Stock assessment for eastern oyster seed production and field grow-out in Louisiana

Justin M. Leonhardt [a], Sandra Casas [b], John E. Supan [a,c], Jerome F. La Peyre [b,*]

[a] School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA, 70803, USA
[b] School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
[c] Louisiana Sea Grant College Program, Louisiana State University, Baton Rouge, LA, USA

ARTICLE INFO

ABSTRACT

There is little information on the performance of oyster populations from Louisiana estuaries limiting the ability to choose stocks for hatchery seed production and field grow-out. The objectives of this study were therefore to compare the mortality, growth, dermo (Perkinsus marinus) infection intensity and condition index of the progeny of wild oysters collected from three Louisiana estuaries differing in salinity regime and oysters specifically selected for dermo resistance. Progeny were deployed in cages in the field, along a salinity gradient in coastal Louisiana. Overall, salinity and temperature had major impacts on the mortality, growth, dermo infection intensity and condition index of oysters of all four stocks and a few differences between stocks could be shown at some sites. At the lowest salinity site, the progeny of wild oysters from Sister Lake, a low salinity estuarine lake, had the lowest mortality suggesting enhanced tolerance to low salinity conditions compared to the other stocks. At the highest salinity site, the progeny of wild oysters from Lake Calcasieu, a high salinity estuarine lake, had the lowest mortality during summer concomitant with increasing dermo infection intensities suggesting a better resistance to dermo disease compared to the other wild stocks and confirming an earlier finding. This initial result suggests that the stocks used are genetically differentiated with respect to low salinity tolerance as well as dermo-related mortality at high salinity and that stock selection for aquaculture grow-out or restoration effort will benefit from being site-specific and dependent on the dominant environmental conditions.

Statement of relevance: Assessment of eastern oyster stocks in Louisiana or other Gulf of Mexico estuaries for seed production and field grow-out is lacking.

1. Introduction

Louisiana leads the nation in the production of oysters, typically accounting for about 34% of the nation's landings and over 55% of the landings along the Gulf of Mexico in 2012 (Louisiana Department of Wildlife and Fisheries, LDWF, 2014). The success of the Louisiana eastern oyster industry is due in large part to an effective public/private partnership in which the LDWF manages the public grounds for the production of seed oysters (25–75 mm) for transplant to private leases where they are cultivated on-bottom and subsequently harvested. While eastern oysters continue to support a viable industry, increased harvest of seed and market oysters from public grounds in recent years has resulted in a net deficit of shell negatively impacting the availability of seed oysters for private leases (Soniat et al., 2012). Moreover, the variability in seed availability from year to year due to natural fluctuations in reproduction and recruitment of wild oysters along with unpredictable mortalities

due to predation and disease during on-bottom grow-out on leases that can reach 50 to 85%, continue to be problematic for oyster farmers (Owen, 1953; Powell et al., 1996; La Peyre et al., 2016).

Intensive oyster aquaculture, which combines hatchery production of seeds and improved grow-out methods, could play an important role in increasing production and sales of oysters from Louisiana and other Gulf States (Maxwell et al. 2008; Walton et al., 2013). Hatchery production can also serve to augment production of public oyster beds as previously attempted in Chesapeake Bay and recently implemented in Louisiana estuaries by LDWF, or in restoration activities (Carlsson et al., 2008; La Peyre et al., 2014). A major advantage with using hatchery produced seed is that it enables selection of broodstocks best adapted to local environmental conditions (Frank-Lawale et al., 2014). Previous studies have shown that optimal temperature and salinity combinations for oyster health and reproduction are population-dependent (Barber et al., 1991 Dittman et al., 1998; Brown et al., 2005; Burford et al., 2014). Moreover, oysters can also be selected on the basis of their increased survival after challenge with Perkinsus marinus, the protistan parasite causing dermo disease that is prevalent in Gulf of Mexico estuaries. The need to develop stocks of locally adapted oysters that are resistant

* Corresponding author at: Animal and Food Sciences Laboratory Building, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA.
E-mail address: jlapeyre@agcenter.lsu.edu (J.F. La Peyre).

to disease has long been recognized (Haskin & Ford, 1979; Matthiessen et al., 1990; Ragone Calvo et al., 2003a).

To date, little information has been gathered on the performance of Louisiana oyster populations from various Louisiana estuaries limiting the ability to choose stocks for hatchery seed production and predict their performance in varying environmental conditions. Given Louisiana estuaries wide range of salinity and the increasing interest in oyster aquaculture and restoration efforts, there is a need to determine the performance of oysters from various Louisiana estuaries. This is especially critical as LDWF policy is to avoid the introduction of out of state oysters into Louisiana waters. The objectives of this study were therefore to compare the mortality, growth, condition index and dermo infection intensity of the progeny of wild oysters collected from public oyster grounds of three Louisiana estuaries differing in salinity regime. In addition, a fourth group of Louisiana oysters consisting of the progeny of a stock selected for increased dermo resistance was included for comparison. The hatchery produced progenies of the four oyster broodstocks were deployed at three sites along a salinity gradient in Breton Sound estuary, LA and at a high salinity site off Grand Isle in Barataria Bay estuary, LA to represent different grow-out environmental conditions. Breton sound is a key public oyster ground that has experienced a significant decline in harvest in recent years and has been targeted for seeding with hatchery propagated oysters by LDWF (Soniat et al., 2012; La Peyre et al., 2013; LDWF, 2014).

2. Materials and methods

2.1. Oysters

The wild stocks used in the study were collected in October and November 2010 from three public oyster grounds, Sister (Caillou) Lake (29.2341°N; 90.9172°W), Breton Sound (Bay Gardene, 29.5910°N 89.6425°W) and Lake Calcasieu (29.5100°N; 93.1900°W). These grounds have different salinity regimes with yearly means (± standard deviation) calculated from 2003 to 2011 (N = 9) of 11.9 ± 2.4 for Sister Lake, 10.4 ± 2.5 for Breton Sound's Bay Gardene and 20.4 ± 2.2 for Lake Calcasieu. Daily salinities for each of these areas were obtained from USGS data recorders (SL-07381349, BG-07374527, LC-08017118) to calculate yearly means. All oysters collected were transported to the Louisiana Sea Grant Oyster Research Hatchery and Demonstration Farm in Grand Isle, LA (29.2380° N, 90.0030° W), where they were placed in labeled aquaculture bags held in an adjustable long line system (ALS, BST Oyster Co., Cowell, South Australia) prior to spawning.

The oyster stock named 'OBOY', selectively bred for dermo-resistance in Grand Isle, consists of the descendants of large oysters, collected in 1999, from a dermo endemic area (i.e., Oyster Bayou, Cameron Parish, 29.7941°N; 93.3872°W). The stock progeny has been challenged in the field (F0) and in the laboratory (F1 and F2) with P. marinus for two subsequent generations.

All four stocks were spawned at the Louisiana Sea Grant Oyster Hatchery in May 2011 to produce an F0 generation for the wild stocks and an F4 generation for the OBOY stock. Each stock was naturally spawned using about 150 oysters and the resulting larvae were reared using methods similar to Dupuy et al. (1977). Pediveliger (~280 μm) larvae were then set on micro-cultch material (~500 μm ground oyster shell) to produce single oyster spat. After 48 h, the resulting oyster spats were transferred to an upwell nursery system, where they were grown to 25 mm in shell height (i.e., seed oysters) prior to placement on the ALS at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle. Seed oysters were held in the ALS until the start of the study.

2.2. Study sites

The oysters were deployed in bags at three different sites along a low to intermediate salinity gradient in the lower Breton Sound estuary in

Southeast Louisiana and at a high salinity site at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle (Fig. 1). The sites were chosen based on nine years (2003 − 2011) of salinity data from USGS real-time monitoring stations located in Breton Sound and Barataria Pass, which is adjacent to Grand Isle. In Breton Sound, Cow Bayou (CB) was selected as the low salinity site (yearly mean of 6.2 ± 2.3, USGS Recorder 073745258), Bay Gardene (BG) as the low-intermediate salinity site (yearly mean of 10.4 ± 2.5, USGS Recorder 07374527), and Mozambique Point (MP) as the intermediate salinity site (yearly mean of 13.1 ± 2.5, LDWF weekly salinity data). Grand Isle (GI), a barrier island bordering the Gulf of Mexico, was selected as the high salinity site (yearly mean of 20.9 ± 1.9, USGS Recorder 073802516).

### 2.3. Study design and measurements

On October 12, 2011, 16 ALS culture bags containing 75 oysters per bag were prepared for each stock at the Louisiana Sea Grant Oyster Research Hatchery and Demonstration Farm. The shell heights of 25 oysters from each bag were sampled haphazardly and the bags were returned to the ALS. On November 4, 2011, 12 bags from each stock were deployed in Breton Sound, four bags of each stock per site. Bags deployed at the three sites in Breton Sound were held just off-bottom by 0.3 m PVC legs. The four bags from each stock that remained at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle were suspended beneath the water surface in the ALS. These bags were not air dried to maintain consistency with the bags deployed in Breton Sound. In summary, four ALS culture bags containing 75 oysters per bag were deployed for each stock at each of the four sites, or three hundred oysters per stock per site, for a total of 1200 oysters deployed at each site. All bags used for the study were fully enclosed to prevent predation mortality. Since predation was largely removed, mortality could be more readily attributed to stressful abiotic conditions and P. marinus. At all four sites, oyster mortality (counts of live/dead) and growth (shell height) data were collected about bimonthly for a total of seven sampling periods (Table 1). In addition, 15 oysters from each stock at each site were sampled in March (CB, BG, MP) or April (GI), July and September 2012 to determine changes in P. marinus infection intensities and condition index from the time of deployment following protocols listed below.

Hourly water salinity and temperature data were obtained from real-time monitoring stations located in Cow Bayou (USGS station 073745258), Bay Gardene (USGS station 07374527) and Barataria Pass at Grand Isle (USGS station 073802516) adjacent to where the oysters were deployed. An YSI-650 sonde (YSI Incorporated, Yellow Springs, OH) was placed at Mozambique Point which lacked a USGS real-time monitoring station, to record salinity and temperature hourly. Daily salinity and temperature means calculated from the hourly data at each site were used to determine interval salinity and temperature (mean ± standard deviation) between sampling about every two months.

Mortality was measured by counting dead oysters and the proportion of dead to total oysters was calculated to determine interval mortality for each stock at each site. Dead oysters were discarded at each sampling. Cumulative mortality was calculated following Ragone Calvo et al. (2003a).

Twenty-five oysters from each bag were sampled haphazardly and their shell heights were measured from shell umbo to distal edge using a digital caliper (ABS Coolant Proof Calipers, Mituyoto Corporation, Japan). Monthly interval growth rate was calculated as the increment in mean shell height between two consecutive sampling times divided by the number of days between sampling and standardized to a 30 day period. Mean growth rate was calculated using the mean shell height of each bag (N = 4).

Perkinsus marinus infection intensity was determined by sampling 15 oysters (i.e., 3–4 oysters per bag) from each stock at each site (Table 1). The number of parasites per gram of oyster wet tissue was
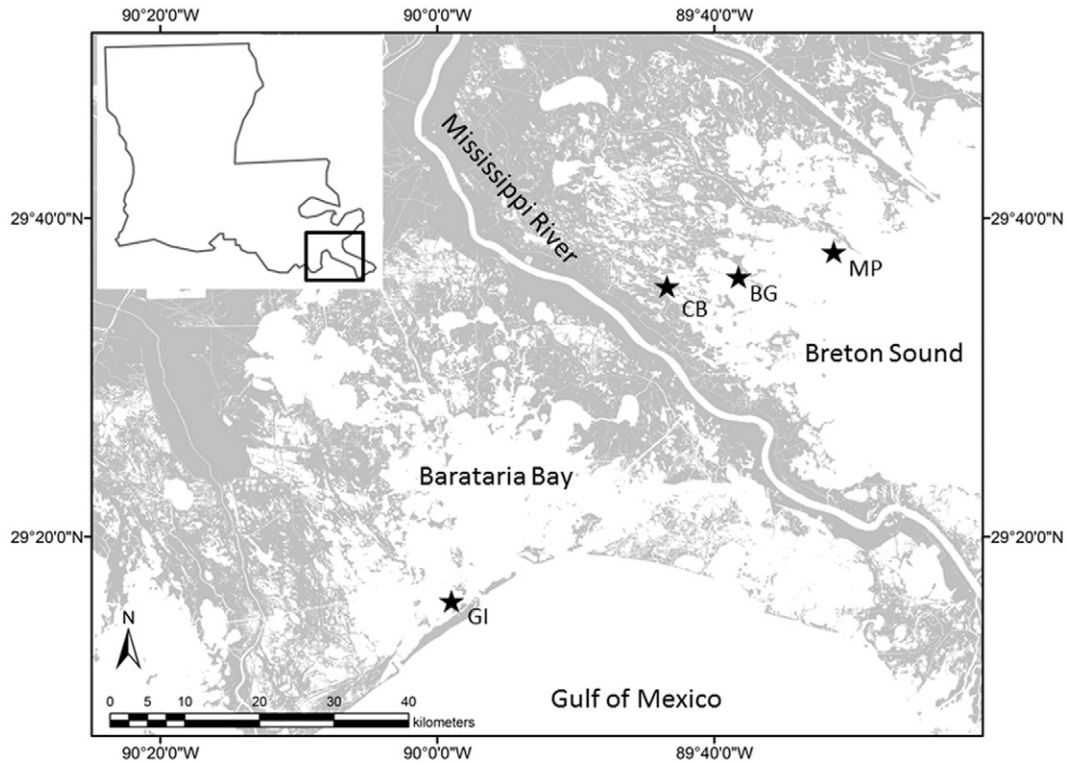
Fig. 1. Map of study area indicating the low salinity site, Cow Bayou (CB), the low-intermediate salinity site, Bay Gardene (BG), the intermediate salinity site, Mozambique Point (MP) and the high salinity site, Grand Isle (GI) where oysters were deployed.

determined using the whole-oyster procedure as described by Fisher & Oliver (1996) and modified by La Peyre et al. (2003).

Condition index (CI) was calculated as the ratio of dry tissue weight to whole weight – shell weight multiplied by 100 using variation of Hopkins (1949) formula as recommended by Lawrence and Scott (1982). For each oyster sampled (Table 1), a 10 ml aliquot of oyster tissue homogenate that was prepared to determine *P. marinus* infection intensity, was dried at 65 °C for 48 h and the dry weight for the whole oyster was calculated based on the total volume of homogenized tissue as described by La Peyre et al. (2003).

2.4. Statistical analyses

SigmaStat version 3.5 (Systat Software Inc. SigmaStat 3.5, San Jose, California, USA) was used to analyze the data. Results of daily salinity and temperature were analyzed using a Kruskal-Wallis one-factor (i.e., site) analysis of variance (ANOVA) on ranks followed by Dunn's multiple comparison procedure. Interval salinity and temperature data at each site were analyzed using a Kruskal-Wallis one-factor (i.e., sampling interval) ANOVA followed by Dunn's multiple comparison procedure. Oyster cumulative mortality (%) at the end of the study was compared using a series of Chi-Square analyses with a significance value of $\alpha = 0.05$ to determine differences between stocks at each site. A one-factor (i.e., stock) ANOVA was used to compare shell heights (mm) at the beginning of the study and at the end of the study at each site followed by post-hoc Tukey-Kramer pairwise comparisons ($\alpha = 0.05$) when significant differences ($p < 0.05$) were found. Growth rates (mm mo^{-1}) at each site were compared using a two-factor (i.e., stock, sampling interval) ANOVA followed by post-hoc Tukey-Kramer pairwise comparisons ($\alpha = 0.05$) when significant differences ($p < 0.05$) were found. Growth rate data were examined using a multiple linear regression with interval salinity, temperature and initial shell height as predictor variables.

Table 1

Sampling schedule for Grand Isle and Breton Sound (Cow Bayou, Bay Gardene, Mozambique Point) sites. On October 12, 2011, 16 culture bags were prepared for each stock at the Louisiana Sea Grant Oyster Research Hatchery and Demonstration Farm and oysters sampled. On November 4, 2011, 12 bags from each stock were deployed at the Breton Sound sites and four bags of each stock remained in Grand Isle. Four bags of each stock were sampled at each site at the date indicated.

Date	Mortality	Shell height	*P. marinus* infection intensity	Condition index
Oct 12, 2011	GI	GI	GI	GI
Jan 21, 2012	GI	GI		
Jan 27, 2012	CB, BG, MP	CB, BG, MP		
Mar 16, 2012	CB, BG, MP	CB, BG, MP	CB, BG, MP	CB, BG, MP
Apr 12, 2012	GI	GI	GI	GI
May 23, 2012	GI	GI		
May 31, 2012	CB, BG, MP	CB, BG, MP		
Jul 5, 2012	CB, BG, MP	CB, BG, MP	CB, BG, MP	CB, BG, MP
Jul 16, 2012	GI	GI	GI	GI
Sept 12, 2012	GI	GI	GI	GI
Sept 19, 2012	CB, BG, MP	CB, BG, MP	CB, BG, MP	CB, BG, MP
Oct 26, 2012	CB, BG, MP	CB, BG, MP		
Nov 28, 2012	GI	GI		

Perkinsus marinus infection intensities (number of parasites per g wet tissue) of each stock during the study were compared at each site using a Kruskal-Wallis one-factor ANOVA on ranks. Condition index of each stock at the beginning of the study was compared using a one-factor (i.e., stock) ANOVA. A two-factor (i.e., stock, sampling time) ANOVA was used to compare condition index at each site followed by post-hoc Tukey-Kramer pairwise comparisons ($\alpha = 0.05$). All data are expressed as means $\pm$ standard deviations.

3. Results

3.1. Water salinity and temperature

Daily salinity over the study period varied among all sites ($p < 0.0001$) with Cow Bayou (5.1 ± 3.0) having significantly lower salinity and Grand Isle (22.2 ± 5.8) having significantly higher salinity than Mozambique Point (12.9 ± 5.2) and Bay Gardene (10.4 ± 4.7). Cow Bayou (CB) was therefore referred to as the low salinity site, Bay Gardene (BG) as the low-intermediate salinity site, Mozambique Point (MP) as the intermediate salinity site and Grand Isle (GI) as the high salinity site. There was a general trend of decreasing salinities from December 2011 to April 2012 and increasing salinity from June to November 2012 at all sites (Fig. 2). The lowest interval salinities were from March to May at the Breton Sound sites and from January to April and April to May in Grand Isle (Fig. 3).

Daily water temperatures over the study period did not vary among sites ($p = 0.514$). There was a general trend of decreasing temperature from November 2011 to February 2012 and September 2012 to November 2012, and increasing temperature from February to May 2012 at all sites (Fig. 2). The lowest interval temperatures were from October to January and January to March at the Breton sites and from October to January and January to April in Grand Isle while the highest interval temperatures were from May to July and July to September at the Breton Sound sites and April to July and July to September in Grand Isle (Fig. 3).

3.2. Mortality

In low salinity, the Sister Lake stock had significantly lower cumulative mortality (59.3%) and the OBOY stock (83.5%) had significantly higher cumulative mortality than both Lake Calcasieu (72.3%) and Breton Sound (75.3%) stocks (Fig. 4). In low-intermediate salinity, the Sister Lake (4.7%) and Lake Calcasieu (8.7%) stocks cumulative mortalities were significantly lower than both Breton Sound (17.2%) and OBOY (15.3%) stocks (Fig. 4). Similar significant differences in cumulative mortalities were observed at intermediate salinity, with the Sister Lake (6.9%) and Lake Calcasieu (7.5%) stocks cumulative mortalities being significantly lower than Breton Sound (13.6%) and OBOY (15.1%) stocks (Fig. 4). At high salinity, the Lake Calcasieu stock (13.4%) had significantly lower cumulative mortality than both Breton Sound (26.4%) and OBOY (23.3%) stocks (Fig. 4) while the Sister Lake stock (18.7%) had significantly lower cumulative mortality than the Breton Sound stock.

While interval mortality could not be analyzed statistically, interval mortality for all stocks appeared highest between March and May at the two lowest salinity sites, between July and September at the intermediate and high salinity sites (Fig. 5). The Sister Lake stock appeared to have the lowest interval mortalities of all stocks at the two lowest salinity sites. The Sister Lake stock, however, appeared to have the highest interval mortality and the Lake Calcasieu stock had the lowest interval mortalities at the highest salinity site (Fig. 5). The spike in interval mortality at the intermediate salinity site between July and September was due to mud smothering of the caged oysters during Hurricane Isaac on August 28, 2012.
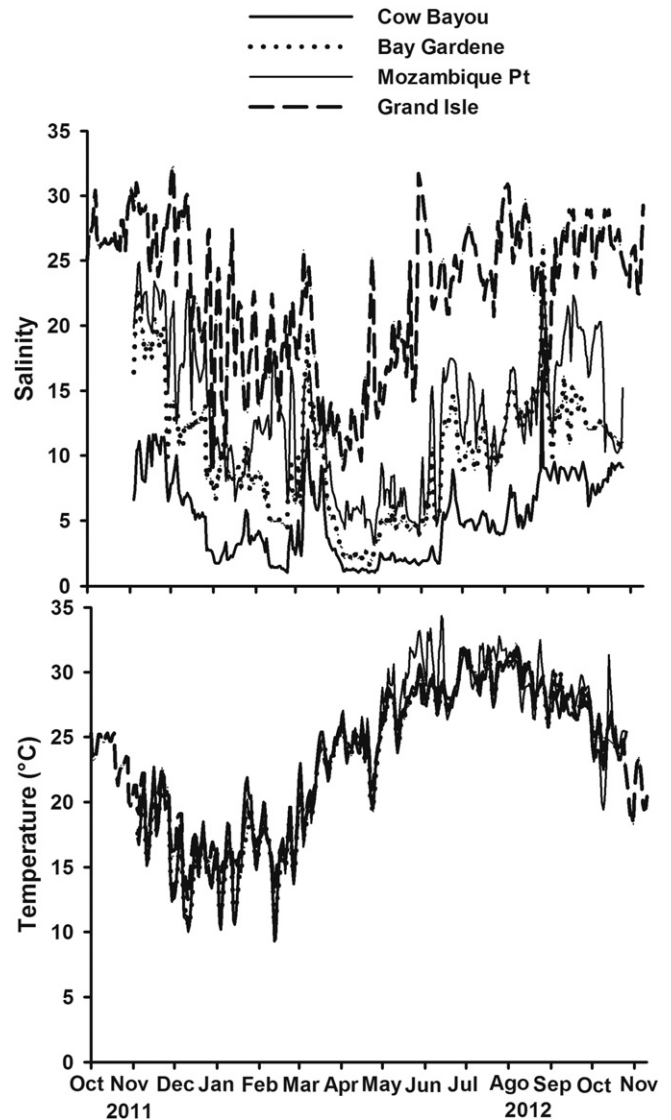

Fig. 2. Daily water temperature and salinity from October 2011 to November 2012 from continuous data recorders at the low salinity site (Cow Bayou, CB, USGS-073745258), the low-intermediate salinity site (Bay Gardene, BG, USGS-07374527), the intermediate salinity site (Mozambique Point, MP, deployed YSI-650 sonde) and at the high salinity site (Grand Isle, GI, USGS-073802516).
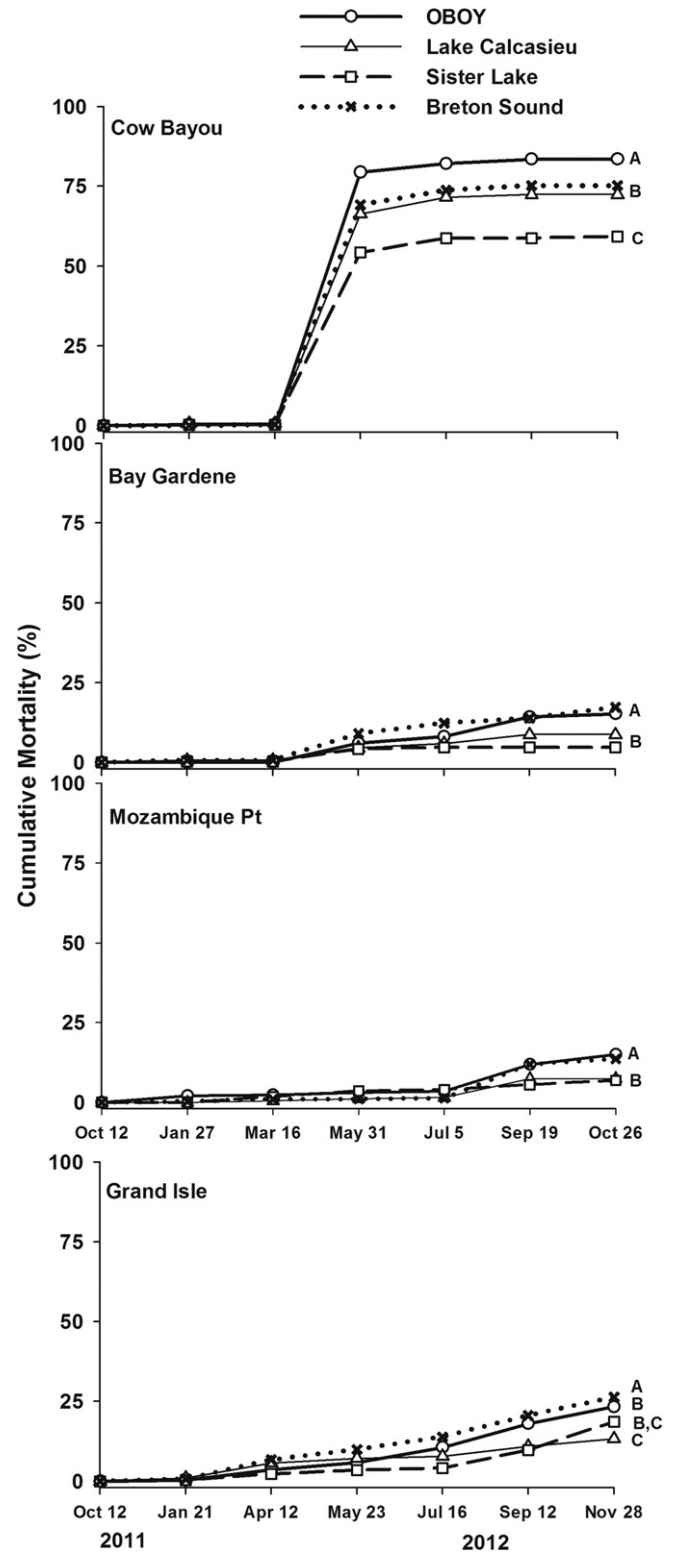
3.3. Growth

At the beginning of the study, no difference could be found in the mean shell heights (mm $\pm$ SD) of oysters from each stock to be deployed at each site (Overall; LC: 46.6 ± 6.9 mm, SL: 47.3 ± 6.7 mm, OBOY: 47.1 ± 7.1 mm; BS: 48.0 ± 8.0 mm). At the end of the study, few significant differences in shell heights could be shown between stocks at each site (Fig. 6). Sister Lake stock had significantly higher shell height than the OBOY stock at the lowest salinity site and, than the OBOY and Breton Sound stocks at the intermediate salinity site. Lake Calcasieu stock had significantly higher shell height than the Breton Sound stock at the intermediate salinity site.

Interval growth rate of oysters at the low-intermediate salinity site was significantly affected by the interaction of stock and interval while interval growth rates at the three other sites differed only by intervals (Fig. 7). Interval growth rates during the first interval of the study, October through January were significantly greater than during other intervals except between September and October at the low salinity site (Fig. 7). In general, interval growth rates tended to be positively
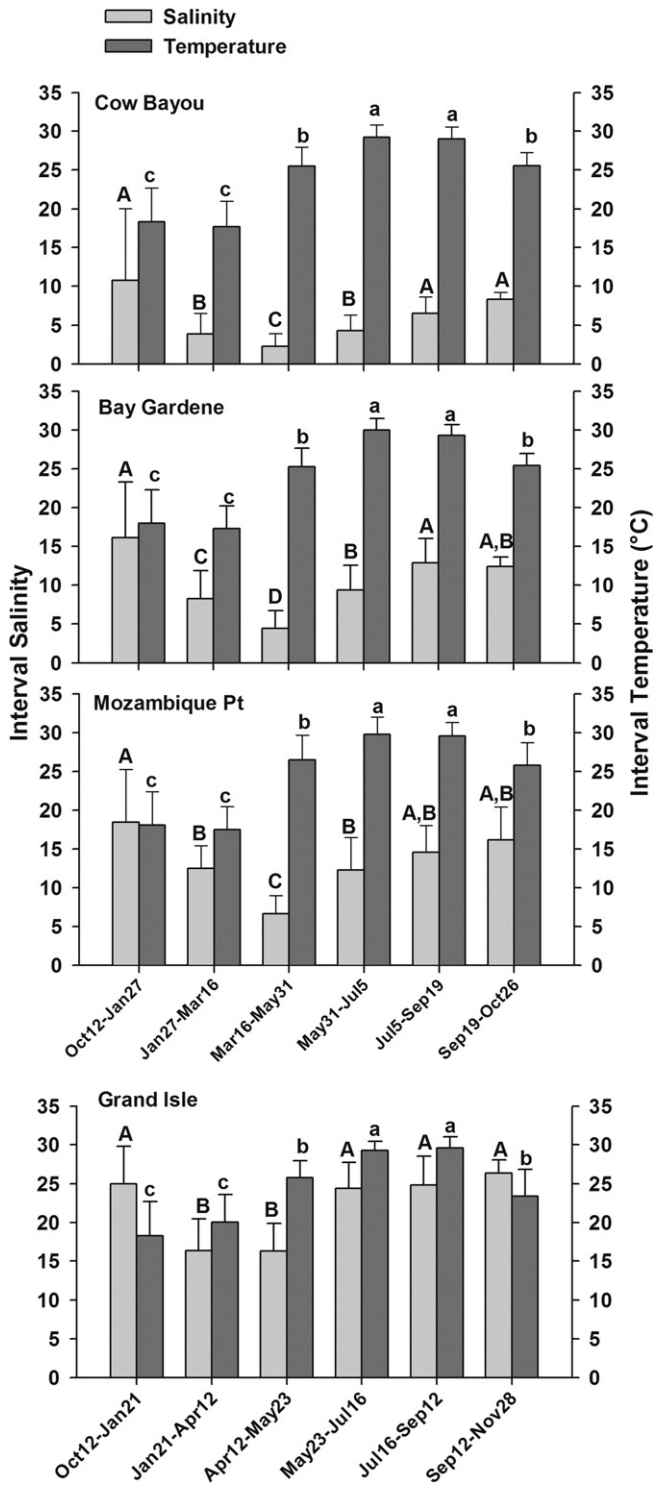
Fig. 3. Mean interval water salinity and temperature ± standard deviation calculated from daily salinity from continuous recorders at the low salinity site (Cow Bayou), the low-intermediate salinity site (Bay Gardene), the intermediate salinity site (Mozambique Point) and at the high salinity site (Grand Isle). Mean interval salinity and temperature from October 12, 2011 to January 27, 2012 for oysters deployed at the Breton Sound sites on November 4, 2011 were calculated by combining salinity and temperature data at Grand Isle from Oct 12 to November 4, and at the Breton Sound sites from November 5 to January 27. Different letters represent statistical differences (p < 0.05).

Fig. 4. Cumulative mortality (%) of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou), the low-intermediate salinity site (Bay Gardene), the intermediate salinity site (Mozambique Point) and at the high salinity site (Grand Isle).

correlated with interval salinities except July through September at the two highest salinity sites. The lowest growth rates occurred January through July at the low salinity site, March through May at the low to intermediate salinity site, January through May and July through

September at the intermediate salinity site and January through March and July through September at the high salinity site (Fig. 7).

Multiple linear regression analysis was used to determine the relationship between interval growth rate and the potential predictors of
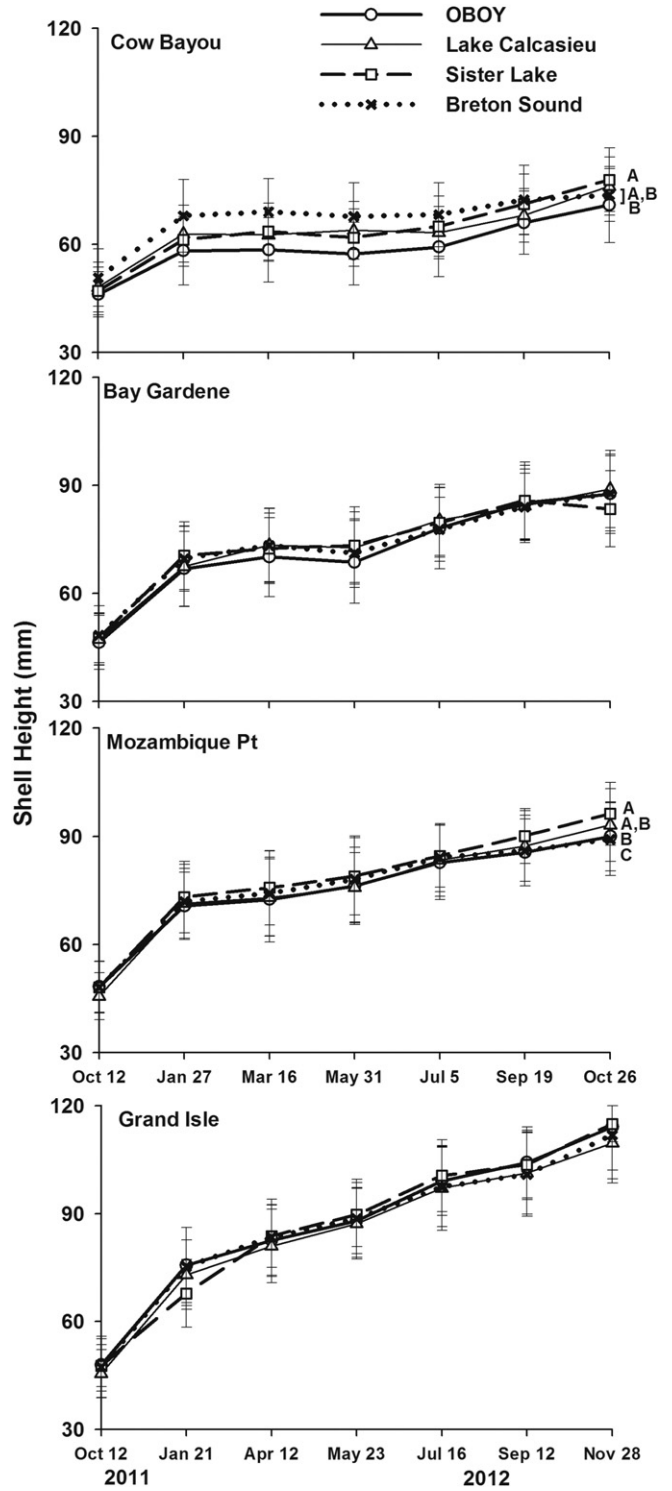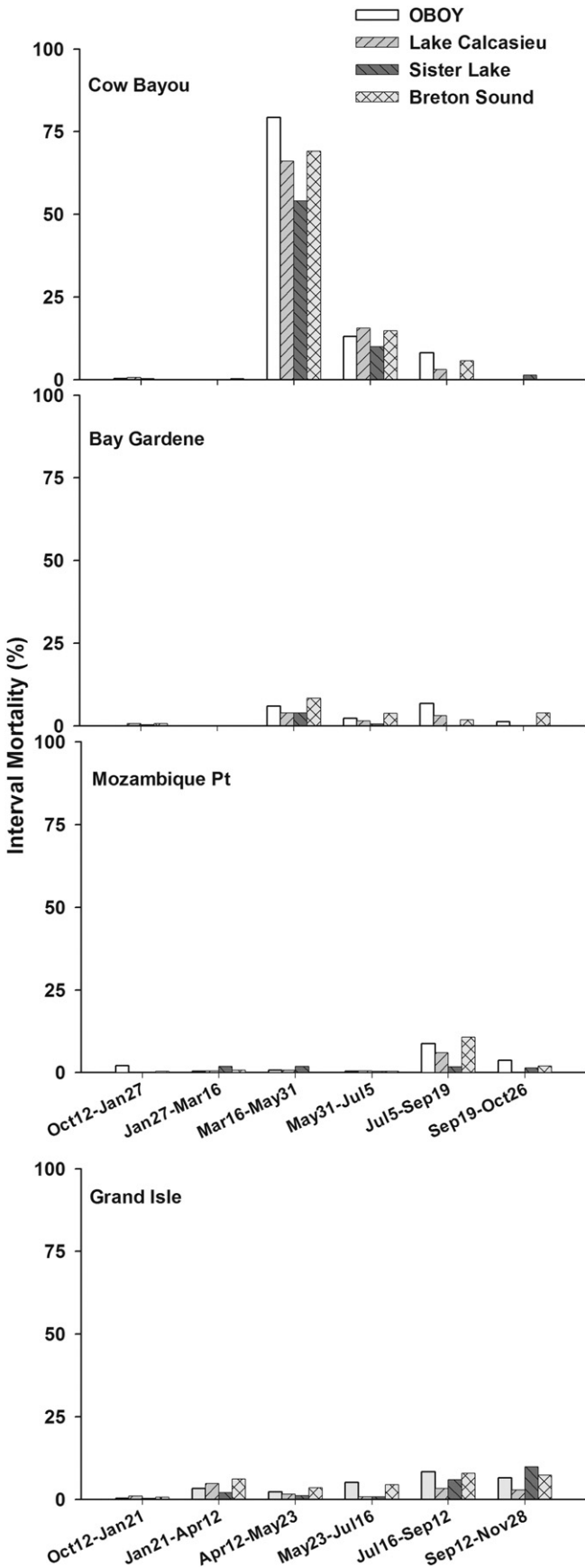
Fig. 6. Mean shell heights (mm) ± standard deviation of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou, CB), the low-intermediate salinity site (Bay Gardene, BG), the intermediate salinity site (Mozambique Point, MP) and at the high salinity site (Grand Isle, GI).

interval salinity, interval temperature and interval initial shell height. All three predictors contributed significantly (interval salinity $p < 0.001$, interval temperature $p < 0.005$, interval initial shell height

Fig. 5. Mean interval mortality (%) of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou), the low-intermediate salinity site (Bay Gardene), the intermediate salinity site (Mozambique Point) and at the high salinity site (Grand Isle).
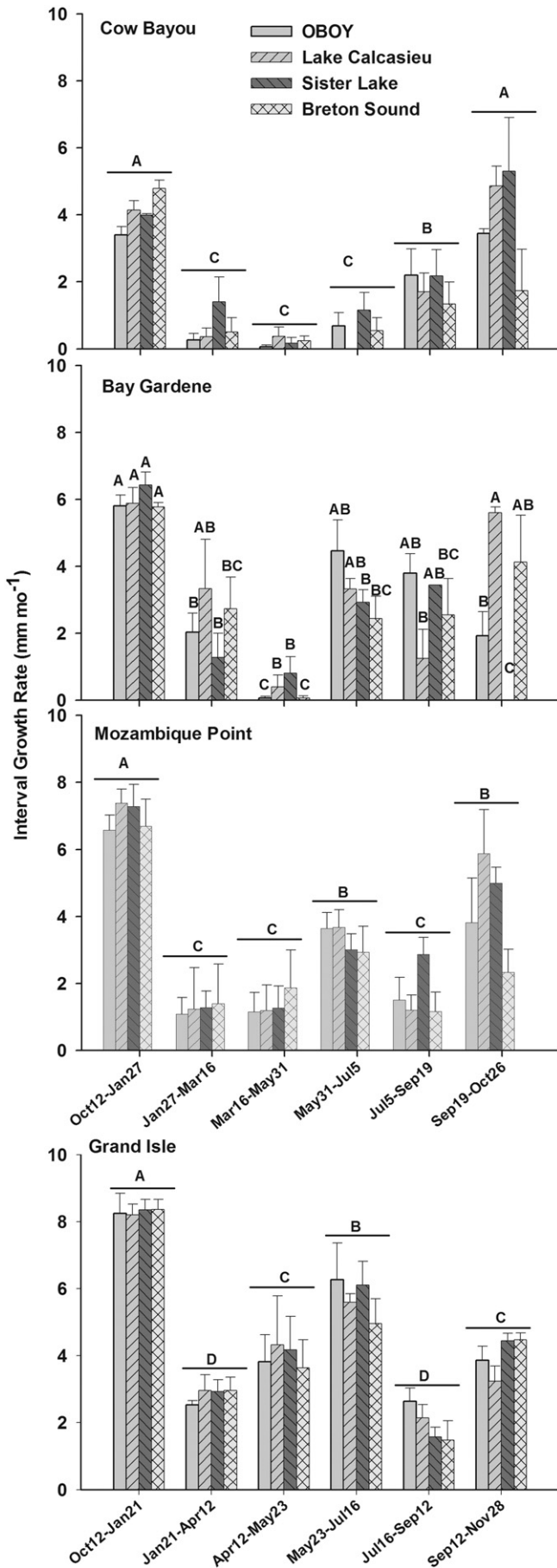
$p < 0.001$) and produced a multiple regression model with growth rate = $4.828 + (0.282 \times$ interval salinity) + $(0.0579 \times$ interval temperature) $- (0.0955 \times$ interval initial shell height) and an R^2 of 0.636.

3.4. Disease

No significant differences could be shown in mean *P. marinus* infection intensity between the four stocks prior to deployment ($p > 0.05$, data no shown) and following deployment at each sampling time and site (Fig. 8). A trend of increasing *P. marinus* infection intensity at the higher salinity sites was however apparent as the study progressed and temperature increased (Fig. 8). At the higher salinity site, three out of four stocks had significantly higher *P. marinus* infection intensities in September compared to April 2012.

3.5. Condition index

No significant differences in mean condition index could be shown between stocks at the time of deployment ($p = 0.1542$, data not shown). Condition index of oysters at the high salinity site was significantly affected by the interaction of stock and interval while condition index at the three other sites differed significantly by stock, interval or both (no interaction, Fig. 9). At the low-intermediate salinity site, the condition index of all stocks significantly decreased from March to September (Fig. 9). At the low salinity site and intermediate salinity site, the condition index of all stocks significantly decreased from March to July and July to September and in addition the condition index of the OBOY stock was significantly greater than that of the other stocks (Fig. 9). At the high salinity site, the condition index of the OBOY stock was significantly higher than that of the other stocks in July. The condition index of the Lake Calcasieu and Sister Lake stocks significantly decreased from April to July and from July to September while the condition index of the Breton Sound stock significantly decreased from April to July and the OBOY stock significantly decreased from July to September and the Breton Sound stock significantly decreased from April to July. The generally higher condition index of the OBOY oysters was due to their greater tissue dry weight relative to whole oyster weight minus oyster shell compared to the other stocks.

4. Discussion

The mortality, growth, dermo infection intensity and condition index of the progeny of wild oysters collected from public oyster grounds of three Louisiana estuaries differing in salinity regime and oysters selected for dermo resistance were compared along a salinity gradient. Overall, salinity and temperature significantly impacted the mortality, growth, *P. marinus* infection intensity and condition index of oysters of all four stocks and a few differences between stocks could be shown at each site. The progeny of oysters from Sister Lake, a low salinity estuarine lake, however, did show higher survival than the other stocks when exposed to periods of low salinity while the progeny of oysters from Lake Calcasieu, a high salinity estuarine lake, showed higher survival than the other stocks when exposed to periods of high salinity. This initial result suggests that the stocks used are genetically differentiated with respect to low salinity tolerance as well as dermo-related mortality at high salinity and implies that stock selection for aquaculture grow-out or restoration effort will benefit from being site-specific.

Oysters suffered >50% mortality at the low salinity site after an extended exposure to low salinity and as temperature increased to ~ 25 °C. The length of low salinity exposure (4 months) combined with

Fig. 7. Mean interval growth rates (mm mo^{-1}) ± standard deviation of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou), the low-intermediate salinity site (Bay Gardene), the intermediate salinity site (Mozambique Point) and at the high salinity site (Grand Isle).
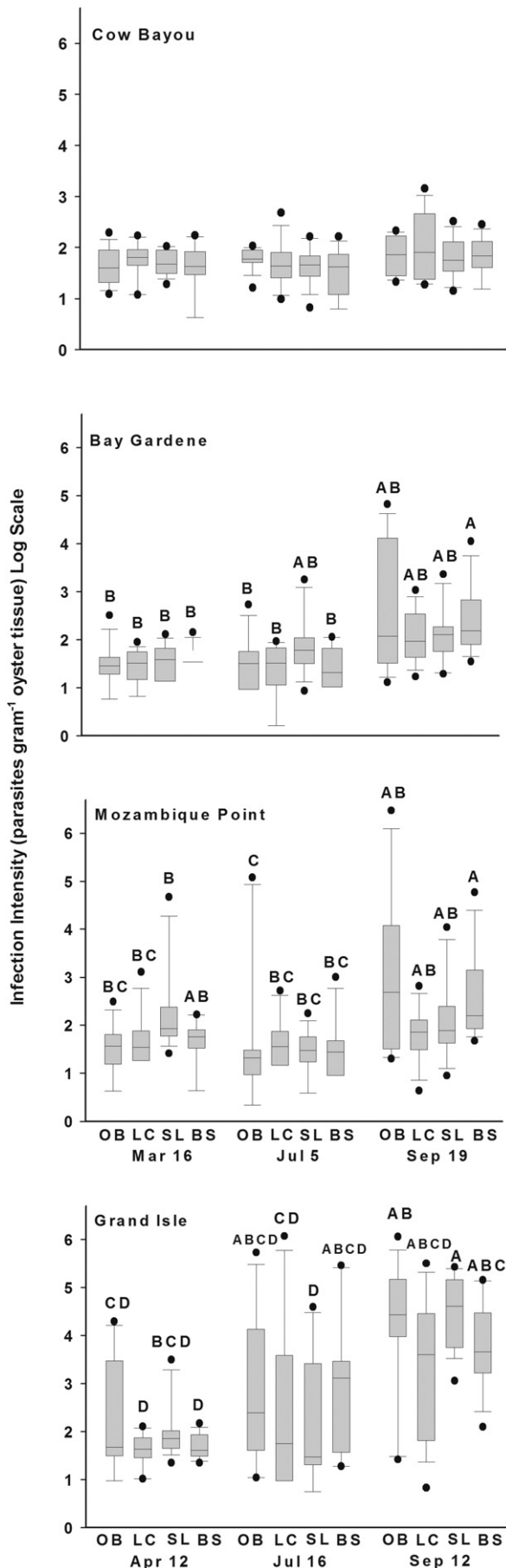
higher temperature was clearly beyond the conditions the oysters could cope with at the low salinity site. A number of laboratory and field studies have shown that eastern oysters can survive extended periods (>2 months) of low salinity (<5) at low temperature but not at elevated temperature (Loosanoff, 1953; La Peyre et al., 2009; Munroe et al., 2013). Interestingly, oysters exposed to a mean salinity of less than 5 during the March 16 to May 31 interval, at the low to intermediate salinity site, suffered less than 10% mortality indicating that even at higher temperature (~25 °C) Louisiana oysters can tolerate low salinity for more than a month in contrast to oysters from higher latitudes (Loosanoff, 1953). Once temperature reaches 30 °C, however, Louisiana oysters suffer heavy mortality at low salinity. In previous studies, oysters experienced 100% mortality at Cow Bayou, the low salinity site in our current study (La Peyre et al., 2013; Rybovich et al., 2016). Laboratory results also indicated that an increase of a few degrees over 30 °C was enough to kill Louisiana oysters within 7 days at low salinity (Rybovitch et al., 2016).

The Sister Lake stock consistently showed the lowest mortality during intervals of low salinity. Moreover, a significantly higher percentage of Sister Lake stock oysters survived salinity < 5 at the low salinity site March through May compared to oysters from the other stocks. Sister Lake stock may be better adapted to low salinity conditions as its broodstock was collected in an estuarine lake characterized by a low salinity regime (yearly mean of 11.9 ± 2.4). Only a few studies have specifically investigated the interaction of oyster genotype and salinity and demonstrated significant genotype-by-environment interactions (Newkirk, 1978; Eierman & Hare, 2013; Frank-Lawale et al., 2014). Interestingly, it has been shown that the intracellular response to osmotic stress can differ significantly between oyster populations (Pierce et al., 1997). Oysters rely on intracellular osmotic regulation as they are poikilosmotic with no ability to regulate the osmotic pressure of their extracellular fluids. The recent identification of candidate genes for osmotic regulation in wild eastern oysters collected from high and low salinity regimes can greatly assist in investigating the genetics of the mechanisms underlying salinity tolerance (Eirman & Hare, 2014). Relating genomic variation in osmoregulation genes with stock performance (e.g., survival, growth) under different salinity regimes may enable the development of markers for salinity tolerance which managers could use to identify stocks best suited to particular outplant environmental conditions. This is particularly relevant for Louisiana because increasing freshwater inflow from current and planned diversions to counter coastal land loss, will significantly lower salinity and reduce the zone of intermediate salinity, between 9 and 14, most favorable to production on oyster grounds (Soniat et al., 2013; La Peyre et al., 2016).

Oysters at the high salinity site showed increased mortality as the study progressed and water temperature and *P. marinus* infection intensities increased. High salinity and temperature are well known to promote the propagation of the parasite *P. marinus* in laboratories studies (Chu et al., 1993; Chu & La Peyre, 1993; Dungan & Hamilton, 1995). In field studies, increased infection intensities of *P. marinus* in eastern oysters have consistently been recorded at salinity higher than 12 and when temperatures exceed 20 °C while lower salinity retarded the progression of the disease (Burreson & Ragone Calvo, 1996; Ragone Calvo et al., 2003b; Bushek et al., 2012). There was limited change in infection intensities of oysters and only a few oysters developed moderate (i.e.,

Fig. 8. Box plots of *P. marinus* infection intensities of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou), the low-intermediate salinity site (Bay Gardene), the intermediate salinity site (Mozambique Point) and at the high salinity site (Grand Isle) and sampled in 2012. The median infection intensity is shown as the center line in the box. The line at the bottom of the box represents the point where 25% of the data are below it. The line at the top of the box represents the point where 75% of the data are below it. The lowest whisker represents the point where 5% of the data are below it. The highest whisker represents the point where 95% of the data are below it. Points that fall outside the limits of the whiskers are outliers.
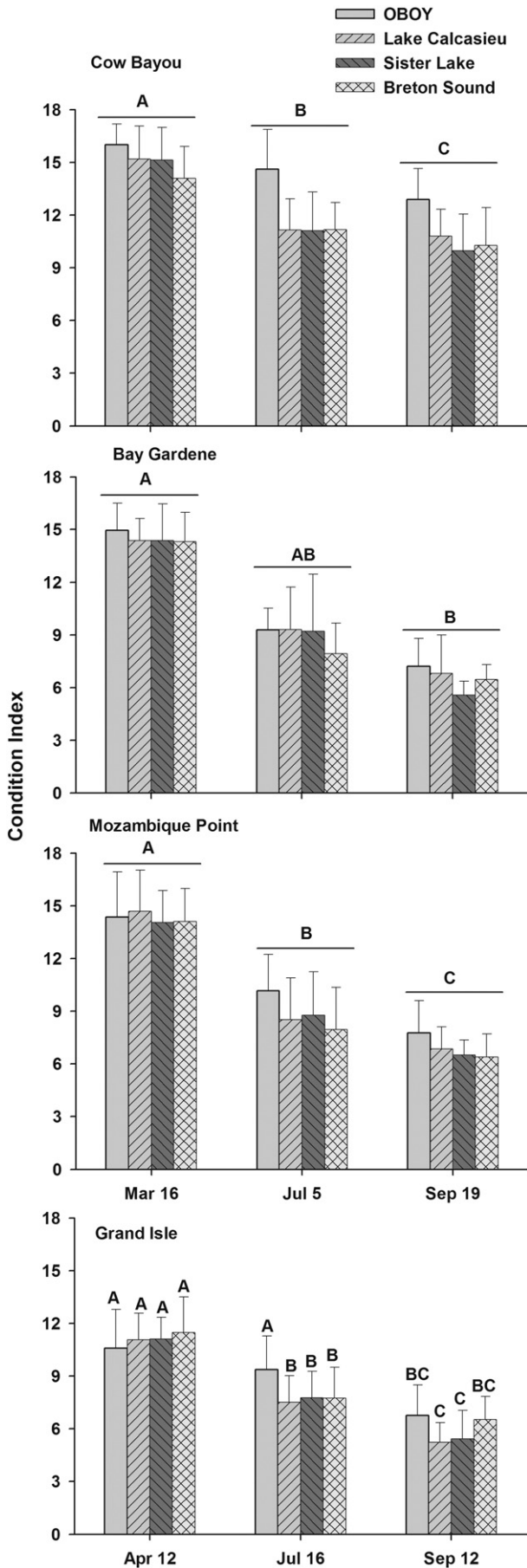
$>10^4 - 5 \times 10^5$ parasites per g tissue) or heavy (i.e., $>5 \times 10^5$ parasites per g tissue) infection from all three sites of Breton Sound where salinity remained below 15 as previously reported (La Peyre et al., 2009, 2013).

Contrary to expectation, the OBOY stock selected for dermo resistance did not show significantly lower mortality and *P. marinus* infection intensities than the other stocks. The infection intensities actually tended to be higher and mortality was significantly higher than the Lake Calcasieu stock at the high salinity site. As with any traditional selective breeding program, inbreeding and the loss of rare alleles in oyster lines are real concerns and may have occurred in the OBOY line following mass spawning at the Grand Isle hatchery (Launey et al., 2001; Yu & Guo, 2005). This possibility, however, seems to be contradicted by results of a study we conducted in Alabama estuaries indicating that the same OBOY progeny used in the current study had lower mortality and *P. marinus* infection intensity than the progeny of wild Alabama and Lake Calcasieu broodstocks (Casas et al., In Preparation). At this time, it is difficult to determine if these mortalities are from inbreeding, genetic trade-offs between growth, survival and reproduction associated with selection or other environmental factors such as food availability (Gaffney & Bushek 1996; Boudry et al., 2004).

Multiple linear regression analysis revealed that the best or most significant predictor of growth rate during a specific interval was salinity ($\beta = 0.282$) followed by initial shell height ($\beta = 0.0955$) and temperature ($\beta = 0.0579$). Oyster growth rate is well known to increase with increasing salinity, as well as with increasing temperature, and is dependent on initial size (reviewed by Kraeuter et al., 2007). Oyster shell heights increased more rapidly at the progressively higher salinity sites. Oysters reached market-size (75 mm) within 10 months at the high salinity site, within 12 to14 months at the intermediate and low intermediate salinity site and within about 18 months at the low salinity site. The rapid growth has been reported in other Gulf of Mexico estuaries such as Apalachicola Bay which is due to a continuous growing season in our rich subtropical estuaries, as temperatures are above 20 °C for more than half of each year and rarely drop below 10 °C, in contrast to more temperate or northern regions, where a period of no growth occurs in winter (Ingle & Dawson, 1952; Paynter & Dimichelle, 1990). Oyster growth rates decreased as oyster size increased as expected and the range in growth rates relating initial sizes of oysters for each interval reflects the ranges reported in Kraeuter et al. (2007).

While growth rate generally increased with temperature, there was a significant decrease in growth rates concomitant with the high temperatures experienced by our oysters July through September at the intermediate and high salinity sites. The decrease in growth rate was likely due to the increase in dermo infection intensities as was described in earlier studies (Ray et al., 1953; Menzel & Hopkins, 1955; Paynter & Burreson, 1991). A number of oysters had moderate and heavy infection intensities starting in July and those numbers increased in September. High temperature which often exceeded 30 °C may also have contributed directly to the decrease in growth rate because of reduction or cessation of pumping and shell closure as reported in past studies (Collier, 1954; Loosanoff, 1958).

Finally, condition index at each site decreased from a high in March to a low in September as would be expected following spawning and higher metabolic rate with increasing temperatures (Mann, 1978; Supan & Wilson, 2001). Low salinity is well known to delay spawning, which explains the delayed decreases in oyster condition index at the low salinity site (Butler, 1949; Loosanoff, 1953). Generally, the condition index of the OBOY stock was significantly greater than those of the other stocks and was due to the OBOY greater tissue dry weight relative to whole oyster weight minus oyster shell. The greater relative dry

Fig. 9. Mean condition index ± standard deviation of oysters from the OBOY, Lake Calcasieu, Sister Lake and Breton Sound stocks deployed at the low salinity site (Cow Bayou, CB), the low-intermediate salinity site (Bay Gardene, BG), the intermediate salinity site (Mozambique Point, MP) and at the high salinity site (Grand Isle, GI) sampled in 2012.

weight may indicate that the OBOY oysters are allocating more resources to reproduction than survival compared to oysters from the other stocks as mortality of the OBOY stock also tended to be highest. Favoring reproductive effort and growth at the expense of survival when resources are abundant, which is the case in our rich estuaries, has been reported in Pacific oysters (Ernande et al., 2004; Boudry et al., 2004). Possible selection of larger individuals for spawning for the propagation of the OBOY line may also have unintentionally selected for high growth and reproductive allocation at the expense of survival and counteracted our selection for disease resistance. Measurements of biochemical indices recommended by Mann (1978) and gonadal index in future studies should help answer whether resource allocation differs between our oyster stocks.

5. Summary

The most significant differences between oyster stocks in our study were their mortalities. The differences in mortalities, however, varied depending on site or interval salinity suggesting genetic × environment interaction. While mortalities were highest at the low and high salinity sites and lowest at the intermediate salinity sites, a different stock had the lowest mortality at the low (i.e., Sister Lake) and high (i.e., Lake Calcasieu) salinity sites. This initial result suggests that the stocks used are genetically differentiated with respect to low salinity tolerance as well as dermo-related mortality at high salinity. Stock selection for aquaculture grow-out or restoration effort will therefore need to be site-specific and dependent on the dominant environmental conditions. The ability of oyster stocks to tolerate extended periods of low salinity will be critical in Louisiana estuaries where large freshwater diversions are planned. In contrast, the ability of a stock to rapidly grow off-bottom to market size in less than a year and be harvested before succumbing to dermo should be selected for in high salinity areas. Selection based on resistance to or tolerance of *P. marinus* infection will still be needed in estuaries where oysters require more than one growing season to reach market size. Differences in oyster responses to environmental conditions due to their genetic background will need to be addressed more thoroughly in future studies considering the diverse and varying environmental conditions encountered in Louisiana and other Gulf of Mexico estuaries.

Acknowledgments

References

Barber, B.J., Ford, S.E., Wargo, R.N., 1991. Genetic variation and the timing of gonadal maturation and spawning of the eastern oyster Crassostrea virginica (Gmelin). Biol. Bull. 181, 216–221.

Boudry, P., Dégremont, L., Taris, N., McCombie, H., Haffray, P., Ernande, B., 2004. Genetic variability and selective breeding for traits of aquacultural interest in the Pacific oyster (Crassostrea gigas). Bull. Aquac. Assoc. Can. 104 (2), 12–18.

Brown, B.L., Butt, A.J., Shelton, S.W., Meritt, D., Paynter, K.T., 2005. Resistance of Dermo in eastern oysters, Crassostrea virginica (Gmelin), of North Carolina but not Chesapeake Bay heritage. Aquac. Res. 36 (14), 1391–1399.

Burford, M.O., Scarpa, J., Cook, B.J., Hare, M.P., 2014. Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 505, 161–175.

Burreson, E.M., Ragone Calvo, L.M., 1996. Epizootiology of Perkinsus marinus disease of oysters in Chesapeake Bay, with emphasis on data since 1985. J. Shellfish Res. 15 (1), 17–34.

Bushek, D., Ford, S.E., Burt, I., 2012. Long-term patterns of an estuarine pathogen along a salinity gradient. J. Mar. Res. 70, 225–251.

Butler, P.A., 1949. Gametogenesis in the oyster under conditions of depressed salinity. Biol. Bull. 96, 263–269.

Carlsson, J., Carnegie, R.B., Cordes, J.F., Hare, M.P., Leggett, A.T., Reece, K.S., 2008. Evaluating recruitment contribution of a selectively bred aquaculture line of the oyster, Crassostrea virginica used in restoration efforts. J. Shellfish Res. 27, 1117–1124.

Chu, F.-L.E., La Peyre, J.F., 1993. Perkinsus marinus susceptibility and defense related activities in eastern oysters (Crassostrea virginica): temperature effects. Dis. Aquat. Org. 16, 223–234.

Chu, F.-L.E., La Peyre, J.F., Burreson, C.S., 1993. Perkinsus marinus infection and potential defense related activities in eastern oysters, Crassostrea virginica: salinity effects. J. Invertebr. Pathol. 62, 226–232.

Collier, A., 1954. A study of the response of oysters to temperature, and some long range ecological interpretations. Addresses Delivered at the Convention of the National Shellfisheries Association. 1953, pp. 13–38.

Dittman, D.E., Ford, S.E., Haskin, H.H., 1998. Growth patterns in oysters, Crassostrea virginica, from different estuaries. Mar. Biol. 132, 461–469.

Dungan, C.F., Hamilton, R.M., 1995. Use of a tetrazolium-based cell proliferation assay to measure the effects of in vitro conditions on Perkinsus marinus (Apicomplexa) proliferation. J. Eukaryot. Microbiol. 42 (4), 379–388.

Dupuy, J.L., Windsor, N.T., Sutton, C.E., 1977. Manual for Design and Operation of an Oyster Seed Hatchery for the American Oyster Crassostrea Virginica. Spec. Rpt. No. 142. V.I.M.S., Gloucester Pt., VA, p. 104.

Eierman, L.E., Hare, M.P., 2013. Survival of oyster larvae in different salinities depends on source population within an estuary. J. Exp. Mar. Biol. Ecol. 449, 61–68.

Eierman, L.E., Hare, M.P., 2014. Transcriptomic analysis of candidate osmoregulatory genes in the eastern oyster Crassostrea virginica. BMC Genomics 15, 503.

Ernande, B., Boudry, P., Clobert, J., Haure, J., 2004. Plasticity in resource allocation based life history traits in the Pacific oyster, Crassostrea gigas. I. Spatial variation in food abundance. J. Evol. Biol. 17 (2), 342–356.

Fisher, W.S., Oliver, L.M., 1996. A whole-oyster procedure for diagnosis of Perkinsus marinus disease using Ray's fluid thioglycollate culture medium. J. Shellfish Res. 15, 109–118.

Frank-Lawale, A., Allen Jr., S.K., Degremont, L., 2014. Breeding and domestication of eastern oyster (Crassostrea virginica) lines for culture in the mid-Atlantic, USA: line development and mass selection for disease resistance. J. Shellfish Res. 33, 153–165.

Gaffney, P.M., Bushek, D., 1996. Genetic aspects of disease resistance in oysters. J. Shellfish Res. 15 (1), 135–140.

Haskin, H.H., Ford, S.E., 1979. Development of resistance to Minchinia nelsoni (MSX) mortality in laboratory-reared native oyster stocks in Delaware Bay. Mar. Fish. Rev. 41 (1–2), 54–63.

Hopkins, A.E., 1949. Determination of condition of oysters. Science 110, 567–568.

Ingle, R.M., Dawson Jr., C.E., 1952. Growth of the American oyster, Crassostrea virginica (Gmelin) in Florida waters. Bull. Mar. Sci. 2 (2), 393–404.

Kraeuter, J.N., Ford, S., Cummings, M., 2007. Oyster growth analysis: a comparison of methods. J. Shellfish Res. 26 (2), 479–491.

La Peyre, M.K., Nickens, A.D., Volety, A.K., Tolley, G.S., La Peyre, J.F., 2003. Environmental significance of freshets in reducing Perkinus marinus infection in eastern oysters Crassostrea virginica: potential management applications. Mar. Ecol. Prog. Ser. 248, 165–176.

La Peyre, M.K., Gossman, B., La Peyre, J.F., 2009. Defining optimal freshwater flow for oyster production: effects of freshet rate and magnitude of change and duration on eastern oysters and Perkinsus marinus infection. Estuar. Coasts 32, 522–534.

La Peyre, M.K., Eberline, B.S., Soniat, T.M., La Peyre, J.F., 2013. Differences in extreme low salinity timing and duration differentially affect eastern oyster (Crassostrea virginica) size class growth and mortality in Breton Sound, LA. Estuar. Coast. Shelf Sci. 135, 146–157.

La Peyre, M., Furlong, J., Brown, L., Piazza, B., Brown, K., 2014. Oyster reef restoration in the northern Gulf of Mexico: extent, methods and outcomes. Ocean Coast. Manag. 89, 20–28.

La Peyre, M.K., Geaghan, J., Decossas, G., La Peyre, J.F., 2016. Analysis of environmental factors influencing salinity patterns, oyster growth and mortality in lower Breton Sound Estuary, Louisiana using 20 years of data. J. Coast. Res. 32, 519–530.

Launey, S., Barre, M., Gerard, A., Naciri-Graven, Y., 2001. Population bottleneck and effective size in Bonamia ostreae-resistant populations of Ostrea edulis as inferred by microsatellite markers. Genet. Res. 78 (3), 259–270.

Lawrence, D.R., Scott, G.I., 1982. The determination and use of condition index of oysters. Estuaries 5 (1), 23–27.

Loosanoff, V.L., 1953. Behavior of oysters on water of low salinities. Proceedings of the National Shellfisheries Association. 43, pp. 135–151.

Loosanoff, V.L., 1958. Some aspects of behavior of oysters at different temperatures. Biol. Bull. 114 (1), 57–70.

Louisiana Department of Wildlife and Fisheries, 2014. Oyster stock assessment report. Oyster Data Report Series No. 20. Louisiana Department of Wildlife and Fisheries, P.O. Box 98000. Baton Rouge, LA 70898.

Mann, R., 1978. A comparison of morphometric, biochemical, and physiological indices of condition in marine bivalve mollusks. In: Thorp, J.H., Gibbons, J.W. (Eds.), Early Environmental Stress in Aquatic Systems. US Department of Energy Symposium Series (771114). Woods Hole Oceanographic Institute, Woods Hole, MA, pp. 484–497.

Matthiessen, G.C., Feng, S.Y., Leibovitz, L., 1990. Patterns of MSX (Haplosporidium nelsoni) infection and subsequent mortality in resistant and susceptible strains of the eastern oyster Crassostrea virginica (Gmelin 1791), in New England. J. Shellfish Res. 9, 359–366.

Maxwell, V.J., Supan, J., Schiavinato, L.C., Showalter, S., Treece, G.D., 2008. Aquaculture parks in the coastal zone: a review of legal and policy issues in the Gulf of Mexico state waters. Coast. Manag. 36, 241–253.

Menzel, R.W., Hopkins, S.H., 1955. The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. J. Parasitol. 41 (4), 333–342.

Munroe, D., Tabatabai, A., Burt, I., Bushek, D., Powell, E.N., Wilkin, J., 2013. Oyster mortality in Delaware Bay: impacts and recovery from Hurricane Irene and Tropical Storm Lee. Estuar. Coast. Shelf Sci. 135, 209–219.

Newkirk, G., 1978. Interaction of genotype and salinity in larvae of the oyster *Crassostrea virginica*. Mar. Biol. 48, 227–234.

Owen, M.H., 1953. Growth and mortality of oysters in Louisiana. Bull. Mar. Sci. 3 (1), 44–54.

Paynter, K.T., Burreson, E.M., 1991. Effects of Perkinsus marinus infection in the eastern oyster, Crassostrea virginica II. Disease development and impact on growth rate at different salinities. J. Shellfish Res. 10, 425–431.

Paynter, K.T., Dimichele, L., 1990. Growth of tray-cultured oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay. Aquaculture 87 (3), 289–297.

Pierce, S.K., Dragolovich, J., Crombie, B.N., 1997. Variations in intracellular choline levels may account for differences in glycine betaine synthesis between conspecific oyster populations responding to hyperosmotic stress. J. Exp. Zool. 278 (5), 283–289.

Powell, E.N., Klink, J.M., Hofmann, E.E., 1996. Modeling diseased oyster populations II. Triggering mechanisms for *Perkinsus marinus* epizootics. J. Shellfish Res. 15, 141–165.

Ragone Calvo, L.M., Calvo, G.W., Burreson, E.M., 2003a. Dual disease resistance in a selectively bred eastern, *Crassostrea virginica*, strain tested in Chesapeake Bay. Aquaculture 220, 69–87.

Ragone Calvo, L.M., Dungan, C.F., Roberson, B.S., Burreson, E.M., 2003b. Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. Dis. Aquat. Org. 56, 75–86.

Ray, S., Mackin, J.G., Boswell, J.L., 1953. Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. Bull. Mar. Sci. 3 (1), 6–33.

Rybovich, M., La Peyre, M.K., Hall, S.G., La Peyre, J.F., 2016. Increased temperatures combined with lowered salinities differentially impact oyster size class growth and mortality. J. Shellfish Res. 35 (1), 101–113.

Soniat, T.M., Klinck, J.M., Powell, E.N., Cooper, N., Abdelguerfi, M., Hoffmann, E.E., Dahal, J., Tu, S., Finigan, J., Eberline, B.S., J.F, L.P., La Peyre, M.K., Quaddoura, F., 2012. A shell-neutral modeling approach yields sustainable oyster harvest estimates: a retrospective analysis of the Louisiana state primary seed grounds. J. Shellfish Res. 31, 1103–1112.

Soniat, T.M., Conzelmann, C.P., Byrd, J.D., Roszell, D.P., Bridevaux, J.L., Suir, K.J., Colley, S.B., 2013. Predicting the effects of proposed Mississippi River diversions on oyster habitat quality; application of an oyster habitat suitability index model. *J. Shellfish Res.* 32 (2), 629–638.

Supan, J.E., Wilson, C.A., 2001. Analyses of gonadal cycling by oyster broodstock, *Crassostrea virginica* (Gmelin), in Louisiana. J. Shellfish Res. 20, 215–220.

Walton, W.C., Davis, J.E., Supan, J.E., 2013. Off-bottom Culture of Oysters in the Gulf of Mexico. SRAC Publication, Southern Regional Aquaculture Center No. 4308.

Yu, Z., Guo, X., 2005. Genetic analysis of selected strains of the eastern oyster (*Crassostrea virginica* Gmelin) using AFLP and microsatellite markers. Mar. Biotechnol. 6, 575–586.