Ecological Applications, 31(4), 2021, e02315

Context-dependent carryover effects of hypoxia and warming in a coastal ecosystem engineer

Sarah C. Donelan ,[1] Denise Breitburg, and Matthew B. Ogburn

Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, Maryland 21037 USA

Citation: Donelan, S. C., D. Breitburg, and M. B. Ogburn. 2021. Context-dependent carryover effects of hypoxia and warming in a coastal ecosystem engineer. Ecological Applications 31(4):e02315. 10.1002/eap.2315

Abstract. Organisms are increasingly likely to be exposed to multiple stressors repeatedly across ontogeny as climate change and other anthropogenic stressors intensify. Early life stages can be particularly sensitive to environmental stress, such that experiences early in life can "carry over" to have long-term effects on organism fitness. Despite the potential importance of these within-generation carryover effects, we have little understanding of how they vary across ecological contexts, particularly when organisms are re-exposed to the same stressors later in life. In coastal marine systems, anthropogenic nutrients and warming water temperatures are reducing average dissolved oxygen (DO) concentrations while also increasing the severity of naturally occurring daily fluctuations in DO. Combined effects of warming and diel-cycling DO can strongly affect the fitness and survival of coastal organisms, including the eastern oyster (*Crassostrea virginica*), a critical ecosystem engineer and fishery species. However, whether early life exposure to hypoxia and warming affects oysters' subsequent response to these stressors is unknown. Using a multiphase laboratory experiment, we explored how early life exposure to diel-cycling hypoxia and warming affected oyster growth when oysters were exposed to these same stressors 8 weeks later. We found strong, interactive effects of early life exposure to diel-cycling hypoxia and warming on oyster tissue : shell growth, and these effects were context-dependent, only manifesting when oysters were exposed to these stressors again two months later. This change in energy allocation based on early life stress exposure may have important impacts on oyster fitness. Exposure to hypoxia and warming also influenced oyster tissue and shell growth, but only later in life. Our results show that organisms' responses to current stress can be strongly shaped by their previous stress exposure, and that context-dependent carryover effects may influence the fitness, production, and restoration of species of management concern, particularly for sessile species such as oysters.

Key words: aquaculture; Chesapeake Bay; climate change; conservation; Crassostrea virginica*; diel-cycling hypoxia; early life; latent effects; multiple stressors; oyster; restoration.*

Introduction

Efforts to predict the responses of organisms to environmental stress such as climate change often focus on the impact of a single stressful event and extrapolate potential effects on populations and communities (e.g., Kroeker et al. 2010). A growing body of work, however, suggests that environmental stress experienced during one stage of life can "carry over" to affect the fitness and performance of organisms later in life (Crean et al. 2011, O'Connor et al. 2014). Within-generation carryover effects have been shown in a diverse array of organisms and in response to a variety of environmental conditions (Harrison et al. 2011). For example, sea urchins that are exposed to low pH during larval development produce fewer spines post-settlement than urchins in control conditions (Byrne et al. 2011), and songbirds with less developed wings prior to fledging have higher mortality after fledging (Jones and Ward 2020). Carryover effects of early life (e.g., embryonic, larval, or juvenile) experiences are especially common (Kasumovic 2013), likely because early life experiences can constrain phenotypic plasticity later in life (i.e., the epiphenotype problem; DeWitt et al. 1998) or affect irreversible early life history decisions (e.g., dispersal; Stamps et al. 2009). Much of the work on carryover effects has focused on how poor environmental conditions early in life have lifelong, negative effects on organism performance (Pechenik 2006) or vice versa (e.g., the silver spoon effect; Madsen and Shine 2000) regardless of an organism's future environment. However, the impact of early life experiences may depend on an individual's future environmental context; specifically, how organisms perform when they re-encounter the same stressors later in life. For example, in *Tribolium* beetles, low larval habitat quality significantly reduced adult survival, but only when adults dispersed

Manuscript received 11 May 2020; revised 6 November 2020; accepted 6 December 2020. Corresponding Editor: Brice X. Semmens.
[1]E-mail: donelans@si.edu

to low, and not high, quality habitats (Van Allen and Rudolf 2013). The importance of such environmental matching has been well-documented in other latent effects (e.g., parental effects; Sheriff and Love 2013), but is rarely tested for within-generation carryover effects (but see Hettinger et al. 2012, Donelan and Trussell 2019). Indeed, we know surprisingly little about the potential for early life stressors to modify the response of organisms to those same stressors later in life, particularly in species of management concern.

Ocean deoxygenation is one of the primary consequences of climate change in marine systems, but efforts to study its effects on marine organisms have lagged behind those for other climate change stressors such as ocean acidification (Levin and Breitburg 2015). Dissolved oxygen (DO) concentrations have declined sharply in recent decades in both coastal and open seas (Diaz and Rosenberg 2008, Breitburg et al. 2018), spurred by local processes such as coastal eutrophication (Rabalais et al. 2010) and global processes including increased atmospheric CO_2 (Bopp et al. 2013). In addition to long-term declines in coastal oxygen (Diaz and Rosenberg 2008), coastal DO commonly fluctuates over shorter (e.g., daily) timescales (Baumann et al. 2015). Diel-cycling hypoxia (typically defined as DO < 2 mg/L, Diaz and Rosenberg 2008) frequently occurs during the summer in poorly mixed, shallow water because of variation in photosynthetic and respiration rates of phytoplankton and algae that corresponds with the day/night cycle (Tyler et al. 2009, Baumann et al. 2015). For example, in shallow water tributaries of the Chesapeake Bay, USA, DO can range from 0 mg/L (anoxic) to over 9 mg/L (supersaturated) over a single day, and these daily cycles can last for weeks during the summer months (Breitburg et al. 2015). Even this relatively brief, episodic exposure to hypoxia can affect organisms' survival (Davidson et al. 2016), while also modifying behavior and growth (Tyler et al. 2009), reproduction (Flint et al. 2018), and pathogen loads (Keppel et al. 2015).

The negative effects of diel-cycling hypoxia may be further exacerbated by warming water temperatures associated with climate change (Altieri and Gedan 2015). Shallow, coastal waters are likely to warm faster than well-mixed, deeper water bodies, especially in relatively enclosed estuaries (Rabalais et al. 2010, Altieri and Gedan 2015), reducing overall oxygen solubility and enhancing water column stratification, which limits oxygen supply at depth (Rabalais et al. 2010). Warm temperatures can also increase metabolic rates of organisms, especially of ectotherms (Deutsch et al. 2008), such that many coastal organisms require more oxygen for basal metabolic processes at the same time as less oxygen is available. The negative effects of hypoxia on survival can be intensified at warm temperatures: marine benthic organisms exposed simultanoeusly to hypoxia and warming had 35% lower survival compared to those exposed to hypoxia alone (Vaquer-Sunyer and Duarte 2011). Because warming and deoxygenation are two key climate change stressors in marine environments that are likely to have interactive effects (Doney et al. 2011), it is critical to explore their combined effects simultaneously. The ability of organisms to acclimate and adapt to these combined stressors, and their potential to carry over across life stages, may substantially affect the structure and function of coastal communities and ecosystems as climate change intensifies.

Climate change and local anthropogenic activities are simultaneously impacting coastal ecosystems, with dramatic effects on critical estuarine species such as the eastern oyster (*Crassostrea virginica*, hereafter "oysters"). Oysters are an important fishery species and key ecosystem engineer throughout their range, and provide a multitude of ecosystem services including shoreline stabilization, water filtration, and habitat for other commercially harvested species (Coen et al. 2007). Oysters are sessile except for a two-week planktonic life stage, so they have limited capacity to behaviorally avoid stressful conditions. Summertime hypoxic events (~5 d at <2 mg/L) can cause mass mortality of oysters on natural reefs (Lenihan and Peterson 1998), and diel-cycling hypoxia may also be a strong selective force for oysters. Diel-cycling hypoxia can reduce oyster valve-gaping behavior (Porter and Breitburg 2016), in turn limiting foraging rates and reducing shell growth (Keppel et al. 2016), but its effect on oyster tissue growth, a strong proxy for oyster fitness (Thompson et al. 1996) and critical trait in aquaculture, is unknown. In contrast, warm water temperatures can increase oyster filtration rates (Loosanoff 1958) and are associated with higher growth rates in the field (Lowe et al. 2017). In some cases, however, warming has little effect on juvenile oyster growth or mortality (Talmage and Gobler 2011), though it can reduce oysters' tolerance of other stressors such as low salinity (Rybovich et al. 2016). Oysters have a wide temperature tolerance (Shumway 1996), so warming temperatures associated with climate change may only prove stressful if temperatures exceed oysters' tolerance or if oysters are simultaneously exposed to another stressor (e.g., hypoxia). While carryover effects have been shown to operate in other species of oyster in response to single climate changes stressors (e.g., pH; Hettinger et al. 2012), the potential for multiple climate change stressors to carryover to affect the response of eastern oysters to these same stressors later in life is unexplored. If operating, multi-stressor carryover effects have the potential to affect the growth of oysters in aquaculture or on reefs restored by coastal managers, thereby affecting oyster production and conservation outcomes.

We conducted a multiphase laboratory experiment to quantify how early life exposure to sublethal diel-cycling hypoxia and warming affected the tissue and shell growth of juvenile oysters when oysters were exposed to these same conditions ~8 weeks later. Our results revealed within-generation carryover effects of diel-cycling hypoxia and warming on oyster tissue : shell growth. Early life exposure to diel-cycling hypoxia and

warming interactively influenced oyster growth, but these effects were context-dependent. Specifically, oysters grew less tissue relative to shell if they were exposed to both hypoxia and warming both early and later in life, but these effects only manifested in the presence of multiple stressors. Moreover, diel-cycling hypoxia and warming did not affect oyster tissue or shell size early in life, but did affect oyster tissue and shell growth later in life. These results suggest that early life exposure to climate change stressors can have context-dependent effects on organisms later in life. Because oyster aquaculture and restoration often occur in shallow-water estuaries where diel-cycling hypoxia is increasingly common, carryover effects may further reduce oyster fitness to the detriment of these important ecological and economic activities.

Materials and Methods

Study system

Three- to four-month-old eastern oysters (*C. virginica*, 3–5 mm shell height) were purchased from Horn Point Oyster Hatchery in Cambridge, Maryland, USA. Oysters were spawned from adult (>7.6 cm shell height, $n_{female} = 39$, $n_{male} = 16$) broodstock collected from two sites (Sandy Hill and Chlora Point) in the Choptank River, a tributary of the Chesapeake Bay. Broodstock were maintained in a single tank on unfiltered, flowing seawater at 20°C for an average of 67 d (range: 61–78 d) prior to spawning. Spawning was induced in a separate, static tank by quickly (1–2 h) raising the water temperature to 30°C. The resulting larvae were held in 32,000-L tanks of filtered, aerated water that was changed every 2–3 d and fed a mixture of algae (*Isochyrsis* sp., *Chaetoceros calcitrans*, *Chaetoceros muelleri*, *Tetraselmis chui*). Once competent (15–18 d post-spawn), larvae were set on microcultch (300-μm ground oyster shell; Baywatch Oyster Seed, Reedville, Virginia, USA) and placed in a downweller system for two weeks with ad libitum food (same algal species as above). They were then transitioned to ambient flowing water for ~3 months until they were purchased and moved to the flow-through facilities at the Smithsonian Environmental Research Center (SERC) on the Rhode River, another tributary of the Chesapeake Bay, in Edgewater, Maryland.

At SERC, oysters were acclimated for 6 d in ambient, flowing water from the Rhode River (salinity 4.5 ppt, temperature 28.5°C) before being placed into Phase 1 of our experiment, described below. During acclimation, salinity and temperature were within 2 ppt and 1.5°C, respectively, of conditions at Horn Point at the time of purchase; this variability should not elicit changes in oyster physiology (Matoo et al. 2013, McFarland et al. 2013). Salinity was unusually low throughout the Chesapeake Bay during our experiment due to high spring rainfall, and while low salinity can affect oyster growth (Shumway 1996; and see *Discussion*), salinity was similarly low across both phases of our experiment.

Experimental design and manipulation of temperature and diel-cycling dissolved oxygen

We conducted a multiphase experiment (Fig. 1) to explore how early life exposure (Phase 1) to diel-cycling dissolved oxygen (DO, normoxic/hypoxic) and temperature (ambient/warm) affected the tissue, shell, and relative tissue : shell growth of juvenile eastern oysters exposed to diel-cycling DO (normoxic/hypoxic) and temperature (ambient/warm) later in life (Phase 2). Both Phase 1 and Phase 2 had four treatment combinations (normoxic/ambient, normoxic/warm, hypoxic/ambient, hypoxic/warm) for a total of 16 treatment combinations and an orthogonal design. Target DO concentrations in the diel-cycling hypoxia treatment level ranged from near 100% saturation (Table 1) to hypoxic (0.5 mg/L, 7–8% saturation) over the course of a day; this low DO concentration was chosen because it affects oyster growth without affecting survival (Keppel et al. 2016) and is similar to the diel-cycling conditions in some shallow water tributaries in the Chesapeake Bay (Breitburg et al. 2015). Target water temperatures in the warm treatment level were 2.5°C above ambient to match the predictions of the 2014 IPCC RCP6.0 scenario (Pachauri et al. 2014). Oysters in the Chesapeake Bay routinely experience temperatures within this range (Table 1; Southworth et al. 2017), and the temperatures in the warm treatment are well within oysters' thermal tolerance (Shumway 1996).

We manipulated temperature and diel-cycling DO in an indoor aquarium facility that received unfiltered, ambient water from the Rhode River. Water flowed into a series of five 568 L fiberglass holding tanks; tanks 1–3 allowed sediment from the unfiltered water to settle before being dispensed into the experimental aquaria. In one of the two remaining holding tanks, we increased the target water temperature to 2.5°C above ambient. Warm water temperature was dynamically maintained through a custom integrated microprocessor feedback control (Rich et al. 2015) that manipulated power to 3,000 watts of aquarium heaters (500 W heaters; Aquatop, Brea, California, USA). Water was pumped from the warm holding tank and the remaining ambient holding tank to 24 experimental aquarium tanks (75 L, $n = 6$ per treatment combination) through vinyl aquarium tubing at a rate of 300 mL/minute. Each aquarium tank received two vinyl tubes to distribute flowing water and gas and was covered with a tight-fitting Plexiglas lid to minimize ambient gas exchange.

DO concentrations were manipulated in the diel-cycling hypoxia treatment level over a 24-h cycle. Each cycle consisted of a 3-h draw down of DO from normoxia (~100% saturation) to hypoxia (0.5 mg/L O_2, 7–8% saturation), a 4-h "low plateau" period when hypoxia

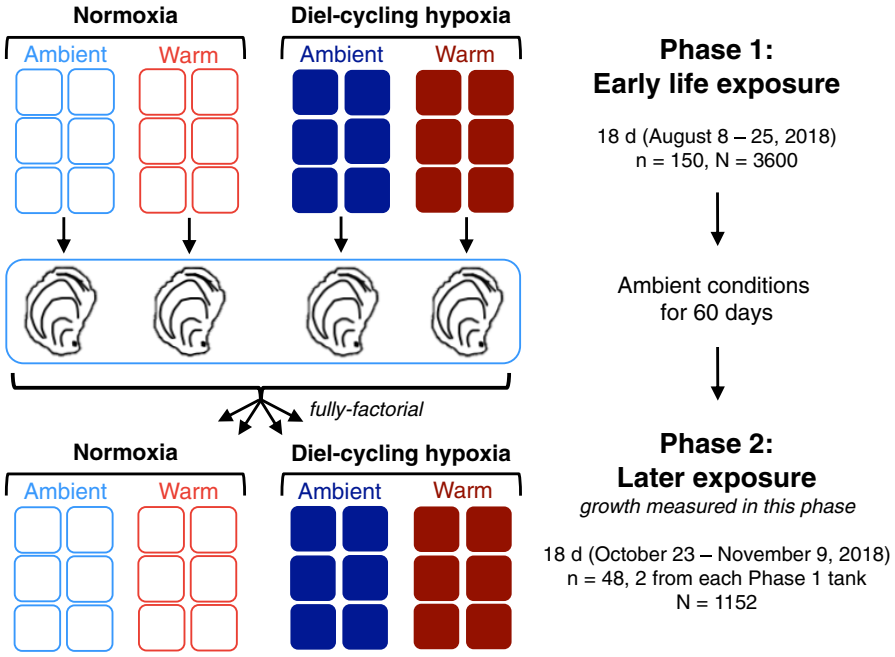

FIG. 1. Schematic of experimental design. Juvenile *Crassostrea virginica* oysters were exposed to diel-cycling dissolved oxygen (DO; normoxic/hypoxic) and temperature (ambient/warm) treatments in a fully factorial design (Phase 1, Early life exposure, August 2018). Oysters were then held in ambient conditions before being placed into the same treatments 60 d later (Phase 2, later exposure, October–November 2018) in a fully factorial design (16 treatment combinations). Two oysters from each Phase 1 aquarium tank replicate ($N = 24$) were placed into each Phase 2 aquarium tank replicate ($N = 24$) such that there were 48 oysters in each Phase 2 replicate ($N = 1152$). See Table 1 for average treatment conditions. Colors match those used in Figs. 2–4.

was maintained, a 3-h ramp up from hypoxia to normoxia, and a 14-h period at normoxia (Fig. 2a,b). DO concentrations in the normoxic treatment levels were maintained at normoxia throughout the day. We manipulated diel-cycling DO using custom designed LabView software (detailed in Burrell et al. 2016) that uses input from dissolved oxygen (Oxyguard, Birkeroed, Denmark) and pH sensors (Durafet III, Honeywell, Fort Washington, Pennsylvania, USA) to mix a combination of four gases (nitrogen, carbon dioxide, air, and CO_2-stripped air) to achieve target DO concentrations. pH was maintained at constant levels in the diel-cycling DO tanks by adding CO_2 gas as needed, and was similar to ambient conditions in the Rhode River during each phase of the experiment (target pH: Phase 1 8.10, Phase 2 8.14). DO and pH levels were constantly monitored through four DO and four pH sensors, one for each treatment combination in both Phase 1 and Phase 2. Based on the readings from these sensors, gases were mixed as necessary to create one gas mixture per treatment combination. Gases were dispensed to each replicate aquarium at a rate of 25 L/minute through mass flow controllers (Dakota Instruments, Orangeburg, New York, USA), gas manifolds, vinyl tubing, and silica air stones.

We checked and calibrated the continuously monitoring DO and pH sensors each morning using an external probe (Orion Star A326, Thermo Scientific, Waltham, Massachusetts, USA). This morning external probe reading was used in our water quality analysis as the daily normoxic reading ($n_{\text{Phase1}} = 13$, $n_{\text{Phase2}} = 14$). We used this same external probe to measure DO concentration, temperature, and pH each day in each tank at the start and end of a hypoxic plateau period (i.e., twice each day, $n_{\text{Phase1}} = 26$, $n_{\text{Phase2}} = 28$) to verify that the target DO concentrations (4 h at 0.5 mg/L O_2, 7–8% saturation) and temperatures (warming, +2.5°C above ambient) were achieved. These readings at the start and end of a hypoxic plateau were used in our water quality analysis as the hypoxic DO and temperature readings. We used pH readings from both the normoxic and hypoxic tank readings (i.e., three times each day, $n_{\text{Phase1}} = 39$, $n_{\text{Phase2}} = 42$) in our water-quality analysis.

For both Phase 1 and 2, DO was cycled 5 d per week in the diel-cycling treatment and kept at normoxia on the remaining 2 d. This cycling pattern was primarily done for logistical reasons, but also mimics natural variability in DO conditions in Chesapeake Bay (Breitburg et al. 2015). Temperature treatments were applied 7 d per week, again mimicking natural patterns. Photoperiod was manipulated on a 14 h light : 10 h dark cycle using incandescent 5 V rope lighting to simulate conditions at ~2 m depth (approximate depth of oyster reefs) in the Rhode River (Keppel et al. 2015). We also measured alkalinity once per week ($n_{\text{Phase1}} = 3$, $n_{\text{Phase 2}} = 2$) in one replicate of the normoxic/ambient and normoxic/warm treatment combinations, and used this to calculate

TABLE 1. Summary of water quality measurements in Phase 1 (early life exposure) and Phase 2 (later exposure) treatment combinations.

	Treatments			
	Normoxia		Diel-cycling hypoxia	
Measurement	Ambient	Warm	Ambient	Warm
Phase 1: Early life exposure				
Dissolved oxygen (mg/L)				
Normoxia	$6.77^{A} \pm 0.16$	$6.51^{B} \pm 0.14$	$6.75^{A} \pm 0.19$	$6.56^{B} \pm 0.10$
Hypoxic plateau	$6.92^{A} \pm 0.21$	$6.52^{B} \pm 0.16$	$0.56^{C} \pm 0.19$	$0.54^{C} \pm 0.27$
Dissolved oxygen (percent saturation)				
Normoxia	$88.9^{A} \pm 2.1$	$89.1^{B} \pm 1.9$	$88.6^{A} \pm 2.5$	$89.5^{B} \pm 1.4$
Hypoxic plateau	$91.1^{A} \pm 2.0$	$89.3^{B} \pm 1.5$	$7.15^{C} \pm 0.07$	$7.12^{C} \pm 0.06$
Temperature (°C)	$28.30^{A} \pm 0.81$	$30.67^{B} \pm 0.79$	$28.27^{A} \pm 0.86$	$30.51^{B} \pm 0.84$
pH	$8.13^{A} \pm 0.03$	$8.11^{B} \pm 0.03$	$8.14^{A} \pm 0.02$	$8.11^{B} \pm 0.05$
Salinity (ppt)	4.00 ± 0.13	4.00 ± 0.13	4.00 ± 0.13	4.00 ± 0.13
Alkalinity (μmol/kg seawater)	$1315.3^{A} \pm 3.1$	$1305.3^{A} \pm 5.6$		
pCO_2 (μatm)	$360.7^{A} \pm 6.4$	$376.8^{A} \pm 20.5$		
Phase 2: Later exposure				
Dissolved oxygen (mg/L)				
Normoxia	$9.10^{A} \pm 0.18$	$8.55^{B} \pm 0.19$	$9.18^{A} \pm 0.19$	$8.52^{B} \pm 0.25$
Hypoxic plateau	$9.22^{A} \pm 0.23$	$8.66^{B} \pm 0.23$	$0.77^{C} \pm 0.15$	$0.71^{C} \pm 0.14$
Dissolved oxygen (percent saturation)				
Normoxia	$94.3^{A} \pm 1.9$	$93.1^{B} \pm 2.1$	$95.0^{A} \pm 2.0$	$92.7^{B} \pm 2.7$
Hypoxic plateau	$97.3^{A} \pm 1.5$	$96.0^{B} \pm 1.7$	$7.80^{C} \pm 0.06$	$7.61^{C} \pm 0.07$
Temperature (°C)	$15.90^{A} \pm 0.70$	$18.31^{B} \pm 0.68$	$15.88^{A} \pm 0.70$	$18.28^{B} \pm 0.69$
pH	$8.15^{A,C} \pm 0.04$	$8.11^{A,D} \pm 0.04$	$8.17^{B,C} \pm 0.05$	$8.16^{B,D} \pm 0.04$
Salinity (ppt)	3.70 ± 0.20	3.70 ± 0.20	3.70 ± 0.20	3.70 ± 0.20
Alkalinity (μmol/kg seawater)	$1144.7^{A} \pm 95.6$	$1133.2^{A} \pm 98.8$		
pCO_2 (μatm)	$291.9^{A} \pm 28.4$	$296.4^{A} \pm 24.2$		

Notes: Values are mean ± SE. Dissolved oxygen (DO) was measured by one method during the normoxic phase and another method during the hypoxic phase of a diel cycle: at normoxia, data were taken from one replicate of each treatment combination once daily using an external probe (Thermo Scientific Orion Star A326, $n_{Phase1} = 13$, $n_{Phase2} = 14$); at hypoxia, data were taken at the beginning and end of a hypoxic plateau in each replicate aquarium tank (both those that cycled to hypoxia and those that did not) with the same external probe ($n_{Phase1} = 26$, $n_{Phase2} = 28$). Dissolved oxygen was measured in mg/L, but we also report average percent saturation. Temperature (°C) was measured twice daily in each tank with the same external probe at the same time as the hypoxic DO readings ($n_{Phase1} = 26$, $n_{Phase2} = 28$), and pH was measured three times daily with the same external probe at the same time as the normoxic and hypoxic DO readings. Salinity (parts per thousand, ppt) data were taken from an external probe (EXO2 sonde, YSI, Yellow Springs, Ohio, USA) mounted in the Rhode River at the same depth (~2 m) as the intake pipe for the flowing water system that recorded conditions every 6 minutes ($n_{Phase1} = 4079$, $n_{Phase2} = 4355$). Finally, water samples were taken for alkalinity (μmol/kg seawater) three times in Phase 1 and two times in Phase 2 and processed according to the methods described in APHA (1992). Different letters indicate significant differences ($P < 0.05$) based on ANOVAs (see Results).

pCO_2 (μatm) using the seacarb package in R (Gattuso et al. 2020).

Oyster tissue and shell growth

In Phase 1 (August 2018), 150 experimental oysters (3–5 mm shell height) were randomly placed together in a perforated plastic container (16 × 16 × 9.5 cm, length × width × height) that was placed individually in an aquarium tank. Plastic containers were held off the bottom of tanks by baskets to minimize sedimentation effects. There were 24 total tanks and each treatment combination was replicated six times ($N = 3{,}600$ oysters, Fig. 1). Temperature and diel-cycling DO were manipulated as previously described. Oysters remained in Phase 1 for 18 d (13 d of diel-cycling DO, 18 d of temperature treatment) after which time they were removed from the tanks and held in ambient, aerated, running water until the start of Phase 2.

Sixty days after the end of Phase 1, half of the oysters from Phase 1 were placed back into the experimental array for a secondary exposure (Phase 2, October–November 2018, Fig. 1). Two oysters from each of the six Phase 1 replicate tanks were placed into each Phase 2 replicate tank to create a fully factorial, nested design with 16 treatment combinations. There were 24 Phase 2 tanks with 12 oysters from each Phase 1 treatment combination in each tank ($n = 48$ oysters per tank, $N = 1{,}152$). Oysters began Phase 2 at the same size regardless of Phase 1 treatment (for measurement methods; initial tissue mass 76.0 ± 1.3 mg [mean ± SE], $P = 0.3$; initial shell mass 200.7 ± 2.5 mg, $P = 0.08$).

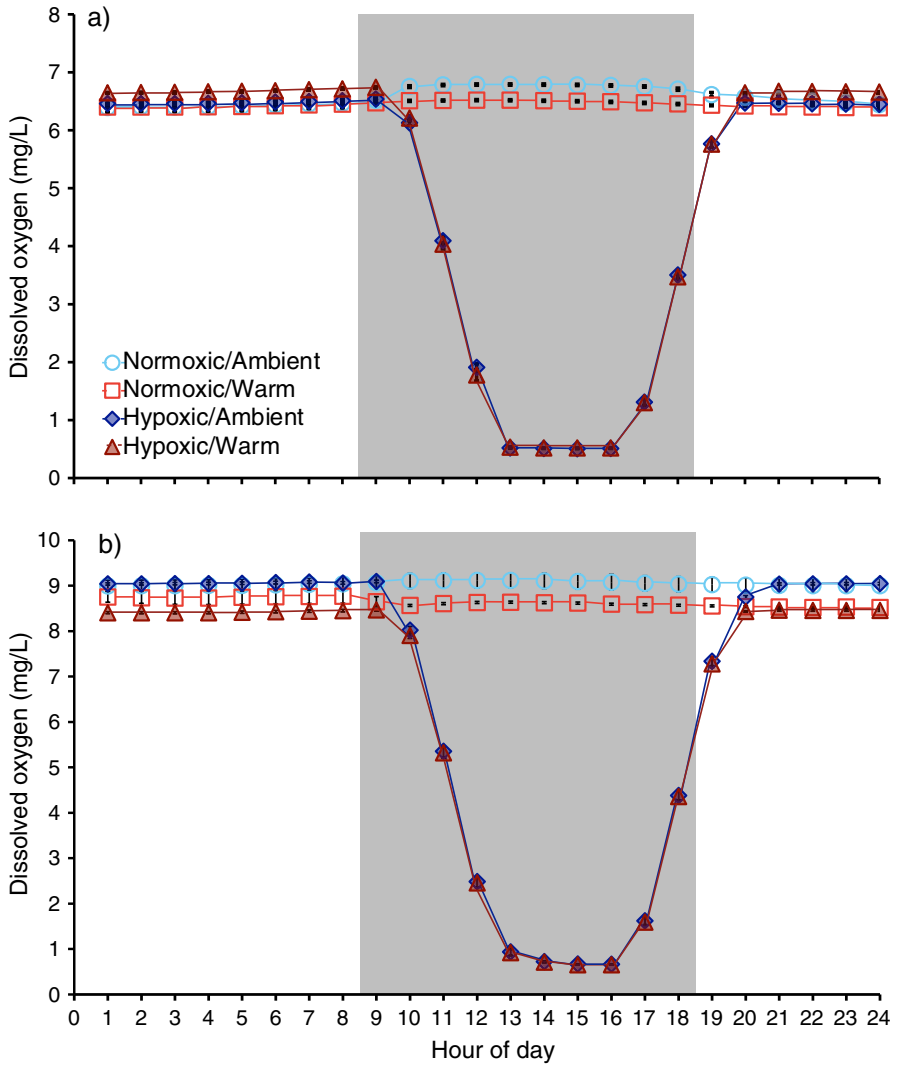

FIG. 2. Hourly dissolved oxygen concentrations (mean ± SE) in (a) Phase 1 (August 2018) and (b) Phase 2 (October–November 2018) of the experiment. Data are from the one replicate of each treatment combination that contained continuously sampling pH and DO probes used by the LabView software program to actively monitor and cycle DO concentrations. The gray box represents the 10 h period of darkness when rope lights in the experimental array were turned off. Error bars are almost always smaller than symbols.

Temperature and diel-cycling DO were manipulated as previously described. Oysters remained in Phase 2 for 18 d (14 d of diel-cycling dissolved oxygen, 18 d of temperature treatment). The duration of (18 d) and time between (60 d) the exposure phases were chosen to maximize the chances of observing an experimental effect while mimicking possible conditions that oysters would be exposed to in the field (Breitburg et al. 2015). See Appendix S3: Fig. S1 for calendar of cycling days.

Immediately prior to the start of Phase 2, we individually tagged each experimental oyster with a plastic bee tag (Betterbee, Greenwich, NY, USA) and measured oyster tissue and shell growth using the buoyant weighing technique (Palmer 1982). Oysters were placed in a hanging basket submerged in room temperature, Rhode River water to measure approximate oyster shell mass. Oysters were then placed on a paper towel to dry at room temperature for 1 h, after which time they were weighed in air to measure their total dry mass (tissue + shell). Using an empirically derived equation (actual shell mass = 1.4315 × submerged mass + 0.0188; R^2 = 0.9875) obtained through a destructive regression, we calculated actual oyster shell mass (mg) from the approximate oyster shell mass obtained through buoyant weighing (see Appendix S1: Section S1 for methods). We calculated oyster tissue mass (mg) by subtracting actual shell mass from total dry mass. Oysters were measured immediately before and after Phase 2, and tissue and shell growth were calculated as final − initial mass. Tissue growth : shell growth ratios were calculated by

dividing individual tissue by individual shell growth. Five oysters were excluded from the analyses due to death during Phase 2 of the experiment ($n = 2$) or measurement error ($n = 3$).

Statistical analyses

We analyzed DO concentration, temperature, and pH to verify differences between our experimental treatments using separate two-way ANOVAs that considered diel-cycling dissolved oxygen and temperature as fixed effects. We also included replicate nested within diel-cycling dissolved oxygen and temperature to account for potential non-independence among readings. Alkalinity and pCO2 were analyzed using a one-way ANOVA that considered temperature as a fixed effect. Phase 1 and Phase 2 conditions were analyzed separately.

We analyzed oyster tissue, shell, and tissue : shell growth using separate four-way nested ANOVAs (with REML variance estimates). Each ANOVA had four fixed effects: Phase 1 diel-cycling dissolved oxygen (Phase 1 DO), Phase 1 temperature (Phase 1 Temp), Phase 2 diel-cycling dissolved oxygen (Phase 2 DO), and Phase 2 temperature (Phase 2 Temp). We also included the following random factors to account for the nested design and non-independence among individuals in Phase 1 and 2: Phase 1 replicate was nested within Phase 1 DO and Phase 1 Temp; Phase 2 replicate was nested within Phase 2 DO and Phase 2 Temp; and Phase 2 replicate was separately crossed with Phase 1 DO, Phase 1 Temp, and Phase 1 DO × Phase 1 Temp and nested within Phase 2 DO and Phase 2 Temp (see Appendix S2: Table S1 for ANOVA table). Analyses were conducted in R (v.3.4.3) and R Studio (v. 1.1.463). We used lme4 (v. 1.1.23, Bates et al. 2015) to run the overall mixed model, car (v. 3.0.7; Fox and Weisburg 2019) to produce the ANOVA table, and emmeans (v. 1.4.6; Lenth 2020) to run the pairwise least square contrasts. Data are available on FigShare (Donelan et al. 2020*b*).

Results

Water quality

The diel pattern of DO concentrations measured by the continuously recording sensors in one replicate of each treatment is shown in Fig. 2. DO concentrations were also separately measured during normoxia and at the start and end of the hypoxic plateau, and we used these data in the water quality analyses here. Table 1 presents means of each individual treatment combination; here, we report analyses across treatments and means of groups that were statistically different. During the hypoxic plateau, DO concentrations were significantly lower in the diel-cycling hypoxia (Phase 1, 0.55 ± 0.19 mg/L; Phase 2, 0.74 ± 0.15 mg/L) than normoxia (Phase 1, 6.65 ± 0.15 mg/L; Phase 2, 8.85 ± 0.22 mg/L) treatment levels (Phase 1 DO, $F_{1,20} = 74149.5$, $P < 0.0001$; Phase 2 DO, $F_{1,19.9} = 117589.1$, $P < 0.0001$). These values closely matched our target hypoxia value (0.5 mg/L, 7–8% saturation). During normoxia, warming treatments (Phase 1, 6.53 ± 0.12 mg/L; Phase 2, 8.54 ± 0.22 mg/L) had significantly lower DO concentrations than ambient temperature treatments (Phase 1, 6.76 ± 0.18 mg/L; Phase 2, 9.14 ± 0.19 mg/L; Phase 1 Temp, $F_{1,40} = 25.08$, $P < 0.0001$; Phase 2 Temp, $F_{1,47} = 106.1$, $P < 0.0001$) regardless of diel-cycling DO (both Phase 1 DO and Phase 2 DO $P > 0.6$). This effect of temperature on DO concentration persisted during the hypoxic plateau, but only in the normoxia treatment tanks (normoxia/ambient, Phase 1, 6.92 ± 0.21 mg/L, Phase 2, 9.22 ± 0.23 mg/L; normoxia/warm, Phase 1, 6.52 ± 0.16 mg/L, Phase 2, 8.66 ± 0.23 mg/L, Phase 1 DO × Phase 1 Temp, $F_{1,20} = 73.5$, $P < 0.0001$, Phase 2 DO × Phase 2 Temp, $F_{1,19.9} = 111.5$, $P < 0.0001$; normoxia least square [ls] contrasts $P < 0.0001$). While statistically significant, these differences in dissolved oxygen were very small (<2% saturation) and therefore unlikely to be biologically meaningful at the high normoxic oxygen levels (~90% saturation; Keppel et al. 2016). DO concentrations were similarly low in both the hypoxic/warm and hypoxic/ambient treatment combinations (hypoxia ls contrasts, $P > 0.1$).

Temperatures in the warming treatment level (Phase 1, 30.6° ± 0.8°C; Phase 2, 18.3° ± 0.7°C) were significantly higher than those in the ambient treatment (Phase 1, 28.3° ± 0.8°C; Phase 2, 15.9° ± 0.7°C) level (Phase 1 Temp, $F_{1,20} = 1042.8$, $P < 0.0001$; Phase 2 Temp, $F_{1,19.9} = 3663.1$, $P < 0.0001$, Table 1). Warm temperature treatment levels were 2.3°C and 2.4°C higher than ambient temperatures in Phase 1 and Phase 2, respectively, and closely matched our target temperature difference (2.5°C).

Temperature also significantly affected pH in both Phase 1 ($F_{1,20.2} = 37.3$, $P < 0.0001$) and Phase 2 ($F_{1,21.8} = 13.6$, $P = 0.001$), with lower pH in the warming vs. ambient temperature treatment levels (Table 1). Diel-cycling DO also affected pH in Phase 2 ($F_{1,21.8} = 22.8$, $P < 0.0001$), with lower pH in the normoxia vs. diel-cycling hypoxia treatment level. However, these differences were in the <0.05 unit range, which is an order of magnitude lower than levels needed to elicit changes in growth in this system (Waldbusser et al. 2011, Keppel et al. 2016). Finally, alkalinity and pCO_2 were similar across all treatments in both Phase 1 (alkalinity, $F_{1,4} = 6.9$, $P = 0.07$; pCO_2, $F_{1,4} = 0.57$, $P = 0.49$) and Phase 2 (alkalinity, $F_{1,2} = 0.01$, $P = 0.92$; pCO_2, $F_{1,2} = 0.01$, $P = 0.91$; Table 1).

Oyster growth

Early life exposure to diel-cycling hypoxia and warming did not affect oyster tissue (Phase 1 DO, $F_{1,14.9} = 0.00$, $P = 0.9$; Phase 1 Temp, $F_{1,17.2} = 1.46$, $P = 0.2$) or shell (Phase 1 DO, $F_{1,10.9} = 0.72$, $P = 0.4$;

Phase 1 Temp, $F_{1,15.7} = 1.61$, $P = 0.2$) growth in any Phase 2 treatment (Fig. 3a–h). However, we found an interactive effect of Phase 2 exposure to diel-cycling hypoxia and temperature on oyster tissue growth (Phase 2 DO × Phase 2 Temp, $F_{1,20.0} = 8.38$, $P = 0.009$). Exposure to diel-cycling hypoxia in Phase 2 reduced oyster tissue growth by 22% at ambient (ls contrast, $P = 0.02$, Fig. 3c), but not warm (ls contrast, $P = 0.13$, Fig. 3d), temperatures. Oysters grew 59% more tissue at warm vs. ambient Phase 2 temperatures (Phase 2 Temp, $F_{1,20.0} = 68.8$, $P < 0.0001$, Fig. 3a–d). Shell growth was not affected by DO concentrations in Phase 2 (Phase 2 DO, $F_{1,20.0} = 3.7$, $P = 0.07$, Fig. 3e–h), though trends suggested a negative effect of diel-cycling hypoxia (Fig. 3e,g). Oysters produced 42% more shell at warm vs. ambient temperatures (Phase 2 Temp, $F_{1,20.0} = 60.9$, $P < 0.0001$, Fig. 3e–h).

While early life exposure to diel-cycling hypoxia and warming did not directly affect oyster tissue or shell growth, we found carryover effects of diel-cycling hypoxia and warming on relative tissue : shell growth. These carryover effects were context-dependent, manifesting only in certain Phase 2 treatment combinations (Phase 1 DO × Phase 1 Temp × Phase 2 DO × Phase 2 Temp, $F_{1,20.0} = 5.37$, $P = 0.03$). There was no effect of early life exposure to diel-cycling hypoxia on oyster tissue : shell growth at ambient temperatures (Fig. 4a, blue circles, ls contrast, $P = 0.52$, Fig. 4c, blue diamonds, ls contrast, $P = 0.63$). However, at warm temperatures (both Phase 1 and 2), early life exposure to diel-cycling hypoxia reduced oyster tissue : shell growth, but only if oysters were re-exposed to hypoxia in Phase 2 (Fig. 4d, red triangles, ls contrast, $P = 0.01$, 106% difference; Fig. 4b,d, filled red symbols, ls contrast, $P = 0.04$, 108% difference). Similarly, there was no effect of early life exposure to warming when oysters were at normoxia (Fig. 4a, open circles, ls contrast, $P = 0.68$; Fig. 4b, open squares, ls contrast, $P = 0.25$). However, in diel-cycling hypoxia treatments (both Phase 1 and 2), early life exposure to warming reduced oyster tissue : shell growth, but only if oysters were re-exposed to warming in Phase 2 (Fig. 4d, filled triangles, ls contrast, $P = 0.03$, 108% difference; Fig. 4c,d, filled red symbols, ls contrast, $P = 0.002$, 104% difference). Full ANOVA table given in Appendix S2: Table S1.

Discussion

We found within-generation carryover effects of early life experience with diel-cycling DO and warming on tissue : shell growth in juvenile oysters, but these effects depended on oysters' subsequent exposure to these conditions. At warm temperatures, early life exposure to diel-cycling hypoxia substantially reduced oyster tissue : shell growth if oysters were re-exposed to diel-cycling hypoxia 8 weeks later (Fig. 4d, red triangles). In contrast, there was no effect of early life exposure to diel-cycling hypoxia at ambient temperatures or if oysters were not re-exposed to diel-cycling hypoxia (Fig. 4a–c). Similarly, in diel-cycling hypoxia treatments, early life exposure to warming substantially reduced oyster tissue : shell growth if oysters were re-exposed to warming 8 weeks later (Fig. 4d, filled symbols). In contrast, there was no effect of early life exposure to warming in normoxic conditions or if oysters were not re-exposed to warming (Fig. 4a–c).

Because oysters began Phase 2 of the experiment at the same size regardless of their previous exposure to hypoxia and warming, these carryover effects do not appear to be driven by changes in overall energetic state. Rather, we hypothesize that early life exposure to these combined stressors may irreversibly affect oyster physiological development such that performance is reduced in response to re-exposure to both stressors. Similar physiological carryover effects have been shown in embryonic salmon: early life exposure to hypoxia reduces subsequent tolerance to hypoxia and to warming, and early life exposure to both hypoxia and warming reduces overall fry condition (Del Rio et al. 2020). Moreover, early life exposure to hypoxia can affect biochemical responses (Bianchini and Wright 2013) in rainbow trout, and limit maximum swimming speed later in life (Johnston et al. 2013). While these experiments manipulated larval exposure to stress and larval development is likely to be an especially sensitive life stage (Burggren and Reyna 2011), our results highlight that even juvenile exposure can have persistent effects on organism performance. Moreover, a potential reduction in physiological functioning affected how oysters allocated their energy toward tissue or shell growth: oysters grew relatively less tissue to shell if they were exposed to multiple stressors early in life and then encountered these stressors later in life. In many calcifying species, tissue growth is more energetically costly than shell growth (Watson et al. 2017) and is more closely tied to fitness (Thompson et al. 1996). Because of its effects on relative tissue growth, any loss of physiological function that may have occurred in oysters as a result of early life exposure to diel-cycling hypoxia and warming may therefore have especially strong effects on subsequent growth and reproductive output. Such physiological changes may occur, for example, through environmentally induced changes in epigenetic patterning (Eirin-Lopez and Putnam 2019) or behavioral changes directly caused by previous experiences (English et al. 2016); future work should explore the potential mechanisms driving this relationship.

The carryover effects we observed on relative oyster tissue : shell growth were context-dependent: early life exposure only affected oysters when they were re-exposed to those conditions 8 weeks later. Context-dependent carryover effects may be particularly influential in systems such as oysters where organisms have little behavioral capacity to avoid stress (e.g., sessile organisms). Moreover, context dependency can be critical in evaluating the role of other types of latent effects (e.g.,

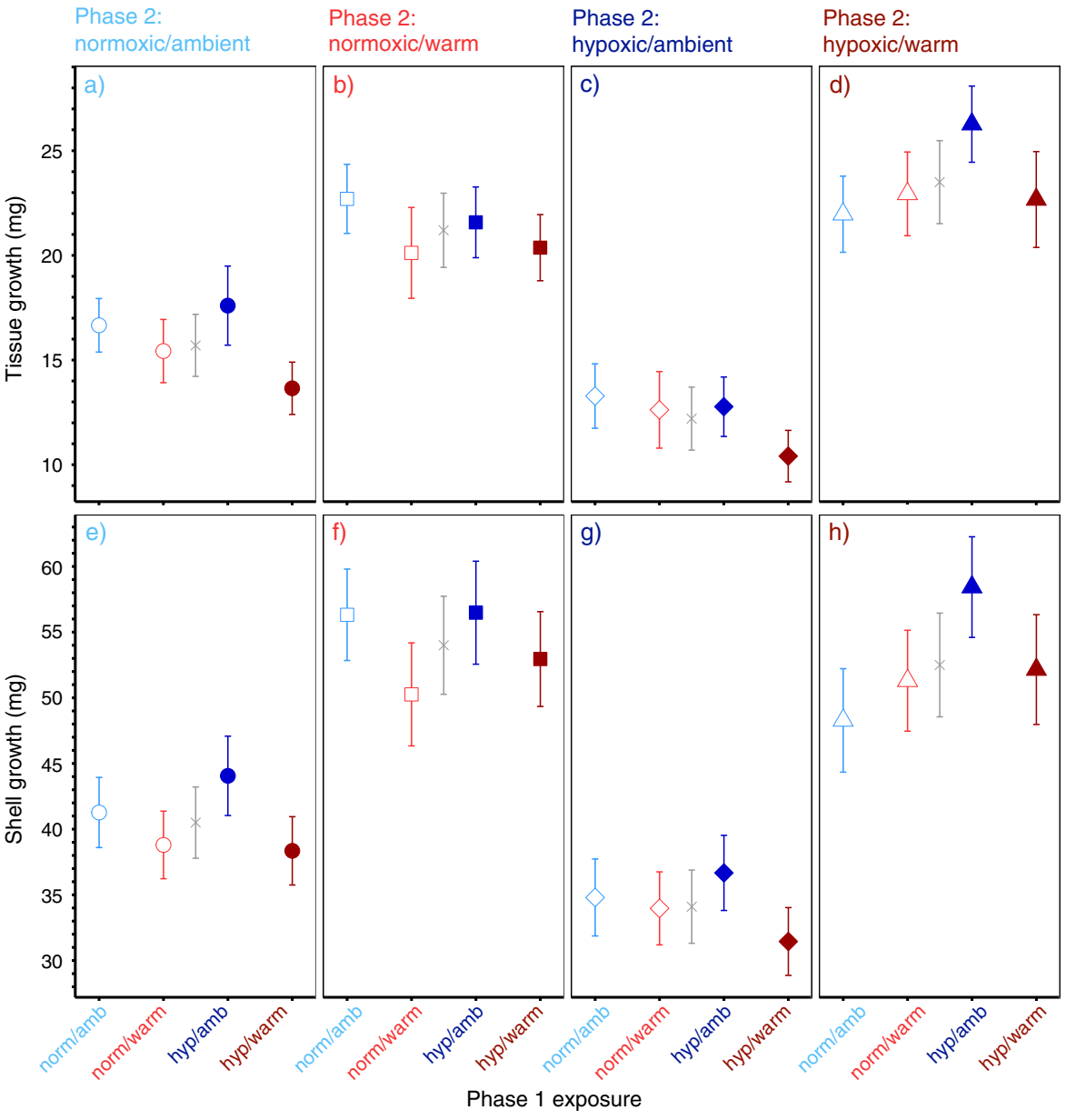

FIG. 3. (a–d) Tissue and (e–h) shell growth (mg, mean ± SE) of juvenile oysters (*Crassostrea virginica*) exposed in Phase 2 to normoxia and ambient temperature (a and e), normoxia and warm temperature (b and f), diel-cycling hypoxia and ambient temperature (c and g), and diel-cycling hypoxia and warm temperature (d and h). Oysters were exposed to these same experimental treatment combinations in Phase 1 (*x*-axis), 60 d prior to the start of Phase 2. The four-way interaction is not significant for either tissue or shell growth, but is shown for ease of comparison to tissue : shell growth, which is affected by the interaction between Phase 1 and Phase 2 diel-cycling dissolved oxygen and temperature (see Fig. 4). Phase 2 means ± SE are shown in gray points.

transgenerational effects; Sheriff and Love 2013), and our results suggest it is also critical here. For example, previous exposure (either direct or indirect) to an environmental stressor can reduce the performance of organisms in later in life if their past and future environments are mismatched, but enhance fitness if the environments are matched (Sheriff and Love 2013, Bateson et al. 2014). While some previous studies have evaluated the potential context-dependency of early life carryover effects (e.g., Marshall et al. 2010, Garcia et al. 2017, Donelan and Trussell 2019), most explore these patterns without considering organisms' later environmental context, particularly whether current conditions match early life conditions (see O'Connor et al. 2014). We therefore have limited understanding of how and when carryover effects are likely to be adaptive (Moore and Martin

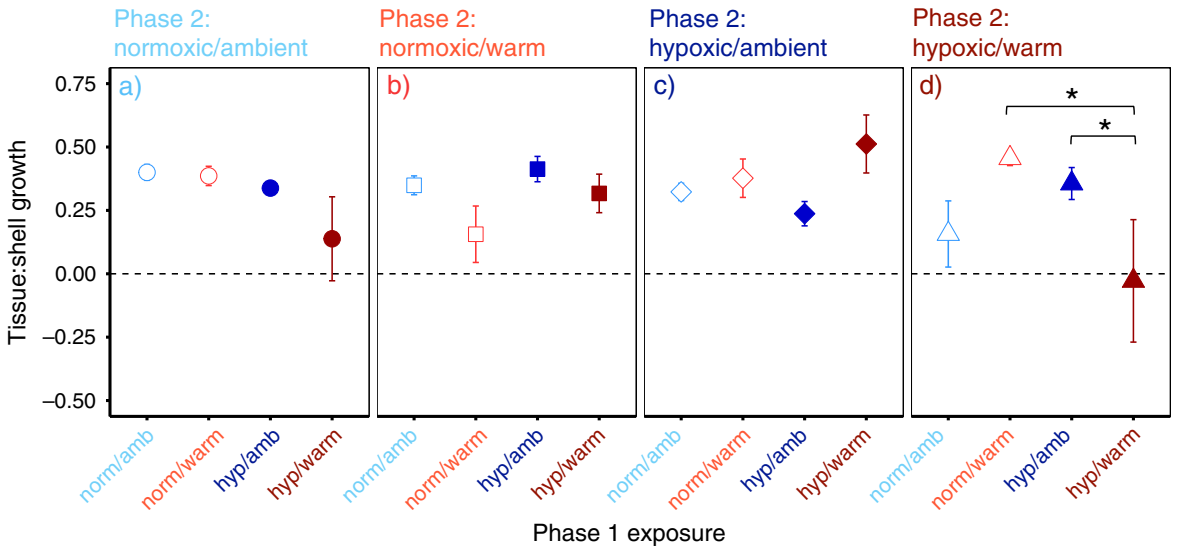

FIG. 4. Tissue : shell growth (mean ± SE) ratios of juvenile oysters (*Crassostrea virginica*) exposed in Phase 2 to (a) normoxia and ambient temperature, (b) normoxia and warm temperature, (c) diel-cycling hypoxia and ambient temperature, and (d) diel-cycling hypoxia and warm temperature. Oysters were exposed to these same experimental treatment combinations in Phase 1 (*x*-axis), 60 d prior to the start of Phase 2. Stars indicate significant differences ($P < 0.05$) between treatments based on least square contrasts.

2019), particularly with respect to stressors associated with climate change. In our system, matching between previous and subsequent environments (e.g., diel-cycling hypoxia and warming in both Phase 1 and 2) *reduced* oyster tissue : shell growth, but only in the most stressful conditions. Hence, the importance of matching/mismatching between early life and future environments may depend on the mechanism through which carryover effects act. If early life exposure to hypoxia or warming creates irreversible changes in phenotype (e.g., through effects on physiological functioning), re-exposure to these stressors may exploit these changes and enhance the negative impacts of effects of early life exposure. In contrast, if carryover effects manifest in traits that are more easily reversed (e.g., behavior), early life exposure to stress may provide information to organisms that better prepares them for future stress and that improves fitness outcomes (Ferrari et al. 2019). While we do not know the mechanism by which carryover effects influence fitness in this system, exploring these hypotheses will improve our understanding of the adaptive potential of carryover effects.

We also only observed carryover effects in our experiment in response to multiple, simultaneously acting stressors: early life exposure to hypoxia affected oyster tissue : shell growth only if oysters were exposed to warming and early life exposure to warming only affected oysters if they were exposed to hypoxia. Because warm water holds less oxygen and limits oxygen supply at depth, diel-cycling hypoxia and warming are stressors that will co-occur frequently (Rabalais et al. 2010) and will simultaneously affect respiration, as ectotherms require more oxygen at higher temperature (Deutsch et al. 2008). Quantifying their synergistic effects is therefore critical to accurately predicting how they will influence coastal and aquatic species.

The importance of testing multiple stressors is well-documented (e.g., Boyd et al. 2015), but rarely considered for context-dependent within-generation carryover effects in which organisms are re-exposed to the same stressors across ontogeny (but see Fischer and Phillips 2014). Manipulating multiple stressors simultaneously is particularly challenging with regard to context-dependent carryover effects because of the logistical constraints of factorial experimental designs involving an initial and subsequent exposure to stress. However, our results highlight that every effort should be made to test multi-stressor carryover effects, especially if those stressors co-occur in the field. Diel-cycling hypoxia and warming do frequently co-occur worldwide and throughout the geographic range of oysters. For example, in Delaware Bay, USA, mean daily water temperature was the strongest predictor of the severity of summertime diel-cycling hypoxia (<2 mg/L DO), and small increases in temperature above 26°C resulted in relatively large impacts on the duration of hypoxia (Tyler et al. 2009). Moreover, summertime monitoring in tributaries of Chesapeake Bay from 2001 to 2011 found daily minimum dissolved oxygen concentrations of <0.5 mg/L DO (the same as used in our experiment) at ~50% of sites, with occurrences at the most severe sites for as much as ~60% of the summer (Breitburg et al. 2015). Temperatures in our warming treatments are routinely reached in tributaries of Chesapeake Bay (Southworth

et al. 2017). Because these shallow-water tributaries are often the location of oyster restoration and aquaculture, our experimental oysters were likely exposed to conditions similar to those they would experience in the field, suggesting that similar carryover effects may be operating in field-reared populations.

While the carryover effects we observed were present 8 weeks after oysters' initial exposure, whether these effects persist throughout oyster ontogeny to affect adult (~1 yr old) fitness is unknown. The growth disparities we observed in oysters as a result of being exposed and re-exposed to diel-cycling hypoxia and warming suggest that these effects could have lifelong impacts. For example, oysters that were exposed to diel-cycling hypoxia and warming both early and later in life had substantially lower tissue : shell growth compared to those that were exposed to normoxia early in life. This substantial decrease in tissue mass may have persistent, detrimental effects on fitness later in life, as has been shown in other systems (Tüzün and Stoks 2018). Moreover, even if smaller sized oysters are able to "catch up" once the stressors subside (Keppel et al. 2016), such compensatory growth can have lifelong negative effects through decreased adult size, fecundity, and longevity (Metcalfe and Monaghan 2001). Finally, carryover effects of early life experiences can persist to affect the response of adult organisms to future stress (Donelan and Trussell 2018), and similar patterns may be operating here.

Carryover effects add complexity to previously reported impacts of diel-cycling DO and warming that were also observed here, namely that oyster growth is also strongly affected by exposure to diel-cycling DO and warming regardless of early life exposure. Oysters grew 59% more tissue and 42% more shell at warm vs. ambient temperatures in Phase 2 of the experiment (Fig. 3a–h). Many ectotherms, including oysters (Jones et al. 2019), increase their metabolic rate as temperature increases, at least until a thermal maximum is reached and performance declines (Torossian et al. 2020), and this increase in metabolic rates could explain the increase in growth since food is not limiting in this system. In our experiments, temperatures in the warm treatment level were well within oysters' thermal tolerance (Shumway 1996). Oysters in the shallow water tributaries of Chesapeake Bay are frequently exposed to temperatures well above those in our experiment (Southworth et al. 2017). Because food was provided ad libitum (via flowing river water), oysters likely had enough food to keep pace with their increased metabolic demands at these warm temperatures, allowing them to convert more energy into tissue and shell growth. In contrast, diel-cycling hypoxia reduced oyster tissue growth and tended to decrease shell growth (though $P = 0.07$), but only at ambient temperatures. These results support previous work that found a negative effect of diel-cycling hypoxia on growth in this (Keppel et al. 2016) and other (Davidson et al. 2016) systems. However, diel-cycling hypoxia had stronger effects on tissue vs. shell growth, suggesting that even short-term exposure to hypoxia (~5 h/d for 2 weeks) can have strong effects on oyster fitness. It is interesting that diel-cycling hypoxia did not affect oyster tissue growth at warm temperatures, and patterns suggested that it actually increased growth (but $P = 0.13$). Previous work suggests that oysters reduce their foraging rates in response to diel-cycling hypoxia, potentially in order to limit their exposure to oxidative stress (Sussarellu et al. 2010), but compensate for these reductions by feeding more as normoxia is restored (Breitburg et al. 2015, Porter and Breitburg 2016). We hypothesize that similar behavior occurred in our experiment, but only affected oysters at warm temperatures, when filtration rates are especially high (Loosanoff 1958), and allowed oysters to make up for the lost foraging incurred during hypoxia. Alternatively, exposure to hypoxia can induce upregulation in genes associated with respiration (Sussarellu et al. 2010), so these growth effects may also manifest through changes in oyster physiology. Overall, our results suggest that the effects of diel-cycling hypoxia on oyster growth may be weaker at warmer temperatures, at least over short timescales such as those we observed in this experiment.

Both phases of our experiment took place in 2018, a year of record rainfall for the Chesapeake Bay region (Chesapeake Bay Foundation 2019). As such, salinities during our experiment were far lower (<4.0 ppt) than average in the summer and fall in the Upper Chesapeake Bay (~10–15 ppt; Maryland Department of Natural Resources 2020). Similarly low salinities (3 ppt) can reduce oyster clearance rates by as much as 95% in some populations (Casas et al. 2018), so it is likely that these low salinities reduced oyster growth overall in our experiment, as found in Keppel et al. (2016). Moreover, low salinity may itself affect how oysters allocate energy to tissue or shell growth. It is possible, for example, that energetic deficits caused by low salinity cause oysters to preferentially grow shell, which is less energetically costly, at the expense of tissue growth, as has been shown in other bivalves (Kumar et al. 2015). We have no evidence that low salinity conditions influenced the context-dependent carryover effects seen in this study. However, if it did, the implication could be important. Low salinity areas provide important refuge habitat from two salinity-sensitive pathogens that cause widespread mortality of oysters in Chesapeake Bay and elsewhere (*Perkinsus marinus* and *Haplosporidium nelson*; Burreson and Ragone Calvo 1996). If these habitats are particularly susceptible to the multiple stressor carryover effects seen here, their value as refuge habitat could be diminished.

Conclusions and implications

Organisms are increasingly likely to encounter multiple, interacting stressors repeatedly throughout their lives as climate change and other anthropogenic stressors intensify. Because exposure to early life stress can

influence subsequent responses of organisms to the same stressors, carryover effects may strengthen the negative impacts of climate change in some systems (Donelan et al. 2020*a*), as we observed here. In our system, carryover effects of hypoxia and warming reduced relative tissue to shell growth. Since oyster aquaculture, harvest, and restoration are often concentrated in shallow-water tributaries where seasonal warming and diel-cycling hypoxia are common (Tyler et al. 2009), early life exposure to these stressors is likely to occur. If carryover effects are operating in these field-raised populations, growth deficits associated with early life exposure and re-exposure to multiple stressors may ultimately reduce oyster size and limit their economic value since oysters with similar shell sizes would contain less meat (tissue). Moreover, because oyster fecundity is correlated with size (Marshall et al. 2020), carryover effects of stress may affect wild oyster population size or hatchery production, while also affecting oysters' ability to provide key ecosystem services such as denitrification (Kellogg et al. 2014). Finally, oysters are protandrous hermaphrodites (larger individuals are female Thompson et al. 1996), so these size effects may impact oyster sex ratios in the field, further influencing population dynamics.

The experimental oysters used here were produced by (wild-caught) broodstock that were spawned in a hatchery setting. Hatchery-produced cohorts are often less genetically diverse than those produced in the wild (e.g., Li et al. 2004, Araki et al. 2007, but see Hornick and Plough 2019), so they may have reduced performance in key traits such as initial size and subsequent survival (Hughes et al. 2019). A smaller size at settlement may, for example, make hatchery-bred cohorts less resilient to stress (Metcalfe and Monaghan 2001) and therefore potentially more likely to exhibit adverse within-generation carryover effects of early life stress than wild-bred stocks. Moreover, stress associated with captive-rearing can itself elicit negative carryover effects that reduce the success of hatchery-reared individuals once they are released into the wild. For example, captive-bred salmon exhibit much lower survival than wild salmon, and these differences are associated with epigenetic modifications that are induced by hatchery rearing (Le Luyer et al. 2017). Hence, hatchery spawning may itself contribute to the prevalence of within-generation carryover effects in bivalves such as oysters; if these carryover effects reduce oyster fitness, they may detrimentally affect the success of aquaculture operations and restoration. As such, efforts should be made to limit early life exposure to stress in hatchery-bred and -reared bivalves, particularly those in which subsequent exposure to stressors is likely to occur such as when wild stocks are supplemented with hatchery-raised organisms.

Adopting longer-term perspectives on the effects of stress may be critical in evaluating the impacts of anthropogenic change on species of conservation concern (O'Connor and Cooke 2015, Spencer et al. 2020). Eastern oysters are a key fishery and species of management concern throughout their range (Beck et al. 2011). Oysters are ecosystem engineers whose populations are near 1% of historic levels (Wilberg et al. 2011) and whose performance is strongly affected by exposure to climate change stress (Keppel et al. 2016). Our results show that early life exposure to climate change stressors can reduce oyster performance when they are exposed to multiple stressors later in life, and may contribute to reductions in oyster growth and production. Overall, we suggest that incorporating carryover effects into our understanding of the responses of organisms to climate change is key to predicting the long-term effects of these persistent stressors in both natural and farmed systems.

Acknowledgments

We thank Yamilla Samara Chacon, Kristina Borst, Sarah Gignoux-Wolfsohn, and Matilda Newcomb for help with experimental maintenance and data collection, Roy Rich for generously lending the temperature manipulation equipment, Shannon Hood and Alex Golding at Horn Point Oyster Hatchery for assistance with oyster spat, and Catherine Matassa for statistics advice. All authors designed the study, S. C. Donelan coordinated the study, carried out statistical analysis, and drafted the manuscript. All authors contributed equally to manuscript revisions. The authors declare no conflicts of interest. This work was generously supported by a Smithsonian Institution Postdoctoral Fellowship to S. C. Donelan, the National Science Foundation (DBI-1659668), and Maryland Sea Grant Award #NA14OAR4170090-SA75281450-P to D. Breitburg and M. B. Ogburn.

Literature Cited

Altieri, A. H., and K. B. Gedan. 2015. Climate change and dead zones. Global Change Biology 21:1395–1406.

APHA 1992. Standard methods for the examination of water and wastewater. Water Environmental Federation, Washington, D.C., USA.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318:100.

Bates, D., M. Maechler, and B. Bolker. 2015. lme4: Linear mixed-effects models using S4 classes. http://CRAN.R-project.org/package=lme4

Bateson, P., P. Gluckman, and M. Hanson. 2014. The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. Journal of Physiology 592:2357–2368.

Baumann, H., R. B. Wallace, T. Tagliaferri, and C. J. Gobler. 2015. Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. Estuaries and Coasts 38:220–231.

Beck, M. W., et al. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. BioScience 61:107–116.

Bianchini, K., and P. A. Wright. 2013. Hypoxia delays hematopoiesis: retention of embryonic hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during chronic hypoxia exposure. Journal of Experimental Biology 216:4415.

Bopp, L., L. Resplandy, J. C. Orr, S. C. Doney, J. P. Dunne, M. Gehlen, P. Halloran, C. Heinze, T. Ilyina, and R. Seferian. 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10:6225–6245.

Boyd, P. W., S. T. Lennartz, D. M. Glover, and S. C. Doney. 2015. Biological ramifications of climate-change-mediated oceanic multi-stressors. Nature Climate Change 5:71–79.

Breitburg, D., et al. 2018. Declining oxygen in the global ocean and coastal waters. Science 359:eaam7240.

Breitburg, D. L., D. Hondorp, C. Audemard, R. B. Carnegie, R. B. Burrell, M. Trice, and V. Clark. 2015. Landscape-level variation in disease susceptibility related to shallow-water hypoxia. PLoS ONE 10:e0116223.

Burggren, W. W., and K. S. Reyna. 2011. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. Respiratory Physiology & Neurobiology 178:13–21.

Burrell, R. B., A. G. Keppel, V. M. Clark, and D. L. Breitburg. 2016. An automated monitoring and control system for flow-through co-cycling hypoxia and pH experiments. Limnology and Oceanography: Methods 14:168–185.

Burreson, E. M., and L. M. Ragone Calvo. 1996. Epizootiology of Perkinsus marinus disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research 15:17–34.

Byrne, M., M. Ho, E. Wong, N. A. Soars, P. Selvakumaraswamy, H. Shepard-Brennand, S. A. Dworjanyn, and A. R. Davis. 2011. Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. Proceedings of the Royal Society B 278:2376–2383.

Casas, S. M., R. Lavaud, M. K. La Peyre, L. A. Comeau, R. Filgueira, and J. F. La Peyre. 2018. Quantifying salinity and season effects on eastern oyster clearance and oxygen consumption rates. Marine Biology 165:1–13.

Chesapeake Bay Foundation 2019. State of the Bay Report: 2018. Chesapeake Bay Foundation, Annapolis, Maryland, USA. https://www.cbf.org/document-library/cbf-reports/2018-state-of-the-bay-report.pdf

Coen, L. D., R. D. Brumbaugh, D. Bushek, R. Grizzle, M. W. Luckenbach, M. H. Posey, S. P. Powers, and S. G. Tolley. 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341:303–307.

Crean, A. J., K. Monro, and D. J. Marshall. 2011. Fitness consequences of larval traits persist across the metamorphic boundary. Evolution 65:3079–3089.

Davidson, M. I., T. E. Targett, and P. A. Grecay. 2016. Evaluating the effects of diel-cycling hypoxia and pH on growth and survival of juvenile summer flounder *Paralichthys dentatus*. Marine Ecology Progress Series 556:223–235.

Del Rio, A. M., B. E. Davis, N. A. Fangue, and A. E. Todgham. 2020. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. Conservation Physiology 7:coy078.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy USA 105:6668–6672.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13:77–81.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926–929.

Donelan, S. C., J. K. Hellmann, A. M. Bell, B. Luttbeg, J. L. Orrock, M. J. Sheriff, and A. Sih. 2020*a*. Transgenerational plasticity in human-altered environments. Trends in Ecology & Evolution 35:115–124.

Donelan, S. C., M. B. Ogburn, and D. Breitburg. 2020*b*. Dataset: Context-dependent carryover effects of hypoxia and warming in a coastal ecosystem engineer. The Smithsonian Institution, Washington, D.C., USA. Data set, Figshare https://doi.org/10.25573/serc.13341614

Donelan, S. C., and G. C. Trussell 2018. Parental and embryonic experiences with predation risk affect prey offspring behaviour and performance. Proceedings of the Royal Society B 285:20180034.

Donelan, S. C., and G. C. Trussell. 2019. The effects of embryonic experience with predation risk vary across a wave exposure gradient. Ecosphere 10:e02676.

Doney, S. C., et al. 2011. Climate change impacts on marine ecosystems. Annual Review of Marine Science 4:11–37.

Eirin-Lopez, J. M., and H. M. Putnam. 2019. Marine environmental epigenetics. Annual Review of Marine Science 11:335–368.

English, S., T. W. Fawcett, A. D. Higginson, P. C. Trimmer, T. Uller, J.-M. Gaillard, and J. L. Bronstein. 2016. Adaptive use of information during growth can explain long-term effects of early life experiences. American Naturalist 187: 620–632.

Ferrari, M. C. O., D. T. Warren, M. I. McCormick, and D. P. Chivers. 2019. The cost of carryover effects in a changing environment: context-dependent benefits of a behavioural phenotype in a coral reef fish. Animal Behaviour 149:1–5.

Fischer, J., and N. E. Phillips. 2014. Carry-over effects of multiple stressors on benthic embryos are mediated by larval exposure to elevated UVB and temperature. Global Change Biology 20:2108–2116.

Flint, N., R. G. Pearson, and M. R. Crossland. 2018. Reproduction and embryo viability of a range-limited tropical freshwater fish exposed to fluctuating hypoxia. Marine and Freshwater Research 69:267–276.

Fox, J., and S. Weisburg 2019. An R companion to applied regression. Third edition. Sage, Thousand Oaks, California, USA.

Garcia, T. S., J. C. Urbina, E. M. Bredeweg, and M. C. O. Ferrari. 2017. Embryonic learning and developmental carry-over effects in an invasive anuran. Oecologia 184:623–631.

Gattuso, J.-P., J.-M. Epitalon, H. Lavigne, and J. Orr. 2020. seacarb: Seawater carbonate chemistry. https://CRAN.R-project.org/package=seacarb

Harrison, X. A., J. D. Blount, R. Inger, D. R. Norris, and S. Bearhop. 2011. Carry-over effects as drivers of fitness differences in animals. Journal of Animal Ecology 80:4–18.

Hettinger, A., E. Sanford, T. M. Hill, A. D. Russell, K. N. Sato, J. Hoey, M. Forsch, H. N. Page, and B. Gaylord. 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology 93:2758–2768.

Hornick, K. M., and L. V. Plough. 2019. Tracking genetic diversity in a large-scale oyster restoration program: effects of hatchery propagation and initial characterization of diversity on restored vs. wild reefs. Heredity 123:92–105.

Hughes, A. R., T. C. Hanley, J. E. Byers, J. H. Grabowski, T. McCrudden, M. F. Piehler, and D. L. Kimbro. 2019. Genetic diversity and phenotypic variation within hatchery-produced oyster cohorts predict size and success in the field. Ecological Applications 29:e01940.

Johnston, E. F., S. L. Alderman, and T. E. Gillis. 2013. Chronic hypoxia exposure of trout embryos alters swimming performance and cardiac gene expression in larvae. Physiological and Biochemical Zoology 86:567–575.

Jones, H. R., K. M. Johnson, and M. W. Kelly. 2019. Synergistic effects of temperature and salinity on the gene expression and physiology of *Crassostrea virginica*. Integrative and Comparative Biology 59:306–319.

Jones, T. M., and M. P. Ward. 2020. Pre- to post-fledging carryover effects and the adaptive significance of variation in wing development for juvenile songbirds. Journal of Animal Ecology 89:2235–2245.

Kasumovic, M. M. 2013. The multidimensional consequences of the juvenile environment: towards an integrative view of the adult phenotype. Animal Behaviour 85:1049–1059.

Kellogg, M. L., A. R. Smyth, M. W. Luckenbach, R. H. Carmichael, B. L. Brown, J. C. Cornwell, M. F. Piehler, M. S. Owens, D. J. Dalrymple, and C. B. Higgins. 2014. Use of oysters to mitigate eutrophication in coastal waters. Estuarine, Coastal and Shelf Science 151:156–168.

Keppel, A. G., D. L. Breitburg, and R. B. Burrell. 2016. Effects of co-varying diel-cycling hypoxia and pH on growth in the juvenile eastern oyster, *Crassostrea virginica*. PLoS ONE 11: e0161088.

Keppel, A. G., D. L. Breitburg, G. H. Wikfors, R. B. Burrell, and V. M. Clark. 2015. Effects of co-varying diel-cycling hypoxia and pH on disease susceptibility in the eastern oyster *Crassostrea virginica*. Marine Ecology Progress Series 538:169–183.

Kroeker, K. J., R. L. Kordas, R. N. Crim, and G. G. Singh. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters 13:1419–1434.

Kumar, V., A. K. Sinha, P. P. Rodrigues, V. K. Mubiana, R. Blust, and G. De Boeck. 2015. Linking environmental heavy metal concentrations and salinity gradients with metal accumulation and their effects: a case study in 3 mussel species of Vitória estuary and Espírito Santo bay, Southeast Brazil. Science of the Total Environment 523:1–15.

Le Luyer, J., M. Laporte, T. D. Beacham, K. H. Kaukinen, R. E. Withler, J. S. Leong, E. B. Rondeau, B. F. Koop, and L. Bernatchez 2017. Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. Proceedings of the National Academy of Sciences USA 114:12964.

Lenihan, H. S., and C. H. Peterson. 1998. How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. Ecological Applications 8:128–140.

Lenth, R. 2020. emmeans: Estimated marginal means, aka least-square means. R package version 1.4.6. https://CRAN.R-project.org/package=emmeans

Levin, L. A., and D. L. Breitburg. 2015. Linking coasts and seas to address ocean deoxygenation. Nature Climate Change 5:401.

Li, Q., C. Park, T. Endo, and A. Kijima. 2004. Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). Aquaculture 235:207–222.

Loosanoff, V. L. 1958. Some aspects of behavior of oysters at different temperatures. Biological Bulletin 114:57–70.

Lowe, M. R., T. Sehlinger, T. M. Soniat, and M. K. La Peyre. 2017. Interactive effects of water temperature and salinity on growth and mortality of eastern oysters, *Crassostrea virginica*: a meta-analysis using 40 years of monitoring data. Journal of Shellfish Research 36:683–697.

Madsen, T., and R. Shine. 2000. Silver spoons and snake body sizes: prey availability early in life influences long-term growth rates of free-ranging pythons. Journal of Animal Ecology 69:952–958.

Marshall, D., K. Monro, M. Bode, M. Keough, and S. Swearer. 2010. Phenotype–environment mismatches reduce connectivity in the sea. Ecology Letters 13:128–140.

Marshall, D. A., S. C. Moore, M. Sutor, J. F. La Peyre, and M. K. La Peyre. 2020. Using reproductive potential to assess oyster population sustainability. Restoration Ecology 28:1621–1632.

Maryland Department of Natural Resources. 2020. Eyes on the Bay. Maryland Department of Natural Resources, Annapolis, Maryland, USA. eyesonthebay.dnr.maryland.gov

Matoo, O. B., A. V. Ivanina, C. Ullstad, E. Beniash, and I. M. Sokolova. 2013. Interactive effects of elevated temperature and CO2 levels on metabolism and oxidative stress in two common marine bivalves (Crassostrea virginica and Mercenaria mercenaria). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164:545–553.

McFarland, K., L. Donaghy, and A. K. Volety. 2013. Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, Perna viridis, and the native oyster, Crassostrea virginica, in Southwest Florida. Aquatic Invasions 8:299–310.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution 16:254–260.

Moore, M. P., and R. A. Martin. 2019. On the evolution of carry-over effects. Journal of Animal Ecology 88:1832–1844.

O'Connor, C. M., and S. J. Cooke. 2015. Ecological carryover effects complicate conservation. Ambio 44:582–591.

O'Connor, C. M., D. R. Norris, G. T. Crossin, and S. J. Cooke. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. Ecosphere 5:1–11.

Pachauri, R. K., M. R. Allen, V. R. Barros, J. Broome, W. Cramer, R. Christ, J. A. Church, L. Clarke, Q. Dahe, and P. Dasgupta. 2014. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Palmer, A. R. 1982. Growth in marine gastropods: a non-destructive technique for independently measuring shell and body weight. Malacologia 23:63–74.

Pechenik, J. A. 2006. Larval experience and latent effects—metamorphosis is not a new beginning. Integrative and Comparative Biology 46:323–333.

Porter, E. T., and D. L. Breitburg. 2016. Eastern oyster, *Crassostrea virginica*, valve gape behavior under diel-cycling hypoxia. Marine Biology 163:218.

Rabalais, N. N., R. J. Díaz, L. A. Levin, R. E. Turner, D. Gilbert, and J. Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7:585–619.

Rich, R. L., A. Stefanski, R. A. Montgomery, S. E. Hobbie, B. A. Kimball, and P. B. Reich. 2015. Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. Global Change Biology 21:2334–2348.

Rybovich, M., M. K. L. Peyre, S. G. Hall, and J. F. L. Peyre. 2016. Increased temperatures combined with lowered salinities differentially impact oyster size class growth and mortality. Journal of Shellfish Research 35:101–113.

Sheriff, M., and O. Love. 2013. Determining the adaptive potential of maternal stress. Ecology Letters 16:271–280.

Shumway, S. E. 1996. Chapter 13: natural environmental factors. Pages 467–514 *in* V. S. Kennedy, R. I. Newell, and A. F. Eble, editors. The eastern oyster: *Crassostrea virginica*. University of Maryland Sea Grant College, College Park, Maryland, USA.

Southworth, M., M. C. Long, and R. Mann. 2017. Oyster (*Crassostrea virginica* [Gmelin, 1791]) mortality at prolonged exposures to high temperature and low salinity. Journal of Shellfish Research 36:335–340.

Spencer, L. H., Y. R. Venkataraman, R. Crim, S. Ryan, M. J. Horwith, and S. B. Roberts. 2020. Carryover effects of temperature and pCO2 across multiple Olympia oyster populations. Ecological Applications 30:e02060.

Stamps, J. A., V. V. Krishnan, and N. H. Willits. 2009. How different types of natal experience affect habitat preference. American Naturalist 174:623–630.

Sussarellu, R., C. Fabioux, G. Le Moullac, E. Fleury, and D. Moraga. 2010. Transcriptomic response of the Pacific oyster *Crassostrea gigas* to hypoxia. Marine Genomics 3:133–143.

Talmage, S. C., and C. J. Gobler. 2011. Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of northwest Atlantic bivalves. PLoS ONE 6:e26941.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy, and R. Mann. 1996. Chapter 9: reproductive processes and early development. Pages 335–370 *in* V. S. Kennedy, R. I. Newell, and A. F. Eble, editors. The eastern oyster: *Crassostrea virginica*. University of Maryland Sea Grant College, College Park, Maryland, USA.

Torossian, J. L., K. E. Hosek, S. C. Donelan, G. C. Trussell, B. S. Helmuth, and M. L. Zippay. 2020. Physiological and biochemical responses to acute environmental stress and predation risk in the blue mussel, *Mytilus edulis*. Journal of Sea Research 159:101891.

Tüzün, N., and R. Stoks. 2018. Pathways to fitness: carry-over effects of late hatching and urbanisation on lifetime mating success. Oikos 127:949–959.

Tyler, R. M., D. C. Brady, and T. E. Targett. 2009. Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries. Estuaries and Coasts 32:123–145.

Van Allen, B. G., and V. H. W. Rudolf. 2013. Ghosts of habitats past: Environmental carry-over effects drive population dynamics in novel habitat. American Naturalist 181:596–608.

Vaquer-Sunyer, R., and C. M. Duarte. 2011. Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. Global Change Biology 17:1788–1797.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, and R. I. E. Newell. 2011. Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuaries and Coasts 34:221–231.

Watson, S.-A., S. A. Morley, and L. S. Peck. 2017. Latitudinal trends in shell production cost from the tropics to the poles. Science Advances 3:e1701362.

Wilberg, M. J., M. E. Livings, J. S. Barkman, B. T. Morris, and J. M. Robinson. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Marine Ecology Progress Series 436:131–144.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2315/full

Data Availability

Data are available in The Smithsonian Institution's Figshare (Donelan et al. 2020): https://doi.org/10.25573/serc.13341614.v1