent years $(2009 - 2014: 19.5,$ 2015 – 2019: 15.2; LDWF 2018). Increased freshening of the estuary along with overfishing contributed to a loss of 90% of the Calcasieu Lake oyster population (LDWF 2018) and may help explain the relatively low mortality of Calcasieu oysters at the low salinity grow out site. It also illustrates how natural and anthropogenic variability, which will likely increase with climate change, can shift the multidirectional selection pressure oysters routinely face in estuarine environments.

Similar to mortality, growth rate was significantly different between the low and moderate salinity grow-out sites. Oysters at the moderate salinity site grew faster than oysters at the low salinity site as previously reported (Kraeuter et al. 2007, La Peyre et al. 2016). Oyster filtration rate is significantly reduced when food quantity and quality are depressed by low salinity (Casas et al. 2018, Lavaud et al. 2017, Riekenberg et al. 2015). At the low salinity site, mean interval growth rate was lowest between mid-June and mid-July $(-0.21 \pm 0.3 \text{ mm} \text{ m}^{-1}$ all stocks combined; Supplementary Table S1), coinciding with highest temperatures and lowest salinity experienced through the study duration. Interestingly, the lack of oyster growth from mid-June to mid-July concomitant with an uptick in mortality preceded the mortality peak from

mid-July through mid-August, possibly acting as an early indicator of unfavorable conditions in the shorter term but with much deadlier consequences in the longer term.

Generally, oysters at the low salinity site had a higher condition index than oysters at the moderate salinity site, which can be attributed to a reduced or a delayed gonad development and spawning due to low salinity (< 10; Butler 1949, Loosanoff 1953, Marshall et al. 2021). At the low salinity site, oysters from the Calcasieu Lake population had a significantly lower condition index than the other populations, most likely because those oysters were slightly older when salinity became more favorable for spawning in late summer (Figure 4). In general, the relatively high condition in all oysters (> 10) was not unexpected for relatively young and small oysters like the ones used in this study, and it is unlikely that the small differences observed between populations impacted overall oyster mortality (Casas et al. 2017).

Oysters at the lower salinity site experienced both lighter infection intensity and lower overall prevalence of infection at the low salinity site compared to the moderate salinity site which can be attributed to limited or delayed development of *P. marinus* at lower salinities (Chu and La Peyre 1993, La Peyre et al. 2003, Ragone Calvo and Burreson 2003, Bushek et al. 2012). Additionally, with higher mortalities seen at the site with lighter infection (LUMCON), we concluded that *P. marinus* infection was not a leading cause of differing mortality between stocks in this study. Infection intensity considered high enough to cause mortality $($ > 500,000 parasites per g wet tissue) did not occur in most individuals in this study (La Peyre et al. 2019b).

Overall, our findings indicate differences in low salinity tolerance between Louisiana oyster populations and provide further evidence that phenotypic differentiation in oysters can occur within relatively small regions. Whether genetic and plastic divergence explain these differences remains to be determined. These findings could be due to local adaptation based on site of origin conditions, but the lack of adequate site-specific water quality data limits our ability to fully support this hypothesis. The progeny of the broodstock collected at the lowest salinity site (based on best available data), Sabine Lake, did have the lowest mortality and highest growth rate at the low salinity grow-out site, suggesting potential local adaptation. Increased monitoring of water quality across open-water areas, including bottom water conditions will be critical to explain these and other findings related to population specific oyster tolerances to various conditions. Furthermore, the identification of additional adapted populations and exploration of the underlying molecular mechanisms and genetics associated with low salinity tolerance may promote the use of adapted stocks in aquaculture, specifically in areas experiencing and predicted to experience low salinity events.

[A]Acknowledgments

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Tables

Table 1. Data source, geographic coordinates, relative position to oyster broodstock collection site, data time period, frequency of sampling, long-term mean \pm SEM salinity, and minimum and maximum salinity reported for bottom environmental data collected at stations close to broodstock collection sites at Sabine Lake (SL), Calcasieu Lake (CL), Point Au Fer (PAF) and Pass a Loutre (PAL). Due to the absence of a monitoring station in close proximity to the Point Au Fer broodstock collection site, data from two stations further away are provided. Available surface salinity data are also provided for the Pass a Loutre station. Data for all collection sites were obtained from the Louisiana Department of Wildlife and Fisheries (LDWF) independent monitoring stations (LDWF 2018), LDWF independent sampling (LDWF 2018), or the Coastwide Reference Monitoring System (CRMS; CPRA 2021).

Table 2. Date of spawning and number of males and females of each broodstock spawned at Louisiana Sea Grant Oyster Research Farm (LASGRF) to produce progeny of populations. Mean ± SEM shell height of progeny oysters at deployment of study 12/12/2019. Sabine Lake (SL), Calcasieu Lake (CL), Point Au Fer (PAF) and Pass a Loutre (PAL).

Stock	Date spawned	Eggs fertilized	$#$ Males	$#$ Females	LASGRF Initial mean \pm SEM shell height	LUMCON Initial mean \pm SEM shell height
SL	7/16/2019	4.48×10^{7}	9	9	11.3 ± 0.2	10.8 ± 0.2
CL	6/4/2019	3.15×10^8	2	5	23.1 ± 0.3	22.2 ± 0.3
PAF	8/6/2019	4.41×10^8	17	15	16.9 ± 0.2	16.4 ± 0.2
PAL	8/6/2019	2.83×10^8	13	13	17.0 ± 0.2	17.3 ± 0.2

Table 3. Mean \pm SEM growth rate (mm mo⁻¹) from the time of deployment at the Louisiana Sea Grant Oyster Research Farm (LASGRF) and the Louisiana Universities Marine Consortium (LUMCON) on 12/12/2019 to the end of the study on 11/19/2021, condition index, infection prevalence (number of infected oysters / total number of oysters sampled * 100), and infection intensity of *Perkinsus marinus* (parasites per g wet tissue) reported for each population, Sabine Lake (SL), Calcasieu Lake (CL), Point Au Fer (PAF) and Pass a Loutre (PAL). Letters reflect statistically significant differences within each parameter $(p<0.05)$.

Site	Stock	Growth rate	Condition index	Infection prevalence	Infection intensity
LASGRF	SL	5.6 ± 0.1^z	11.8 ± 0.3 ^{zx}	90	$10,9981 \pm 53,825$ ^z
	CL	5.5 ± 0.05^z	10.3 ± 0.5^z	100	$400,528 \pm 169,432$ ^z
	PAF	6.1 ± 0.1 y	10.0 ± 0.5^z	95	$62,270 \pm 40,033$ ^z
	PAL	5.9 ± 0.03 ^{zy}	10.0 ± 0.3^z	100	$132,720 \pm 82,985$ ^z
LUMCON	SL	3.0 ± 0.04 ^x	19.6 ± 0.7 ^y	35	17 ± 6 ^y
	CL	2.6 ± 0.1^x	13.6 ± 0.5^x	30	10 ± 5 ^y
	PAF	2.6 ± 0.1^x	18.1 ± 0.6 ^y	15	4 ± 2 y
	PAL	2.7 ± 0.2^x	19.7 ± 0.7 ^y	30	$5 \pm 2y$

Figures

Figure 1. Locations of broodstock collection sites (Sabine Lake, Calcasieu Lake, Point Au Fer, and Pass a Loutre) and grow-out sites (Louisiana Sea Grant Oyster Research Farm (LASGRF) in Grand Isle, LA and the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie, LA). Zoomed panels depict each broodstock collection site and associated environmental data recorder.

Figure 2. Daily water salinity and temperature (°C) from December 12, 2019 to November 19, 2020 from continuous recorders at the Louisiana Universities Marine Consortium (LUMCON) (LUMCON 2021) and the Louisiana Sea Grant Oyster Research Farm (LASGRF) at Grand Isle (Barataria Pass at Grand Isle, LA, 073802516; USGS 2021), and Barataria Bay near Grand Terre Island, LA 291929089562600, USGS 2021). Solid horizontal line represents salinity of 5.

Figure 3. Cumulative mortality (%) (mean \pm SEM) of the progeny of oysters collected from Sabine Lake, Calcasieu Lake, Point Au Fer, and Pass a Loutre. Different letters denote statistical differences $(p<0.05)$. LASGRF = Louisiana Sea Grant Oyster Research Farm, LUMCON = Louisiana Universities Marine Consortium.

Figure 4. Mean \pm SEM shell height (mm) of the progeny of oysters collected from Sabine Lake, Calcasieu Lake, Point Au Fer, and Pass a Loutre. LASGRF = Louisiana Sea Grant Oyster Research Farm, LUMCON = Louisiana Universities Marine Consortium

Table S.1. Interval data (mean ± SEM) at high salinity (LASGRF, Louisiana Sea Grant Research Farm, LA) and low salinity (LUMCON, Louisiana Universities Marine Consortium, LA) grow out sites, reported for four tested populations of oysters (Sabine Lake, Calcasieu Lake, Point Au Fer, and Pass a Loutre). Interval data are mean ± SEM data obtained between field sampling dates. For each interval, initial shell height (SH, mm) was taken from 25 randomly sampled oysters at each sampling, mortality rate (%/mo) is $[((# dead#total)*100)/# days$ since previous sampling) * 30], and growth rate (mm/mo) is [(change in SH from previous sampling) / (days since previous sampling) * 30].

Table 1. Data source, geographic coordinates, relative position to oyster broodstock collection site, data time period, frequency of sampling, long-term mean ± SEM salinity, and minimum and maximum salinity reported for bottom environmental data collected at stations close to broodstock collection sites at Sabine Lake (SL), Calcasieu Lake (CL), Point Au Fer (PAF) and Pass a Loutre (PAL). Due to the absence of a monitoring station in close proximity to the Point Au Fer broodstock collection site, data from two stations further away are provided. Available surface salinity data are also provided for the Pass a Loutre station. Data for all collection sites were obtained from the Louisiana Department of Wildlife and Fisheries (LDWF) independent monitoring stations (LDWF 2018), LDWF independent sampling (LDWF 2018), or the Coastwide Reference Monitoring System (CRMS; CPRA 2021).

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Local populations of eastern oyster from Louisiana differ in low salinity tolerance

Suggested running head: low-salinity tolerance of Louisiana oyster populations

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					Initial mean \pm	Initial mean \pm
					SEM shell	SEM shell
					height	height
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