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Effects of flood-associated stressors on growth and survival of early life stage oysters (*Crassostrea virginica*)

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ABSTRACT

Oyster reefs provide essential ecosystem services but are severely degraded worldwide. Extreme flooding events, which can be intensified by water management decisions, reduce water quality in estuaries and further threaten oyster populations. Restoration and conservation of oysters is dependent on the success of early oyster life stages. This study examined the effect of water quality stressors associated with flooding events on the growth and survival of larval and juvenile oysters (*Crassostrea virginica*). In 96-h assays, we exposed D-stage larvae to a range of dissolved oxygen, microcystin-LR, pH, and salinity concentrations. These conditions were selected based on water quality data from the Mississippi Sound during a 2019 freshwater flooding event caused by the Bonnet Carré Spillway opening. There was no negative effect of microcystin-LR or pH on early veligers at the concentrations tested, but low salinity significantly reduced shell growth, and hypoxia (< 2 mg L^{-1} O_2) decreased both larval growth and survival. Post-metamorphosis juvenile oysters were exposed to the same water quality stressors for 24 days in the lab. Low DO, pH, and salinity treatments reduced juvenile change in wet weight and shell growth rates, but had no effects on survival. These laboratory-exposed juveniles were subsequently deployed into the field to assess the ability of juveniles to recover from short-term exposure to simulated flooding-associated stressors. After deployment to natural conditions in the Mississippi Sound, juvenile oysters were able to compensate for reduced growth during the lab exposure, even though survival was reduced for juveniles previously exposed to low pH during the first two weeks in the field. In general, early oyster life stages were relatively tolerant of the duration and stressor concentrations tested, but negative sublethal impacts of flood-associated stressors must be considered in the face of increasing frequency and duration of flooding events due to climate change.

1. Introduction

Eastern oysters (*Crassostrea virginica*) are an ecologically and economically important species (Grabowski et al., 2012). Oyster reefs create three-dimensional biogenic structures that supply essential ecosystem services, such as water filtration, shoreline stabilization, crucial nursery habitat, and increased biodiversity (Newell, 2004; Scyphers and Powers Jr, 2011; Tolley and Volety, 2005; Wells, 1961). However, oyster populations are threatened worldwide due to overharvesting and habitat degradation (Beck et al., 2011). Recognition of the loss of oyster reefs has led to large-scale efforts to restore both the fisheries production and ecological function of these critical ecosystems, particularly on the Atlantic and Gulf coasts of the United States (Bersoza Hernández et al., 2018; Coen and Luckenbach, 2000). Oyster reef habitat restoration approaches include deploying hard substrate for larval settlement or out-planting live juveniles into suitable coastal zones to establish new reefs or enhance existing local populations (Brumbaugh and Coen, 2009; Kennedy et al., 2011). Success of these restored oyster reefs is dependent upon sustained recruitment of free-swimming larvae and their subsequent post-settlement survival (Powers et al., 2009; Thorson, 1950).

Oysters have a wide salinity tolerance, but exposure to large amounts of freshwater for extended periods reduces adult growth, reproduction, and survival (Butler, 1949; La Peyre et al., 2013; Loosanoff, 1952). Oyster mortality due to rapid reductions in salinity frequently occurs in areas prone to freshet events caused by spring flooding and storms

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(Shumway, 1996). Flooding events also increase nutrient input into coastal waters, leading to eutrophication, which can be associated with algal blooms and hypoxia (Diaz and Rosenberg, 2008; Sinha et al., 2017). Some flood-induced algal blooms can be toxic (i.e., harmful algal blooms [HABs]; Hallegraeff, 2010), and cause mass mortalities in bivalve populations (Shumway, 1990). Reports of microcystin detected in estuaries due to the presence of salt tolerant freshwater cyanobacteria species have risen worldwide (see Preece et al., 2017). The death and decay of algal blooms create hypoxic conditions (< 2 mg L^{-1} O_2) and reduce pH levels (Rabalais et al., 2010; Wallace et al., 2014), which can detrimentally impact oysters (Lenihan and Peterson, 1998; Pace et al., 2020). More frequent and intense freshwater events are predicted to rise with climate change due to extreme rainfall caused by alterations in precipitation patterns (IPCC, 2014; Scavia et al., 2002). Flood-associated water quality stressors can be exacerbated by flooding control practices, which increase the intensity and duration of freshwater inputs to estuaries (Gillanders and Kingsford, 2002; Parker et al., 2013).

Oyster recruitment is also reduced during extreme flooding events (La Peyre et al., 2013; Pollack et al., 2011). Flood-associated abiotic stressors can negatively impact larval survival and development in the water column (Baker and Mann, 1992; Clark and Gobler, 2016; Davis, 1958), as well as reduce growth rates and increase mortality in post-metamorphosis juveniles on the benthos (Dickinson et al., 2012; Loosanoff, 1952; Stevens and Gobler, 2018). Multiple marine HAB-forming species also have negative effects on early oyster life stages (Griffith et al., 2019; Luckenbach et al., 1993; Rolton et al., 2015), but the impacts of freshwater cyanobacteria blooms are unknown. Although larvae and post-settlement juveniles are known to be more sensitive to environmental stressors than adult oysters, the tolerance ranges of early life stages are not as well studied as in adults (His et al., 1999; Shumway, 1996). Negative growth effects and reduced survival in early oyster life stages can cause declines in adult populations and limit reef resilience to freshwater inflow events and other disturbances (Gosselin and Qian, 1997; Thompson et al., 1996).

The Gulf of Mexico is the largest remaining wild oyster fishery in the world (Beck et al., 2011), but catches have declined in the 21st century due to numerous environmental disasters, including freshwater intrusion events (Grabowski et al., 2017; NOAA Fisheries Office of Science and Technology, 2020). Freshwater diversions in response to the *Deepwater Horizon* oil spill created very low salinities in Louisiana estuaries that resulted in substantial oyster mortality (Powers et al., 2017). In the Mississippi (MS) Sound, openings of the Bonnet Carré spillway (BCS), to reduce the threat of the MS River flooding New Orleans, release large volumes of freshwater that reduce salinity and cause oyster losses (Butler, 1952; Pace et al., 2020; Turner, 2006). Most recently, nearly 100% oyster mortality on MS oyster reefs was reported following two spillway openings in 2019 that lasted for a combined 123 days (Gledhill et al., 2020; MDMR, 2019). Substantial oyster reef restoration efforts are ongoing in this region (La Peyre et al., 2014), with millions of dollars available for future projects (Gulf Coast Ecosystem Restoration Council, 2016), but the success of these, and similar projects worldwide, relies on the ability of early oyster life stages to survive and thrive in the conditions that occur during and after increasingly frequent flooding events.

This study addressed the overall question of which flood-associated stressors caused more adverse outcomes in larval and juvenile oyster (*C. virginica*) growth and survival. First, in single stressor laboratory experiments, we exposed D-stage larvae to concentrations of dissolved oxygen, microcystin, pH, and salinity relevant to flood conditions to determine the larval tolerance range for each stressor. Using concentrations informed by results of the larval assays, we then assessed growth and survival of juvenile oysters exposed to the same stressors for 24 days in the lab. These laboratory-exposed juveniles were then transferred to the field to evaluate how prior exposure to flood-associated stressors affects juvenile recovery under natural conditions.

2. Methods

2.1. Laboratory larval exposures

Oyster larvae were obtained from the Auburn University Shellfish Laboratory (AUSL; Dauphin Island, AL, USA) and shipped overnight to the University of Mississippi (UM) as embryos. Larvae were held in aerated artificial seawater at 25 °C and salinity of 15 for 24 h prior to experiments. Upon arrival, larvae were fed Shellfish Diet 1800® (Reed Mariculture, Campbell, CA, USA) with added ClorAm-X® (0.12 g mL^{-1} Shellfish Diet; AquaScience, Richmond, MO, USA) to prevent ammonia buildup (Rikard and Walton, 2010). To examine the tolerance limits of oyster larvae to flood-associated stressors, we exposed 48-h old larvae to ranges of dissolved oxygen (DO), microcystin, pH, and salinity concentrations in 96-h single stressor exposures. Exposures consisted of four stressor concentrations and a control. Test concentrations of each abiotic stressor (DO, pH, salinity) were chosen based on values recorded in the MS Sound during a BCS opening in the summer of 2019 (Fig. S1; Gledhill et al., 2020). Microcystin-LR (MC-LR) is a common and toxic congener of microcystin that is used to monitor toxin levels during blooms of microcystin-producing cyanobacteria (WHO, 2003). MC-LR concentrations used to examine the effects of microcystin on larvae fell within the range of reported environmental concentrations in estuaries and coastal waters during freshwater intrusion events (Preece et al., 2017), including BCS openings (Bargu et al., 2011). This range also reflects World Health Organization (WHO) microcystin guidelines for safe drinking water (1 μg L^{-1}) and recreational waters (20 μg L^{-1}) (WHO, 2003). The DO and MC-LR exposures and the pH and salinity exposures took place on different dates with larvae spawned from different broodstock oysters. Broodstock for the DO and MC-LR exposures represented a F2 generation with original lineage from Apalachicola and Cedar Key, FL. Larvae used in the pH and salinity exposures were spawned from wild broodstock from Matagora Bay, TX.

Stressor treatments were prepared in 2 L Nalgene containers filled with artificial seawater (ASW). ASW was prepared by mixing deionized water with Crystal Sea® Bioassay Mixture (Pentair, Minneapolis, MN, USA) to reach a salinity of 15. DO treatments were obtained by bubbling pure N_2 gas into containers with ASW until the desired oxygen levels of 1, 2, 3, or 5.5 mg L^{-1} were reached. The desired MC-LR concentrations (1, 3, 8, or 20 μg L^{-1}) were generated by adding MC-LR purchased from MilliporeSigma (MA, USA; purity >95%) and dissolved in DMSO (2.5 μg μL^{-1}) into the 2 L containers. The pH treatments of 7.1, 7.3, 7.5, or 7.9 were obtained by bubbling pure CO_2 gas into containers of control seawater until designated pH levels were achieved. Salinity treatments of 3, 6, 9, or 12 were made by diluting ASW with the appropriate amount of deionized water to produce 2 L of treatment water. Controls consisted of ASW with no MC-LR added. Forty-five μL of Shellfish Diet 1800® with ClorAm-X® were mixed into containers, as recommended by Rikard and Walton (2010) based on larval stage and density. Seawater conditions in each container were measured and verified immediately after preparation. DO levels were quantified with a fiber optic oxygen probe (OXROB10) connected to a FireSting O_2 meter (FSO2–4, PyroScience, Aachen, Germany). pH and temperature were measured using a HOBO® pH and temperature logger (MX2501, Onset Computer, Bourne, MA, USA), which was calibrated using NIST-traceable buffer solutions. Salinity was measured with a refractometer. Water samples (1 mL) were collected from MC-LR treatment containers prior to the addition of food to analyze MC-LR concentrations using liquid chromatography tandem mass spectrometry (UPLC-MS/MS; Alliance UPLC with Xevo TQD, Waters Corporation, Milford, MA, USA) methods (EPA, 2015) at the University of Mississippi's Chemistry and Drug Metabolism Pharmacokinetics Core.

Exposures were performed in glass Mason jars (473 mL) stocked with an initial concentration of 10 larvae mL^{-1}. Stressor treatment water was transferred without bubbling to experimental jars ($n = 4$ per stressor treatment) and immediately capped. Jars were held in an incubator set

at 25 °C to simulate spring temperatures when BCS openings typically occur (Parra et al., 2020). Water in jars was replaced daily with freshly prepared stressor treatment water. Prior to daily water changes, seawater conditions in jars were measured as described above. Exposures ran for 96 h.

For the pH and salinity exposures, larvae from each jar were filtered onto 25 μm mesh and then concentrated into 50 mL of control seawater after 96 h. The number of live (i.e., swimming or active velum) and dead (i.e., lack of pigment or empty shell) larvae in three 1 mL subsamples of the concentrated jar water were counted using a Sedgwick-Rafter counting chamber and compound microscope equipped with a digital camera. The remaining larvae from each treatment jar were preserved using 75% ethanol. A subset of preserved larvae ($n = 10$–15) from each jar was photographed to measure shell area using ImageJ software (version 1.52, National Institutes of Health). In addition, 30 random larvae from the stock tank were preserved at the start of the exposures to measure initial shell area. Shell growth rate (μm d^{-1}) was calculated as the difference between average shell area in each jar at the end of the experiment and average initial shell area divided by four days. Two replicates, one control jar and one from the pH 7.6 treatment, were removed from the analysis due to low larval numbers (< 5% of starting concentration). An error in preservation for one replicate of the salinity 6 treatment resulted in no larvae available for shell growth measurements and was subsequently dropped from analysis.

For the DO and MC-LR exposures, the methods for measuring larval growth and survival were slightly modified. Larvae were preserved immediately at the end of the experiment by pouring larvae from each jar onto a 25 μm mesh and then concentrating them into 20 mL of 75% ethanol. The number of shells with tissue (i.e., alive) and without tissue (i.e., dead) were counted in three 1 mL subsamples of the preserved samples. The first 10 live larvae in each 1 mL subsample were photographed to measure shell area ($n = 30$ per jar), and shell growth rates relative to 30 random larvae from the stock tank preserved at the start of the exposure were calculated as described for the pH and salinity stressor exposures.

A generalized linear model (GLM) with binomial error and logit link for each stressor exposure was used to test whether larval survival was a function of stressor concentration. The relationship between shell growth rate and stressor concentration was analyzed with separate linear regressions for each stressor. Bonferroni adjusted p values for the GLMs and regression analyses are reported to account for the possibility of type I error due to multiple analyses. All statistical analyses were performed in R 3.4.2 (R Core Team, 2017). Normality and homogeneity of variance were confirmed prior to analyses using Shapiro-Wilk and Levene's tests in the car package for R (Fox and Weisberg, 2011).

2.2. Laboratory juvenile exposures

Juvenile oysters (4–5 weeks post-settlement, 5–8 mm shell length) were shipped overnight from AUSL and acclimated for 7 days in aerated artificial seawater (salinity 15, 24-26 °C) prior to the juvenile stressor exposure. These juveniles were from the same spawn as the larvae used in the pH and salinity exposures, but they were settled and reared at the AUSL hatchery before shipping to UM. Stressor concentrations for the juvenile exposure were informed by larval responses in the individual stressor exposures (see *Results; Laboratory larval exposures*). Briefly, the low DO treatment was maintained between 1 and 2 mg L^{-1} to represent hypoxic conditions, which showed negative effects on larval growth and survival. There was no negative effect of MC-LR on larval growth or survival, so the MC-LR treatment concentration was maintained at 3 μg L^{-1} to reflect maximum reported concentrations in Gulf of Mexico estuaries during freshwater inflow events (1.7 μg L^{-1}, Bargu et al., 2011; 2.9 μg L^{-1}, Riekenberg et al., 2015). pH had no negative effects on larval responses, so the pH treatment was maintained at the lowest pH tested in the larval experiment (7.1). The low salinity treatment was 6, which resulted in ~50% reduction in larval growth rates, but is within the lower threshold of reported juvenile tolerances (Shumway, 1996).

Stressor treatments were prepared in 8 L high-density polyethylene buckets filled with ASW using the same methods as the larval exposures (see above). Seawater conditions were measured and verified immediately after preparation using the same instruments as the larval exposures (see above). There were five replicate buckets for each of the four stressor treatments and the control. All juveniles, including controls, were individually labeled with color-coded and numbered bee tags (Betterbee, Greenwich, NY, USA) attached with super glue gel prior to the experiment. Twenty juveniles were added to each bucket, and buckets were either sealed to be airtight for the low DO treatment or loosely covered for the other treatments. Buckets were maintained in a temperature-controlled room set at 25 °C. Seawater conditions in buckets were monitored daily and 0.1 mL of Shellfish Diet 1800® with ClorAm-X® was added. Conditions remained stable between water changes. Water was changed every three days, at which time juvenile survival was assessed. Water samples (1 mL) were collected immediately before and after water changes in the MC-LR treatment buckets to analyze MC-LR concentrations using the same methods as for the larval MC-LR exposure. The experiment ran for 24 days.

At the start and end of the experiment, wet weights and photographs of individually labeled juveniles were taken. Photographs were used to measure initial and final shell areas with ImageJ software. Shell growth rate was calculated as the difference between final and initial shell area divided by the number of days between photos. There was no juvenile mortality during the experiment. One-way ANOVAs were used to test for the effect of stressor treatments on change in wet weight and shell growth rate. Pairwise comparisons for significant ANOVAs were assessed using Tukey's post hoc test.

2.3. Field juvenile deployment

Juveniles exposed to stressor treatments in the lab were deployed one week later into the field to assess how prior exposure to flood-associated stressors affects growth and survival under natural environmental conditions. Individuals from each replicate bucket were enclosed in separate baskets (20 cm × 20 cm × 8 cm; L x W x H) constructed out of 10 mm^2 vexar mesh. Baskets were suspended from a dock in Bay St. Louis, MS (30°18′18" N, 89°19′30" W) ~0.5 m above the bottom in ~3 m water depth. In addition to the lab-exposed oysters, each basket also contained 5 individually labeled, weighed and photographed oysters from the same spawn as the experimental juveniles, but they had been maintained in flow-through conditions at the AUSL hatchery during the duration of the laboratory experiment. These individuals served as controls for any laboratory handling effects on the experimental oysters. Field deployment began on 1 September 2020 and ended on 27 October 2020. Baskets were shaken weekly to remove sediment buildup and epibionts and opened every other week to record survival. Baskets were removed from the field for 3 days during Hurricane Sally (14–17 September 2020) and 4 days during Hurricane Delta (8–12 October 2020). Juveniles were maintained indoors in coolers with aerated MS Sound seawater during these times. Juvenile wet weights and photographs were obtained at the end of the field deployment. Photographs were used to measure final shell area of the surviving juveniles.

Continuous water quality data were also collected during the juvenile field deployment. HOBO® loggers were used to record DO (U26–001), pH (MX2501), and salinity (U24–002-C) at 5-min intervals. All three loggers recorded temperature but only temperature from the pH logger was reported. Loggers were attached to an oyster sensor platform (Gledhill et al., 2020) that was deployed on the bottom at the site of the juvenile deployment. The sensor platform was removed from the water between 14 and 17 September 2020 and 8–12 October 2020 due to hurricanes. Data were also not collected between 30 September-6 October 2020 due to sensor platform maintenance.

Biweekly juvenile survival was compared among stressor treatments and sampling weeks using a GLM with binomial error and logit link. Post

hoc comparisons were performed using the 'lsmeans' package (Lenth, 2016). One-way ANOVAs were used to analyze the effect of prior exposure to stressors on changes in wet weight and shell growth rate during the field deployment period.

3. Results

3.1. Laboratory larval exposures

Oyster larval survival decreased with declining DO concentrations (LR $\chi^2 = 9.10$, $p = 0.020$). Survival was ~12% lower under hypoxic conditions (DO <2 mg L^{-1}) compared to the control DO level (~7 mg L^{-1}) (Fig. 1A, Table S1). Larval survival was not affected by MC-LR concentration and remained similarly high across treatments (Fig. 1B, Table S2; LR $\chi^2 = 0.641$, $p = 1.0$). Larval survival increased as both pH (Fig. 1C, Table S3; LR $\chi^2 = 12.8$, $p = 0.003$) and salinity (Fig. 1D, Table S4: LR $\chi^2 = 11.8$, $p = 0.005$) decreased.

Growth rate also had a positive relationship with DO concentration (Fig. 2A, Table S1; $r^2 = 0.359$, $F_{1,18} = 10.1$, $p = 0.042$). Oyster larval shell growth was ~35% lower in the hypoxic treatments relative to the control. The MC-LR (Fig. 2B, Table S2; $r^2 = 0.311$, $F_{1,18} = 8.14$, $p = 0.085$) and pH treatments (Fig. 2C, Table S3; $r^2 = 0.028$; $F_{1,16} = 0.464$, $p = 1.0$) did not have any effects on shell growth, whereas growth rates decreased with declining salinity (Fig. 2D, Table S4; $r^2 = 0.878$, $F_{1,16} = 116$, $p < 0.001$). Larval growth was ~50% lower at a salinity of 6 and ~84% lower at a salinity of 3 relative to controls.

3.2. Laboratory juvenile exposures

After 24 days of exposure to individual stressors (Table 1), there was no juvenile mortality in any of the stressor treatments. However, there were treatment effects on juvenile oyster growth (Fig. 3). Change in juvenile wet weight during the laboratory exposure ($F_{4,20} = 26.8$, $p < 0.001$) was reduced by 23.0%, 27.4%, and 29.4% compared to the control in the low DO, low pH, and low salinity treatments, respectively, whereas the presence of MC-LR had no effect on wet weight (Fig. 3A). Similarly, juvenile shell growth rates differed between the single stressor treatments (Fig. 3B; $F_{4,20} = 21.2$, $p < 0.001$). Exposure to low DO, low pH, or low salinity reduced juvenile shell growth by an average of 46%, to 0.077 ± 0.006 mm^2 d^{-1} (mean ± SE) in the stressor treatments, compared to 0.15 ± 0.01 mm^2 d^{-1} under control conditions.

3.3. Field juvenile deployment

During the first two weeks of the juvenile field deployment in the MS Sound, ambient DO concentrations and pH levels were lower than in the subsequent six weeks (Table S5), with DO dropping below 2 mg L^{-1} and pH dropping to nearly 7.1 on two days in September (Fig. S2). Temperature also averaged 5.51 ± 0.05 °C higher during the first two weeks of the deployment compared to the subsequent six weeks (Table S5, Fig. S2). Survival of juveniles previously exposed to low pH in the lab was reduced by ~18% relative to lab-reared controls, but only after the first two-week period of the two-month field deployment (Table S6, Fig. S3). At that time, the average number of live juveniles previously exposed to low pH was 14 ± 1 per basket, and the average number of dead juveniles was 4 ± 1. Otherwise, overall proportional juvenile survival in the field following prior laboratory exposure to stressors was high and averaged 0.96 ± 0.01 across all times and treatments (Fig. S3). There was no mortality of the AUSL hatchery-reared control juveniles in any of the baskets, and they were four times larger than the control lab-reared juveniles at the beginning of the deployment, with an initial shell area of 225 ± 5.0 mm^2 compared to 42.6 ± 0.7 mm^2 for the lab controls.

After two months under natural conditions, there was no effect of prior exposure to any stressor treatment on juvenile growth in terms of either change in wet weight ($F_{4,20} = 0.884$, $p = 0.503$) or shell growth ($F_{4,20} = 0.237$, $p = 0.914$). Change in wet weight averaged 1.14 ± 0.02 g across all treatments, and shell growth rate was 2.91 ± 0.7 mm^2 d^{-1} during the field deployment. Final shell area for the hatchery-reared

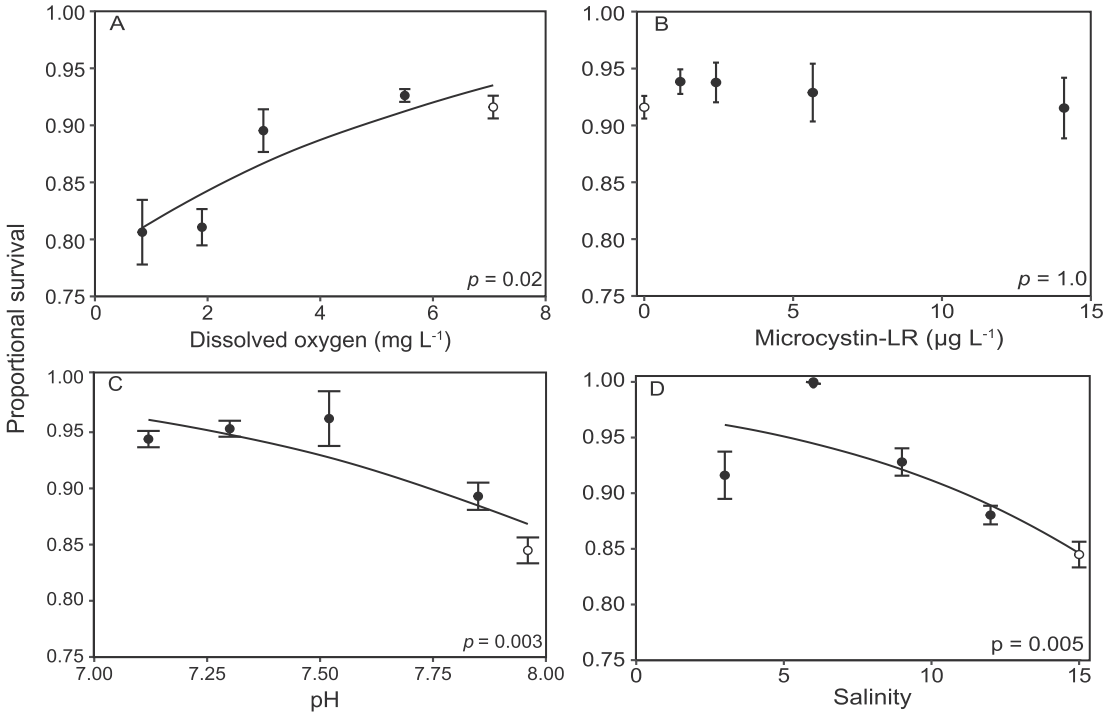

Fig. 1. Proportional larval survival (mean ± SE) as a function of A) dissolved oxygen (Table S1; $n = 4$ jars per treatment), B) microcystin-LR (Table S2; $n = 4$ jars per treatment), C) pH (Table S3; $n = 4$ jars per treatment except $n = 3$ jars for control and pH 7.6), and D) salinity (Table S4; $n = 4$ jars per treatment except $n = 3$ jars for control and salinity 6) after 96-h stressor exposure. Open symbol indicates control treatment. Best fit curve was included if generalized linear model (binomial error; logit link) was significant ($p < 0.05$).

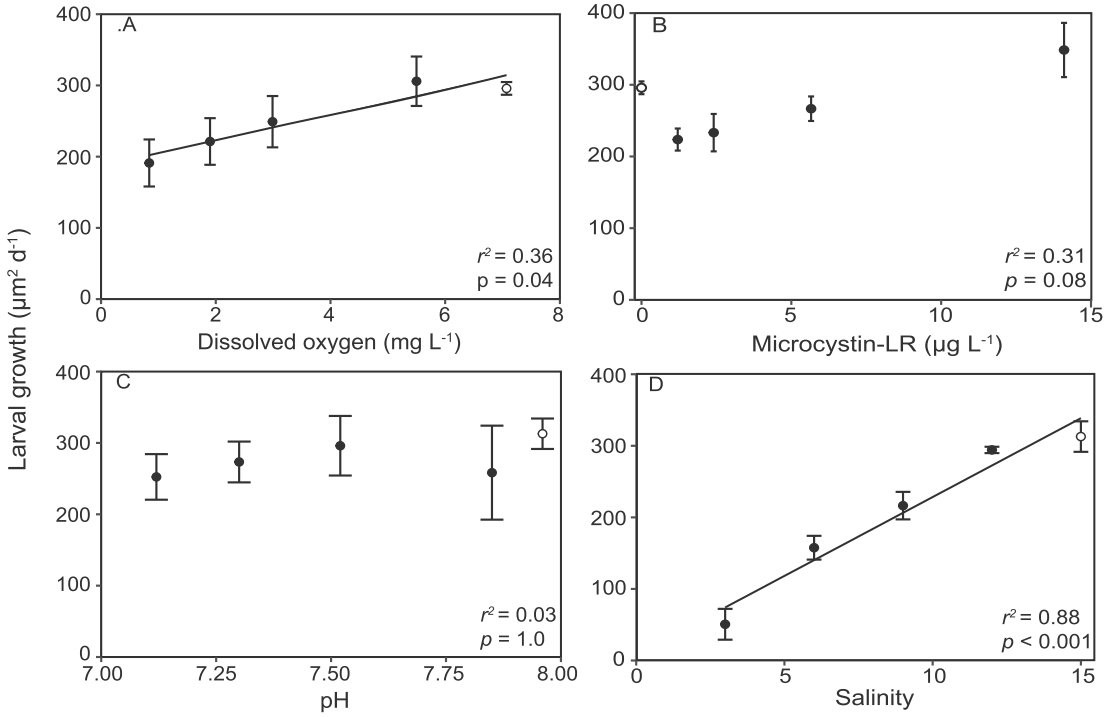

Fig. 2. Mean (± SE) shell growth rate as a function of A) dissolved oxygen (Table S1; $n = 4$ jars per treatment), B) microcystin-LR (Table S2; $n = 4$ jars per treatment), C) pH (Table S3; $n = 4$ jars per treatment except $n = 3$ jars for control and pH 7.6), and D) salinity (Table S4; $n = 4$ jars per treatment except $n = 3$ jars for control and salinity 6) after 96-h stressor exposure. Open symbol indicates control treatment. Best fit line was included if linear regression was significant ($p < 0.05$).

Table 1
Mean seawater conditions (± SD) in buckets during the 24-day single stressor juvenile experiment.

Stressor treatment	DO (mg L^{-1})	Microcystin-LR (MC-LR; μg L^{-1})	pH	Salinity	Temperature (°C)
Control	7.24 ± 0.36	n/a	7.91 ± 0.17	15 ± 0	24.6 ± 0.69
DO	1.37 ± 0.60	n/a	7.99 ± 0.19	15 ± 0	24.6 ± 0.66
MC-LR	7.13 ± 0.45	2.11 ± 0.78[a]	7.96 ± 0.15	15 ± 0	23.5 ± 0.60
pH	7.32 ± 0.40	n/a	7.21 ± 0.13	15 ± 0	24.7 ± 0.66
Salinity	7.50 ± 0.39	n/a	7.74 ± 0.20	6 ± 0	24.6 ± 0.66

n/a = not measured and presumed zero.
[a] MC-LR concentration in a 1 mL sample from a randomly selected MC-LR treatment replicate was quantified before and after each water change using UPLC-MS/MS methods.

juveniles was 575 ± 13.2 mm^2 compared to 235 ± 6.8 mm^2 for the control lab-reared juveniles.

4. Discussion

Extreme flooding events reduce water quality in estuaries, which can affect oyster recruitment processes (La Peyre et al., 2013; Pollack et al., 2011). We found that survival of early oyster life stages was relatively tolerant to the duration and concentrations tested for each flooding-associated stressor, which were based on water quality measurements during a long-term freshwater intrusion event (Fig. S1; Gledhill et al., 2020). Larval survival and shell growth responses were unique to each stressor exposure, whereas abiotic stressors (i.e., low DO/pH/salinity) uniformly reduced juvenile growth with minimal effects on survival in the lab. Once transferred to a natural setting, juveniles were able to compensate for reduced growth during the lab exposure, although prior exposure to low pH decreased juvenile survival under suboptimal conditions in the field (i.e., dramatic fluctuations of DO and pH during the first two weeks of deployment in September). Overall, negative sublethal impacts on early life stages may limit the recovery and resilience of oyster reefs during and after exposure to flooding events.

Developing embryos and larvae are considered to be more sensitive to environmental stressors due to the conflicting energetic demands of early development coupled with limited energy availability (Hamdoun and Epel, 2007; Sokolova et al., 2012). In our laboratory larval experiments, only exposure to low DO had significant negative effects on early veliger larvae survival (Fig. 1A), particularly at hypoxic levels (< 2 mg L^{-1}). Prior work found that under anoxic conditions, early oyster larval stages experienced complete mortality in less than 24 h due to their inability to reduce heat loss and their need to maintain feeding rate, whereas pediveligers and juveniles were more tolerant of anoxia because they were able to reduce metabolism in low DO conditions (Widdows et al., 1989). We also found that larval growth rates decreased in hypoxic conditions (Fig. 2A), which is consistent with other studies demonstrating negative growth responses in bivalve larvae to low DO (Gobler et al., 2014; Wang and Widdows, 1991). Hypoxia commonly occurs in estuaries worldwide during the summer after spring phytoplankton blooms begin to die off, and is exacerbated by increased freshwater discharge (Diaz and Rosenberg, 2008; Rabalais and Turner, 2019). Hypoxic events that coincide with oyster spawning season may limit larval supply due to reduced growth or increased mortality, resulting in lower recruitment (Baker and Mann, 1992; Johnson et al., 2009).

pH naturally fluctuates in estuaries, but can be significantly reduced during flooding events (Fig. S1; Salisbury et al., 2008; Wallace et al., 2014). Low pH conditions reduce oyster larval survival in some studies (Clark and Gobler, 2016; Kurihara et al., 2007; Talmage and Gobler, 2009), while others report null effects of acidification on survival of oyster larvae (Clements et al., 2020; Durland et al., 2019; Ginger et al., 2013). In our larval pH exposures, survival increased as pH decreased

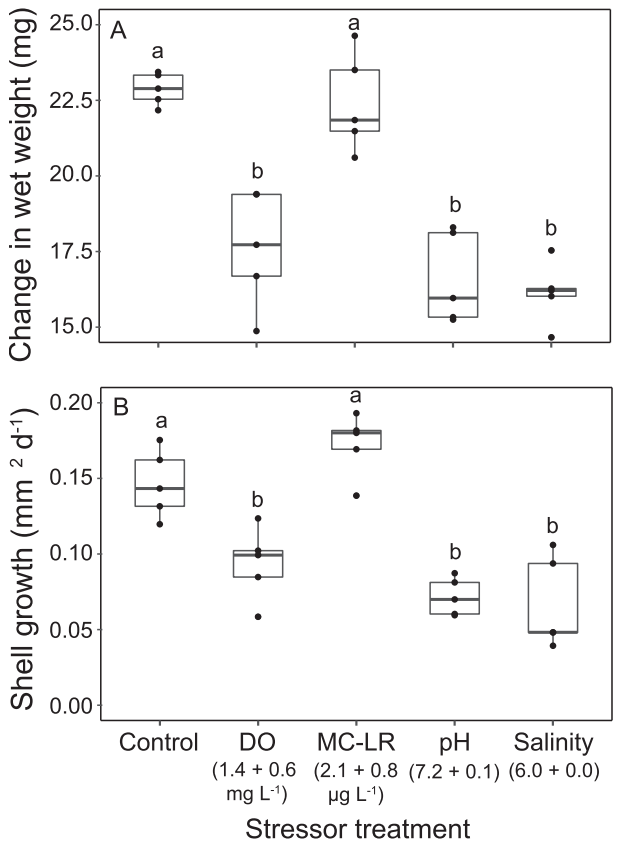

Fig. 3. Boxplots with raw values showing juvenile oyster growth as (A) change in juvenile wet weight and (B) shell area in each single stressor treatment at end of the 24-day juvenile laboratory exposure ($n = 5$ buckets per treatment with 20 individuals per bucket). Boxes show upper and lower quartiles with median line inside box, and whiskers extend to 1.5 times the interquartile range from the upper and lower quartiles, respectively. Mean ± SD values for each stressor treatment are shown (see Table 1 for detailed conditions). Different letters denote means that are significantly different based on Tukey's post hoc tests ($p < 0.05$).

(Fig. 1C), and there was no relationship with shell growth (Fig. 2C). Higher larval survival at reduced pH conditions (~7.5) was reported in 48-h assays with fertilized *C. gigas* (Durland et al., 2019) and *C. virginica* (Clements et al., 2020) embryos. These studies suggested that local adaptations in the broodstock enhanced survival, which is supported by evidence that parental exposure to low pH can provide carry-over benefits for offspring (Durland et al., 2019; Parker et al., 2012). Larvae may be able to compensate for negative pH effects on growth and survival through reallocation of metabolic energy (Pan et al., 2015). In the present study, larvae used for the pH and salinity stressor exposures were spawned from wild broodstock populations from Matagorda Bay, TX, which is characterized by frequent reductions of freshwater inflow (Marshall et al., 2019) and declines in pH over the past few decades (Hu et al., 2015). Thus, oyster populations from this location may produce larvae that are more resilient to low pH. However, the duration of our larval exposures was relatively short and did not incorporate major shell-building stages, so negative pH effects might have occurred if larval exposure continued until metamorphosis (Miller et al., 2020).

Freshwater flooding events are predicted to rise due to increases in climate-induced variability in global precipitation patterns (IPCC, 2014). Intense precipitation events lead to sustained freshwater discharge that lowers salinities in coastal areas and can cause oyster mortality (Munroe et al., 2013; Cheng et al., 2016). In our larval exposures, survival increased as salinity decreased (Fig. 1D), similar to the pH results, yet salinity was confounded with pH levels. In the lower salinity treatments (3–6), pH was ~7.6, compared to ~8.0 in the controls (Table S4), simply as a result of mixing neutral freshwater and seawater. This pH level corresponded to the pH with the highest larval survival (~7.5) in the pH exposures (Fig. 1C, Table S3). In contrast to larval survival, decreased salinities caused significant declines in growth rate (Fig. 2D), which may have severe consequences for pediveliger survival and larval metamorphosis. Davis (1958), using *C. virginica* D-stage larvae from a low salinity broodstock, found 100% mortality after six days of exposure to a salinity of 2.5, and no growth, followed by complete mortality, after 12 days of exposure to a salinity of 5. Additionally, decreased growth rates at intermediate salinities may not inhibit metamorphosis, but may still have latent effects on post-metamorphosis juvenile shell growth and survival (Hettinger et al., 2012; Pechenik et al., 1998).

Freshwater microcystin-producing cyanobacterial blooms are becoming increasingly common in estuaries due to the increased number of coastal flooding events (Paerl and Paul, 2012; Preece et al., 2017). Management practices to mitigate flooding damage can create water conditions that support microcystin-producing blooms in estuaries with oyster populations, such as freshwater diversions from Lake Okeechobee in southeast Florida (Salewski and Proffitt, 2016; Kramer et al., 2018) and BCS openings in Mississippi (Bargu et al., 2011; MDMR, 2019). Early oyster life stages can be vulnerable to toxins produced by marine HAB species (Griffith and Gobler, 2020), but their responses to freshwater HAB toxins have not been studied. There was no effect of the microcystin congener, MC-LR, on larval survival (Fig. 1B) or shell growth (Fig. 2B) at any of the concentrations tested, which reflect concentrations in marine waters reported during bloom events (Preece et al., 2017). Likewise, juvenile oysters were unaffected by MC-LR in the lab exposure (Fig. 3). Adult marine bivalves also appear to be tolerant to freshwater microcystin-producing cyanobacterial blooms and can bioaccumulate MC-LR in tissues, with potential negative implications for higher trophic levels and human health (Gibble et al., 2016; Miller et al., 2010).

Marine invertebrates can tolerate disturbance events and associated environmental stressors by altering metabolic processes, but this is often at the cost of reduced growth and reproduction (Sokolova et al., 2012). We found no effects of abiotic flood-associated stressors on juvenile survival in the lab, while juvenile growth was reduced following exposure to low DO, low pH, or low salinity (Fig. 3). Oysters decrease metabolism and energy production in suboptimal environmental conditions to maintain homeostasis (Dickinson et al., 2012; Willson and Burnett, 2000), but prolonged exposures can result in reduced growth rates (Baker and Mann, 1992; Stevens and Gobler, 2018). Our field juvenile deployment was designed to assess recovery of previously stressed juvenile oysters once conditions return to normal (e.g., following a short-duration flooding event). There were no carry-over effects of previous stressor exposure on juvenile growth rates during the two months in the field. Juvenile oysters exhibiting reduced growth rates when initially exposed to hypoxia or low pH in the lab can acclimate or compensate later in the lab exposure or when deployed into field conditions (Keppel et al., 2016). We observed reduced survival in oysters previously exposed to low pH, but only during the first two weeks in the field (Fig. S3), a period that also coincided with higher water temperatures and lower and more variable DO and pH levels than the other six weeks of deployment (Table S5, Fig. S2), which may have compounded effects of laboratory stressor exposures. Shell deposition and maintenance can be more costly under elevated CO_2 and reduced pH due to lower calcification rates and increased shell dissolution (Beniash et al., 2010; Gazeau et al., 2007; Waldbusser et al., 2011). This increased metabolic cost during the lab exposure may have depleted energy reserves and incurred fitness costs in juveniles previously exposed to low pH that made them less resilient to suboptimal conditions in the field.

5. Conclusions

Oyster reefs are degraded ecosystems (Beck et al., 2011) that occur in estuaries subjected to dramatic fluctuations in environmental conditions due to natural and anthropogenic influences. In the face of climate change and water management decisions, these habitats are increasingly susceptible to extreme flooding events that deteriorate water quality (Gledhill et al., 2020; Parker et al., 2013; Scavia et al., 2002). While significant resources have been invested to restore oyster reef habitat and function (Bersoza Hernández et al., 2018; La Peyre et al., 2013), restoration success is reliant on the survival of early oyster life stages. Our results demonstrate that oyster larvae and juveniles are generally tolerant to flood-associated stressors in isolation, but negative sublethal effects of these stressors may reduce recruitment and population resilience. In addition, these flood-associated stressors generally occur simultaneously during freshwater inflow events and may cause interactive effects on early oyster life stages (Stevens and Gobler, 2018). The negative impacts of flooding disturbances on oyster recruitment need to be considered in oyster reef restoration planning and flooding control mitigation strategies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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