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RESEARCH ARTICLE

Legacy of past exposure to hypoxia and warming regulates an ecosystem service provided by oysters

Sarah C. Donelan | Matthew B. Ogburn | Denise Breitburg

Smithsonian Environmental Research Center, Edgewater, Maryland, USA

Correspondence
Sarah C. Donelan, Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA
Email: donelans@si.edu

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Abstract

Climate change is having substantial impacts on organism fitness and ability to deliver critical ecosystem services, but these effects are often examined only in response to current environments. Past exposure to stress can also affect individuals via carryover effects, and whether these effects scale from individuals to influence ecosystem function and services is unknown. We explored within-generation carryover effects of two coastal climate change stressors—hypoxia and warming—on oyster (*Crassostrea virginica*) growth and nitrogen bioassimilation, an important ecosystem service. Oysters were exposed to a factorial combination of two temperature and two diel-cycling dissolved oxygen treatments at 3-months-old and again 1 year later. Carryover effects of hypoxia and warming influenced oyster growth and nitrogen storage in complex and context-dependent ways. When operating, carryover effects of single stressors generally reduced oyster nitrogen bioassimilation and relative investment in tissue versus shell growth, particularly in warm environments, while early life exposure to multiple stressors generally allowed oysters to perform as well as control oysters. When extrapolated to the reef scale, carryover effects decreased nitrogen stored by modeled oyster reefs in most conditions, with reductions as large as 41%, a substantial decline in a critical ecosystem service. In some scenarios, however, carryover effects increased nitrogen storage by modeled oyster reefs, again highlighting the complexity of these effects. Hence, even brief exposure to climate change stressors early in life may have persistent effects on an ecosystem service 1 year later. Our results show for the first time that within-generation carryover effects on individual phenotypes can impact processes at the ecosystem scale and may therefore be an overlooked factor determining ecosystem service delivery in response to anthropogenic change.

KEYWORDS
acclimatization, carryover effect, climate change, eutrophication, nitrogen, nutrient removal, oyster reef

1 | INTRODUCTION

Climate change is altering patterns and processes across all levels of biological organization (Pinsky et al., 2020). Phenotypic plasticity is an important component of organismal responses to rapid environmental change (Fox et al., 2019) and can facilitate individual survival in response to changing and variable environments (Merilä & Hendry, 2014). Plasticity in traits such as growth, fecundity, and foraging influence not only individual performance and population growth (Green et al., 2022; Snell-Rood et al., 2018) but can also scale

up to affect ecosystem processes such as productivity, energy flux, and nutrient cycling (Castilla et al., 2017; Mccary & Schmitz, 2021). For example, warming affects foraging behavior and nutrient assimilation efficiency in stream detritivores, causing them to retain less and excrete more nitrogen, with downstream impacts on nutrient cycling (Mas-Martí et al., 2015). Through effects on individuals, plasticity can thereby influence species' capacity to provide ecosystem services and exacerbate or alleviate the effects of anthropogenic change (Burge et al., 2016; Rader et al., 2013).

Assessments of the role of plasticity in species' responses to environmental stress have focused primarily on responses to current environments (e.g., Riddell et al., 2018; Vargas et al., 2022). However, organism phenotypes are also shaped by past environments such as those experienced during previous life stages. These within-generation carryover effects are widespread across taxa (Sutton et al., 2021; Trontin et al., 2020) and affect functional traits similar to those that respond plastically to current environmental conditions, including growth (Donelan & Trussell, 2019), fecundity (Stuligross & Williams, 2021), foraging (Van Allen et al., 2010), and disease transmission (Roux et al., 2015). Mounting evidence suggests that phenotypic changes caused by within-generation carryover effects in individuals cascade to impact population (Stuligross & Williams, 2021) and community dynamics (Van Allen & Rudolf, 2016). But whether carryover effects scale beyond populations and communities to influence ecosystem-level processes remains untested.

In marine systems, foundation species such as oysters provide critical ecosystem services, including improving water quality through nutrient reductions (Grabowski et al., 2012). Oysters can remove substantial amounts of nitrogen (hundreds of metric tons; Bricker et al., 2020) from estuaries and help ameliorate downstream effects of eutrophication such as algal blooms and hypoxic zones that can result in ecosystem collapse (Altieri et al., 2017; Watson et al., 2016). Oysters remove nitrogen by filtering nitrogen-rich plankton and seston as they feed (Kellogg et al., 2014; Smyth et al., 2013). This nitrogen is either bioassimilated into oyster tissue and shell or excreted onto the surrounding sediment as biodeposits that promote processes (e.g., denitrification, anammox) that allow nitrogen to escape from the system as N_2 gas (Kellogg et al., 2014; Smyth et al., 2013). While bioassimilation is only a component of this ecosystem service, it tends to remove nitrogen for especially long periods of time (months to years; Kellogg et al., 2014) or even permanently for harvested oysters (Parker & Bricker, 2020). Because oyster filtration and growth rates vary plastically across current environmental contexts (Hoellein et al., 2015), oysters' ability to store and transform nitrogen can vary similarly with current contexts (Smyth et al., 2015; Westbrook et al., 2018) and may also vary based on past experiences. Oysters are a model system for exploring the ecosystem-level impacts of within-generation carryover effects not only because of their role in the coastal nitrogen cycle but also because they are relatively long lived (10–15 years; Powell & Cummins, 1985) and sessile. These traits make oysters especially likely to re-encounter similar abiotic conditions throughout their lives, and exposure to environments early in life can adaptively prime individuals for future encounters with the same conditions (English et al., 2016).

Dissolved oxygen (DO) is declining systemically in marine systems due in part to rising temperatures as well as increased nutrient loading into coastal systems (Breitburg et al., 2018). DO also fluctuates over shorter timescales such as over the course of a day due to changes in photosynthetic rates of phytoplankton and submerged aquatic vegetation (Baumann et al., 2015). The duration and magnitude of this diel-cycling hypoxia (<2 mg L^{-1}, Breitburg et al., 2018) are exacerbated by warming water temperatures associated with climate change (Altieri & Gedan, 2015). Direct exposure to hypoxia and warming affects key oyster functional traits. Prolonged severe hypoxia (~5 days) results in mass mortality (Lenihan & Peterson, 1998), and diel-cycling hypoxia can reduce oyster growth and valve gaping (necessary for feeding) by 40% and 90%, respectively (Keppel et al., 2016; Porter & Breitburg, 2016). Warm water temperatures, in contrast, generally increase oyster filtration rates (Casas et al., 2018) and growth (Donelan et al., 2021), although only to a certain point (Lowe et al., 2017). Oysters have a wide temperature tolerance (Shumway, 1996), so modest increases in temperature associated with climate change may only prove stressful if they exceed oysters' tolerance or if oysters are simultaneously exposed to other stressors such as hypoxia. Previous work has found carryover effects of hypoxia and warming on oyster growth over short timescales: early life exposure to both hypoxia and warming resulted in a substantial reduction in relative tissue to shell growth 3 months later (Donelan et al., 2021). But whether carryover effects persist and alter oyster bioassimilation, and hence a coastal ecosystem service, has not been explored.

We examined within-generation carryover effects of hypoxia and warming on growth and nitrogen bioassimilation in eastern oysters (*Crassostrea virginica*) across different environmental contexts. We show that past exposure to hypoxia and warming synergistically affects oyster growth and nitrogen storage 1 year after initial exposure. When extrapolated to a reef scale, carryover effects have the potential to reduce nitrogen stored by oysters by as much as 41%, a substantial decline in a key ecosystem service, although carryover effects also increased nitrogen storage potential in some modeled scenarios. These results provide, to our knowledge, the first evidence that carryover effects can impact individual traits in ways that influence processes at the ecosystem scale, suggesting they may be an overlooked factor influencing ecosystem service delivery in natural systems in response to climate change.

2 | MATERIALS AND METHODS

2.1 | Experimental design and set-up

We conducted a multiyear fully factorial experiment to explore how early life exposure (Year 1) to diel-cycling DO (normoxia/hypoxia; hypoxia target: 0.5 mg L^{-1}, 7%–8% saturation) and temperature

(ambient/warm; warm target: 2.5°C above ambient) affected oyster tissue, shell, and relative tissue:shell growth as well as nitrogen bioassimilated in tissue and shell growth when oysters were exposed to the same treatment combinations 1 year later (Year 2). There were a total of 16 treatment combinations (Figure 1). The Year 1 exposure ran in August 2018 and the Year 2 exposure in August 2019.

Three- to four-month-old oysters, spawned from broodstock (n_{female} = 39, n_{male} = 16) collected from the Choptank River, a tributary of the Chesapeake Bay, were purchased from Horn Point Oyster Hatchery (Cambridge, Maryland). They were then acclimated for 6 days in flow-through aquarium facilities at the Smithsonian Environmental Research Center (SERC) on the Rhode River, Maryland prior to the Year 1 exposure. Conditions were similar between the two locations (within 2 psu salinity and 1.5°C), limiting oysters' physiological stress (Mcfarland et al., 2013).

The experiment took place in an indoor aquarium facility at SERC. Water is pumped from the Rhode River into a 568 L fiberglass holding tank set in series with four other identical tanks. The first three tanks allowed sediment to settle while the final two were the water sources for the ambient or warming treatments. Water in the warming treatment tank was heated to a target temperature of 2.5°C above ambient to align with the 2014 IPCC RCP6.0 scenario (Pachauri et al., 2014). Warming was dynamically maintained through a custom microprocessor feedback control (Rich et al., 2015) that controlled power to 3000 W of aquarium heaters (500 W heaters). Water in the ambient treatment tank was unmanipulated. Water was dispensed from the ambient and warm tanks into the 24 experimental aquaria (75 L) through vinyl tubing at a rate of 300 mL min^{-1}.

DO concentrations in the normoxic treatment level were held at normoxia (~100% saturation). DO concentrations in the diel-cycling hypoxia treatment level were manipulated over a 24-h cycle using custom LabView software that uses input from DO (Oxyguard) and pH (Honeywell Durafet III) sensors placed in one replicate tank of each treatment combination (Burrell et al., 2016). The program controls the flow of a mixture of four gases (nitrogen, carbon dioxide, air, and CO_2-stripped air) as necessary to achieve target DO concentrations for a given timepoint. Gases were dispensed to each replicate aquarium at a rate of 25 L min^{-1} through mass flow controllers, gas manifolds, vinyl tubing, and silica air stones. A hypoxic cycle consisted of a 3-h draw down from normoxia to hypoxia (Δ30% saturation h^{-1} to target of 0.5 mg L^{-1}, 7%–8% saturation), a 4-h plateau period when hypoxia was maintained, a 3-h ramp up from hypoxia to normoxia (Δ30% saturation h^{-1}), and a 14-h period at normoxia, mimicking DO concentrations and cycle durations in tributaries of the Chesapeake Bay (Breitburg et al., 2015). pH remained constant by adding CO_2 gas as needed and was similar to ambient conditions during the experiment. Each aquarium was covered with a tight-fitting plexiglass lid to maintain target DO, temperature, and pH, which were verified in each tank once or twice during a daily hypoxic plateau using an external water quality probe (see Supplemental Information for more details). DO cycled on weekdays in the diel-cycling treatment and stayed at normoxia on weekends, except for 1 week in Year 2 when it cycled for the entire week (Figure S1). Temperature treatments were applied each day of the experiment. This schedule was used for logistical reasons but also mirrored natural variability (Breitburg et al., 2015).

2.2 | Growth and nitrogen storage

At the start of the Year 1 exposure, oysters (N = 3000, 3–5 mm shell height) were placed into the experimental array. There were six replicate tanks of each of the four treatment combinations (N = 24, n = 150 oysters $tank^{-1}$). In each tank, oysters were held in a perforated plastic container (16 × 16 × 9.5 cm, l × w × h) that was placed on a semi-open platform to minimize sedimentation. Oysters remained in these conditions for 18 days (13 days of diel-cycling DO, 18 days of temperature treatment, Figure S1). At the end of the Year 1 exposure, oysters were placed in a tank of aerated, ambient Rhode River water for 2 months before being moved (within their experimental mesocosms) to large vexar mesh bags (84 × 48 × 10 cm, l × w × h; 9 mm mesh) suspended off the SERC dock in the Rhode River until the start of the Year 2 exposure. Bags were rinsed throughout the winter to prevent mortality.

At the start of the Year 2 exposure, we placed approximately half of the oysters (N = 960) from Year 1 back into the experimental array. To ensure that oysters were the same size at the start of Year 2 regardless of their Year 1 treatment, we excluded all oysters from one replicate of each of the four Year 1 treatment combinations that on average contained the largest oysters. We chose to exclude

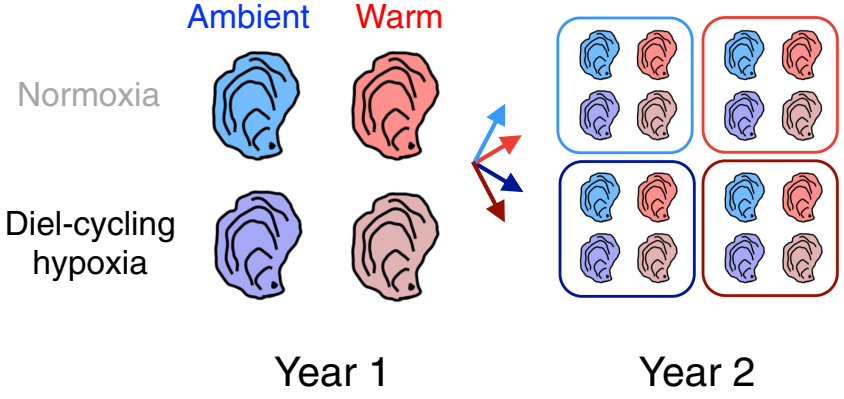

FIGURE 1 Schematic of the experimental design. Juvenile oysters (*Crassostrea virginica*) were exposed to diel-cycling dissolved oxygen (normoxia/hypoxia) and temperature (ambient/warm) treatments at 3-months-old (Year 1, n = 6 replicates with 150 oysters $replicate^{-1}$) and again 1 year later (Year 2, n = 6 replicates with 40 oysters $replicate^{-1}$, N = 960) in a fully factorial design (treatment combinations). See Table S1 for average treatment conditions [Colour figure can be viewed at wileyonlinelibrary.com].

oysters from one replicate per Year 1 treatment combination rather than the largest oysters in each treatment regardless of the replicate they were from to keep the sample sizes among the remaining Year 1 replicates similar (1–3 oysters Year 1 replicate^{-1}), which should reduce Type II error. Hence, oysters from five replicates of each Year 1 treatment combination were used in Year 2 and oysters were the same initial size across all 16 treatment combinations based on ANOVAs ($\bar{x} \pm$ SE; shell mass: 412.8 ± 5.1 mg, $p = .9$, tissue mass: 176.1 ± 2.7 mg, $p = .3$). While size standardization prior to the Year 2 exposure removes a potential pathway by which carryover effects may act (size differences), it allows us to explore other potential mechanistic pathways (e.g., physiology) that would otherwise be confounded by oyster size. There were 24 tanks with 10 oysters from each Year 1 treatment combination in each tank ($n = 40$ oysters tank^{-1}, 1–3 oysters Year 1 replicate^{-1}). Oysters remained in the Year 2 exposure for 20 days (16 days of diel-cycling DO, 20 days of temperature treatment).

To track individual oyster tissue and shell growth, we tagged each oyster ($n = 60$ treatment combination^{-1}, $N = 960$) with a plastic numeric tag and weighed them using the buoyant weighing technique (Donelan et al., 2021; Palmer, 1982) immediately prior to the start and following the end of the Year 2 exposure (Supplementary Information). Shell and tissue growth (mg) were calculated as final − initial mass. The growth metric encompasses only the shell and tissue added during the Year 2 exposure. To determine if oysters partitioned resources differently into shell or tissue growth, we also calculated a ratio of relative tissue to shell growth by dividing individual tissue growth by shell growth.

Immediately after the final weighing, a subsample of oysters ($n = 6$ treatment combination^{-1}, $N = 96$) was placed in a −80°C freezer. Oysters were later removed, dissected to separate tissue and shell, and oven dried at 60°C. Each shell and tissue sample was ground into a fine powder using a ball mill grinder, weighed to a standard weight, packed individually into tin capsules, and analyzed for the percent of total (organic + inorganic) nitrogen by weight on a Thermo Delta V Advantage mass spectrometer coupled to an Elemental vario ISOTOPE Cube Elemental Analyzer at the Smithsonian MCI Stable Isotope Mass Spectrometry Laboratory. This generated an average percent nitrogen in oyster tissue and shell for each separate treatment combination (Table S4), which was multiplied by the tissue and shell growth of each oyster as appropriate to determine the nitrogen contained in tissue and shell growth (mg).

2.3 | Nitrogen storage projections for an oyster reef

We used the nitrogen content of the experimental oysters to model how carryover effects could impact nitrogen bioassimilation of oysters in the field if exposed to conditions similar to those in our experiment. We used a restored reef in Harris Creek, a tributary of the Choptank River in Maryland, as a model system because it was restored with oysters from the same population as our experimental oysters and has been regularly monitored. Data on Harris Creek oyster shell length and density of live oysters were obtained from the 2020 Maryland Oyster Monitoring Report using reefs restored with live oysters in 2013 and monitored for shell length and density in 2020 (Maryland Oyster Restoration Interagency Working Group, 2021).

Harris Creek oysters were substantially larger ($\bar{x}$ shell length ± SE; 73.6 ± 0.62 mm) than the experimental oysters (13.6 ± 0.09 mm) and we wanted our projections to account for this larger size. However, the relationship between shell length and mass can change as oysters grow (Powell et al., 2016) and oyster mass also varied among our experimental treatments (see Section 3). To integrate these differences into our projections, we used allometric scaling to extrapolate the tissue and shell mass of potential Harris Creek oysters based on relationships between shell length (mm) and tissue and shell mass (mg) of our experimental oysters. Specifically, we used log–log plots to generate separate linear relationships between shell length and tissue and shell mass of the experimental oysters from each of the 16 treatment combinations (equations in Table S5). We then used these equations to calculate potential tissue and shell masses of two groups of Harris Creek oysters: (1) those classified as spat (<40 mm shell height) and (2) oysters of all sizes using shell length data provided in the 2020 Monitoring Report. This data set contains the lengths of 4058 oysters, 1270 classified as spat (Figshare), that were randomly resampled via bootstrapping (1000 iterations, boot package; Canty & Ripley, 2021) to generate concomitant tissue and shell masses separately for both groups (spat and all). The tissue and shell masses were then multiplied by the percent nitrogen content in tissue and shell of oysters from that treatment combination (Table S4) to find the mean and 95% confidence intervals of nitrogen in a potential Harris Creek oyster. These values were multiplied by the density of live spat (38.76 oysters m^{-2}) or all (121.42 oysters m^{-2}) oysters on Harris Creek (Maryland Oyster Restoration Interagency Working Group, 2021) as appropriate and converted to an acre scale. While calculating the nitrogen stored in spat-sized Harris Creek oysters separately may allow more direct comparisons with our experimental oysters, prior work suggests that the percent nitrogen in oyster tissue does not vary with oyster size (Dalrymple & Carmichael, 2015) such that differences between our experimental oysters would be maintained throughout ontogeny, particularly as the majority (≥97%) of nitrogen stored in the experimental oysters was in tissue (see Section 4).

2.4 | Statistical analyses

2.4.1 | Water quality

To determine that our treatment applications met our target conditions, we analyzed DO concentrations during both the normoxic and hypoxic plateaus, temperature, and pH using separate two-way ANOVAs with diel-cycling DO and temperature as fixed effects. We included replicate nested within diel-cycling DO and temperature to

account for potential non-independence among readings and used REML variance estimates. Alkalinity and pCO_2 were analyzed using a one-way ANOVA that considered temperature as a fixed effect. Year 1 and Year 2 were analyzed separately.

2.4.2 | Oyster growth and nitrogen storage

Shell and tissue growth encompasses only that grown during the Year 2 exposure. We analyzed oyster tissue growth (mg), shell growth (mg), tissue:shell growth, and nitrogen stored in oyster tissue and shell growth (mg) using separate factorial linear mixed models with Year 1 DO, Year 1 temperature, Year 2 DO, Year 2 temperature, and all interactions as fixed effects and REML variance estimation. Initial shell mass (mg) was included as a covariate and was significant for all analyses (Tables S2 and S3). To account for non-independence among oysters within the same tank, the following nested factors were included as random effects: Year 1 replicate nested within Year 1 DO and Year 1 temperature, Year 2 replicate nested within Year 2 DO and Year 2 replicate, and Year 2 replicate was separately crossed with Year 1 DO, Year 1 temp, and Year 1 DO × Year 1 Temp and nested within Year 2 DO and Year 2 temperature. *F*-ratios and *p*-values were generated using Type III ANCOVAs (Tables S2 and S3). Data met the assumptions of ANCOVA except the residuals were not normal (Figure S2), but ANCOVA is robust to violations of normality. Post hocs were calculated using least square means ($\alpha = 0.05$). Analyses were conducted in R (v. 3.6.3, R Core Team, 2020). Three oysters died during the experiment, so were excluded from analyses.

3 | RESULTS

3.1 | Water quality

Complete water quality results are provided in Table S1. Briefly, DO concentrations were lower in the diel-cycling hypoxia (0.55 mg L^{-1}, 7.7% saturation on average) than normoxia (7.0 mg L^{-1}, 94.6% saturation on average) treatment level during the hypoxic plateau in both years. Warming reduced DO during both the normoxic and hypoxic plateaus, but these differences were very small (~1% saturation) and likely not biologically meaningful, particularly at normoxia (Keppel et al., 2016). Temperatures in the warming treatment level were 2.3 and 2.4°C above ambient (Year 1 and 2, respectively), and temperatures in both the ambient and warm treatments were within oysters' thermal tolerance (Shumway, 1996). Temperature also affected pH, but differences were <0.04 units, an order of magnitude lower than levels needed to affect oyster growth (Keppel et al., 2016).

3.2 | Oyster growth

Early life exposure to diel-cycling DO and warming affected oyster tissue growth regardless of Year 2 exposure ($F_{1,9.8} = 7.80$, $p = .02$, Figure S3a), with oysters exposed to both hypoxia and warming early in life growing the same amount of tissue as control oysters (normoxia DO, ambient temperature, $p = .07$), but more tissue than oysters exposed to warming alone (8%, $p = .01$) or hypoxia alone (9%, $p = .01$) early in life. Oysters also grew 10% more tissue if they were in warm versus ambient Year 2 temperatures ($F_{1,20.0} = 5.56$, $p = .03$, Figure S3b). Carryover effects did not impact shell growth, but Year 2 exposure to hypoxia reduced shell growth ($F_{1,20.0} = 5.35$, $p = .03$, Figure S4) by 8% relative to Year 2 control ($p = .01$) and 13% relative to Year 2 normoxia/warm environments ($p = .0001$).

Carryover effects impacted relative tissue to shell growth and these effects varied across Year 2 environments ($F_{1,20.1} = 5.04$, $p = .03$, Figure 2). In Year 2 control conditions (normoxia, ambient), oysters exposed to warming alone early in life grew 14% less tissue relative to shell than oysters exposed to both warming and hypoxia early in life ($p = .03$). When oysters were exposed to multiple stressors (hypoxia and warming) in Year 2, those exposed only to hypoxia early in life tended to grow less tissue versus shell than oysters exposed to both hypoxia and warming early in life ($p = .08$, 9%). Carryover effects did not impact oyster relative tissue to shell growth when oysters were exposed to only one stressor (warming or hypoxia) in Year 2.

3.3 | Nitrogen storage

We found carryover effects of diel-cycling DO and warming on nitrogen stored in oyster tissue and shell growth and these effects varied with Year 2 temperature (tissue: $F_{1,20.1} = 9.98$, $p = .005$, Figure 3a; shell: $F_{1,20.0} = 12.34$, $p = .002$, Figure 3b). In warm Year 2 temperatures (regardless of Year 2 DO treatment), oysters stored 14% less nitrogen in their tissue if they were exposed to warming alone ($p = .01$) or hypoxia alone ($p = .004$) early in life compared with

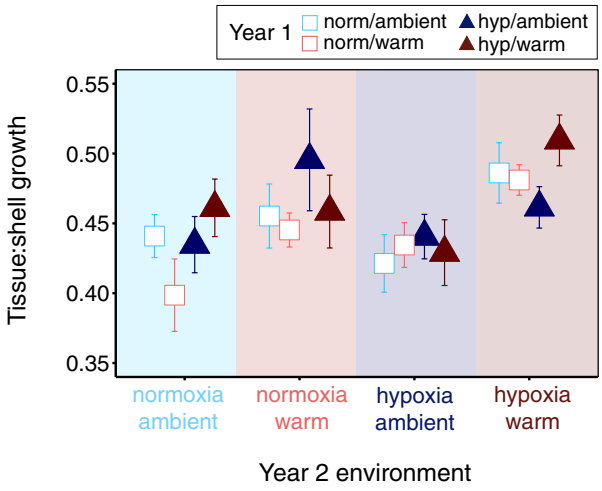

FIGURE 2 Tissue:shell growth (mean ± SE) for oysters exposed to diel-cycling dissolved oxygen (normoxia/hypoxia) and temperature (ambient/warm) treatments at 3-months-old (Year 1) and again 1 year later (Year 2). $n = 60$ oysters treatment combination^{-1} [Colour figure can be viewed at wileyonlinelibrary.com]

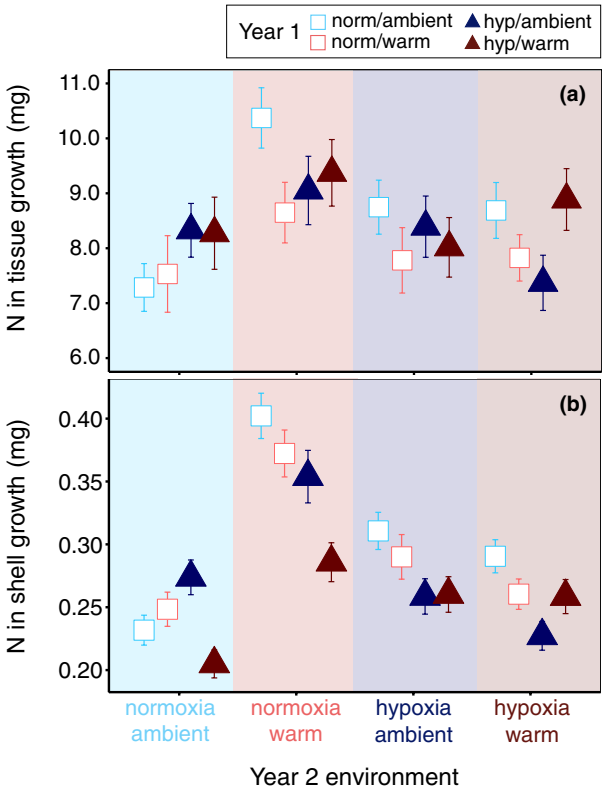

FIGURE 3 Nitrogen bioassimilated into (a) tissue and (b) shell growth (mean ± SE) of oysters exposed to diel-cycling dissolved oxygen (normoxia/hypoxia) and temperature (ambient/warm) at 3-months-old (Year 1) and again 1 year later (Year 2). The four-way interaction is not significant for either (a) or (b), but is shown for ease of comparison with Figures 2 and 4; instead, the three-way interaction between Year 1 DO, Year 1 temperature, and Year 2 temperature is significant here. Empirically derived percent nitrogen by weight provided for each treatment combination in Table S4. $n = 60$ oysters treatment combination^{-1} [Colour figure can be viewed at wileyonlinelibrary.com]

oysters exposed to control conditions or both hypoxia and warming early in life (Figure 3a). In ambient Year 2 temperatures (regardless of Year 2 DO treatment), oysters exposed to hypoxia alone early in life tended ($p = .06$) to store more nitrogen in their tissue than control oysters, although effects were quite small (4%, Figure 3a). Early life exposure to warming alone reduced nitrogen stored in oyster tissue by 8% compared with oysters exposed to hypoxia alone and 5% compared with oysters exposed to hypoxia and warming simultaneously early in life (both $p < .05$, Figure 3a). Year 2 temperature also affected nitrogen in oyster shell growth: in warm Year 2 temperatures, oysters exposed to hypoxia early in life stored 14% less nitrogen in their shell than oysters exposed to normoxia early in life ($p = .0001$, Figure 3b) and these effects were exacerbated if oysters were exposed to both hypoxia and warming early in life (18%, $p = .0003$). At ambient Year 2 temperatures, oysters exposed to both hypoxia and warming early in life had 13% less nitrogen in their shell growth compared with oysters from all other early life environments ($p = .01$, Figure 3b).

Carryover effects also operated differently across Year 2 DO environments, but only for nitrogen stored in oyster shell growth (Table S3, Figure 3b). In normoxic Year 2 environments, early life exposure to both hypoxia and warming reduced nitrogen in oyster shell growth by 22% compared with all other early life environments ($p < .0001$). In hypoxic Year 2 environments, oysters exposed to hypoxia early in life stored 13% less nitrogen in their shell compared with oysters exposed to normoxia early in life, regardless of early life temperature ($p = .004$).

Finally, carryover effects had implications for nitrogen storage projections on a restored oyster reef whether we considered all oysters (Figure 4) or spat-sized oysters only (Figure S5). For both groups of oysters, early life exposure to hypoxia, warming, or both decreased the amount of nitrogen stored on a potential reef in most conditions compared with control early life environments (7/12 and 6/12 conditions for all vs. spat oysters, respectively, Table S5). For both groups of oysters, the largest change occurred in a hypoxic potential reef environment for oysters exposed to both hypoxia and warming early in life, which resulted in a 41% reduction (101 kg acre^{-1}) in nitrogen stored for all oysters compared with warm early life environments (Figure 4c) and a 20% reduction (2.6 kg acre^{-1}) in nitrogen stored for spat-sized oysters compared with all other early life environments (Figure S5c). Substantial declines were also observed in other conditions; for all oysters, early life exposure to warming reduced nitrogen storage by as much as 30% (96 kg acre^{-1}) in potential normoxic/ambient reef environments (Figure 4a) and in potential warm reef environments, early life exposure to hypoxia, warming, or both reduced nitrogen storage by as much as 22% (38 kg acre^{-1}, Figure 4b). These patterns and effect sizes were similar in warm environments for spat-sized oysters (17%, Figure S5b). Interestingly, early life exposure to hypoxia or warming also increased nitrogen stored in some, albeit fewer, potential reef conditions compared with control early life environments (3/12 and 1/12 conditions for all vs. spat oysters, respectively Table S5). The largest increase occurred in a hypoxic potential reef environment for all oysters (Figure 4c), where early life exposure to warming alone increased potential nitrogen storage by 22% (42 kg acre^{-1}). For spat-sized oysters, the largest increase in nitrogen storage occurred in a normoxic/ambient potential reef environment (Figure S5a), where oysters exposed to hypoxia early in life stored 20% more nitrogen (2.6 kg acre^{-1}) than control oysters. This same pattern also occurred among all oyster sizes classes (Figure 4a) but was less impactful (10% increase).

4 | DISCUSSION

Climate change is increasing the likelihood that organisms—particularly sessile organisms like oysters—will be exposed to multiple stressors repeatedly over their lifetimes (Ummenhofer & Meehl, 2017). Within-generation carryover effects may, therefore, be increasingly common and important if they alter phenotypes in ways that are adaptive in those environments (Putnam, 2021). We found that early life exposure to multiple climate change conditions has persistent carryover effects on growth and nitrogen storage in an important coastal ecosystem engineer, thus demonstrating, for

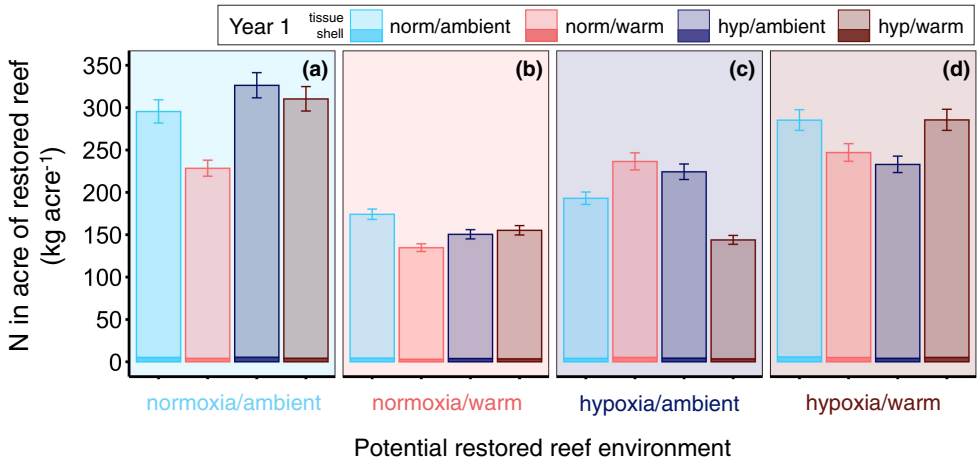

FIGURE 4 Model estimates of nitrogen assimilated by oysters restored to Harris Creek, Maryland if oysters were exposed to different combinations of diel-cycling DO (normoxia/hypoxia) and temperature (ambient/warm) at 3 months old (Year 1) and outplanted to reefs that experience different hypoxia and temperature exposures 1 year later (a–d). Means and 95% CI (error bars) were calculated via bootstrapping using the boot R package (Canty & Ripley, 2021), 1000 iterations, and a normal approximation using oyster shell lengths and density (121.42 m^{-2}) from surveys completed in 2020 of reefs restored in 2013 (Maryland Oyster Restoration Interagency Working Group, 2021). See Section 2 for detailed description of calculations, Table S5 for allometric equations relating experimental oyster size to the size of Harris Creek oysters, and Figure S5 for projections based on spat-sized oysters only (<40 mm shell height). [Colour figure can be viewed at wileyonlinelibrary.com]

the first time, that carryover effects can have persistent impacts on individual phenotypes in ways that scale up to affect ecosystem processes. While the specific results are complex and may vary based on idiosyncrasies of our experiment (e.g., specific treatment levels used), the overall finding that within-generation carryover effects influence ecosystem service delivery 1 year later is novel and suggests that carryover effects can have profound impacts on ecosystems in a changing climate that are currently unaccounted for.

Carryover effects influenced nitrogen stored in oyster tissue and shell. Effects on oyster nitrogen storage were driven by a combination of small, non-significant changes in percent nitrogen by weight (Table S4) and differences in tissue and shell growth and are therefore reflective of multiple traits that can show variation based on early life experiences. The majority of nitrogen (97%–99%) was stored in oyster tissue (despite being only 28% of total growth), so total nitrogen storage was dominated by these differences. Carryover effects on nitrogen tissue storage operated most strongly in Year 2 warm environments—regardless of hypoxia—that reflect potential increases in summertime water temperature for Chesapeake Bay in the coming decades (Hinson et al., 2021). In these warm conditions, early life exposure to both hypoxia and warming allowed oysters to store as much nitrogen as oysters exposed to control conditions (normoxia, ambient) early in life, while oysters exposed to *either* hypoxia or warming only early in life stored 12% less than these oysters (Figure 3a).

It is interesting that oysters exposed to multiple stressors early in life performed as well as control oysters in warm Year 2 environments (with and without hypoxia). Our experiment did not test the mechanisms underlying these patterns. But because these differences were not influenced by growth alone (i.e., they differ from growth patterns), they are likely not driven solely by feeding or clearance rates. However, oysters do feed more at warmer temperatures (to a certain point; Casas et al., 2018) and oysters exposed to both hypoxia and warming early in life may retain more nitrogen from their food because of nitrogen-intensive metabolic processes activated early in life. For example, early life exposure to hypoxia and warming together may necessitate the initiation of metabolic pathways that ameliorate physiological stress effects (Gunderson et al., 2016) such as enhanced protein synthesis to repair damaged tissue or maintain homeostasis (Hawkins, 1985). These nitrogen-intensive processes may then become canalized and remain active later in life, particularly in warm environments where metabolic and growth rates are higher and thus cellular damage is more likely (Abele et al., 2002), and increase oyster nitrogen assimilation efficiency and thus tissue nitrogen content. Exposure to single stressors early in life may fail to activate these stress pathways, reducing overall nitrogen content. Alternatively, the oyster microbiome community affects denitrification rates (conversion of bioavailable nitrogen to nitrogen gas; Arfken et al., 2017) within oysters and is influenced by both hypoxia (Khan et al., 2018) and warming (Scanes et al., 2021). Differences in microbial community diversity driven by early life exposure to both hypoxia and warming could permanently alter nitrogen processing rates, thereby affecting oysters' long-term ability to assimilate and store nitrogen. Carryover effects on nitrogen tissue storage may only manifest at warm Year 2 temperatures because of changes in microbial physiology associated with warming (e.g., faster at warm temperatures, Price & Sowers, 2004) or because changes in oyster nitrogen processing caused by early life environments are more evident in higher growth environments (Figure S3; Bayne, 2009). Temperatures in our warming treatment are within oysters' thermal tolerance (Shumway, 1996), so may only have been stressful to oysters when they were exposed to hypoxia simultaneously. Regardless of the mechanism, early life environments continue to affect oysters' ability to store nitrogen 1 year

later, suggesting the importance of carryover effects in ecosystem service delivery.

While carryover effects of hypoxia and warming combined allowed oysters to store as much nitrogen in their tissue, and thus overall, as control oysters, these oysters stored the least nitrogen in their shell growth on average compared with other oysters in warm Year 2 temperatures (with and without hypoxia, Figure 3b). Nitrogen is an important component of the proteinaceous organic matrix in shells of calcifying organisms such as oysters that is central to the biomineralization process (Joubert et al., 2014). Declines in shell nitrogen content that we observed in oysters exposed to early life hypoxia (with or without warming) may therefore have important consequences for oyster shell structure and exacerbate the negative effects of climate change on marine calcifiers (Ries et al., 2009). Whether nitrogen is stored in tissue or shell also has important implications for the efficacy of oysters as a mechanism for nitrogen removal in coastal systems (Higgins et al., 2011). Nitrogen stored in tissue is likely removed for a shorter time than nitrogen in shell because oyster shells maintain their integrity even after death while tissue readily decomposes, releasing nitrogen back into the system (Kellogg et al., 2014).

The nitrogen content of our experimental oysters (Table S4) is similar to those found in previous studies (Kellogg et al., 2014). Hence, our results show that hypoxia and warming have similar effects on oyster nitrogen storage as other environmental conditions, but that past experiences continue to be influential. These carryover effects were also context-dependent, which may be especially likely in sessile species such as oysters that lack the behavioral capacity to alter their environment if they help organisms acclimate in ways that are adaptive (Moore & Martin, 2019). Indeed, we found that oysters exposed to multiple stressors early in life (hypoxia *and* warming) grew more tissue relative to shell than oysters exposed to a single stressor early in life across multiple Year 2 environments (Year 2 control and hypoxia, warm; Figure 2). This positive carryover effect of multiple stress exposure opposes patterns found in our earlier work that found negative carryover effects of hypoxia and warming on relative tissue growth when oysters were re-exposed to those conditions 3 months later (Donelan et al., 2021). These contrasting patterns may have emerged due to changes in physiological functioning caused by carryover effects (sensu Bianchini & Wright, 2013) that impact oysters differently as they age. Oysters have varying energetic demands throughout ontogeny—as they mature, storing energy for future gonad production becomes more critical for fitness (Thompson et al., 1996). A change in physiological functioning that reduces relative tissue growth early in life may instead reduce relative shell growth later in life because of the importance of adding tissue mass as oysters approach maturity. This also could explain why Year 1 warming reduced oyster tissue growth while Year 2 warming increased tissue growth (Figure S3). At ~14 mm shell length on average, our experimental oysters were small for their age, likely due to abnormally low salinity in Chesapeake Bay across experimental years (Maryland Department of Natural Resources, 2022), and hence still sexually immature. But if the positive effects we observed here continue to influence oysters as they become mature, early life exposure to multiple climate change stressors may actually increase oyster fecundity and alter oyster sex ratios (oysters are protandrous hermaphrodites; Thompson et al., 1996) in particular environments.

Interestingly, exposure to certain stressors had a greater effect on growth and nitrogen storage if it occurred earlier versus later in life. Organisms are often particularly sensitive to stress early in life ("sensitive windows," Fawcett & Frankenhuis, 2015), so exposure to stress during this stage may trigger physiological or energetic changes that cannot be easily reversed, as discussed above. Oysters in Chesapeake Bay spawn in spring and early summer (Mann et al., 2014) when water temperatures are warming especially quickly, likely due to climate change (Hinson et al., 2021), which may simultaneously speed the onset of seasonal coastal hypoxia (Fennel & Testa, 2019). Oysters may therefore be increasingly likely to experience these stressors simultaneously when they are young, with important fitness consequences later in life.

When extrapolated to the reef scale, our results suggest the potential for carryover effects to alter ecosystem services at a landscape level. Differences in early life environments affected estimated reef storage capacity by as much as 41%, with up to 101 fewer kilograms of nitrogen stored per acre in some environments (Figure 4c). Compared with early life control environments, carryover effects decreased potential nitrogen storage in most scenarios for both oyster size classes considered. These reductions were especially prevalent in warm potential reef environments, where exposure to any stressor early in life (hypoxia, warming, or both) reduced nitrogen storage compared with control early life conditions. Average summertime temperatures in Chesapeake Bay are likely to be 2.5°C higher in the coming decades (Hinson et al., 2021); in these conditions, our results suggest that carryover effects may reduce oysters' ability to provide a critical ecosystem service. Interestingly, carryover effects also increased oyster nitrogen storage, but only for potential reefs at ambient temperatures (for both size classes), suggesting that temperature is an important driver of carryover effects on oyster nitrogen bioassimilation. These effects could operate via physiological changes in oysters themselves or in their associated microbiome, as suggested above, and future work should explore these potential metabolic mechanisms.

Harris Creek is one of the largest oyster restoration projects in the world, with 348 acres of reef restored (Bersoza Hernández et al., 2018; Maryland Oyster Restoration Interagency Working Group, 2021). On this reef scale, our results suggest that carryover effects have the potential to alter nitrogen stored long-term in oyster tissue and shell by ~35,100 kg (35.1 metric tons), a profound change in ecosystem service potential. While Harris Creek is an oyster sanctuary that prohibits oyster harvest, it provides an illustrative example of the potential for carryover effects to impact an ecosystem service in the field; these results could be similarly applied to systems where oysters are harvested, including aquaculture. Because oysters have the potential to remove hundreds of metric tons of nitrogen from coastal estuaries (Bricker et al., 2020), in-water removal of nitrogen by bivalves is

often cited as a key benefit of habitat restoration projects and aquaculture (Parker & Bricker, 2020) and increasingly considered in watershed management plans enacted to improve water quality (Reichert-Nguyen, 2018). For aquaculture operations, there is interest in compensating growers for nutrients removed via their leases through nutrient trading programs (Depiper et al., 2016; Parker & Bricker, 2020). But estimates of nitrogen bioassimilation by oysters are often based only on oyster size (Higgins et al., 2011) and/or periodic measurements (e.g., monthly) of relevant environmental parameters (Parker & Bricker, 2020). Our results show that past environmental conditions may further affect oyster nitrogen storage via carryover effects, so are critical to consider when estimating nutrient reduction provided by oyster restoration and aquaculture.

Using the nitrogen content of our experimental oysters to model potential differences in nitrogen stored in oyster reefs in the field of course has limitations. For example, carryover effects impacted nitrogen storage in spat-sized oysters (<40 mm shell height) that were more similar in size to our experimental oysters (Figure S5), and previous work has shown that the percent nitrogen in oyster tissue does not vary with oyster size (Dalrymple & Carmichael, 2015). But we do not know whether carryover effects impact oyster nitrogen storage differently as oysters age or become reproductive and theory predicts that early life information should decline in value over ontogeny as new information about the environment becomes available (Fawcett & Frankenhuis, 2015). However, even without these reef-wide projections, our experimental data show the potential for early life experiences to affect nitrogen stored in oysters up to a year later (Figure 3). The estimates we provide here for Harris Creek are meant only to demonstrate the potential large-scale effects that within-generation carryover effects can have on an ecosystem service via changes in individual traits, and more work is necessary to ground truth our reef-scale estimates. Moreover, bioassimilation is only one avenue through which oysters remove nitrogen from coastal systems. Denitrification, DNRA (dissimilatory nitrate reduction to ammonium), and anammox are other pathways of nitrogen conversion by oysters that can have even stronger effects on the coastal nitrogen cycle (Ayvazian et al., 2021; Ray & Fulweiler, 2021). Our work thus highlights only a piece of how carryover effects can impact oysters' ability to deliver ecosystem services. This is an important first step, and future work should explore whether carryover effects influence these other pathways via, for example, altering oyster feeding and egestion rates, to provide a more complete picture of contribution of carryover effects to coastal ecosystem services. Furthermore, extrinsic drivers of oyster nitrogen bioassimilation are not well quantified; for example, nitrogen bioassimilation rates are correlated with changes in site (Higgins et al., 2011; Westbrook et al., 2018) and season (Reitsma et al., 2017) in the field, but mechanistic explorations of environmental effects on nitrogen content via experimentation are lacking. Hence, the potential for carryover effects to impact processes at the ecosystem scale and to persist across multiple years should also be explored across a wider range of environmental conditions to assess how they might be operating in natural systems.

Phenotypic plasticity in individual functional traits in response to current environments has long been known to have higher-level ecological effects, and studies linking the two have revealed previously unknown mechanisms of ecological change (Miner et al., 2005; Schmitz, 2006). Despite their potential to affect similar traits, within-generation carryover effects have yet to be incorporated into this eco-evolutionary framework. Our results demonstrate the need to include within-generation carryover effects not only in assessments of individual and population performance as others have done (Donelan & Trussell, 2019; Stuligross & Williams, 2021) but also in assessments of ecosystem-level processes. Mounting evidence suggests that carryover effects operate in a diversity of species (Donelan & Trussell, 2019; Stuligross & Williams, 2021; Sutton et al., 2021; Trontin et al., 2020), so their ecological impacts are likely widespread and affecting ecosystem processes in countless other systems. While challenging, our data suggest that incorporating carryover effects into assessments of ecosystem service delivery is a critical step to accurately assess how phenotypic variation among individual organisms affects the functioning of natural ecosystems.

AUTHOR CONTRIBUTIONS

Sarah C. Donelan conceived and designed the study, collected, analyzed, and visualized the data with input from Matthew B. Ogburn and Denise Breitburg, and drafted the manuscript. All authors contributed equally to manuscript revisions.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at http://doi.org/10.25573/serc.21702062 (Donelan et al., 2022).

ORCID

Sarah C. Donelan https://orcid.org/0000-0002-4066-7884
Matthew B. Ogburn https://orcid.org/0000-0001-5417-555X
Denise Breitburg https://orcid.org/0000-0002-5453-6973

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SUPPORTING INFORMATION

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