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Effect of environmental history on the physiology and acute stress response of the eastern oyster *Crassostrea virginica*

Jill Ashey[1,3,*], Emily B. Rivest[2]

[1]Department of Biology, William & Mary, Williamsburg, VA 23185, USA
[2]Department of Biological Sciences, Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA 23062, USA

[3]*Present address:* Department of Biological Sciences, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI 02881, USA

ABSTRACT: Environmental history (regimes of water quality to which an organism has been exposed in the past) may influence how the physiology of eastern oysters *Crassostrea virginica* responds to future environmental conditions caused by climate change. Previous research has examined environmental history in a 1-dimensional framework, failing to capture environmental history complexity through space and time. In this study, we examined environmental history as a multi-faceted parameter, incorporating abiotic water quality components, such as temperature, pH, and salinity, that differ among locations. We also assessed how different lengths of environmental histories, defined as proximal and distal, affected oyster physiology and stress response. Finally, we compared the relative influence of abiotic components of environmental history on oyster physiology. We found that physiology and stress response are differentially affected by proximal and distal environmental history, demonstrating the importance of examining environmental history as a multi-faceted and dynamic parameter. Specifically, distal environmental history primarily influenced condition index and total antioxidant potential, while proximal environmental history primarily influenced glycogen content. Salinity of distal environmental history significantly shaped condition index, establishing salinity as a principal factor when considering acclimatization to variable environments. No water quality components were significant influences on glycogen and total antioxidant potential, providing opportunities for research on other components of environmental history. Identifying the temporal portion of oysters' environmental history that influences physiology supports future efforts to predict population tolerance to climate change. Additionally, examining multiple abiotic and biotic components of environmental history can elucidate means of acclimatization to future environmental change.

KEY WORDS: Oyster · Environmental history · Condition index · Glycogen · Antioxidants · Salinity · Acclimatization

1. INTRODUCTION

Environmental history may influence how populations respond to climate change, so it is critical to evaluate how environmental histories shape the physiological processes of species, like the eastern oyster *Crassostrea virginica* (Gmelin, 1791), that play important roles within their ecosystem. For our purposes, environmental history comprises the regimes of water quality to which an oyster has been exposed in the past. Eastern oysters provide numerous ecosystem services, which include stabilizing shorelines, filtering water, and creating a habitat and food source for other animals (Karp et al. 2018, Salvador

*Corresponding author: jillashey@uri.edu

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de Paiva et al. 2018). Eastern oysters are also a major component of the shellfish aquaculture industry in the Chesapeake Bay (USA), with cultured oysters generating US $14.5 million for Virginia in 2018 (Hudson & VA SGMAP 2019). Currently, eastern oyster populations are at <1% of their historic abundance due to overfishing, disease, habitat degradation, and pollution (Beck et al. 2011, Zu Ermgassen et al. 2012). Understanding the connection between physiology and environmental history can inform both restoration efforts and predictions about how oysters will respond to future conditions.

In the Chesapeake Bay, environmental histories of eastern oysters comprise a wide range of habitat conditions that vary spatially and temporally: <5–35 psu (Southworth et al. 2017), 5–32.5°C (Cherkasov et al. 2007), 6.9–8.2 pH units (Matoo et al. 2013), and minimum dissolved oxygen content of <2 mg l^{-1} (Wilson et al. 2005). Among temporal scales, environmental history can influence physiological performance through several mechanisms. Short-term (i.e. 4–8 h) thermal lab acclimatization of intertidal oysters (*Isognomon nucleus*) can affect feeding rates, respiration, and heat shock protein production (Giomi et al. 2016). An oyster's environmental history of 5 mo related to low or high salinity affected mortality but did not have an effect on other physiological metrics (Méthé et al. 2015). Acclimatization to *in situ* environments over multiple years is also ecologically relevant. Over 2 yr, oysters in high salinities and shallow depths had higher glycogen content and growth rates than oysters in low salinities or high depths (Bataller et al. 1999), suggesting that certain environmental histories facilitated an increased ability to grow and sustain glycogen content in oysters, while other environmental histories impeded those physiological functions. Additionally, environmental history can impact new generations through adaptation and carryover effects. For example, offspring whose parents were exposed to elevated pCO_2 had faster shell growth and development rates compared to offspring with no parental history of pCO_2 exposure (Parker et al. 2015). Although this prior work has demonstrated multiple timescales of environmental history that shape oyster physiology, few have examined the relative contributions of time periods of environmental history towards oyster physiological performance.

In the eastern oyster, environmental history can influence growth rates, recruitment, survival, and metabolic rate, among other parameters. For example, certain environmental histories with low salinities (<5 psu) and high temperatures (>25°C) negatively affected eastern oyster growth, recruitment, and mortality, while environmental histories with low salinities (<5 psu) and low temperatures (<25°C) had minimal impacts on growth and survival (La Peyre et al. 2013). Another study found that oysters from sites with higher temperatures had a greater shell thickness than those from lower temperature sites, although their metabolic rates were similar (Lord & Whitlatch 2014). In addition to the physiological parameters above, environmental history can affect density, size, biomass, and infection intensity and disease prevalence (Brown 1988, Drexler et al. 2014). Given the previous research, it is reasonable to hypothesize that environmental history could also affect stress responses in eastern oysters, but little is known about the connection.

Environmental history could have disproportionate effects on juvenile oysters during their first growing season, when they are rapidly growing (Munroe et al. 2017). Here, we examined juvenile oysters to address 3 key knowledge gaps in the existing understanding of how environmental history shapes physiology. Firstly, we approached environmental history as a multi-faceted parameter that incorporates many aspects of water quality that often differ among locations. Secondly, we examined several lengths of environmental history that could influence physiological responses to future conditions, referred to here as proximal and distal environmental history. Proximal environmental history refers to the regime of water quality that a juvenile oyster has most recently experienced (e.g. 4 wk), whereas distal environmental history refers to all of the water quality regimes a juvenile oyster has experienced over its lifetime (e.g. 4 mo). Thirdly, we examined how environmental history could affect the response of an oyster to acute stress. Low salinity is one of many factors (including e.g. temperature, pollution) that can cause acute stress (Zanette et al. 2011, Zhao et al. 2012, Zhang et al. 2016). Additionally, the salinity in the York River region of the Chesapeake Bay (where this study was conducted) is predicted to become more variable over time, leading to increased acute exposure to low salinity for eastern oysters (Muhling et al. 2018, Ross et al. 2021).

The objectives of this study were to (1) investigate the relative importance of proximal and distal environmental history on oyster physiology by evaluating glycogen content and condition index, (2) assess the relative importance of proximal and distal environmental history in shaping physiological stress responses by evaluating total antioxidant potential under acute low salinity, and (3) evaluate the compo-

nent(s) of environmental history that underpin oyster responses by comparing the relative influences of abiotic water parameters. A better understanding of the relative importance of each portion of environmental history, as well as their relation to physiology and stress responses, can inform place-based predictions about how oysters might respond to future climate change. Knowledge of the effects of proximal and distal environmental history can also aid in selecting specific locations or genetic strains for oyster restoration and aquaculture.

2. MATERIALS AND METHODS

2.1. Study sites and deployments

An overview of deployment and sampling approaches is provided in Fig. 1. In July 2018, 50 juvenile eastern oysters were deployed for 4 mo at each of 4 sites along the Chesapeake Bay: Merroir, Piankatank River, Urbanna Creek, and the Virginia Institute of Marine Science (VIMS) Pier (Fig. 2). VIMS Pier served as a control site whose water conditions were likely most representative of the growing conditions at the research hatchery where the oysters were raised. The goal of these deployments was to establish different distal environmental histories. The oysters were (±SE) 14.20 ± 2.86 mm in size and a few months old at the time of deployment (see Fig. S1 and Table S13 in the Supplement at www.int-res.com/articles/suppl/m647p115_supp.pdf). The oysters were obtained from the Aquaculture Genetics and Breeding Technology Center at VIMS (diploid, broodstock family LOLA18) and had all experienced the same growing conditions prior to the start of the experiment. We hypothesized that the 4 mo deployment would be sufficient to create a unique distal history for oysters at each site. This time period in the life history of an oyster also encompasses the majority of the first growing season, where oysters increase in size manyfold and where impacts of environmental history could have disproportionate effects. Oysters were placed in mesh bags (mesh size:

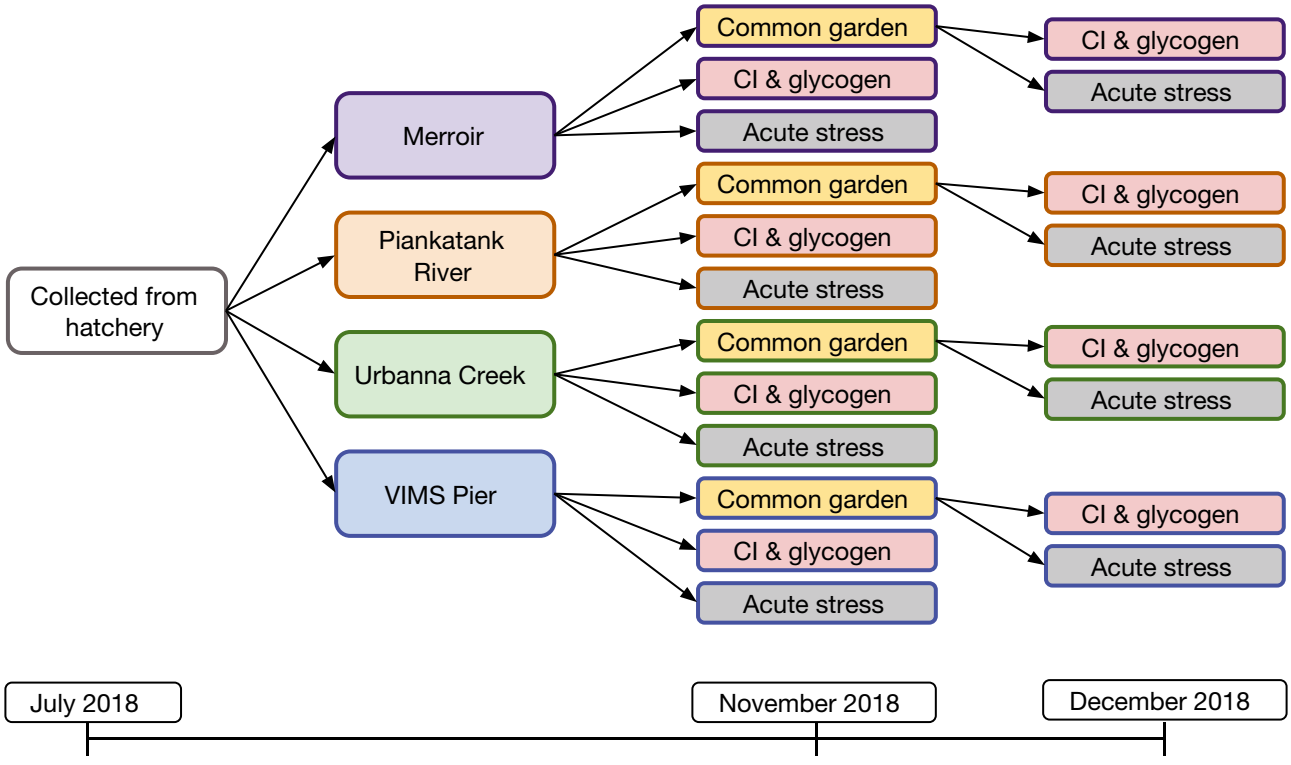

Fig. 1. Overview of distal and proximal deployments. To establish different distal environmental histories, juvenile oysters were deployed in July 2018 at 1 of 4 distal sites: Merroir, Piankatank River, Urbanna Creek, and VIMS Pier. In November 2018, oysters from each site were either redeployed in common garden conditions (yellow background), shucked and preserved for condition index (CI) and glycogen content analysis (red background), or subjected to an acute stress experiment (grey background). In December 2018, oysters were collected from common garden conditions and were either shucked and preserved for CI and glycogen content analysis or subjected to an acute stress experiment

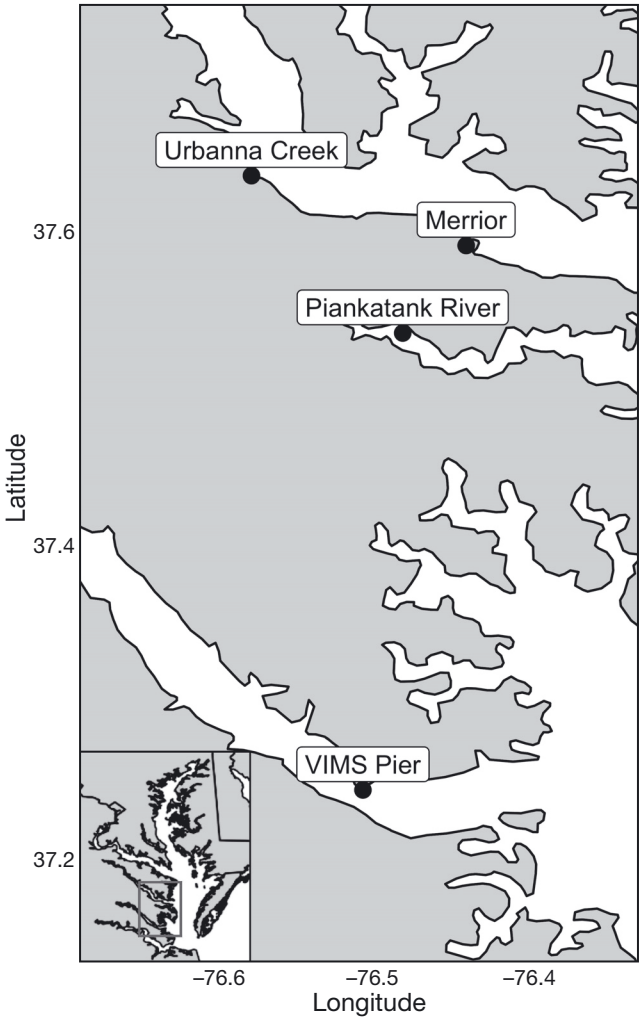

Fig. 2. Sites in Chesapeake Bay where oysters were deployed from July to November 2018: Merroir, Piankatank River, Urbanna Creek, and VIMS Pier

5 mm × 5 mm), which were suspended inside weighted milk crates to prevent oyster burial from shifting sediments. The milk crates were tied to an adjacent dock or pier at a depth of 0.5–2 m. During this deployment, water quality, as well as oyster survival and growth, were measured. After 4 mo, half of the oysters from each site were collected. Some of the collected oysters were shucked for analysis of glycogen content and condition index (N = 7–9). Whole animal tissue was preserved at −80°C in preweighed Falcon tubes, and shells were saved for measurements of condition index. Other oysters from these collections were immediately placed in an acute salinity stress experiment (N = 14, see Section 2.3). The remaining half of the oysters from each site (N = 21–29) entered the common garden phase of the experiment.

To evaluate the importance of the proximal environmental history relative to the distal environmental history in shaping the physiology and stress response of an oyster, individuals from deployments at each of the field sites were re-deployed in mesh bags and milk crates at VIMS Pier, the control site, in November 2018. The oysters remained in these common garden conditions for 1 mo and were then collected. The common garden deployment defined the proximal environmental history of the animals (1 mo), while the prior deployments comprised the distal history (4 mo). It is important to note here that 'proximal' and 'distal' are relative terms, and other lengths of environmental histories should be examined in future work. To describe the proximal history of the redeployed oysters at VIMS Pier, water quality was measured weekly as described in Section 2.2. Survival and growth were assessed at the end of the common garden period, as more frequent measurements were not needed to characterize slower growth under the colder ambient temperature. After 1 mo, whole animal tissue and shells of oysters from each site were preserved for analysis of glycogen content and condition index as described above (N = 7–15). The remaining oysters from each site were immediately subjected to an acute salinity stress experiment to assess the relative influence of distal and proximal environmental history on the stress response of an oyster (N = 14, see Section 2.3).

2.2. Characterizing environmental history

In order to evaluate how multiple aspects of water quality influenced oyster physiology, measurements of temperature, salinity, and dissolved oxygen were taken every 2 wk at each site using a YSI ProPlus multi-parameter water quality instrument from July to November 2018. At the same time, water samples were collected for measurements of carbonate chemistry following best practices (Dickson et al. 2007). At remote sites, water was collected in acid-washed borosilicate glass bottles, immediately poisoned with saturated mercuric chloride, and stored at 4°C until later analysis of pH and total alkalinity. At VIMS Pier, spectrophotometric measurements of pH were performed within 1 h of water collection, and water was stored at −20°C for later analysis of total alkalinity. pH was determined spectrophotometrically using *m*-cresol purple (Sigma Aldrich, Cat. No. 211761-5G, Lot#MKBR3, following SOP 6b, Dickson et al. 2007). Total alkalinity of preserved water samples was determined using an open-cell potentiometric titration

method (following SOP 3b, Dickson et al. 2007) and an automatic titrator (Metrohm 855 Robotic Titrosampler). Samples were titrated using a certified acid titrant (0.1 M HCl acid in 0.6 M NaCl; A. Dickson laboratory) with 2 technical replicates per water sample. For each day of sample analysis, the accuracy of the titrations was confirmed to be within 1% of the certified value of reference material (Batch no. 165, A. Dickson laboratory). The pH at *in situ* temperature, pCO_2, and $\Omega_{Calcite}$ were calculated using the 'seacarb' package in R version 3.2.12 (Gattuso & Lavigne 2009).

2.3. Acute stress exposure

To assess the relative influence of distal and proximal environmental history on the stress response of an oyster, individuals collected after the initial deployment and oysters collected after the common garden deployment were each subjected to an acute exposure to low salinity water. Oysters from each group (i.e. distal site) were exposed to either ambient or low salinity for 48 h (N = 7 for both treatments). Ambient salinity was defined as the average salinity experienced by oysters at their most recent *in situ* location and was determined by averaging the salinity measurements collected over the prior deployment period (Schrandt et al. 2018). For the oysters evaluated after deployment at the 4 distal sites, ambient salinity levels were 11 psu for Merroir, 9 psu for both Piankatank River and Urbanna Creek, and 16 psu for VIMS Pier. For the oysters collected after the month-long common garden deployment, ambient salinity was 11 psu. For both sets of acute exposures, 5 psu was used as the low salinity level. At 5 psu, a suite of oyster physiological functions can become compromised (Fulford et al. 2007, M. Brush pers. comm.), including growth (La Peyre et al. 2013, Hauton 2016), energy acquisition and storage (Bataller et al. 1999, Dickinson et al. 2012), and metabolism (Heilmayer et al. 2008, Dickinson et al. 2012). To generate the low and distal ambient salinity treatments (with the exception of VIMS Pier), distilled water was mixed with coarsely filtered water from the York River to achieve the desired salinity. No distilled water was added to the VIMS Pier distal and common garden salinity treatments.

For the acute salinity exposures, each oyster was placed in a plastic container filled with 800 ml of treatment water. The containers were placed in a tank with continuous flow-through of river water to hold oysters at uniform, environmentally relevant temperatures throughout the exposure. Atmospheric air was continuously bubbled into each container using air stones to sustain and standardize suitable oxygenation and carbonate chemistry. The oysters were not fed beyond what was in the 800 ml cups at the start of the exposure. To minimize build-up of nitrogenous waste, water in the containers was replaced every 12 h with freshly prepared treatment water. After 48 h of total exposure time, gill tissue was dissected from each oyster and preserved at −80°C in pre-weighed Falcon tubes for later analysis of total antioxidant potential and total protein content. Feces and pseudofeces were observed in multiple containers with oysters, indicating that the oysters were actively ventilating during the acute exposures, and their tissues were exposed to the treatment water.

To document water conditions experienced by the oysters during the experiment, temperature, salinity, pH, and dissolved oxygen were measured in each container prior to each water change using a YSI ProPlus. Water was also collected from 1 random container from each treatment per site for later analysis of carbonate chemistry (N = 12 per water change; see Section 2.2).

2.4. Physiological analyses

Whole animal tissue collected for glycogen content analysis was freeze-dried (Labconco Freezone 4.5 Plus), weighed, and then homogenized using a mortar and pestle. Dried, homogenized tissue per oyster was sub-sampled for glycogen content analysis (20–30 mg) with 3 technical replicates. Glycogen content analysis was performed following Guévélou & Allen (2016). Briefly, glycogen was extracted using 15% trichloroacetic acid and was precipitated using ethanol. Following resuspension in ultrapure water, glycogen was quantified at 450 nm using a plate reader (SpectraMax iD3, Molecular Devices) and a standard curve of serial dilutions of pure glycogen from an oyster (Sigma-Aldrich, Lot SLBV8773).

Total antioxidant potential was measured using the ferric reducing/antioxidant potential (FRAP) assay following Griffin & Bhagooli (2004). First, gill tissue samples were homogenized in Tris-HCl buffer using sonication. Protease inhibitor cocktail (Sigma P8340) was added to the homogenate. Total antioxidant potential was measured at 595 nm using a plate reader (SpectraMax iD3, Molecular Devices) and a standard curve of concentrations of $FeSO_4 \cdot 7H_2O$. Total antioxidant potential was then normalized to wet gill tissue weight.

Shells from the oysters that were analyzed for glycogen content were dried to a constant weight and weighed. Shell weight and total dry tissue weight (freeze-dried) from glycogen-sampled oysters was used to calculate condition index: Condition Index = Dry Tissue Weight (g) / [Dry Shell Weight (g) + Dry Tissue Weight (g)] × 100% (Rainer & Mann 1992).

To compare the relative influences of proximal and distal environmental history on oyster physiology and stress response, changes in glycogen content, condition index, and total antioxidant potential over the common garden period were calculated by comparing respective values at the beginning and end of deployment under common garden conditions.

2.5. Statistical analyses

Data analyses were done in R (R version 4.0.2; RStudio version 1.3.959). Levene's test and Q-Q plots were used to confirm that dependent variables met assumptions for normal distribution and homogeneity of variance, following log or reciprocal transformations, if needed.

For comparisons of water quality, dependent variables were temperature, salinity, dissolved oxygen, total alkalinity, pH, pCO_2, and $\Omega_{Calcite}$. To characterize differences in water quality comprising distal environmental history among sites, 1-way ANOVAs were used. To characterize differences between proximal (i.e. common garden conditions) and distal environmental history within each site, 1-way ANOVAs were used. Tukey's post hoc tests were performed when relevant. To characterize differences in water quality among treatments during the acute salinity exposure, 1-way ANOVAs followed by Tukey's post hoc tests were performed for variables that satisfied assumptions for parametric testing. For all other dependent variables, the Kruskal-Wallis test was performed, followed by Dunn's test for multiple comparisons (Quinn & Keough 2002).

To assess the relative importance of proximal vs. distal environmental history, physiology after the distal period and the change in oyster physiology (i.e. condition index, glycogen content, total antioxidant potential) over the common garden period were compared by distal site using 1-way ANOVAs, followed by Tukey's post hoc tests. Student's *t*-tests were used to assess if the changes in oyster stress response over the common garden period differed significantly from zero. To evaluate the relative importance of proximal and distal environmental history in shaping physiological stress responses, total antioxidant potential following the acute salinity exposure was compared using 2-way ANOVAs, with distal site and treatment as factors. When the interaction term in the full model was insignificant, model reduction was performed following Faraway (2009). Tukey's post hoc tests were performed when relevant.

To identify the component(s) of environmental history that were predictors of the observed changes in oyster physiology, multiple linear regression models were used. First, all possible pairs of water quality variables (temperature, salinity, dissolved oxygen, pH, total alkalinity, pCO_2, and $\Omega_{Calcite}$) were plotted to assess correlation. When 2 variables were highly correlated, 1 was removed from further analysis, and the analyses proceeded using the condensed variable. Accordingly, pCO_2 and $\Omega_{Calcite}$ were condensed with pH, total alkalinity with salinity, and dissolved oxygen with temperature. The condensed water quality parameters (pH, salinity, and temperature) were subsequently used in the multiple regression analyses. Full multiple linear regression models were fit using the 'lm' procedure in R for all physiological metrics and included the condensed water quality parameters (salinity, temperature, and pH). Acute salinity treatment was included in full models for analyses of total antioxidant potential. If a water quality parameter was not significant in the model, model reduction was performed following Faraway (2009). The selected model was then evaluated for normality through histograms of residuals and Q-Q plots. Physiological variables were log-transformed to achieve normality as needed.

3. RESULTS

3.1. Relative importance of proximal and distal environmental history

3.1.1. Comparison among distal environmental histories

Significant differences in water quality among distal sites were identified, illustrating that oysters from these sites had different distal environmental histories. To characterize differences in distal environmental histories, temperature, salinity, dissolved oxygen, pH, total alkalinity, pCO_2, and $\Omega_{Calcite}$ were compared by distal site (Table 1, Table S1). Salinity was significantly higher at VIMS Pier than at all other sites (1-way ANOVA, Tukey's HSD; Table 1, Table S1). Total alkalinity was significantly higher at

Table 1. Water quality conditions (mean ± SE) during oyster deployment. Data from Merroir (MR), Piankatank River (PR), Urbanna Creek (UC), and Virginia Institute of Marine Science Pier (VP) were collected fortnightly from July to November 2018. Data from common garden conditions at VP were collected weekly from November to December 2018. Within each water quality parameter, significantly different sites are marked with different superscript letters (Tukey's HSD, ANOVA, see Tables S1–S5 in the Supplement)

Site	Temperature (°C)	Salinity (psu)	pH_{Total}	Dissolved oxygen (mg ml^{-1})	Total alkalinity (µmol kg^{-1})	pCO_2 (µatm)	$\Omega_{Calcite}$
MR	24.98± 1.84^{a}	11.03 ± 0.51^{a}	7.73 ± 0.05^{a}	6.34 ± 0.81^{a}	1415 ± 25^{a,b,c}	823 ± 105^{a}	1.19 ± 0.16^{a,b}
PR	26.03 ± 1.82^{a}	8.63 ± 0.97^{a}	7.53 ± 0.09^{a}	6.67 ± 1.11^{a}	1348 ± 79^{a,c}	1381 ± 252^{a}	0.97 ± 0.30^{a}
UC	24.75 ± 1.69^{a}	9.07 ± 0.82^{a}	7.74 ± 0.06^{a,b}	6.50 ± 0.73^{a}	1289 ± 55^{a,c}	769 ± 121^{a,b}	1.04 ± 0.12^{a,b}
VP	24.81 ± 1.59^{a}	15.71 ± 0.39^{b}	7.78 ± 0.07^{a,b}	5.93 ± 1.00^{a}	1571 ± 15^{b}	739 ± 137^{a}	1.65 ± 0.26^{b}
Common garden	11.20 ± 1.35^{b}	13.33 ± 0.52^{c}	7.90 ± 0.03^{b}	11.78 ± 0.73^{b}	1402 ± 30^{c}	449 ± 28^{b}	1.00 ± 0.10^{a,b}

VIMS Pier than at Piankatank River and Urbanna Creek, and $\Omega_{Calcite}$ was significantly higher at VIMS Pier than that at Piankatank River (1-way ANOVA, Tukey's HSD; Table 1, Table S1). Temperature, dissolved oxygen, pH, and pCO_2 did not differ by site (1-way ANOVA; Table 1, Table S1).

3.1.2. Comparison between distal and proximal environmental histories

Common garden conditions differed from those at the oysters' previous sites, establishing distinct proximal and distal environmental histories. Common garden conditions at VIMS Pier were compared to the conditions at the 4 distal sites during the prior 4 mo deployment in order to evaluate whether water quality regimes comprising proximal and distal environmental histories differed (Table 1, Tables S2–S5). Temperature, salinity, and dissolved oxygen differed significantly in all pairwise site comparisons (1-way ANOVA; Table 1, Tables S2–S5). Temperature was lower and dissolved oxygen was higher during deployment under common garden (proximal history) than during deployment at the 4 field sites (distal history; Tukey's HSD; Table 1, Tables S2–S5). Salinity of common garden (proximal history) was higher than at Merroir, Piankatank River, and Urbanna Creek, but lower than at VIMS Pier (distal history; Tukey's HSD; Table 1, Tables S2–S5). pH of common garden (proximal history) was higher than pH at Merroir and Piankatank River (distal history), but pCO_2 of common garden was lower than pCO_2 at Merroir and Piankatank River (distal history; Tukey's HSD; Table 1, Tables S2 & S3). Additionally, the total alkalinity of common garden (proximal history) was lower than that at VIMS Pier (distal history; Tukey's HSD; Table 1, Table S5).

3.1.3. Influence of proximal and distal environmental history on physiology

Initial shell height of oysters deployed in July 2018 did not significantly differ among sites (Fig. S1, Table S13). In order to characterize the physiological state of the oysters collected at the end of their initial deployment, condition index and glycogen content were compared by distal site. Condition index differed significantly by site (1-way ANOVA; Fig. 3a, Table S8). Oysters from VIMS Pier had the lowest average condition index among sites, which was significantly different from the condition indices of oysters at all other sites (Tukey's HSD; Fig. 3a, Table S8). Oysters from Merroir, Piankatank River, and Urbanna Creek all had similar condition indices following the initial deployment (Tukey's HSD; Fig. 3a, Table S8). Glycogen content did not differ significantly among oysters by site (1-way ANOVA; Fig. 4a, Table S8).

To evaluate the relative effects of proximal and distal environmental history on oysters, changes in condition index and changes in glycogen content over the common garden period were compared by distal site. If proximal history had a relatively larger effect on oyster conditions, we expected changes over the common garden period to be similar among oysters from different sites. If distal history had a relatively larger effect on oyster conditions, we expected changes over the common garden period to differ among oysters from different sites. Change in condition index was significantly different between VIMS Pier oysters and oysters from all other distal sites (1-way ANOVA; Fig. 3b, Table S8). Oysters from VIMS Pier were the only group to show an increase in condition index over the common garden period; oysters from all other sites decreased in condition index (Tukey's HSD; Fig. 3b, Table S8). Piankatank River oysters had

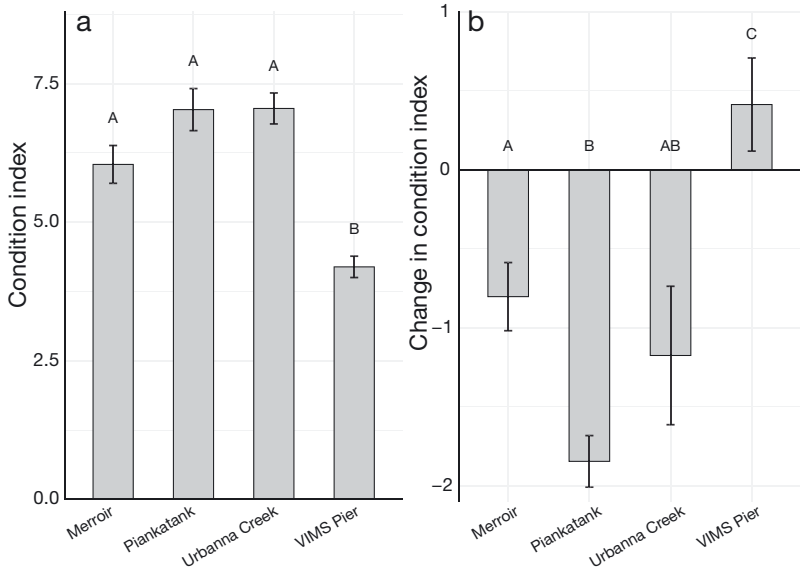

Fig. 3. Condition index and comparisons of condition index content by distal site (mean ± SE). (a) Condition index from oysters collected at the end of the period of distal environmental history. (b) Change in condition index during the common garden period. Changes were calculated by comparing respective values of condition index before and after exposure to common garden conditions. Different letters indicate significant differences among sites. These differences are expanded upon in Table S8

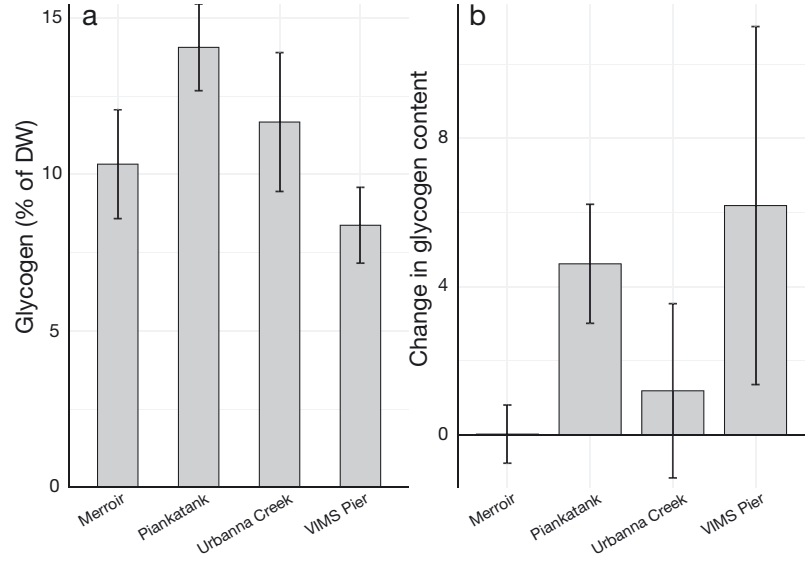

Fig. 4. Glycogen content of bulk dry tissue (% of dry weight, DW) and comparisons of glycogen content by distal site (mean ± SE). (a) Glycogen content from oysters collected at the end of the period of distal environmental history. (b) Change in glycogen content during the common garden period. Changes were calculated by comparing respective values of glycogen content before and after exposure to common garden conditions (i.e. proximal environmental history). No significant differences in glycogen content or change in glycogen content were detected. This information is expanded upon in Table S8

a significantly larger decrease in condition index than oysters from Merroir (Tukey's HSD; Fig. 3b, Table S8). There were no significant differences in the change in glycogen content by distal site (1-way ANOVA; Fig. 4b, Table S8). Student's *t*-tests were used to evaluate if there were significant differences between the mean change in physiological variables for each site and zero. Changes in condition index in response to common garden conditions were significantly different from zero in oysters from Merroir, Piankatank River, and Urbanna Creek, but not for oysters from VIMS Pier (Student's *t*-test; Table S9). Oysters from Piankatank River were the only group whose mean change in glycogen content was significantly different from zero during the common garden period (Student's *t*-test; Table S9).

3.2. Relative importance of proximal and distal environmental history in shaping stress response to salinity

3.2.1. Water conditions during acute salinity exposures

Although the goal of the acute stress exposures was to manipulate salinity alone, the experimental design and resources available did not allow for other water quality variables to be directly controlled. Nevertheless, we characterized differences in water quality variables among treatments, and the low salinity treatment in this study was still deemed the more 'stressful' treatment based on the relative values of all of the water quality variables (Tables 2 & 3). For the acute salinity exposure using oysters collected after the initial deployment, salinity, pH, total alkalinity, pCO_2, and $\Omega_{Calcite}$ differed among all treatments (1-way ANOVA, Kruskal-Wallis; Tukey's HSD, Dunn test; Table 2, Table S6). Temperature and dissolved oxygen did not significantly differ among treatment groups (one-way ANOVA, Kruskal-Wallis; Tukey's HSD, Dunn test; Table 2, Table S6). For the acute salinity exposure using oysters collected after the common garden, all water quality variables differed

Table 2. Treatment conditions (mean ± SE) during the acute (48 h) salinity exposure for oysters that were collected after the initial deployment. Water quality measurements were taken every 12 h during water changes for the experimental containers. PR and UC had the same average salinity *in situ* and therefore shared the same treatment conditions during the acute exposure. Within each water quality parameter, significantly different treatments are marked with different superscript letters (Tukey's HSD, ANOVA, Table S6)

Salinity treatment	Temperature (°C)	Salinity (psu)	pH_{Total}	Dissolved oxygen (mg ml^{-1})	Total alkalinity (µmol kg^{-1})	pCO_2 (µatm)	$\Omega_{Calcite}$
Low	17.24 ± 0.08^{a}	4.98 ± 0.03^{a}	7.49 + 0.00^{a}	9.22 ± 0.05^{a}	442 ± 4^{a}	479 ± 8^{a}	0.23 ± 0.02^{a}
MR	16.86 ± 0.13^{b}	10.97 ± 0.00^{b}	7.69 ± 0.01^{b}	9.15 ± 0.06^{b,c}	1039 ± 10^{b}	609 ± 24^{b}	0.56 ± 0.02^{b}
PR	16.86 ± 0.09^{b}	8.94 ± 0.02^{c}	7.60 ± 0.01^{c}	9.15 ± 0.04^{a}	842 ± 11^{c}	649 ± 21^{c}	0.39 ± 0.01^{c}
UC	16.86 ± 0.09^{b}	8.94 ± 0.02^{d}	7.60 ± 0.01^{d}	9.15 ± 0.04^{a}	842 ± 11^{d}	649 ± 21^{d}	0.39 ± 0.01^{d}
VP	16.83 ± 0.12^{b}	16.17 ± 0.02^{e}	7.79 ± 0.02^{e}	8.87 ± 0.06^{c}	1552± 2^{e}	680 ± 56^{e}	1.23 ± 0.04^{e}

Table 3. Water quality conditions (mean ± SE) during the acute (48 h) salinity exposure for the oysters that were collected from common garden conditions. The exposure consisted of 2 salinity treatments. Water quality was measured every 12 h during water changes in the experimental containers. Within each water quality parameter, significantly different treatments are marked with different superscript letters (Tukey's HSD, ANOVA, Table S7)

Salinity treatment	Temperature (°C)	Salinity (psu)	pH_{Total}	Dissolved oxygen (mg ml^{-1})	Total alkalinity (µmol kg^{-1})	pCO_2 (µatm)	$\Omega_{Calcite}$
Low	12.11 ± 0.24^{a}	4.91 ± 0.02^{a}	7.54 ± 0.00^{a}	11.60 ± 0.07^{a}	548 ± 2^{a}	484 ± 4^{a}	0.16 ± 0.00^{a}
Ambient	11.07 ± 0.17^{b}	11.92 ± 0.01^{b}	7.78 ± 0.00^{b}	10.89 ± 0.05^{b}	1247 ± 11^{b}	548 ± 7^{b}	0.70 ± 0.01^{b}

significantly between low and ambient salinity treatments, even though salinity was the only manipulated variable (one-way ANOVA, Kruskal-Wallis; Table 3, Table S7).

3.2.2. Influence of proximal and distal environmental history on stress response

Acute stress responses were compared among distal sites, for oysters collected at the end of their initial deployment. Total antioxidant potential in gill tissue did not differ significantly by site or treatment (2-way ANOVA; Fig. 5a, Table S10).

To evaluate the relative influence of proximal vs. distal history on stress response, the change in total antioxidant potential was compared among the groups of oysters with distinct distal histories. The change in total antioxidant potential differed significantly by distal site, but not by treatment (2-way ANOVA; Fig. 5b, Table S10). Total antioxidant potential in oysters from Piankatank River decreased during the common garden period, differing significantly from the changes in total antioxidant potential in oysters from Merroir and VIMS Pier (Tukey's HSD; Fig. 5b; Table S10). Oysters from Piankatank River were the only group whose mean change in total antioxidant potential was significantly different from zero during the common garden period (Student's *t*-test; Table S11).

3.3. Relative influences of the component(s) of environmental history on oyster physiology

The relative influence of water quality parameters (distal pH, distal salinity, distal temperature; acute salinity treatment included for total antioxidant potential) as predictors of changes in physiological variables over the common garden period was evaluated. Specifically, changes in condition index and changes in total antioxidant potential were considered, the 2 physiological variables that differed significantly by distal site. As described above, pCO_2 and $\Omega_{Calcite}$, total alkalinity, and dissolved oxygen were condensed into pH, salinity, and temperature, respectively. Distal salinity was the only significant predictor of change in condition index ($p < 0.001$, $R^2 = 0.530$; Table S12). No water quality variables were significant predictors for change in total antioxidant potential (Table S12).

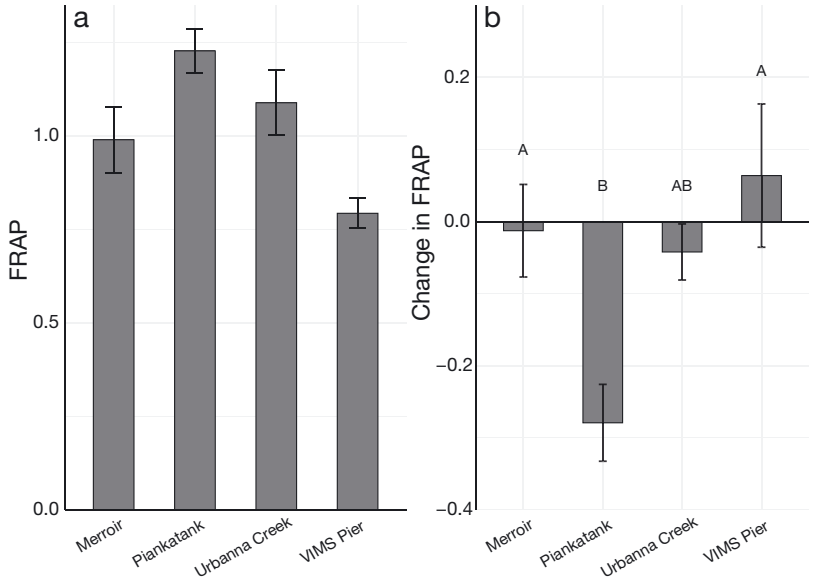

Fig. 5. Total antioxidant potential and comparisons of total antioxidant potential by distal site (mean ± SE). (a) Total antioxidant potential from oysters collected at the end of the period of distal environmental history following acute stress exposure. (b) Change in total antioxidant potential during the common garden period. Total antioxidant potential was measured using the ferric reducing/antioxidant potential (FRAP) assay following Griffin & Bhagooli (2004). Changes were calculated by comparing respective FRAP values before and after exposure to common garden conditions and acute salinity exposure. Different letters indicate significant differences among sites; total antioxidant potential did not differ significantly among distal sites. Since acute salinity exposure treatment did not have a significant effect on total antioxidant potential or the change in total antioxidant potential, values include measurements from oysters exposed to control and low acute salinity treatments for each site. These differences are expanded upon in Table S10

4. DISCUSSION

We investigated how proximal and distal environmental history affect physiology and stress response of juvenile eastern oysters. If proximal environmental history was more important in shaping oyster physiology and stress response, then we hypothesized that physiological parameters, including stress response, would change in similar ways under common garden conditions. However, if distal environmental history was more important, then physiological parameters, including stress response, would change in different ways under common garden conditions. We found that proximal and distal environmental history differentially affect physiology and stress response, with proximal history having the largest impact on glycogen content, and distal history having the largest impact on condition index and total antioxidant potential. These findings underscore the importance of understanding environmental history as a temporally dynamic element. Additionally, water quality parameters comprising both proximal and distal environmental history were evaluated to elucidate their relative impacts on physiology and stress response. We hypothesized that stress response, approximated using total antioxidant potential, and condition index, would be influenced primarily by pH, temperature, and salinity (Heilmayer et al. 2008, Dickinson et al. 2012, Matoo et al. 2013). Interestingly, we found that salinity was the only component of distal environmental history we examined that had a significant influence on condition index.

4.1. Relative importance of proximal and distal environmental history for oyster physiology

Distal environmental history had a greater impact on condition index than proximal history, based on the observation that condition indices of oysters from different distal sites changed in unique ways in response to common garden conditions. During the common garden period, condition indices in oysters from Merroir, Piankatank River, and Urbanna Creek all decreased, while condition indices in oyster originally from VIMS Pier did not change significantly. However, distal history did not explain changes in glycogen content. Under common garden conditions, glycogen content increased similarly in oysters from all sites, suggesting that proximal environmental history was more influential in shaping changes in glycogen content.

Condition index and glycogen content generally follow similar seasonal and reproductive patterns in adult eastern oysters. Both parameters increase throughout the spring and peak in summer prior to spawning as the adult oysters prepare for and undergo gametogenesis. After spawning, there is usually a substantial decrease in both metrics, as much of the energy stores and tissue mass are used to develop and release gametes. In the fall and winter, adult oysters begin to build up their mass and energy reserves in preparation for another reproductive cycle (Austin et al. 1993, Thompson et al. 1996).

Few studies have examined how condition index and glycogen content vary throughout the juvenile stage in eastern oysters, and it is unlikely that the juvenile oysters used in this study were following seasonal reproductive patterns in regards to condition index and glycogen content, as they had not yet reached sexual maturity (Allen & Downing 1986). Instead, we found that distal history explained patterns of condition index and proximal history of glycogen content, likely representative of different physiological processes in juvenile oysters.

The sensitivity of condition indices and glycogen content to different components of environmental history is likely dependent on the response times of their corresponding physiological pathways to novel environmental conditions. It may be easier to detect temporal changes in glycogen reserves, as this form of energy storage can be mobilized for immediate utilization and can be rapidly replaced when energy assimilation exceeds consumption (Lucas & Beninger 1985, Dickinson et al. 2012). In the present study, 1 mo under common garden conditions was enough time to generate detectable changes in glycogen content in oysters from all distal sites. It is important to note that changes in other biochemical substrates could have affected the calculated change in glycogen content as a percent of dry tissue weight. Condition index, a ratio of dry tissue weight to total dry weight, represents the sum of numerous tissue components, such as glycogen, proteins, enzymes, lipids, and carbohydrates, and also takes into account changes in shell weight. Given the variety of constituents and processes that contribute to the condition index of an individual, a significant change in condition index may take more time to emerge (Lucas & Beninger 1985) or otherwise follow different patterns than individual biochemical substrates (Fitzgerald et al. 2020).

Some components of condition index, such as glycogen and protein, may change at similar rates, but in different directions. For example, over the course of a 2 yr field deployment, protein content in Pacific oysters *Crassostrea gigas* increased during the spring and summer months and decreased during the fall and winter; however, glycogen content followed the opposite pattern, increasing during the winter months and decreasing during the summer months (Patrick et al. 2006). Another study found that oysters in a polluted, urban site had significantly lower condition indices than the control site, but protein concentrations were similar between sites (Fitzgerald et al. 2020). If representative of the many other physiological processes that occur across a range of time scales, the different patterns of glycogen content and condition index observed here highlight the opportunity for unique roles of proximal and distal environmental histories in oyster physiology. Prior research conducted in other contexts has identified similar changes in condition index and glycogen content. Both eastern and Pacific oyster adults exhibit similar seasonal patterns in condition index and glycogen, aligned with reproductive cycles (Encomio et al. 2005, Liu et al. 2020). Glycogen, a primary energy source for gametogenesis, changed more quickly than condition index between fall and spring months (Encomio et al. 2005, Liu et al. 2020). In Pacific oysters, glycogen content and condition index follow a similar temporal pattern in response to food deprivation and feeding (Li et al. 2009). The results of the present study suggest that changes in environmental conditions, independent of reproductive cycles and feeding status, can produce changes in condition index. Glycogen content, as a metric of oyster health, is more reflective of near-term environmental conditions. Particular parts of environmental history can have disproportionate effects on condition index and glycogen content of juvenile oysters during their first growing season, which may influence predictions about how these populations will respond to environmental change (Munroe et al. 2017).

It is unlikely that the changes in condition index and glycogen content observed were affected by the acute change in water conditions at the time of transition from distal to common garden sites (Giomi et al. 2016, Parker et al. 2017). Oysters from Merroir, Piankatank River, and Urbanna Creek all experienced an acute increase in salinity and pH when they entered the common garden (Table 1, Tables S2–S4). Based on historical data on eastern oysters and water quality conditions, the common garden conditions were likely more favorable than water quality conditions at their distal site (Wells 1961, Lowe et al. 2017). No studies have examined acclimatization to the less stressful, but novel environmental regime during common garden from prior stressful conditions. The majority of prior work investigates what happens when organisms transition to more stressful conditions (Butt et al. 2006, Rodolfo-Metalpa et al. 2011, Jarrold et al. 2019). However, transplant studies on eastern oysters demonstrate that oysters that have lived in low salinity conditions can be transferred abruptly to high salinity conditions with low rates of mortality (Audemard et al. 2011, Parveen et al. 2017). Therefore, the changes in oyster physiology during the common garden period were likely more representative of environment–organism interactions over

the duration of the entire common garden period rather than at the beginning of the common garden period.

Considering the observed changes in condition index and glycogen content, it can be inferred that different physiological attributes of an oyster can be influenced by different portions of its environmental history. Glycogen content was primarily shaped by proximal environmental history, and thus, glycogen content could be a good indicator to assess the physiological response of an oyster to recent (i.e. 1 mo) environmental change. However, it may be inappropriate to use condition index to measure direct effects of short-term environmental change, as the present study has illustrated that distal history significantly influenced the change of condition index. Future research efforts should explore whether distal environmental history could be used to predict how the condition indices of oyster populations in specific locations may respond to future environmental conditions, and therefore the vulnerability or resilience of those populations.

4.2. Relative importance of proximal and distal environmental history for total antioxidant potential, an indicator of stress response

The present study used total antioxidant potential as a proxy for an oyster's stress response. Antioxidants protect cells from damage associated with oxidative stress caused by reactive oxygen species (ROS), which can occur if an organism experiences an environmental stress. High levels of antioxidants may indicate that an oyster is experiencing oxidative stress from an environmental stressor and is upregulating antioxidant pathways in response (Lesser 2006, Whiteley & Mackenzie 2016). With respect to oysters evaluated before and after the common garden period in this study, antioxidant defense and inferred oxidative stress were not significantly affected by acute changes in water quality conditions during laboratory exposures, at least not by the treatment conditions we imposed. The levels of temperature, salinity, pH, and pCO_2 present in the acute stress treatment were previously shown to negatively impact oyster physiology (Heilmayer et al. 2008, Dickinson et al. 2012), and in bivalves, the production of particular antioxidant enzymes can be induced in response to acute stress treatment (Tomanek et al. 2011, Boudjema et al. 2014). It is possible that the acute stressor levels used in the present study did not generate enough ROS to induce antioxidant production or that a longer exposure duration was required to detect a response (Heilmayer et al. 2008). Potential explanations for the lack of induced oxidative defense include a sufficient abundance of constitutively produced antioxidants (i.e. antioxidants that are always being produced at a basal level) (e.g. Barshis et al. 2013, Zhang et al. 2016) and altered induction thresholds of stress response due to prior acclimatization (e.g. Giomi et al. 2016).

When considering the role of environmental history, constitutive expression of antioxidants, rather than inducible defense (i.e. antioxidants produced in response to a stressor), may play a disproportionate role in the oxidative defense strategy of eastern oysters. We observed changes over longer time periods but not during acute exposures (i.e. 1 mo in common garden conditions rather than 48 h of acute exposure). In temporally variable environments like the Chesapeake Bay estuary, constitutive expression of antioxidants may be more energetically efficient for acclimatization. Longer-term changes in antioxidant levels have also been reported by several other studies. For example, the antioxidant catalase increased activity in response to elevated temperatures and specific copper ion concentrations over a 30 d exposure (Wang et al. 2016). Eastern oysters exposed to elevated pCO_2 and temperature for 8 wk had elevated total antioxidant capacity, but after 15 wk at treatment conditions, total antioxidant capacity had stabilized, indicating acclimation to the new environment over time (Matoo et al. 2013). In the Hooghly estuary in India, antioxidants in the rock oyster *Saccostrea cucullata* fluctuated throughout the year, with maximum levels occurring in March to July and minimum levels occurring in November to February (Niyogi et al. 2001), correlating with seasonal environmental stress levels. Changes in the potential constitutive production of antioxidants in eastern oysters represents a mechanism of resilience by which oysters might acclimatize to future changes in environmental conditions, such as climate change.

4.3. Relative influence of water quality parameters on oyster physiology

Salinity at the distal sites was a predictor of changes in condition index during common garden conditions. Ideal habitat salinity for eastern oysters is 15–25 psu; in this range, populations are dense, and reproductive output and growth rates are high (Bataller et al. 1999, Linhoss et al. 2016). Oysters from Merroir, Piankatank River, and Urbanna Creek all

experienced distal salinities that fell below the ideal salinity range. In common garden conditions, oysters from these sites experienced a significantly higher salinity, though still below the ideal range (Table 1, Tables S2–S4), and their condition indices all significantly decreased. Decreasing condition indices may suggest that there is an energetic cost to acclimatization to a higher salinity environment (Andrews et al. 1959, Méthé et al. 2015, Southworth et al. 2017). Although local adaptation is a known potential mechanism for lower salinity tolerance in oyster populations in southern Chesapeake Bay (Southworth et al. 2017), the oysters used in the present study were all from the same hatchery-based spawn, so salinity tolerance associated with distal history is likely due to acclimatization. Our results build on previous findings that, although other water quality factors such as temperature and pollution can have significant effects (Shpigel et al. 1992, Heilmayer et al. 2008), salinity is a primary driver of condition index in juvenile oysters (Encomio et al. 2005, Gullian & Aguirre-Macedo 2009, La Peyre et al. 2013). Moreover, the present study compares specific temporal portions of an oyster's environmental history that may contribute to its acclimatization to novel environmental conditions. We must note that the year in which this study was conducted (2018) was atypical in its high precipitation and low salinity levels, so future work should examine whether salinity remains the primary driver under other baseline regimes of water quality. With precipitation throughout Chesapeake Bay projected to become more variable over the next century, which will lead to larger salinity fluctuations (Muhling et al. 2018), the physiological responses of oysters to this increased salinity variability may largely depend on environmental history.

Oxidative stress response of oysters during common garden conditions was likely shaped by abiotic or biotic components of distal history beyond salinity, temperature, and carbonate chemistry. Although other work has shown that pCO_2, salinity, and temperature, all of which were measured in the present study, can affect antioxidants in eastern oysters (Tomanek et al. 2011, Dickinson et al. 2012, Matoo et al. 2013), these environmental parameters were not significant predictors of change in total antioxidant potential in this study. It is possible that an environmental factor we did not measure shaped changes in total antioxidant potential (e.g. heavy metals, pollution, and harmful algal blooms (Sanni et al. 2008, Prego-Faraldo et al. 2017). Additionally, the distal water quality components may not have reached or remained at the extreme values necessary to induce oxidative stress and the subsequent production of antioxidants. Furthermore, the oysters initially deployed in July may have had an adequate number of antioxidants present to combat the stresses they may have experienced in their distal environmental histories. Future research should explore other relevant components of the environmental history of an organism, and also the environmental variability or stress they experienced, their physiological state at different time points, and the amount of time it might take for an organism to physiologically respond to environmental changes.

5. CONCLUSIONS

Distal and proximal histories of eastern oysters influence physiological processes in different ways. Multiple parts of environmental history should be considered to accurately predict the vulnerability of oyster populations to climate change. Additionally, we identified salinity as an important driver of physiological responses to changes in environmental conditions over the temporal and spatial scales used in this study. Specifically, acclimatization to distal salinity regimes over 4 mo influenced how oysters performed under common garden conditions. Other parameters of environmental history, like temperature and pH, were not found to be important in shaping physiological responses over the time period examined. Future work should evaluate whether temperature and carbonate chemistry may be significant in shaping other physiological components not measured in this study, such as metabolism and immune response (Perez & Fontanetti 2011, Ivanina et al. 2013). Identifying the consequences of acclimatization to specific components of environmental history, such as salinity or dissolved oxygen, can aid in interpreting physiological responses to variable environmental conditions and informing place-based predictions of physiological outcomes of future environmental change.

The results of this study can be used as building blocks for future research on proximal and distal environmental history. Resolving other relevant distal and proximal histories for oysters, within the context of their life history, will be an important next step. Other abiotic and biotic factors that could contribute to proximal and distal environmental history, such as pollutants and food availability, should be thoroughly examined. Future research should evaluate stress response capabilities under a suite of multistressor scenarios in the context of proximal and dis-

tal environmental history. Knowledge about how environmental history shapes oyster resilience can support smarter management, restoration, and aquaculture practices throughout Chesapeake Bay.

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