Vol. 9: 169–180, 2017
https://doi.org/10.3354/aei00222

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published May 5

Performance of oysters selected for dermo resistance compared to wild oysters in northern Gulf of Mexico estuaries

Sandra Casas[1], William Walton[2], Glen Chaplin[2], Scott Rikard[2], John Supan[1,3], Jerome La Peyre[1,*]

[1]School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
[2]Auburn University Shellfish Laboratory, Dauphin Island, AL 36528, USA
[3]Louisiana Sea Grant College Program, Louisiana State University, Baton Rouge, LA 70803, USA

ABSTRACT: The performance of the progeny of eastern oysters *Crassostrea virginica* from Louisiana selected for resistance to dermo, caused by *Perkinsus marinus* (referred to as 'OBOY') and of wild oysters collected from Louisiana (Calcasieu Lake) and Alabama (Cedar Point, Perdido Pass), USA, estuaries was compared for their potential use in aquaculture. Seed oysters from each stock were deployed in September 2011 at 2 dermo-endemic sites, Dauphin Island and Sandy Bay, Alabama, using an adjustable longline system, and their survival and shell heights were monitored bimonthly. *P. marinus* infection intensity and condition index were measured at deployment and in March, July and September 2012. The OBOY stock showed lower mortality than the unselected stocks (Cedar Point, Perdido Pass, Calcasieu Lake) at Dauphin Island, and both Louisiana stocks had lower mortality than the Alabama stocks at Sandy Bay, a slightly more saline site. Mortality increased in summer, especially between July and September, concomitant with increasing *P. marinus* infection intensities at the higher temperatures and favorable salinities. At the higher salinity site, both Louisiana stocks had lower *P. marinus* infection intensities than the Perdido Pass stock, the stock with the highest percentage of oysters with moderate and heavy infection and cumulative mortality. The OBOY stock reached greater mean shell height than Calcasieu Lake and Perdido Pass stocks. Condition index of the oyster stocks decreased by more than half between March and July following expected spawning. Differences in stock performance highlight the importance of stock selection for aquaculture in dermo-endemic estuaries of the northern Gulf of Mexico.

KEY WORDS: *Crassostrea virginica* · Off-bottom aquaculture · Mortality · Growth · Dermo disease · *Perkinsus marinus*

INTRODUCTION

Off-bottom oyster aquaculture is a nascent and fast-expanding industry in the northern Gulf of Mexico (Maxwell et al. 2008, Walton et al. 2013a). Since 2010, at least 20 commercial oyster farms have begun operation in Louisiana, Alabama and Florida, USA, collectively, with interest in Mississippi where regulations are being developed to allow this type of oyster farming (Petrolia et al. 2017). Off-bottom oyster aquaculture could play an important role in stabilizing production and increasing the sales of premium oysters from Gulf States.

In northern Gulf of Mexico estuaries, most traditional eastern oyster *Crassostrea virginica* production occurs on-bottom between salinities of 5 and 15 because of excessive mortality due to dermo — a disease caused by the protistan parasite *Perkinsus marinus* and directly transmitted from one oyster to another through the water — and predation from southern oyster drill

*Corresponding author: jlapeyre@agcenter.lsu.edu

© The authors 2017. Open Access under Creative Commons by Attribution Licence. Use, distribution and reproduction are unrestricted. Authors and original publication must be credited.

Publisher: Inter-Research · www.int-res.com

snails *Stramonita haemastoma* at salinities >15 (Breithaupt & Dugas 1979, Craig et al. 1989, La Peyre et al. 2016). Off-bottom oyster culture virtually eliminates predation, can improve growth and shell shape and provides opportunities to control fouling and to farm vast areas of previously unsuitable bottom (e.g. mud) and waters at higher salinity where oyster growth is greatest. Moreover, using hatchery-produced seed enables selection of brood stocks for increased resistance to dermo and which are adapted to local environmental conditions (Frank-Lawale et al. 2014, Proestou et al. 2016, Leonhardt et al. 2017).

The need to develop stocks of locally adapted eastern oysters that are resistant to disease has long been recognized (Haskin & Ford 1979, Matthiessen et al. 1990). Previous research studies in the northeastern and mid-Atlantic regions have successfully bred stocks of eastern oysters with increased resistance to the effects of *Haplosporidium nelsoni* (MSX) (Haskin & Ford 1979, Matthiessen et al. 1990) as well as to the effects of both MSX and dermo (Ragone Calvo et al. 2003a) and *Roseovarius* oyster disease (Davis & Barber 1999). Following these findings, selective breeding programs were organized and have significantly contributed to the expansion and success of oyster aquaculture in those regions (Guo et al. 2008, Frank-Lawale et al. 2014, Proestou et al. 2016).

In the northern Gulf of Mexico, a Louisiana eastern oyster stock named 'OBOY' has been selectively bred for dermo resistance since 1999 (Stickler et al. 2001), as *P. marinus* is the only parasite causing significant mortalities in this region. The objective of the current study was to compare the performance of this locally selected stock to wild oysters from Louisiana and Alabama and to determine the true potential of the OBOY stock for use in the rapidly expanding numbers of oyster farms in Alabama estuaries. Specifically, the mortality, growth, *P. marinus* infection intensity, and condition index of the progeny of wild oysters collected in Louisiana and Alabama, and of the OBOY stock, were compared in a common garden experimental design at 2 dermo-endemic Alabama sites. The wild oysters were collected from waters of intermediate to high salinities where dermo is typically endemic.

MATERIALS AND METHODS

Oysters

The eastern oyster *Crassostrea virginica* stock named 'OBOY' consists of the descendants of large oysters, collected in 1999 from a dermo-endemic area (i.e. Oyster Bayou, Cameron Parish, Louisiana, USA, 29° 47′ 39″ N, 93° 23′ 14″ W) and whose progeny have been challenged in the field (i.e. generations F0 and F3) and in the laboratory (i.e. F1 and F2) by *Perkinsus marinus*. Wild market-size eastern oysters from Calcasieu Lake (Cameron Parish, 29° 51′ 00″ N, 93° 18′ 40″ W, salinity yearly mean ± SD of 20.4 ± 2.2, USGS recorder 08017118) were collected in the fall of 2010. OBOY (F3) and Calcasieu Lake oysters were maintained in labeled aquaculture bags (50 oysters per bag) hanging on an adjustable longline system (ALS; BST Oyster Co.) at the Oyster Research and Demonstration Farm in Grand Isle, Louisiana (29° 14′ 20″ N, 90° 00′ 11″ W, 2003–2011 salinity yearly mean ± SD of 20.9 ± 1.9, USGS recorder 073802516) prior to spawning.

The Louisiana stocks were spawned at the Louisiana Sea Grant Oyster Hatchery in Grand Isle in May 2011 to produce a F0 generation of Calcasieu Lake and a F4 generation of the OBOY stock. Each stock (2 separate spawning events) was naturally spawned (150 oysters per stock) and the resulting larvae reared using methods similar to Dupuy et al. (1977). Pediveliger (~280 µm) larvae were set on micro-cultch material (~500 µm ground oyster shell) to produce single oyster spats that were shipped to the Auburn University Shellfish Laboratory (AUSL), Dauphin Island, Alabama, for rearing.

Alabama Cedar Point stock was produced from a cross of a F1 Cedar Point brood stock (CP09; 21 females, 3 males) line with a F0 Heron Bay brood stock (HB09; 17 females, 6 males); Heron Bay, 30° 19′ 55″ N, 88° 8′ 25″ W, is in close proximity to Cedar point, 30° 18′ 33″ N, 88° 8′ 14″ W. No effort was made to select for dermo resistance in these brood stocks, which were 24 mo (CP09) and 22 mo (HB09) old at the time of spawning. Alabama Perdido Pass stock was produced from Perdido Pass wild-stock oysters (32 females, 14 males) collected from Perdido Pass (30° 16′ 25″ N, 87° 33′ 20″ W) in Orange Beach, Alabama. Yearly mean salinity ± SD for 2011–2015 was 14.5 ± 6.9 in Cedar Point and 28.1 ± 5.3 in Perdido Pass (Mobile Bay National Estuary Program, www.mymobilebay.com, data collection for both sites started in 2011). The brood stock oysters were spawned at AUSL in May 2011 and the larvae were reared and set to produce single spat as described above.

Spat from all 4 oyster stocks were reared in individual flow-through upwellers at a density of ~5000 oysters per upweller at AUSL. Once the majority of oysters were retained on a 6 mm grading screen, all stocks were placed in 4.5 mm mesh oyster bags and reared in off-bottom floating cages (OysterGro®) in Sandy Bay (Fig. 1), Alabama, until experimental deployment.

Study sites

The study was conducted in Dauphin Island waters (Billy Goat Hole, 30° 15′ 4″ N, 88° 4′ 46″ W) and in Sandy Bay (30° 22′ 59″ N, 88° 18′ 45″ W), both located in Mobile Bay National Estuary, Alabama (Fig. 1). Daily mean ± SD water salinity and temperature at Dauphin Island Sea Lab station, located ~150 m from our Dauphin Island study site, were 20.3 ± 7.7 and 21.9 ± 7.0°C for the 2007–2011 period (Dauphin Island Sea Lab station 30° 15′ 4″ N, 88° 4′ 40″ W, Mobile Bay National Estuary Program, www.mymobilebay.com). Point Aux Chenes is the closest station to the Sandy Bay study site with daily monitoring (30° 20′ 54″ N, 88° 25′ 6″ W, NOAA NERR National Estuarine Research Reserve System Centralized Data Management Office), and its salinity data showed a strong correlation ($r = 0.904$, $p < 0.001$) with ADPH 176 Alabama Department of Public Health station, which is even closer to our Sandy Bay site but does not continuously record environmental conditions (Fig. 1). Point Aux Chenes daily mean water salinity and temperature for the 2007–2011 period were 21.8 ± 6.1 and 22.1 ± 6.9°C, respectively.

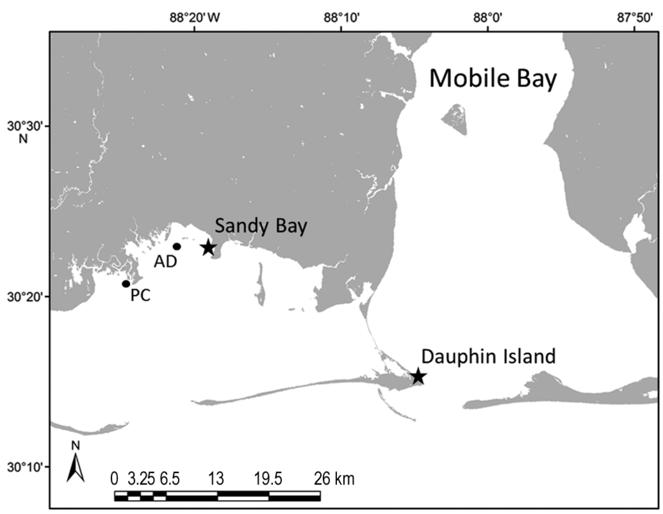

Fig. 1. Mobile Bay estuary, Alabama, USA, with Dauphin Island and Sandy Bay study sites (stars) where oysters were deployed. Water salinity and temperature recorders were located at the Dauphin Island Sea Lab station located ~150 m from our Dauphin Island study site, and at (black dots) Point Aux Chenes (PC) and Alabama Department of Public Health station 176 (AD)

Experimental design

In September 2011, 4 ALS aquaculture bags of each stock containing 75 oysters per bag were deployed at each study site, for a total of 300 oysters per stock and site. ALS bags were fully enclosed to prevent the risk of predation mortality and suspended beneath the water surface. Since predation was largely removed, mortality could be more readily attributed to stressful abiotic conditions and *P. marinus* infection. At both sites, oyster growth (shell height) and mortality (counts of live/dead) data were collected every other month, starting in November 2011 and ending in November 2012, for a total of 7 sampling periods. Condition index and *P. marinus* infection intensity data were collected at the time of deployment to establish pre-deployment baselines for each stock (N = 15 oysters per stock × 4 stocks, total of 60) and in March, July, and September 2012 (N = 15 oysters × 4 stocks × 2 sites, total of 120 per sampling time).

Water quality

Daily mean water salinity and temperature data from September 2011 to November 2012 were obtained from the Dauphin Island Sea Lab station and Point Aux Chenes monitoring stations. Mean water temperature and salinity for each sampling interval were calculated for use as predictor variables in multiple linear regression analyses to examine the relationships between water quality and interval mortality or growth of oysters.

Mortality

At each sampling time the numbers of live and dead oysters in each bag were recorded and the dead oysters were discarded. Interval mortality and cumulative mortality were calculated as described by Ragone Calvo et al. (2003b).

Growth

Shell height, the greatest distance between hinge and growth edge, was measured with digital calipers (Absolute Coolant Proof Calipers, series 500, resolution 0.01 mm, Mituyoto America Corporation) in 100 oysters selected haphazardly per stock, site, and sampling time (25 oysters per bag, 4 bags). Monthly interval growth rate was calculated as the increment in mean shell height between 2 consecutive sampling times divided by the number of days between samplings and normalized to a 30 d period. Mean growth rate was calculated using the mean shell height of each bag (N = 4).

P. marinus infection intensity

The number of *P. marinus* parasites per gram of wet oyster tissue was determined using the whole-oyster procedure as described by Fisher & Oliver (1996) and modified by La Peyre al. (2003). Oysters were further classified as either uninfected, lightly infected (i.e. $<10^4$ parasites g^{-1} wet tissue), moderately infected (i.e. 1×10^4 to 5×10^5 parasites g^{-1} wet tissue), or heavily infected (i.e. $>5 \times 10^5$ parasites g^{-1} wet tissue) (Bushek et al. 1994).

Condition index

Condition index was calculated as the ratio of dry tissue weight to the whole oyster weight minus its shell weight (i.e. filled cavity weight) multiplied by 100 using a variation of Hopkins' (1949) formula as recommended by Lawrence & Scott (1982). For each oyster, a 10 ml aliquot of oyster tissue homogenate that had been 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).

Statistical analyses

All statistical analyses were done using SigmaStat version 3.5 (Systat Software). Results for daily salinity and temperature from continuous data recorders were analyzed with a 1-factor (site) ANOVA followed by Tukey's multiple comparison procedure. Interval salinity and temperature were compared with Kruskal-Wallis 1-factor (sampling interval) ANOVA on ranks followed by Dunn's multiple comparison procedure. Cumulative mortalities (%) at the time oysters reached commercial size and at the end of the study were compared using a series of chi-square analyses to determine differences between stocks at each site. Interval mortality (%) at each sampling interval was analyzed with a 2-factor (stock, site) ANOVA followed by Tukey's multiple comparison procedure. Interval mortality (%) of each stock was analyzed with a 2-factor (site, sampling interval) ANOVA followed by Tukey's multiple comparison procedure. Interval mortality data of all stocks and sites were pooled together and analyzed using a multiple linear regression with interval temperature and interval salinity as predictor variables. Shell height was compared with a 2-factor (stock, site) ANOVA at time of deployment (September 2011) and at the end of the study (November 2012) followed by Tukey's multiple comparison procedure. Shell height was also compared by a 1-factor (stock) ANOVA at the time oysters reached commercial size at each site. Interval growth rate (mm mo^{-1}) of each stock was analyzed with a 2-factor (site, sampling interval) ANOVA followed by Tukey's multiple comparison procedure. Interval growth rate data of all stocks and sites were pooled together and analyzed using a multiple linear regression with interval temperature, interval salinity and interval initial shell height as predictor variables. *P. marinus* body burden and CI were compared at the beginning of the study with 1-factor (stock) ANOVA and data collected in March, July and September were compared with a 2-factor (stock, sampling interval) ANOVA followed by Tukey's multiple comparison procedure. The number of oysters with heavy and moderate infections in the 4 stocks at each site was also compared with a Chi-square test. To achieve normality and homogeneity of variance, shell height and *P. marinus* body burden were log transformed. All data are reported as mean ± SD.

RESULTS

Water quality

Mean daily salinity was significantly higher at Sandy Bay (i.e. Point Aux Chenes, 23.4 ± 4.5, range 5.3–31.4) than at Dauphin Island (21.3 ± 4.9, range 7.6–31.7). Daily salinity at both sites throughout the year tended to be >18 except for the January–March interval. (Fig. 2). Interval salinity at Dauphin Island was significantly lower than at Sandy Bay during the November–January, January–March, and July–September intervals (Fig. 3).

Temperature followed expected seasonal trends and ranges for this region (Fig. 2), and differences between both sites were not found (Dauphin Island: 22.4 ± 5.7°C, range 9.5–31.3°C; Sandy Bay: 23.1 ± 5.7°C, range 10.3–31.8°C). The lowest interval temperatures were during the November–January and

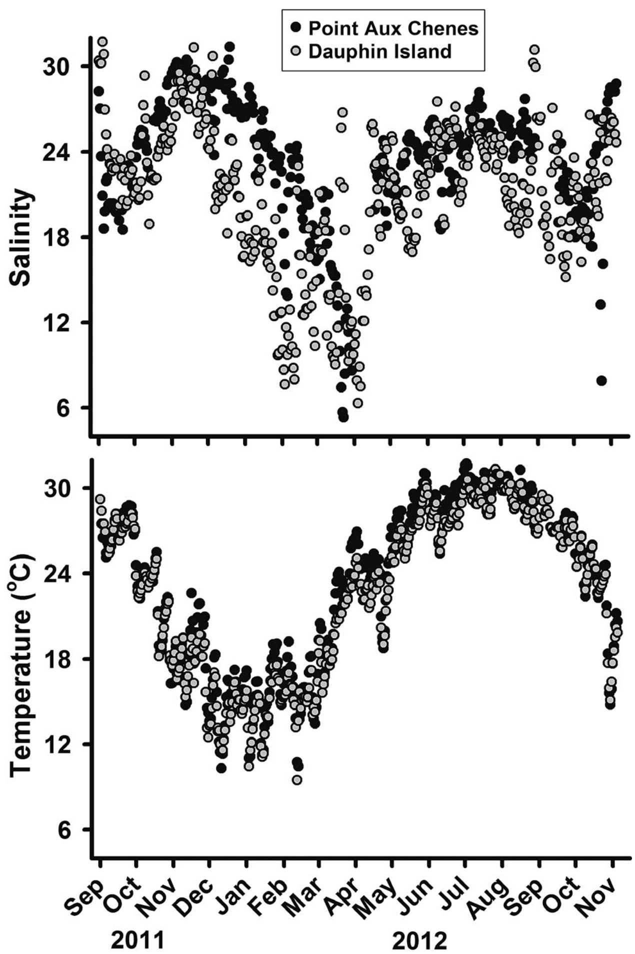

Fig. 2. Daily water temperature and salinity from September 2011 to November 2012 from continuous recorders at Dauphin Island Sea Lab and Point Aux Chenes stations (see Fig. 1)

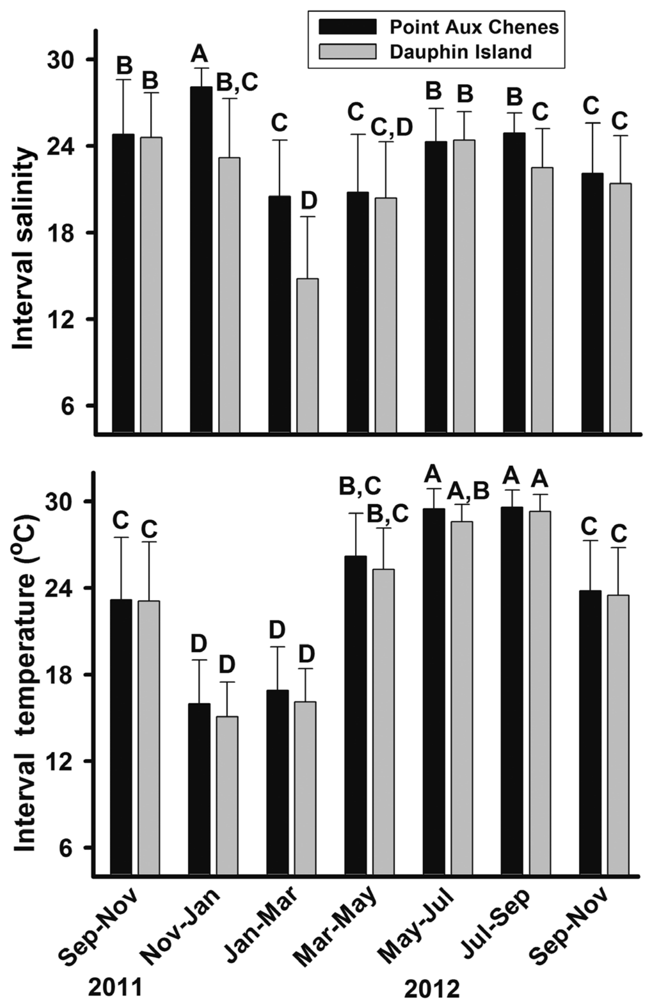

Fig. 3. Mean (±SD) interval water salinity and temperature from September 11, 2011 to November 12, 2012 calculated from daily salinity and temperature recorded at Dauphin Island Sea Lab and Point Aux Chenes stations. Groups that do not share letters have means that are significantly different ($p < 0.05$)

January–March intervals while the highest interval temperatures were during the May–July and July–September intervals (Fig. 3).

Mortality

At the end of the study, significant differences in cumulative mortality were found between stocks at Sandy Bay ($p < 0.001$) and Dauphin Island ($p < 0.001$). At Sandy Bay, the cumulative mortalities of both Louisiana stocks (Calcasieu Lake: 13%, OBOY: 11%) were significantly lower than the cumulative mortalities of either Alabama stocks (Perdido Pass: 29%, Cedar Point: 20%), and Cedar Point stock had significantly lower cumulative mortality than Perdido Pass stock (Fig. 4). At Dauphin Island, the cumulative mortality of the OBOY stock (9%) was significantly lower and the cumulative mortality of Perdido Pass stock (26%) was significantly higher than the cumulative mortalities of either Cedar Point (18%) or Calcasieu Lake (17%) stocks.

In March 2012, when oysters reached commercial size at Sandy Bay, no difference in cumulative mortality between stocks could be shown (0.7–1.0% cumulative mortality, Fig. 4). At the time point when oysters reached commercial size at Dauphin Island in May 2012, cumulative mortality was significantly higher ($p = 0.001$) in Perdido Pass stock (6.2%) than in Cedar Point (1.1%) and OBOY (1.1%) stocks (Fig. 4).

Interval mortality (%) at each sampling interval was only significantly different between stocks during the March–May ($p < 0.05$) and July–September

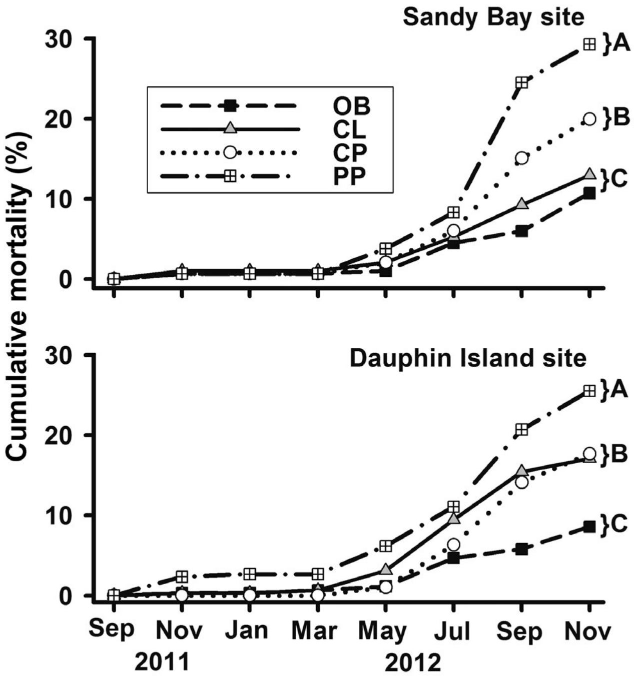

Fig. 4. Cumulative mortality of Louisiana eastern oysters from the OBOY (OB) and Calcasieu Lake (CL) stocks, and Alabama oysters from the Cedar Point (CP) and Perdido Pass (PP) stocks at the Sandy Bay and Dauphin Island study sites. Stocks that do not share a letter have cumulative mortalities that are significantly different at the end of the study ($p < 0.05$)

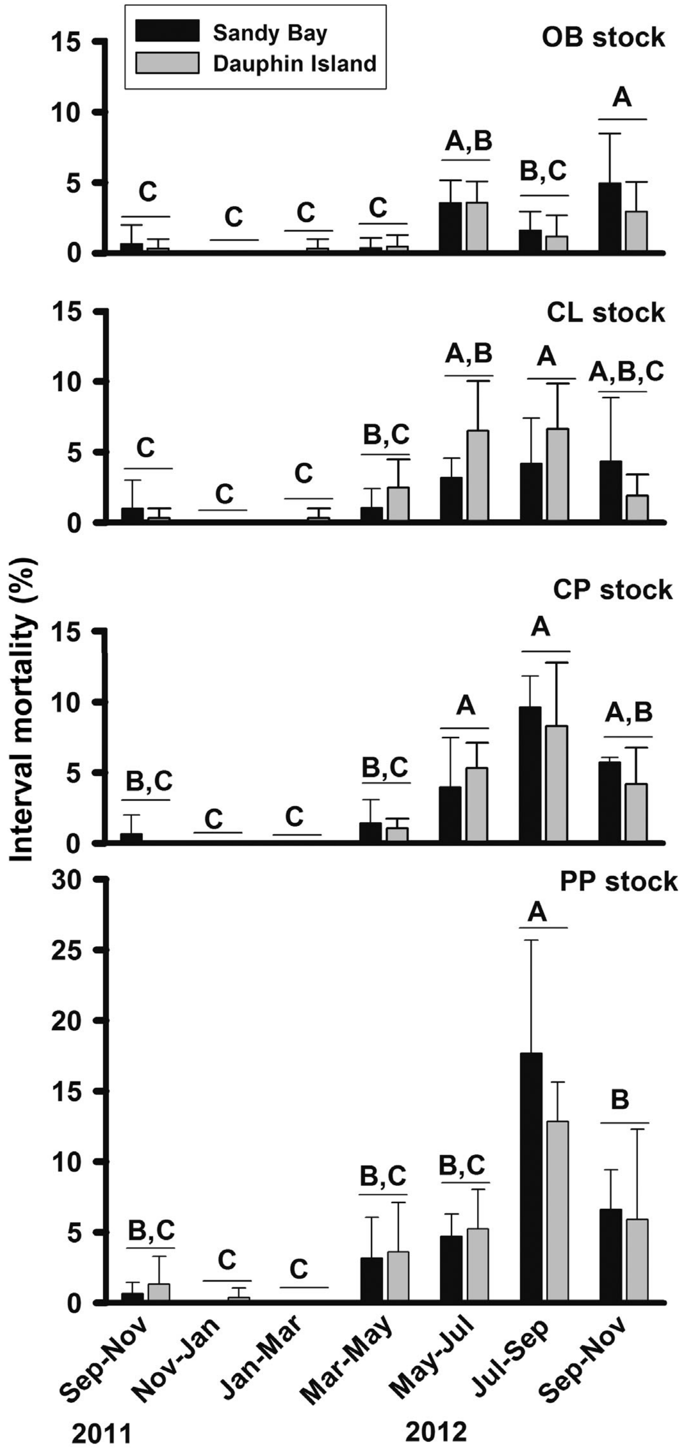

Fig. 5. Mean (±SD) interval mortality of Louisiana eastern oysters from the OBOY (OB) and Calcasieu Lake (CL) stocks, and Alabama oysters from the Cedar Point (CP) and Perdido Pass (PP) stocks at the Sandy Bay and Dauphin Island study sites. Interval mortality was significantly affected by sampling interval but not site. Groups that do not share letters have means that are significantly different ($p < 0.05$). Lines over bars indicate that no significant differences in means could be shown between sites

($p < 0.0001$) intervals and differences between sites were not found. Interval mortality of the Perdido Pass stock was higher than the OBOY stock during the March–May interval, and than the OBOY, Calcasieu Lake and Cedar Point stocks in the July–September interval. The interval mortality of Cedar Point stock was also higher than of OBOY stock in the July–September interval.

When analyzed by stock, interval mortality was only significantly different between intervals ($p < 0.001$) and differences between sites were not found (Fig. 5). Overall mortalities of 4 mo old oysters deployed in September 2011 was very low (< 5%) during fall and winter intervals and increased significantly in summer intervals at both sites (Fig. 5).

Multiple linear regression analysis was used to determine the relationship between interval mortality and the potential predictors of interval temperature and interval salinity. Interval temperature ($p < 0.001$) but not interval salinity ($p = 0.944$) contributed significantly and produced a multiple regression model with interval mortality = −7.054 + (0.413 × interval temperature) + (0.009 × interval salinity) and an R^2 of 0.397.

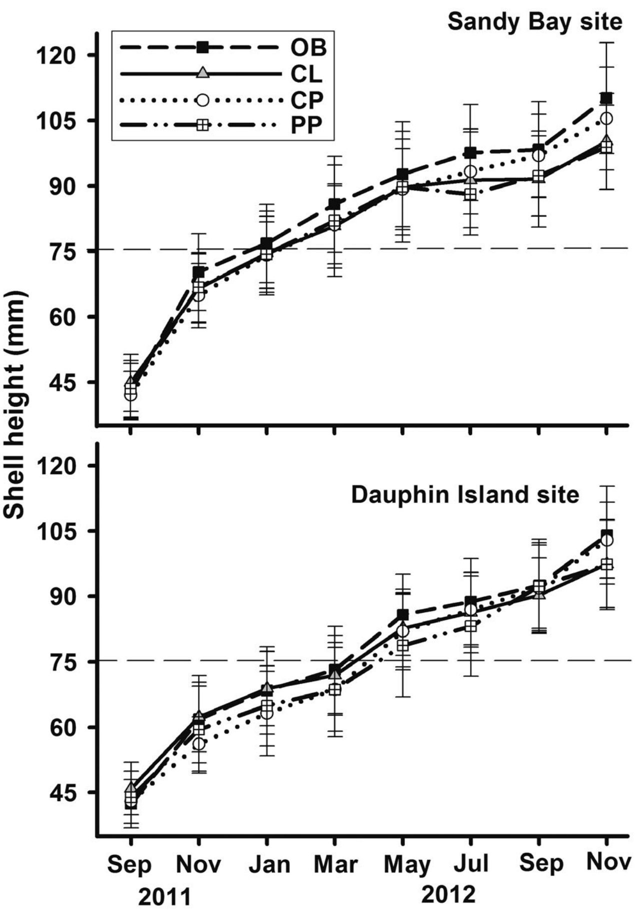

Fig. 6. Mean (±SD) shell heights of Louisiana eastern oysters from the OBOY (OB) and Calcasieu Lake (CL) stocks, and Alabama oysters from the Cedar Point (CP) and Perdido Pass (PP) stocks at the Sandy Bay and Dauphin Island study sites. Dashed lines at 75 mm represent harvest threshold shell height

Growth

At the time of deployment, in September 2011, no differences in shell height were found between stocks (in mm; OBOY: 42.7 ± 6.0, Calcasieu Lake: 45.4 ± 6.3, Cedar Point: 42.5 ± 5.2, Perdido Pass: 43.7 ± 6.3) or sites (in mm; Sandy Bay: 43.3 ± 6.3, Dauphin Island: 43.8 ± 5.8). Stocks reached commercial size (75 mm) in March 2012 at Sandy Bay and May 2012 at Dauphin Island, when the oysters were

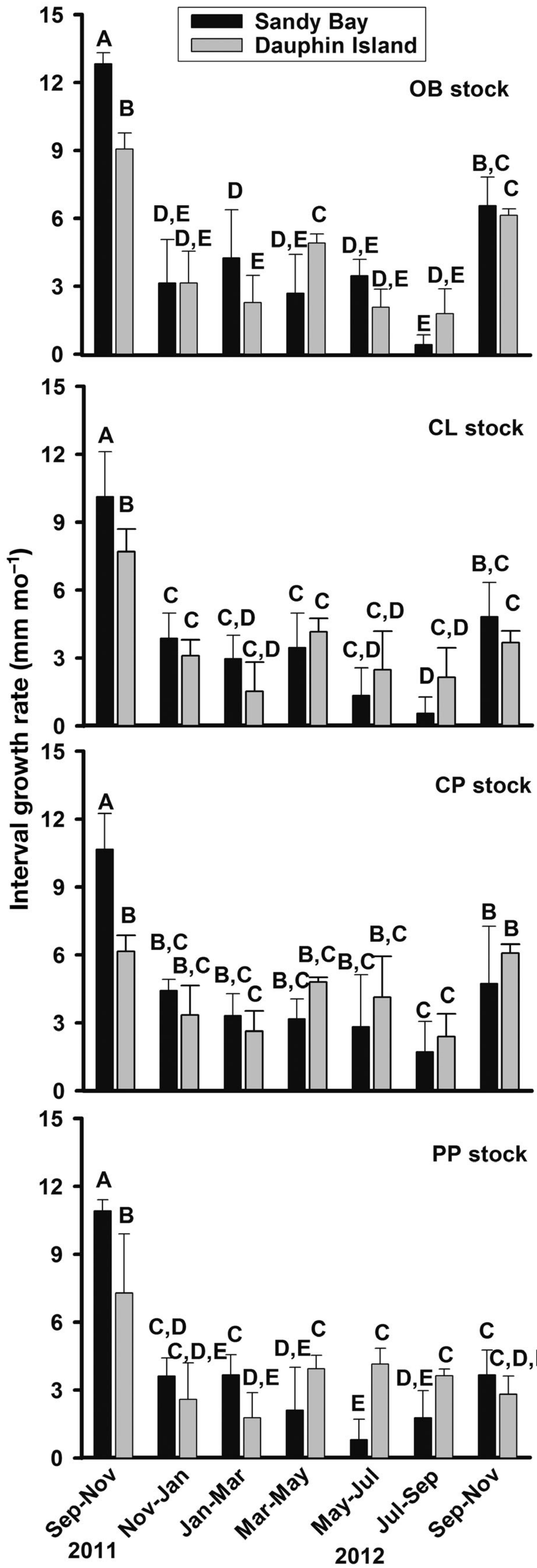

Fig. 7. Mean (±SD) interval growth rates of Louisiana eastern oysters from the OBOY (OB) and Calcasieu Lake (CL) stocks, and Alabama oysters from the Cedar Point (CP) and Perdido Pass (PP) stocks at the Sandy Bay and Dauphin Island study sites. Interval growth rate of each stock was significantly affected by the interaction of site and sampling interval. Groups that do not share letters have means that are significantly different ($p < 0.05$)

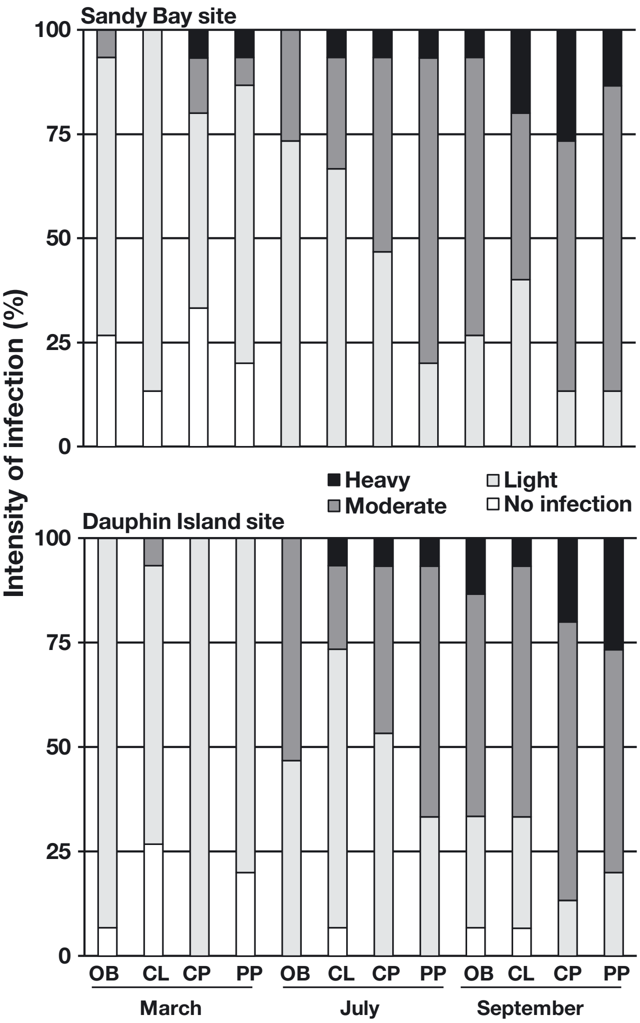

Fig. 8. Percentage of OBOY (OB), Calcasieu Lake (CL), Cedar Point (CP) and Perdido Pass (PP) oysters with no *Perkinsus marinus* infection and with light (<10^4 parasites g^{-1} wet tissue), moderate (1×10^4 to 5×10^5 parasites g^{-1} wet tissue) and heavy (> 5×10^5 parasites g^{-1} wet tissue) infections sampled in March, July, and September 2012 at the Sandy Bay and Dauphin Island study sites

10 and 12 mo old, respectively (Fig. 6). In March 2012, OBOY stock (85.8 ± 10.9 mm) was significantly larger than Calcasieu Lake (80.8 ± 9.6 mm) and Cedar Point (81.1 ± 8.9 mm) stocks at Sandy Bay ($p = 0.004$). In May 2012, OBOY stock (85.8 ± 9.3 mm) was significantly larger than Cedar Point (82.0 ± 8.8 mm) and Perdido Pass (78.8 ± 11.8 mm) stocks at Dauphin Island, and Calcasieu Lake stock (82.7 ± 8.9 mm) was also larger than Perdido Pass stock ($p < 0.001$). At the end of the study in November 2012, there was a significant effect of stock ($p < 0.001$) and site ($p < 0.001$) with no stock × site interaction ($p = 0.149$); OBOY (106.8 ± 12.4 mm) and Cedar Point (104.1 ± 10.5 mm) oysters were significantly larger than Calcasieu Lake (98.8 ± 11.1 mm) and Perdido Pass (98.1 ± 10.0 mm) oysters, and oysters at Sandy Bay (103.7 ± 12.4 mm) were significantly larger than at Dauphin Island (100.3 ± 10.5 mm).

Interval growth rate of each stock was significantly affected by the interaction of site and sampling interval (OBOY, Cedar Point, Perdido Pass: site × sampling interval interaction; $p < 0.001$, Calcasieu Lake: site × sampling interval interaction, $p = 0.017$; Fig. 7). Interval growth rates in OBOY stock were the highest (>6 mm mo^{-1}) in the September–November 2011 and September–November 2012 intervals and the lowest in the July–September interval. In the other stocks, there was only 1 high growth rate interval (>6 mm mo^{-1}) at the beginning of the study in the September–November 2011 interval, and the lowest growth rates were also in the July–September interval. Interval oyster growth rate was significantly higher at Sandy Bay than at Dauphin Island during the September–November 2011 interval and tended to be also higher in the January-March interval, while it tended to be lower than at Dauphin Island in the period between March and September 2012 (Fig. 7).

Multiple linear regression analysis was used to determine the relationship between interval growth rate and the potential predictors of interval temperature, interval salinity and initial shell height at the beginning of each interval. Interval temperature ($p < 0.056$) and initial shell height ($p < 0.001$), but not interval salinity ($p = 0.337$), contributed significantly and produced a multiple regression model with interval growth rate = 7.969 + (0.120 × interval temperature) + (0.0895 × interval salinity) − (0.117 × initial shell height) and an R^2 of 0.474.

P. marinus infection intensity

No significant differences in *P. marinus* infection intensity between stocks were detected at the time of deployment (in $\log_{10}$ parasites g^{-1} wet tissue; OBOY: 1.7 ± 1.4, Calcasieu Lake: 1.0 ± 1.2, Cedar Point: 1.5 ± 1.2, Perdido Pass: 1.3 ± 1.3). At Sandy Bay, there were significant effects of sampling times ($p < 0.001$) and stocks ($p = 0.001$) on *P. marinus* infection intensities after deployment. Infection intensities increased significantly from March (1.5 ± 1.5) to July (3.7 ± 1.6) and again from July to September (4.5 ± 1.5). Infection intensities of Calcasieu Lake (2.7 ± 1.9) and OBOY stocks (2.9 ± 1.8) were significantly lower than Perdido Pass stock (3.8 ± 2.0). Calcasieu Lake stock also had significantly lower infection intensity than Cedar Point stock (3.6 ± 2.1). Moreover, the combined percentage of oysters with moderate and heavy infections was significantly lower in the Louisiana stocks, OBOY ($p = 0.035$) and Calcasieu Lake ($p = 0.011$) stocks, than in the Perdido Pass stock. At Dauphin Island, *P. marinus* infection intensity was only significantly affected by sampling time ($p = 0.047$), with higher infection intensities in September (4.5 ± 1.6) compared with March (1.5 ± 1.0). No differences in the combined percentage of oysters with moderate and heavy infections could be shown between stocks at Dauphin Island (Fig. 8).

Condition index

No differences in mean condition indices between stocks were found at the time of deployment (OBOY: 16.9 ± 1.8, Calcasieu Lake: 15.1 ± 2.8, Cedar Point: 17.2 ± 3.1, Perdido Pass: 15.7 ± 1.8). After deployment, there was a significant effect of stock ($p = 0.036$) and sampling time ($p < 0.001$) in Dauphin Island. Specifically, Perdido Pass (10.5 ± 5.0) stock had a significantly greater condition index than Calcasieu Lake (9.5 ± 5.0) stock, and condition index in March (16.5 ± 1.5) was significantly greater than in July (7.0 ± 1.5) and September (7.0 ± 1.9). Oyster condition index in Sandy Bay was only affected by sampling time, with a significant decrease, by more than half, in condition index of all stocks from March (15.7 ± 2.0) to July (5.8 ± 1.3) and September (7.1 ± 2.3).

DISCUSSION

The mortality, growth, *Perkinsus marinus* infection intensity and condition index of 1 oyster stock selected for dermo resistance in Louisiana and 3 unselected stocks, 1 from Louisiana and 2 from Alabama estuaries, were compared at 2 Alabama sites. The Louisiana stock selected for dermo resistance showed significantly lower mortality than the unselected stocks over the course of the study at Dauphin Island, and both Louisiana stocks had lower mortality than the Alabama stocks at Sandy Bay, a slightly more saline site. Mortality peaked in summer concomitant with increasing *P. marinus* infection intensities and higher temperatures. The selected stock reached greater mean shell height than the unselected Louisiana stock and the Alabama oyster stock with the highest mortality and *P. marinus* infection intensity (i.e. Perdido Pass stock). The shell height of oysters at the higher salinity site was slightly but significantly greater than at the lower salinity site. Growth rates decreased during intervals with higher temperatures and as shell heights at the beginning of each interval increased. Condition indices of the oyster stocks decreased by more than half between March and July, following expected oyster spawning, and stayed depressed throughout the summer. Differences in stock performance especially in dermo-related mortality highlight the importance of stock selection for intensive aquaculture in dermo-endemic estuaries of the northern Gulf of Mexico.

Less than 10% of the OBOY stock oysters selectively bred for dermo-resistance died by the end of the study compared to >25% of the worst-performing Alabama stock oysters (i.e. Perdido Pass). The greater oyster mortality in the worst-performing stock was associated with higher *P. marinus* infection intensities, similarly described in previous studies (Ray 1954, Mackin 1962). Specifically, in Sandy Bay, Perdido Pass stock oysters had significantly higher infection intensity and a greater percentage of moderately and heavily infected oysters with *P. marinus* than Louisiana stock oysters. In Dauphin Island the performance of Perdido Pass stock oysters followed similar trends but significant differences with the other stocks were not found. Mean daily salinity at Sandy Bay (23.4) was significantly greater than at Dauphin Island (21.3); still, differences were small and average salinities at both sites were well within the range favorable for parasite propagation (Chu et al. 1993, Dungan & Hamilton 1995, La Peyre et al. 2006). The salinities prevalent at Dauphin Island between the end of January and the end of March, however, were much lower than at Sandy Bay and lower than the optimum conditions for *P. marinus* proliferation (>15). Those salinities impeded the proliferation of *P. marinus*, as indicated by finding all oysters in March lightly or not-infected at Dauphin Island while some Alabama stock oysters (7–20%) had moderate and heavy infections at Sandy Bay.

The highest susceptibility to dermo of Perdido Pass stock oysters was unexpected as this population inhabits a high-salinity area and was anticipated to have developed some dermo resistance. For all the stocks, but especially for Perdido Pass stock, mortality peaked in summer concomitant with increasing *P. marinus* infection intensities (7–27% heavily infected oysters) at the higher temperatures. High temperatures are well known to promote the propagation of this parasite *in vitro* and *in vivo* in laboratories studies (Chu & La Peyre 1993, Dungan & Hamilton 1995, La Peyre et al. 2008). In field studies, increased infection intensities of *P. marinus* in eastern oysters have consistently been recorded when temperatures exceed 20°C and at salinity >12–15, leading to peak mortalities following summer high temperatures (Ray 1954, Mackin 1962, Ragone Calvo et al. 2003b).

The unselected Louisiana oyster stock, consisting of the progeny of wild oysters collected from Calcasieu Lake public oyster grounds, showed lower mortalities than the Perdido Pass stock at Dauphin Island and both Alabama stocks at the Sandy Bay site. The progeny of Calcasieu Lake oysters also showed the lowest mortality when compared to the progeny of oysters collected on oyster public grounds from other Louisiana estuaries (Stickler et al. 2001, Leonhardt et al. 2017). This is the reason why oysters from Oyster Bayou on the southern edge of Calcasieu Lake were used as the founding brood stock for the OBOY line. Oysters from this location are exposed to higher salinities than oysters of other Louisiana public grounds, which may have favored selection for increased survival upon *P. marinus* infection. Differences in disease susceptibility between oyster populations along both the Atlantic and Gulf of Mexico coasts have been reported and should continue to serve as the basis for brood stock development for local use (Bushek & Allen 1996, Stickler et al. 2001, Brown et al. 2005, Frank-Lawale et al. 2014).

At the end of the study, the selected OBOY stock which showed the lowest mortality also reached the highest shell height while the Perdido Bay stock with the highest mortality had the lowest shell height. As with mortality, the mean *P. marinus* infection intensities and percentage of oysters with moderate and heavy infection intensities which were greatest in Perdido Pass stock oysters than in OBOY stock oysters might explain the lower shell height; increase in *P. marinus* infection intensities can lead to a decrease in oyster growth rates (Menzel & Hopkins 1955, Paynter & Burreson 1991). Oyster growth rates were the lowest during the July–September interval and might be due to increased *P. marinus* infection intensities and the elevated temperatures, which often exceeded 30°C. High temperatures may 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). Interestingly, temperatures tended to be higher and oyster growth rate lower at Sandy Bay compared to Dauphin Island during the July–September interval. Overall, however, temperature and initial shell height were the major predictors of growth rates in this and previous studies (reviewed in Kraeuter et al. 2007).

The differences in shell heights and in salinities between sites were small but significant. Although salinity is a major factor controlling growth rates (Kraeuter et al. 2007), the favorable salinity (generally ≥20) at both sites, along with relatively small differences in interval salinities between sites explain why salinity was not identified as a predictor of growth rates under our study's environmental conditions. While salinities at both sites were >20 during most intervals, it is interesting to note that when salinity was lower (~15) at Dauphin Island compared to Sandy Bay (~20) during the January–March interval, the oyster growth rates at Dauphin Island were also significantly lower than at Sandy Bay. Other factors such as phytoplankton quantity and quality or suspended sediment, which were not measured, may have also had some differential impact on oyster growth at both sites.

The condition index is a prime indicator of how well an oyster uses the shell cavity available for somatic and gonadal tissue growth and reflects physiological or nutritive status (Haven 1961, Rainer & Mann 1992). Oyster condition index is often used to estimate meat quality and yield (Lawrence & Scott 1982). The only significant difference found in condition index was between Perdido Pass and Calcasieu Lake stock oysters, but it is unlikely that the small difference impacted oyster meat quality. Quantitative comparison of oyster meat quality between stocks will be needed to confirm this assertion (Zhang et al. 2016)

The most significant differences between oyster stocks in our study were their mortalities, specifically between July and September during peak *P. marinus* infection intensities. It is therefore recommended that local growers preferably use stocks with greater dermo resistance at sites with comparable or higher salinity regime or harvest oysters prior to this time. In our current study, oysters could have been harvested before summer because they were spawned early (i.e. May) and reached market size within 12 mo, but this strategy may not always work in years where local environmental conditions may not be as favorable for growth as exemplified in an earlier study (Walton et al. 2013b), or where harvest is not allowed due to environmental conditions (e.g. prolonged closures due to rainfall or harmful algal blooms). Better characterization or understanding of the processes involved in disease resistance to dermo may lead in the future to the development of specific markers for use in candidate gene or marker-assisted selection (Cancela et al. 2010, La Peyre et al. 2010). This is important for the development of off-bottom aquaculture at high salinity, an environmental condition that is well known to increase eastern oyster growth rate but also dermo-related mortalities (Ragone Calvo et al. 2003b, Kraeuter et al. 2007, Bushek et al. 2012).

Acknowledgements. We thank Julie Davis at Auburn for field help and Jacqueline Tai, Jaren Lee, Alexis Allen, and April Chow at the Louisiana State University for laboratory help. This research was funded by the National Oceanic and Atmospheric Administration Aquaculture Program.

LITERATURE CITED

Breithaupt RL, Dugas RJ (1979) A study of the southern oyster drill (*Thais haemastoma*) distribution and density on the oyster seed grounds. Louisiana Wildlife and Fisheries Commission, Tech Bull 30, New Orleans, LA

Brown BL, Butt AJ, Shelton SW, Meritt D, Paynter KT (2005) Resistance of dermo in eastern oysters, *Crassostrea virginica* (Gmelin), of North Carolina but not Chesapeake Bay heritage. Aquacult Res 36:1391–1399

Bushek D, Allen SK Jr (1996) Host–parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus.* Mar Ecol Prog Ser 139:127–141

Bushek D, Ford SE, Allen SK Jr (1994) Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica.* Annu Rev Fish Dis 4:201–217

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

Cancela ML, Bargelloni L, Boudry P, Boulo V and others (2010) Genomic approaches in aquaculture and fisheries. In: Cock JM, Viard F, Tessamar-Raible K, Boyen C (eds) Introduction to Marine Genomics. Springer-Verlag, Heidelberg, p 213–286

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

Chu FLE, La Peyre JF, Burreson CS (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. In: Papers delivered at the Convention of the National Shellfisheries Association, New Orleans, LA, June 22–25, 1953. Fish and Wildlife Service, US Department of Interior, Washington, DC, p 13–38

Craig A, Powell EN, Fay RR, Brooks JM (1989) Distribution of *Perkinsus marinus* in gulf-coast oyster populations. Estuaries 12:82–91

Davis CV, Barber BJ (1999) Growth and survival of selected lines of eastern oysters, *Crassostrea virginica* (Gmelin 1791) affected by juvenile oyster disease. Aquaculture 178:253–271

Dungan CF, Hamilton RM (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 42:379–388

Dupuy JL, Windsor NT, Sutton CE (1977) Manual for design and operation of an oyster seed hatchery for the American oyster *Crassostrea virginica.* Spec Rep No 142, Virginia Institute of Marine Science, Gloucester Point, VA

Fisher WS, Oliver LM (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 SK Jr, 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

Guo X, Wang Y, DeBrosse G, Bushek D, Ford SE (2008) Building a superior oyster for aquaculture. Jersey Shoreline 25:7–9

Haskin HH, Ford SE (1979) Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. Mar Fish Rev 41:54–63

Haven D (1961) Seasonal cycle of condition index of oysters in the York and Rappahannock rivers. Proc Natl Shellfish Ass 52:42–66

Hopkins AE (1949) Determination of condition of oysters. Science 110:567–568

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

La Peyre JF, Xue QG, Itoh N, Li Y, Cooper RK (2010) Serine protease inhibitor cvSI-1 potential role in the eastern oyster host defense against the protozoan parasite *Perkinsus marinus.* Dev Comp Immunol 34:84–92

La Peyre MK, Nickens AD, Volety AK, Tolley GS, La Peyre JF (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, Casas S, La Peyre J (2006) Salinity effects on viability, metabolic activity and proliferation of three *Perkinsus* species. Dis Aquat Org 71:59–74

La Peyre MK, Casas SM, Villalba A, La Peyre JF (2008) Determining the effects of temperature on two *Perkinsus* species viability, metabolic activity and proliferation and its significance to understanding seasonal cycles of perkinsosis. Parasitology 135:505–519

La Peyre MK, Geaghan J, Decossas G, La Peyre JF (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

Lawrence DR, Scott GI (1982) The determination and use of condition index of oysters. Estuaries 5:23–27

Leonhardt JM, Casas S, Supan JE, La Peyre JF (2017) Stock assessment for eastern oyster seed production and field grow-out in Louisiana. Aquaculture 466:9–19

Loosanoff VL (1958) Some aspects of behavior of oysters at different temperatures. Biol Bull 114:57–70

Mackin JG (1962) Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publ Inst Mar Sci Univ Tex 7:132–229

Matthiessen GC, Feng SY, Leibovitz L (1990) Patterns of MSX (*Haplosporidium nelsoni*) infection and subsequent mortality in resistant and susceptible strains of the eastern oyster *Crassostrea virginica* (Gmelin, 1971), in New England. J Shellfish Res 9:359–366

Maxwell VJ, Supan J, Schiavinato LC, Showalter S, Treece GD (2008) Aquaculture parks in the coastal zone: a review of legal and policy issues in the Gulf of Mexico state waters. Coast Manage 36:241–253

Menzel RW, Hopkins SH (1955) The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus.* J Parasitol 41: 333–342

Paynter KT, Burreson EM (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

Petrolia DR, Walton WC, Yehouenou L (2017) Is there a market for branded Gulf of Mexico oysters? J Agric Appl Econ 49:45–65

Proestou DA, Vinyard BT, Corbett RJ, Piesz J and others (2016) Performance of selectively-bred lines of eastern oyster, *Crassostrea virginica*, across eastern US estuaries. Aquaculture 464:17–27

Ragone Calvo LM, Calvo GW, Burreson EM (2003a) Dual disease resistance in a selectively bred eastern, *Crassostrea virginica*, strain tested in Chesapeake Bay. Aquaculture 220:69–87

Ragone Calvo LM, Dungan CF, Roberson BS, Burreson EM (2003b) Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. Dis Aquat Org 56:75–86

Rainer JS, Mann R (1992) Comparison of methods for calculating condition index in eastern oysters, *Crassostrea virginica* (Gmelin, 1791). J Shellfish Res 11:55–58

Ray SM (1954) Biological studies of *Dermocystidium marinum.* Rice Institute Pamphlet 41, Rice Institute, Houston, TX

Stickler S, Wagner E, Supan J, Allen S, La Peyre J (2001) Natural Dermo resistance and its role in the development of hatcheries for the Gulf of Mexico. J Shellfish Res 20: 557 (Abstract)

Walton WC, Davis JE, Supan JE (2013a) Off-bottom culture of oysters in the Gulf of Mexico. SRAC Publication-Southern Regional Aquaculture Center No. 4308, Stoneville, MS

Walton WC, Rikard FS, Chaplin GI, Davis JE, Arias CR, Supan JE (2013b) Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: survival, growth, shape, condition index and *Vibrio* abundances. Aquaculture 414–415:260–266

Zhang J, Walton WC, Wang Y (2016) Quantitative quality evaluation of eastern oyster (*Crassostrea virginica*) cultured by two different methods. Aquac Res, doi:10.1111/are.13126

Editorial responsibility: Marianne Holmer, Odense, Denmark

Submitted: November 21, 2016; Accepted: March 9, 2017
Proofs received from author(s): April 10, 2017