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HYPOXIA AND ANOXIA TOLERANCE IN DIPLOID AND TRIPLOID EASTERN OYSTERS AT HIGH TEMPERATURE

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ABSTRACT Increasing reliance on the use of triploid oysters to support aquaculture production relies on their generally superior growth rate and meat quality over that of diploid oysters. Reports of elevated triploid mortality have generated questions about potential trade-offs between growth and tolerance to environmental stressors. These questions are particularly relevant as climate change, coastal activities, and river management impact water salinity, temperature, nutrients, pH, and oxygen levels within key estuarine oyster growing areas. In particular, the co-occurrence of warm water temperatures and low dissolved oxygen concentration (DO) events are increasingly reported in estuaries, with potentially lethal impacts on sessile, oyster resources. To investigate potential differences in DO tolerance, diploid and triploid market-sized or seed oysters were exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) at 28°C and their mortalities were monitored. The hemolymph of the market-sized oysters was collected to measure cellular and biochemical changes in response to hypoxia and anoxia, whereas their valve movements were also measured. In general, about half of market-sized oysters died within about 1 wk under anoxia (LT_{50}: 5.7–8.9 days) and within about 2 wk under hypoxia (LT_{50}: 11.9–19.4 days) with diploid oysters tending to die faster than triploid oysters. Seed oysters took longer to die than market-sized oysters under both anoxia (LT_{50}: 9.5–12.1 days) and hypoxia (LT_{50}: 21.8–25.0 days) with diploid oysters (LT_{50}: 9.5–11.8 days) dying slightly faster than triploid oysters (LT_{50}: 11.8–12.1 days) under anoxia. Hemolymph pH decreased and plasma calcium and glutathione concentrations increased with decreasing DO, with values under anoxia being different than those under normoxia. Hemocyte density was also lower under anoxia than under either normoxia or hypoxia. Overall, few differences in physiological responses to hypoxia and anoxia were found between diploid and triploid oysters suggesting that ploidy (2N versus 3N) had limited effect on the tolerance and response of eastern oysters to low DO.

KEY WORDS: *Crassostrea virginica*, ploidy, low dissolved oxygen, valve movement, hemolymph pH, calcium, glutathione

INTRODUCTION

The eastern oyster, *Crassostrea virginica* is a commercially valuable fisheries product for coastal communities along the U.S. east coast and the northern Gulf of Mexico (GoM) with the GoM region currently sustaining the largest harvest by volume (Fisheries 2020). Despite its success, significant variation in the GoM oyster production due to overharvesting, habitat destruction, and more recently, environmental conditions tied to more extreme climate and weather events (Beseres Pollack et al. 2012, La Peyre et al. 2013, Soniat et al. 2014, Gledhill et al. 2020, Moore et al. 2020, Du et al. 2021) highlights the need for aquaculture to complement and stabilize production. With the use of hatchery produced seedstock and improved off-bottom grow-out methods, oyster aquaculture is now fast expanding in the GoM estuaries (Walton et al. 2013, Leonhardt et al. 2017, Wadsworth et al. 2019a, Swam et al. 2022).

Triploid oysters are preferred seedstocks and a key factor in the growth of the aquaculture industry, both in the GoM and globally (Callam et al. 2016, Wadsworth et al. 2019a, Hudson & Virginia Sea Grant Marine Advisory Program 2019, Yang 2022). Triploid oysters (3N) possess three sets of chromosomes instead of the normal two sets (2N), a condition which disrupts the development of reproductive tissue and drastically reduces the ability to spawn (Allen & Downing 1986, 1990, Guo & Allen 1994). As a result, triploid oysters generally grow faster (Stanley et al. 1984) and maintain superior meat quality during the reproductive season (Hand & Nell 1999). Under certain environmental conditions limiting gonad development, such as low salinity or low food concentrations, the advantages of triploidy are reduced or disappear (Davis 1994, Callam et al. 2016, Wadsworth et al. 2019b). Davis (1994), for example, found that diploid and triploid Pacific oyster (*Crassostrea gigas*) growth rates were comparable at sites experiencing lower temperatures and reduced food supply. In another study, Callam et al. (2016) reported that triploid eastern oysters grew slower than diploids in low salinity. Higher rates of eastern oyster triploid mortality compared with diploid were also recently observed in Alabama and Louisiana at low salinity (Wadsworth et al. 2019b, Bodenstein et al. 2023). These studies suggest that triploids may be more vulnerable to certain environmental stressors and raise questions about potential trade-offs between oyster growth and environmental tolerance.

As eutrophication and climate change continue to impact coastal regions, including the GoM, the co-occurrence of warm water and low oxygen is becoming increasingly common in areas where oysters grow (Diaz & Rosenberg 2008, Breitburg et al. 2018). Dissolved oxygen concentration (DO) low enough to cause a stress response is known as hypoxia,

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a term generally reserved for DO less than 2.0 mg L^{-1}. As the spatial and temporal extent of hypoxia increases in estuaries and coastal ecosystems (Tilman et al. 2001, Rabalais et al. 2009), understanding its impacts on marine species, including on the production of eastern oysters, is critical. In particular, understanding how different ploidies may respond to hypoxia and high temperature stress could enable the selection or development and improvement of seedstocks to ensure consistent production. The generally larger size of triploids combined with predicted larger cell volumes (from increased chromosome numbers) both acting to lower the availability of oxygen could reduce the ability of triploids to maintain aerobic metabolism at higher temperature and decrease their tolerance to low DO compared with diploids.

To explore potential difference in tolerance between diploid and triploid *Crassostrea virginica*, we compared their lethal and sublethal responses during exposure to varying levels of DO. In two studies, we exposed market-sized (>75 mm; Study 1) and seed (26–75 mm; Study 2) oysters to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) at an elevated temperature (28 °C) for several weeks. Cumulative mortality, as well as valve movement, and changes in potential sublethal biomarkers (hemolymph pH, hemocyte density, granulocyte percentage, and plasma calcium, protein, and glutathione concentrations) were assessed as potential indicators of response to hypoxia and anoxia. Triploid oysters were hypothesized to be less tolerant of low DO conditions, with faster mortality, and more evidence of disruption in the physiological changes that comprise the stress response to hypoxia and anoxia.

MATERIALS AND METHODS

Oysters

Study 1

In November 2021, 28-mo-old diploid oysters grown in mesh baskets suspended on long lines (BST Oyster Co., Cowell, South Australia) at the Louisiana Sea Grant Oyster Research Farm, adjacent to the Louisiana Sea Grant Oyster Research Laboratory and Mike C. Voisin Oyster Hatchery in Grand Isle, LA, were collected within a similar size range. Shell height was measured in a sample of 50 diploid and 50 triploid oysters. Condition index and *Perkinsus marinus* infection intensity were determined in a sample of 18 diploid and 18 triploid oysters. Condition index was calculated as the ratio between dry meat weight to the whole oyster weight minus its shell weight (i.e., filled cavity weight) multiplied by 100 (Abbe & Albright 2003). The *P. marinus* infection intensity was determined using the whole oyster procedure detailed in Casas et al. (2017). Oysters were further classified as uninfected, lightly infected ($<1 \times 10^4$ parasites g^{-1} wet tissue), moderately infected ($1 \times 10^4 - 5 \times 10^5$ parasites g^{-1} wet tissue), or heavily infected ($>5 \times 10^5 g^{-1}$ wet tissue) (Bushek et al. 1994, Casas et al. 2017).

The oysters were transported to the LSU Animal and Food Science Laboratory (AFL) in Baton Rouge, LA, and placed into six 800-L tanks (30 2N and 30 3N per tank) equipped with biofilters and filled with aerated artificial seawater (Crystal Sea Marinemix, Marine Enterprises International, Baltimore, MD) adjusted to a salinity of 17 and temperature of 22 °C, similar to field conditions. Salinity, temperature, and DO were measured daily with a YSI-Pro30 handheld multimeter (YSI Incorporated, Yellow Springs, OH). Water quality (ammonia, nitrite, and nitrate) in the tanks was checked once a week using test strips (Lifeguard Aquatics 5-way Test Strips and Ammonia Test Strips, Santa Fe Springs, CA).

The diploid oysters were the progeny of wild (2N) oysters collected from Sister Lake, LA (29° 14′ 45.0″ N, 90° 54′ 35.0″ W; Bodenstein et al. 2023). They were produced at the Mike C. Voisin Oyster Hatchery in June 2019 by fertilizing the eggs of three female oysters with the sperm of four male oysters. At the same time, the triploid oysters were produced by fertilizing the eggs of six Sister Lake (2N) female oysters with the sperm of two male oysters from the 4DGN17 tetraploid (4N) line. Sperm were verified to be from tetraploid males by flow cytometry prior to fertilization (Allen & Bushek 1992). Larvae were reared and set on microcultch material to produce single oyster spat using standard hatchery techniques (Wallace et al. 2008). Spat were grown in upwelling nursery systems from July to September 2019 until oysters were large enough to be deployed in mesh baskets suspended on long lines adjacent to the hatchery for further growth.

Study 2

In December 2021, about 250 diploid (42 ± 5 mm) and triploid (41 ± 4 mm) 7-mo-old, half-sibling oysters grown in mesh baskets suspended on long lines at the Grand Bay Oyster Park (GBOP), Alabama, were obtained from the Auburn University Shellfish Laboratory in Dauphin Island, AL. The diploid and triploid oysters were transported to AFL and placed into six 400-L tanks (40 2N and 40 3N per tank) equipped with biofilters and filled with aerated artificial seawater adjusted to a salinity of 20 and temperature of 17 °C, similar to field conditions. All water-related parameters were measured as described for Study 1.

The diploid oysters (2M2LAFT21) were the progeny of the 2MLAFT19 oyster line and were produced by fertilizing the eggs of 56 diploid females with the sperm of 26 diploid males in May 2021. On the same day, the triploid oysters (3MLAFTFL21) were produced by fertilizing the eggs from the same 56 2MLAFT19 diploid females with the sperm of 8 tetraploid males of the 4MAPCK19 tetraploid oyster line.

Experimental Design

For both Studies (1 and 2), two replicate tanks were used for each DO level (2 replicate tanks × 3 DO levels = 6 tanks total). One week after placing oysters in tanks at the AFL, water salinity was adjusted to 20 and temperature to 25 °C at a rate of 1 °C per day using submersible heaters (Hygger Saltwater Tank Titanium Tube Submersible 200 W). After an additional week of acclimation at 25 °C, temperature was raised to 28 °C at a rate of 1 °C per day. From the start of each study, oysters were fed approximately 5% of their dry meat weight with Shellfish Diet 1800® (Reed Mariculture Inc, Campbell, CA) once a day.

Once the target temperature of 28 °C was reached, oysters were acclimated for 36 h before DO was adjusted to achieve the three target levels. For both studies, aeration continued as before in two of the tanks to serve as controls (normoxia). Aeration was reduced in two of the other tanks enabling adjustment of DO to below 2.0 mg L^{-1} (hypoxia). In the remaining two tanks, the air stones were removed, and gas was injected to

reduce DO to below 0.5 mg L^{-1} (anoxia). For Study 1, a nitrogen (95%) and carbon dioxide (5%) gas mixture was used to achieve anoxia (hypercapnic), whereas in Study 2, only nitrogen gas (hypocapnic) was used because of issues obtaining the gas mixture. The transition from normoxia to hypoxia or anoxia took 1–2 days, depending on the tank. For hypoxia and anoxia tanks, Day 0 was designated as the first day the DO reached the target level. For normoxia tanks, Day 0 was designated as the first day that all hypoxia and anoxia tanks had achieved their target DO level. Water salinity, temperature, DO, and pH were measured twice daily, along with the number of live and dead oysters. Dead oysters were immediately removed upon observation. The pH of water samples was measured using a tabletop pH meter equipped with a microelectrode (Thermo Scientific™ Orion™ PerpHecT™ ROSS™ combination pH Micro electrode, Fisher Scientific, Suwanee, GA).

In Study 1, 15 diploid and triploid oysters were sampled from each of the six tanks (15 oysters × 2 ploidies × 6 replicate tanks = 180 samples) on Day 3 of hypoxia and anoxia exposure. For each oyster, hemolymph was slowly withdrawn with a syringe from the adductor muscle sinus immediately after notching the shell dorsal side and hemolymph pH, hemocyte density, and granulocyte percentage (%) were measured as described in the following. Each hemolymph sample was then centrifuged at 400 g for 15 min and the supernatant or plasma was collected and stored at −80 °C until protein, calcium, and glutathione concentrations were measured as described in the following.

Assays

Hemolymph pH was measured using a tabletop pH meter equipped with a Thermo Scientific Orion PerpHecT ROSS™ combination pH Micro electrode. Hemocyte density (cells mL^{-1}) and granulocyte % were determined with improved Neubauer hemocytometers (Reichert, Buffalo, NY) as described by La Peyre et al. (1995). Plasma protein concentration (mg mL^{-1}) was measured using Pierce Biotech Micro BCA Protein Assay Kit (Rockford, IL) with bovine serum albumin as a standard. Plasma calcium ion concentration (mM) was measured using Sigma-Aldrich MAK022 Calcium Colorimetric Assay Kit (St. Louis, MO). Plasma glutathione concentration (μM) was measured using BioAssay systems, DIGT-250 QuantiChrom™ Glutathione (GSH) Assay kit (Bioassay systems, Hayward, CA) with all plasma samples adjusted to 2.5 mg mL^{-1} with distilled water prior to measurement (La Peyre et al. 2014). All measurements were done in duplicate.

Valve Movement

At the end of Study 1, four diploid oysters and triploid oysters in each of the two normoxia tanks (4 oysters × 2 ploidies × 2 tanks = 16 oysters) were used to measure valve movement first under continuous normoxia, followed by continuous hypoxia in one of the two tanks and continuous anoxia in the other tank, as described in the following. Valve movement was measured with a noninvasive system, detailed by Casas et al. (2018). For each oyster, a small magnet was glued to one valve at the maximum distance from the hinge, and a Hall element sensor (HW-300a, Asahi Kasei, Japan) coated in epoxy was glued directly across from the magnet on the other valve. The magnetic field in the form of output voltage (μV) was recorded every 30 sec by dynamic strain recording devices (DC 204R, Tokyo Sokki Kenkyujo Co., Shinagawa-ku, Tokyo, Japan).

Oysters were initially exposed to 3 days of normoxia, at which point DO was decreased to a hypoxic level in one tank (Tank 1) and to an anoxic level in the other tank (Tank 2) as described in Study 1. After 1 day of transition from normoxia to hypoxia or anoxia, DO levels in both systems were maintained for 3 additional days. After 3 days of hypoxia or anoxia exposure, recording stopped, oysters were opened, calibration wedges of known width (1–6 mm) were placed between the oyster valves, and the output voltage was measured with each wedge to create a voltage-to-width linear regression equation specific to each oyster. The relationships between voltage and wedge width (i.e., valve opening) were strong ($r^2 > 0.85$). Valve movement data were converted into gape angles (θ in degrees) as described in Wilson et al. (2005). Gape angle values were expressed as a percentage of the maximum gape angle recorded during the 7-day exposure and categorized as closed for values less than or equal to 10% (Comeau et al. 2018) or otherwise opened. For two oysters, this threshold was not an accurate representation of closing behavior, so "closed" was defined as 25% for oyster 2N–4 (Fig. 1) and 15% for oyster 2N–7 (Fig. 2). These thresholds were defined because of the difficulty of defining a "fully closed" or "fully open" state for these oysters due to variations at both ends of valve amplitude. Using this categorization, the percentage of time spent open (% time open) was calculated, along with the number of times an oyster opened (# times opened), the average duration of an opening period (average time open), and the mean gape angle when open for each individual oyster during the 3 days of normoxia and 3 days of hypoxia or anoxia.

Statistical Analyses

In both studies, ratio tests (Wheeler et al. 2006) from the R package "ecotox" were used to compare oyster mortality by DO level, and by ploidy (2 replicates × 3 DO levels = 6 tanks). For each ploidy within each tank, LT_{50} with 95% confidence intervals were also calculated using the same R package.

In Study 1, shell height, condition index, and body burden were analyzed with a one-factor (ploidy) analysis of variance (ANOVA). Data on hemolymph pH, hemocyte density, granulocyte % and plasma protein, calcium, and glutathione concentrations were grouped by DO level and ploidy so that replicate tanks were combined (generally 15 oysters × 2 replicates = 30 samples per group). As some samples contained no hemolymph as indicated by very low protein concentration (<0.5 mg mL^{-1}) and few hemocytes, so the sample size for each group (3 DO levels × 2 ploidies = 6 groups) ranged from 26 to 30. Data were examined for normality and homogeneity of variance. Data that fulfilled ANOVA requirements (granulocyte %, plasma protein concentration, and plasma glutathione concentration) were analyzed with a two-factor (DO level, ploidy) ANOVA. When significant differences were found ($P < 0.05$), Tukey's HSD test was used for pairwise multiple comparisons. Data that did not fulfill the ANOVA requirements (hemolymph pH, hemocyte density, and

plasma calcium concentration) were instead analyzed with a Kruskal–Wallis nonparametric rank-sum test, followed by a pairwise *t*-test with a Bonferroni correction when significant differences were found.

Valve movement data were examined for normality and homogeneity of variance. Since oysters were exposed to normoxia followed by either hypoxia (Tank 1) or anoxia (Tank 2), one-factor repeated measure ANOVAs from the R package "rstatix" were used to compare oyster valve movement parameters (% open, # times opened, average time open, and mean gape angle) between DO levels within each tank. To compare valve movements between normoxia exposures, and between hypoxia and anoxia exposures, where all measurements were independent, one-factor ANOVAs were used. One-factor ANOVAs were also used to compare between diploids and triploids within each tank and DO level. All analyses were performed using R 4.0.1 (R Foundation for Statistical Computing 2022).

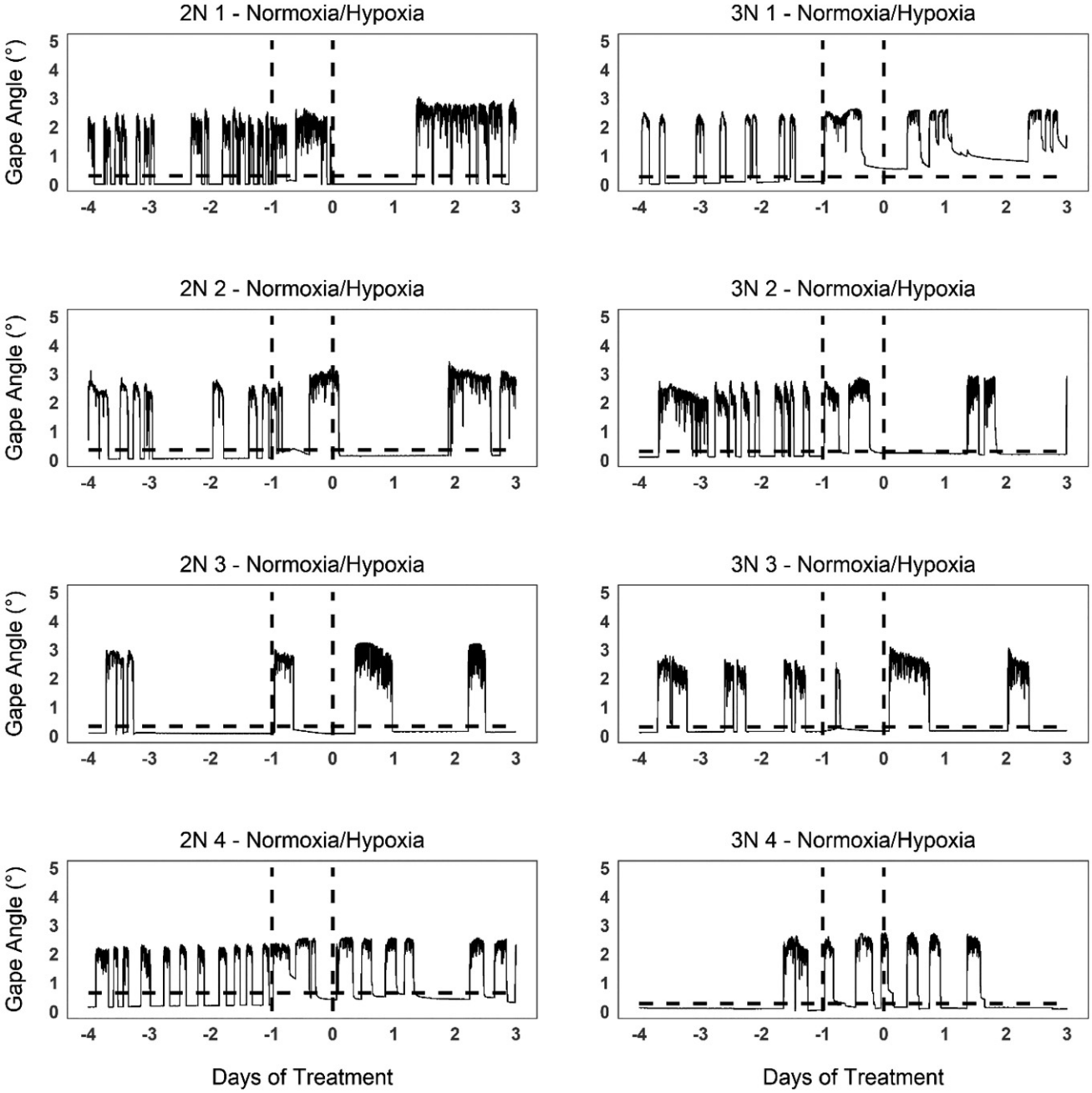

Figure 1. Valve movement (Gape Angle, °) of market-sized diploid (2N) and triploid (3N) oysters during exposure to 3 days of normoxia (DO > 5.0 mg L^{-1}; Day -4 to -1) followed by 3 days of anoxia (DO < 0.5 mg L^{-1}; Day 0–3) in Study 1 follow-up. Air stones were removed and bubbling of a nitrogen (95%) and carbon dioxide (5%) gas mixture began on Day 1. Anoxia was achieved on Day 0 of "Days of Treatment." Vertical dashed lines indicate the beginning and end of the transition from normoxia to anoxia, and the horizontal dashed line indicates the threshold used to categorize closed (below) and open (above) state for each oyster. Oyster "3N–5" died after 1.5 days of anoxia exposure.

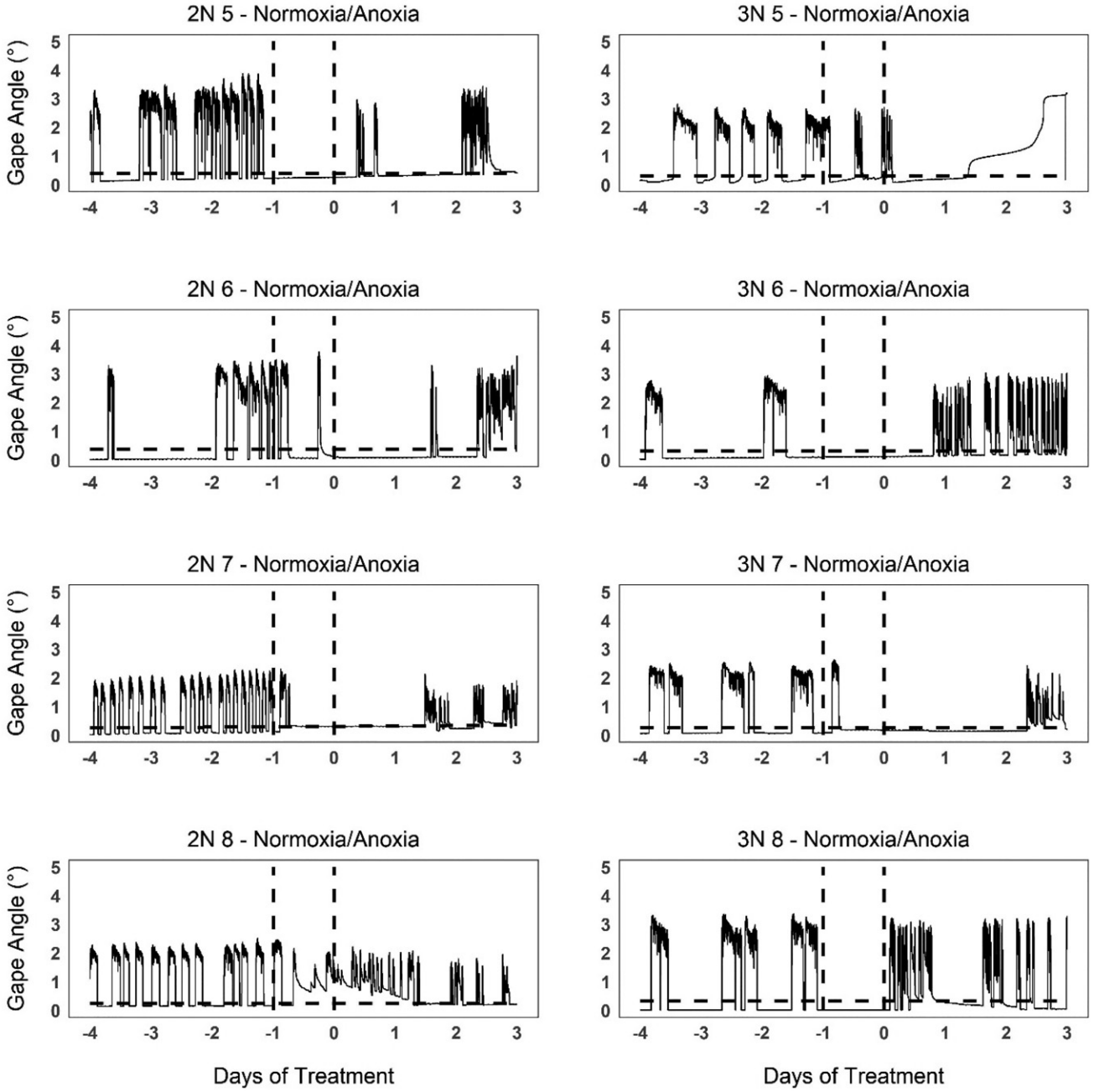

Figure 2. Cumulative mortality (%) of diploid (square) and triploid (triangle) market-sized oysters and dissolved oxygen concentration (filled circles) during Study 1. Hypoxia and anoxia were reached on Day 0, 2 days after all air stones delivering air to the replicate tanks were removed (hypoxia and anoxia) and a nitrogen (95%) and carbon dioxide (5%) gas mixture began to be bubbled into the water (anoxia). Normoxia replicate tanks were supplied with continuous aeration for the duration of the experiment. Dashed lines indicate the maximum desired dissolved oxygen concentration for hypoxia and anoxia treatments.

RESULTS

Study 1

Oyster Shell Height, Condition Index, and *Perkinsus marinus* Infection Intensities

No differences in shell height ($P = 0.075$) were found between diploid and triploid oysters (mean ± SD in mm, 2N:118 ± 11, 3N:123 ± 11). Condition index of the triploid oysters (12.9 ± 2.4) was greater ($P < 0.001$) than that of diploid oysters (7.4 ± 2.0). *Perkinsus marinus* infection intensity was similar ($P = 0.441$) in diploid and triploid oysters (in $\log_{10}$ parasites, g^{-1} wet meat, 2N:3.3 ± 1.1, 3N: 2.9 ± 1.7). In diploids, 67% of the oysters were lightly infected (<10^4 parasites g^{-1} wet tissue) and the remaining 33% had moderate infections ($10^4 - 5 \times 10^5$ parasites g^{-1} wet tissue). In triploids, 83% of the oysters were lightly infected, 5% had moderate infections, and 11% had heavy infections (>5×10^5 parasites g^{-1} wet tissue).

TABLE 1.

Mean ± SD water temperature (°C), salinity, dissolved oxygen (DO, mg L^{-1}), and pH of replicate (Rep) tanks of oysters during continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) in Studies 1 and 2.

	Normoxia		Hypoxia		Anoxia	
Study 1	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
Temperature (°C)	27.8 ± 0.1	28.0 ± 0.1	27.8 ± 0.2	27.8 ± 0.2	27.8 ± 0.2	28.3 ± 1.2
Salinity	20.0 ± 0.1	20.0 ± 0.1	20.2 ± 0.1	20.2 ± 0.2	20.1 ± 0.1	20.1 ± 0.1
DO (mg L^{-1})	5.28 ± 0.12	5.14 ± 0.05	1.34 ± 0.37	1.52 ± 0.57	0.56 ± 0.43	0.22 ± 0.08
pH	7.87 ± 0.04	7.82 ± 0.80	7.30 ± 0.50	7.41 ± 0.50	7.03 ± 0.30	6.99 ± 0.24
	Normoxia		**Hypoxia**		**Anoxia**	
Study 2	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
Temperature (°C)	28.0 ± 0.2	27.7 ± 0.4	28.1 ± 0.2	28.2 ± 0.2	27.9 ± 0.2	27.8 ± 0.6
Salinity	20.2 ± 0.1	20.4 ± 0.1	20.3 ± 0.1	20.3 ± 0.1	20.2 ± 0.1	20.2 ± 0.1
DO (mg L^{-1})	5.47 ± 0.16	5.45 ± 0.23	1.42 ± 0.34	1.51 ± 0.29	0.42 ± 0.16	0.36 ± 0.11
pH	7.60 ± 0.07	7.66 ± 0.08	7.25 ± 0.11	7.31 ± 0.10	7.58 ± 0.08	7.60 ± 0.06

In Study 1, anoxia conditions were obtained by bubbling a nitrogen (95%) and carbon dioxide (5%) gas mixture into the water, whereas in Study 2, anoxia conditions were obtained using nitrogen (100%) gas.

Water Quality

Throughout the study, water temperature, salinity, and DO remained relatively constant (Table 1), except in the anoxia tanks, where DO increased above 0.5 mg L^{-1} for about ½ day to 2½ days depending on the replicate tank (Fig. 3). Levels of ammonia, nitrite, and nitrate remained at or below 1.0, 1.0, and 25 ppm, respectively and within nonharmful ranges at all times (Epifanio & Srna 1975).

Throughout the second part of the experiment to measure valve movement, the temperature, salinity, and DO during normoxia, hypoxia, and anoxia exposure were maintained at the desired levels (Table 2).

Mortality

After 3 days of hypoxia and anoxia exposure, at the time of sampling, cumulative mortalities among diploids and triploids in all tanks were less than or equal to 10% (Fig. 3). By Day 12, all diploids and triploids exposed to anoxia were dead. At the end of the study on Day 21, 86% and 100% of the diploids, and 75% and 100% of the triploids exposed to hypoxia in the two replicate tanks were dead. In contrast, 14% and 38% of the diploids and 10% and 12% of the triploids exposed to normoxia in two of the other replicate tanks were dead by Day 21.

Mortality significantly differed by DO level ($P < 0.001$; Appendix Table 1). Regardless of ploidy, the LT_{50} of oysters under anoxia was about 8 days earlier than oysters under hypoxia, and 22 days earlier than oysters under normoxia (Table 3).

Mortality significantly differed by ploidy with triploid oysters dying slower than diploids under normoxia ($P < 0.001$) and under hypoxia ($P < 0.001$), but only for one replicate tank at each DO level (Table 3, Appendix Table 2).

Hemolymph and Plasma Properties

Oysters under hypoxia had lower granulocyte % than those under normoxia and anoxia ($F_{2,165} = 5.982$, $P = 0.0031$) and triploids had higher granulocyte % than diploids ($F_{1,165} = 74.690$, $P < 0.001$; Table 4). Oysters exposed to hypoxia had higher plasma protein concentration than those exposed to normoxia and anoxia ($F_{2,166} = 5.216$, $P = 0.006$; Table 4). Oysters exposed to anoxia had higher glutathione concentration than those exposed to normoxia and hypoxia regardless of treatment ($F_{2,166} = 5.704$, $P = 0.004$; Table 4).

Under normoxia, pH was greater in diploids [H (5) = 44.185, $P < 0.001$; Table 4]. Regardless of ploidy, oysters under anoxia had lower hemolymph pH than oysters under normoxia [H (5) = 44.185, $P < 0.001$], lower hemocyte density [H (5) = 90.652, $P < 0.001$], and higher calcium concentrations (H (5) = 106.12, $P < 0.001$) than oysters under normoxia and hypoxia.

Valve Movement

In the normoxia–hypoxia treatment tank (Tank 1), oysters under normoxia opened more times ($F_{1,16}$, $P = 0.03$) but remained open for much shorter periods than when under hypoxia ($F_{1,16}$, $P = 0.01$; Table 5). In the normoxia–anoxia treatment tank (Tank 2), oysters under normoxia had larger mean gape angles than when under anoxia ($F_{1,15}$, $P < 0.001$; Table 5).

Oysters under hypoxia opened fewer times ($F_{1,15}$, $P = 0.03$) and had larger mean gape angles ($F_{1,15}$, $P = 0.001$) than those under anoxia (Replicate 2; Table 5). There were no differences in any valve movement parameters between the normoxia exposures of each tank. Under normoxia, prior to changing DO, the number of times oysters opened differed significantly by ploidy ($F_{1,8}$, $P = 0.03$) but only in one of the two tanks (Tank 2), with triploids opening fewer times than diploids (Table 5). Additionally, in the same tank, the average amount of time oysters remained open significantly differed by ploidy ($F_{1,8}$, $P = 0.003$). Triploid oysters were opened on average about 1.6 h longer during an opening period than diploids (Table 5). There were no differences in any valve movement parameter between diploid and triploids during hypoxia or anoxia exposure (Table 5).

Study 2

Water Quality

Throughout the study, water temperature, salinity, and DO remained relatively constant and close to targeted values (Table 1).

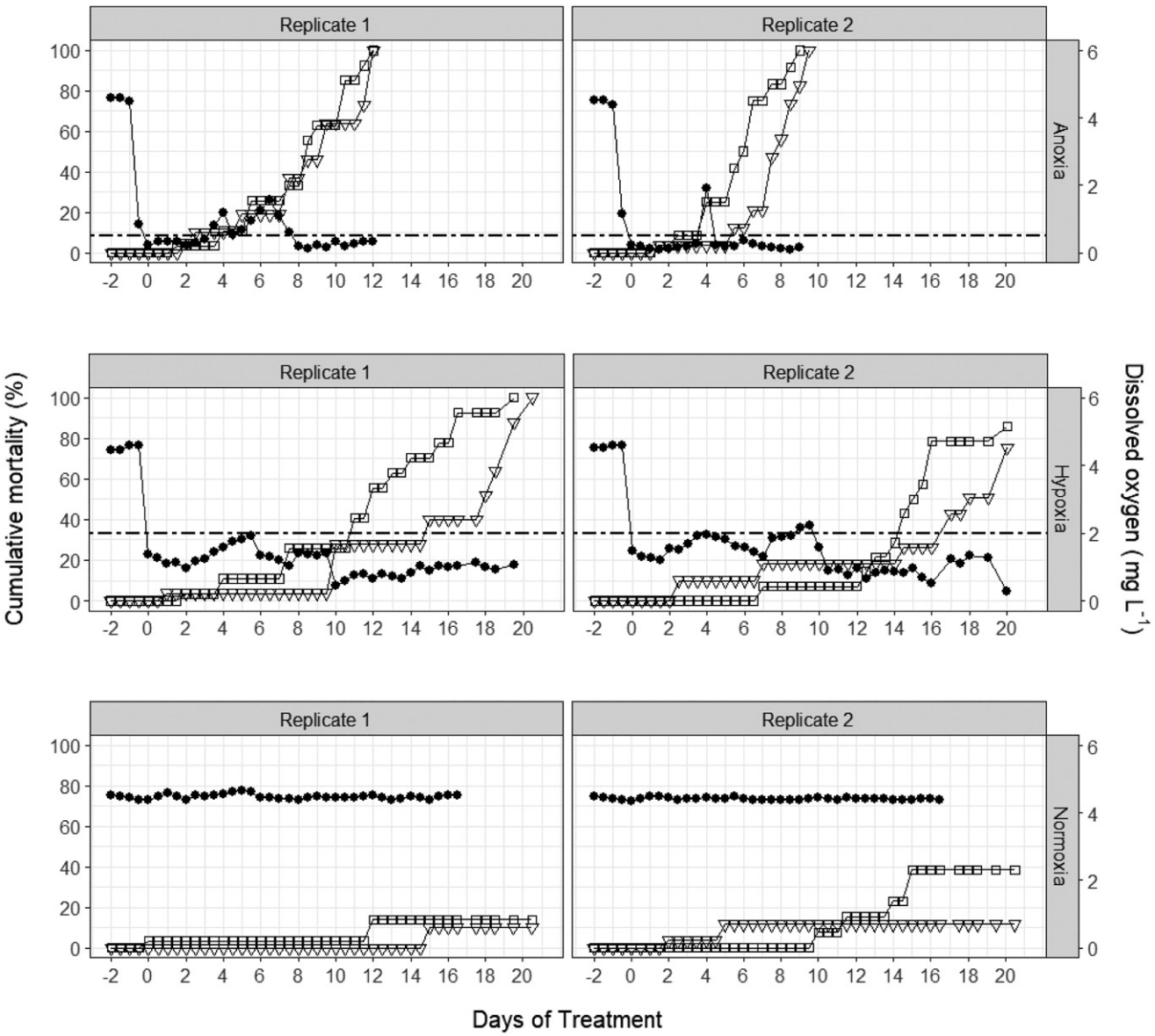

Figure 3. Valve movement (Gape Angle, °) of market-sized diploid (2N) and triploid (3N) oysters during exposure to 3 days of normoxia (DO > 5.0 mg L^{-1}; Day -4 to -1) followed by 3 days of hypoxia (DO < 2.0 mg L^{-1}; Day 0–3) in Study 1 follow-up. Air stones were removed on Day 1, and hypoxia was achieved on Day 0 of "Days of Treatment." Vertical dashed lines indicate the beginning and end of the transition from normoxia to hypoxia, and the horizontal dashed line indicates the threshold used to categorize closed (below) and open (above) state for each oyster.

Mortality

Regardless of ploidy, the LT_{50} of seed oysters significantly differed by DO level ($P < 0.001$; Appendix Table 1). The LT_{50} of oysters under anoxia was about 12 days earlier than oysters under hypoxia, and 58 days earlier than oysters under normoxia (Table 6).

Diploid and triploid seed oysters under anoxia experienced 100% cumulative mortality by Day 17 (Fig. 4). By the end of the experiment on Day 32, diploids and triploids under hypoxia experienced 100% mortality, whereas diploids under normoxia experienced no mortality, and triploids experienced 2.5%–5% mortality.

Mortality only differed by ploidy in the anoxia replicates ($P = 0.003$, $P = 0.018$), where triploid oysters died more slowly than diploids (Fig. 4, Table 6, Appendix Table 3).

DISCUSSION

The objectives of these studies were to compare the tolerance and physiological responses of diploid and triploid seed and market-sized oysters exposed to continuous hypoxia and anoxia at an elevated temperature (28 °C). Overall, seed oysters were more tolerant to low DO compared with market-sized oysters, regardless of ploidy. Sublethal markers indicated similar effects of decreasing DO regardless of ploidy with decreased hemolymph pH and hemocyte density, and increased plasma calcium and glutathione concentrations. Oysters under hypoxia opened fewer times but for longer periods, each time they opened compared with oysters under normoxia or anoxia resulting in no differences in overall percentage of time oysters stayed opened between DO levels. The

TABLE 2.

Mean ± SD water temperature (°C), salinity, dissolved oxygen (DO, mg L^{-1}), and pH of tanks during the measurement of oyster valve movement under normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) in Study 1 follow-up.

Tank 1	Normoxia	Hypoxia
Temperature (°C)	27.8 ± 0.2	27.8 ± 0.2
Salinity	20.1 ± 0.0	20.2 ± 0.0
DO (mg L^{-1})	5.04 ± 0.18	1.43 ± 0.24
pH	7.73 ± 0.04	7.36 ± 0.06
Tank 2	**Normoxia**	**Anoxia**
Temperature (°C)	28.0 ± 0.6	27.7 ± 0.1
Salinity	20.1 ± 0.0	20.2 ± 0.1
DO (mg L^{-1})	5.19 ± 0.22	0.25 ± 0.13
pH	7.66 ± 0.12	7.23 ± 0.04

The valve movement of market-sized oysters under normoxia were measured for 3 days (Day −4 to −1; Figs. 1 and 2) followed by 3 days of either hypoxia (Tank 1) or anoxia (Tank 2) (Day 0–3; Figs. 1 and 2).

TABLE 3.

Median lethal time (LT_{50}; days) with 95% confidence intervals (95% CI) of the market-sized diploid (2N) and triploid (3N) oysters exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) at 28°C in Study 1.

	Normoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	39.0	(27.8, 78.5)	18.9[b]	(17.5, 21.0)
3N	25.3	(21.7, 39.5)	37.6[a]	(27.4, 71.1)
	Hypoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	11.9	(11.0, 13.2)	15.6[b]	(14.6, 17.0)
3N	17.4	(15.4, 20.5)	19.4[a]	(17.0, 23.3)
	Anoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	8.4	(7.6, 9.4)	5.7	(5.2, 5.6)
3N	8.9	(8.0, 10.2)	8.1	(7.3, 9.5)

[a,b] Indicate significant differences in LT_{50} between ploidies within each replicate (Rep) tank as calculated by ratio tests (Appendix Table 1).

gape angle of oysters was smaller under anoxia compared with under normoxia or hypoxia. Overall, few differences between diploid and triploid oysters were found in response to low DO, suggesting tolerance to low DO is not related to ploidy level under the conditions tested.

Our results indicated that triploid oysters were slightly more tolerant to low DO level than diploid oysters of similar shell heights. Both diploid and triploid oysters used in our study were collected to be within the same shell height range to eliminate the confounding effect of size. This approach was taken because of the well-established decreased resilience of larger animals to environmental stressors, including low DO and elevated temperature, reported in oysters, which is attributed to size-related scaling effects on energetics (Sukhotin et al. 2003, Peck et al. 2009, Rybovich et al. 2016, Peralta-Maraver & Rezende 2021). In our study, seed oysters exposed to low DO were more tolerant than market-sized oysters as would be predicted, although differences in pH between Studies 1 and 2 and in oyster genetic background may have had some influence on the results. At elevated temperature, oxygen supply to tissues becomes increasingly limiting to larger animals, resulting in a decrease in tissue oxygen concentration and increasing transition from aerobic to anaerobic metabolism, ultimately resulting in death (Pörtner 2010, Pörtner et al. 2017, Eymann et al. 2020). Although our results indicate that triploid oysters are more

TABLE 4.

Mean ± SD hemolymph pH, hemocyte density, granulocyte %, plasma protein, calcium, and glutathione concentrations of diploid (2N) and triploid (3N) market-sized oysters exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), or anoxia (DO < 0.5 mg L^{-1}) at a temperature of 28°C in Study 1.

	Normoxia		Hypoxia		Anoxia	
Biomarker	**2N**	**3N**	**2N**	**3N**	**2N**	**3N**
Hemocyte density (10^6 mL^{-1})	2.87 ± 2.26[ab]	1.90 ± 1.09[b]	3.76 ± 2.97[a]	2.03 ± 0.82[b]	0.78 ± 0.61[c]	0.61 ± 0.31[c]
Granulocyte %†	30 ± 16	49 ± 16	20 ± 11	40 ± 20	26 ± 14	50 ± 16
Hemolymph pH	7.10 ± 0.30[a]	6.85 ± 0.40[b]	6.86 ± 0.35[ab]	6.73 ± 0.36[bc]	6.52 ± 0.15[c]	6.56 ± 0.12[c]
Calcium (mM)	5.6 ± 0.5[b]	6.3 ± 1.6[b]	6.7 ± 1.0[b]	7.0 ± 1.9[b]	8.6 ± 1.7[a]	9.6 ± 1.9[a]
Protein (mg mL^{-1})*	5.3 ± 2.4	4.9 ± 2.5	5.6 ± 2.6	7.6 ± 3.4	4.8 ± 2.5	5.5 ± 3.0
Glutathione (μM)*	36 ± 24	38 ± 26	36 ± 28	33 ± 21	49 ± 34	52 ± 31

Oysters were sampled after 3 days of normoxia, hypoxia, or anoxia exposure.

[a,b] Indicate significant differences ($P < 0.05$) among treatment groups (DO level × ploidy) as determined by Kruskal–Wallis tests followed by pairwise *t*-tests with a Bonferroni correction.

* Indicates single effect differences by ploidy or DO level.

† Indicates single effect differences by ploidy and DO level as determined by ANOVA tests.

TABLE 5.

Mean ± SD percentage of time spent open (% Open), number of times opened (Times Opened), average amount of time (h) of each opening period (Average Time Open, h), and mean gape angle (Gape angle, °) of 28-mo-old diploid (2N) and triploid (3N) market-sized oysters during exposure to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) at a temperature of 28°C in Study 1 follow-up.

DO Level	Ploidy	% Open	Times Opened	Average Time Open (h)	Gape angle (°)
Tank 1					
Normoxia	2N	34 ± 12	12 ±6	2.3 ± 0.5	2.3 ± 0.3
	3N	33 ± 15	8 ±5	3.4 ± 1.1	2.2 ± 0.0
	All	33 ± 14	10 ±6[A]	2.8 ± 1.0[B]	2.2 ± 0.2
Hypoxia	2N	39 ±7	6 ±3	6.0 ± 2.8	2.6 ± 0.2
	3N	44 ± 33	3 ±1	23.1 ± 28.4	2.1 ± 0.5
	All	41 ± 24	4 ±3[A]	14.6 ± 21.9[A]	2.4 ± 0.5
Tank 2					
Normoxia	2N	44 ± 10	15 ±6[A]	2.3 ± 0.5[B]	2.4 ± 0.5
	3N	37 ±9	5 ±1[B]	5.4 ± 0.9[A]	2.4 ± 0.3
	All	40 ± 10	10 ±7	3.9 ± 1.7	2.4 ± 0.4[A]
Anoxia	2N	37 ± 13	11 ±6	2.6 ± 0.5	1.3 ± 0.5
	3N	33 ±8	14 ± 10	6.0 ± 6.5	1.4 ± 0.4
	All	35 ± 11	12 ±8	4.1 ± 4.6	1.3 ± 0.5[B]

Oysters were exposed to 3 days of normoxia (Day −4 to −1; Figs. 1 and 2), followed by 3 days of hypoxia (Day 0–3; Tank 1) or 3 days of normoxia followed by 3 days of anoxia (Tank 2). Oysters that died during the time of recording were excluded from the analysis; four oysters from each group were used to calculate the mean values, except for triploids in the anoxia exposure where only 3 oysters survived. A single factor repeated measure ANOVA was used to compare the effect of DO level on valve movement within each tank. A single factor ANOVA was used to compare the effect of ploidy on valve movement within each DO level and tank.
[A,B] Indicate significant differences in parameters measured between DO levels within a tank.

TABLE 6.

Median lethal time (LT_{50}, days) with 95% confidence intervals (95% CI) of diploid (2N) and triploid (3N) seed oysters in replicate (Rep) tanks exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) at 28°C in Study 2.

	Normoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	NA	NA	NA	NA
3N	76.0	(57.0, 133.0)	62.2	(50.8, 86.5)
	Hypoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	24.5	(23.1, 26.2)	25.0	(23.7, 26.6)
3N	23.0	(22.0, 24.2)	21.8	(20.9, 22.9)
	Anoxia			
	Rep 1		Rep 2	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI
2N	11.8[b]	(10.9, 13.1)	9.5[b]	(9.0, 10.1)
3N	12.1[a]	(11.6, 12.7)	11.8[a]	(11.5, 12.2)

Both diploid and triploid oysters were included in each replicate tank.
[a,b] Indicate significant differences in LT_{50} between ploidies within each replicate tank as calculated by ratio tests (Appendix Table 1).
NA indicates mortality was too low for CI to be properly calculated.

tolerant to low DO than diploid oysters of similar shell height, it is likely that under favorable field conditions, the opposite might be found between diploid and triploid oysters of similar age as triploid oysters can grow as much as 30% larger than diploid oysters (Shpigel et al. 1992, Dégremont et al. 2012, Walton et al. 2013).

One reason triploid oysters were more tolerant to low DO than diploid oysters in our study, might be related to their greater condition index. The condition index assesses the proportion of available internal cavity capacity occupied by soft tissues and indicates the nutritional status (or meat quality for the industry) of oysters. As such, it is also indicative of a greater energetic reserve in triploid oysters, which may enable them to survive longer than diploids when using less efficient anaerobic energy production under low DO. Another factor known to increase mortality in oysters is pathogen infection by *Perkinsus marinus*, a protist parasite causing dermo disease. Infection intensity was mostly light and similar in triploid and diploid oysters used in Study 1. This level of infection is generally not considered to have much impact on host physiological processes (Paynter 1996). Some of the diploid (33%) and triploid (16%) oysters, however, had moderate (>10^4) and heavy (>5×10^5) infection intensities, which could have affected their response to low DO and would also explain the slow mortality observed in control oysters maintained under normoxia.

In a previous study, Lombardi et al. (2013) measured the number of days until death in 80 mm diploid and triploid eastern oysters exposed to anoxia at 20 °C and salinity of 10. Although no differences in rate of mortality were found in their study, diploid oysters tended to die more slowly (17.0 ± 1.8 days) than triploid oysters (14.8 ± 1.1 days) in contrast to our findings. Comparison between studies, however, is difficult as many exogenous and endogenous factors, including shell height, condition, season, pathogen infection, and genetics, likely influence the tolerance of oysters to low DO. Our studies, for example, were conducted with Fall-collected oysters that may be more resilient than Summer-collected oysters that are reproducing. The primary factor impacting low DO tolerance is increasing temperature, as it not only decreases DO levels in water, but also increases oyster metabolic rate, compounding its effect. At 10 °C, for example, eastern oysters have been shown to tolerate anoxia for several weeks (Stickle et al. 1989). Stickle et al. (1989) showed that their LT_{50} declined rapidly from greater than 28 days to 18 and 4 days when temperature increased from 10 °C to 20 °C and 30 °C under continuous anoxia at a salinity of 20 (Stickle et al. 1989). Overall, our results confirm that oysters, regardless of ploidy, like many other bivalve species, have high tolerance to low DO, especially when temperatures are not excessive.

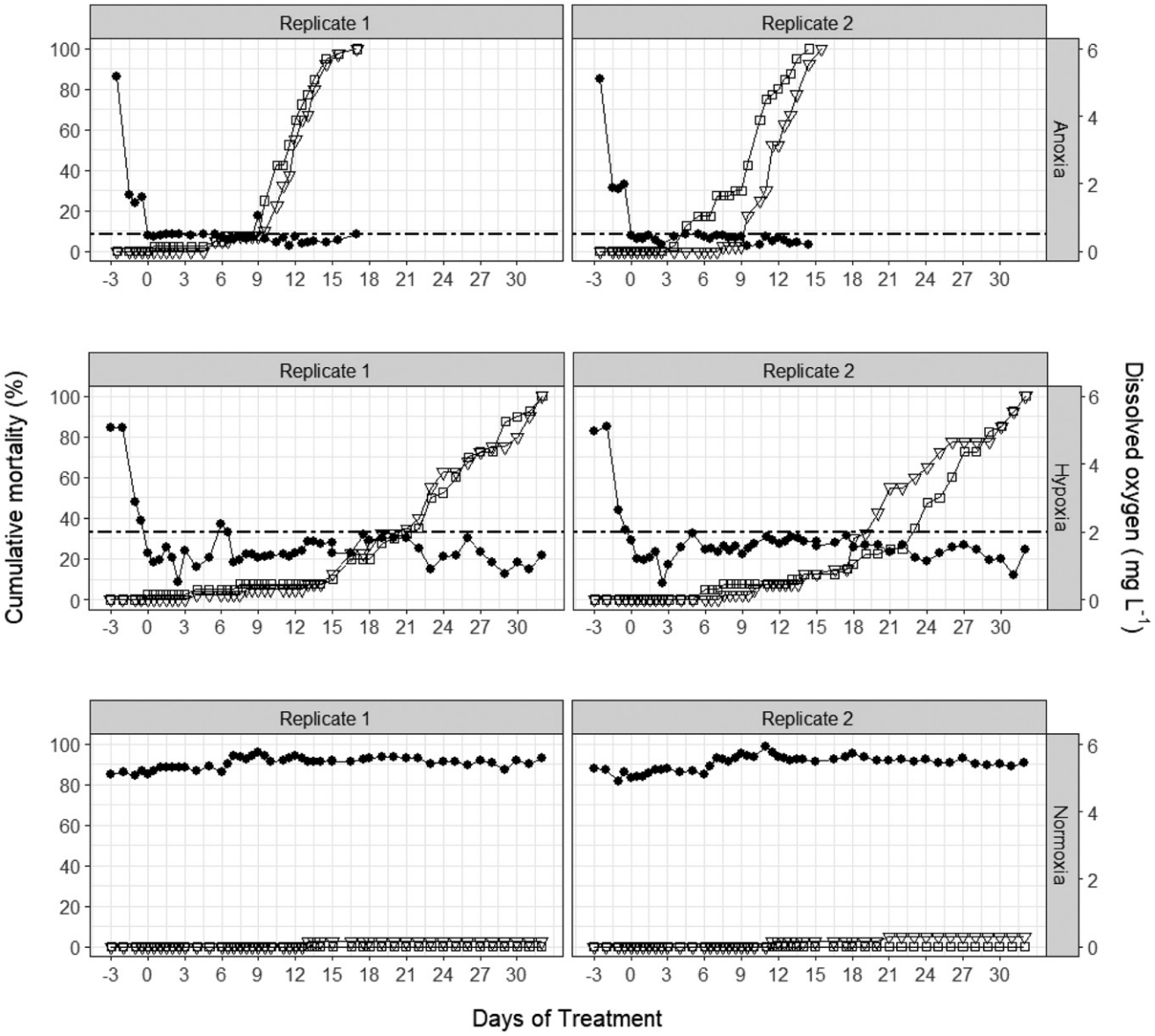

Figure 4. Cumulative mortality (%) of diploid (square) and triploid (triangle) seed oysters and dissolved oxygen concentration (filled circles) in replicate tanks during Study 2. Hypoxia and anoxia were reached on Day 0, 3 days after all air stones delivering air to the tanks were removed (hypoxia, anoxia) and a nitrogen gas began to be bubbled into the water (anoxia). Normoxia tanks were supplied with continuous aeration for the duration of the experiment. Dashed lines indicate the maximum desired dissolved oxygen concentration for hypoxia and anoxia treatments.

Diploid and triploid market-sized oysters under hypercapnic anoxia had similar decreases in hemolymph pH and elevated concentrations of plasma calcium compared with oysters exposed to normoxia and hypoxia. Declining tissue pH (i.e., acidosis) can occur when oysters, as in other bivalves, encounter unfavorable environmental conditions, such as high temperature, low DO, and high CO_2, and undergo anaerobiosis or close their valves (Booth et al. 1984, Boyd & Burnett 1999, Lombardi et al. 2013). In response, calcium can be mobilized into the oyster hemolymph to buffer against acidosis and mitigate its harmful effects (Booth et al. 1984, Dwyer & Burnett 1996, Burnett 1997). In our study, oysters opened less often but for much longer times when opened under hypoxia compared with under normoxia. Moreover, no differences could be shown in the overall percentage of time oysters were opened among DO levels suggesting the decrease in plasma pH was the result of anaerobiosis. Oyster valve movement, however, was highly variable among individuals at all DO levels. Declining hemolymph pH and increased calcium concentrations may simply reflect the change in water conditions as pH decreased and CO_2 increased. Boyd & Burnett (1999) reported a similar effect in hemolymph pH in oysters exposed to hypoxic water.

Plasma glutathione concentrations in diploid and triploid oysters under anoxia were greater than under normoxia with no effect of ploidy. Glutathione is the most abundant antioxidant in living cells (Kelly et al. 1998), and one primary function is to scavenge harmful reactive oxygen species (Meister & Anderson 1983) produced during oxygen stress (Chandel et al.

1998, Clanton 2007). Khan and Ringwood (2016) found that exposure to continuous hypoxia increased digestive gland glutathione levels in eastern oysters by 25%–30% after 4 days compared with normoxia. In marine brown mussels (*Perna perna*) exposure to air for various amounts of time (6–48 h) increased the levels of glutathione in gills and digestive glands (Nogueira et al. 2017). Increased concentration of glutathione only in oysters exposed to anoxia suggests anoxia, but not hypoxia, was stressful enough to elicit an antioxidant response in our experiment. In a previous study (Coxe et al. 2023), we reported that glutathione increased in one oyster population after 2 days of hypoxia exposure (0.9 mg DO L^{-1}) and 4 days of anoxia exposure (0.4 mg DO L^{-1}) compared with normoxia; however, in that study, the DO in hypoxia tanks was about 0.4–0.6 mg L^{-1} lower than those in the present study, and the exposure temperature was higher at 32 °C. This may have increased oysters' propensity for glutathione accumulation compared with the present study.

The most striking effect of anoxia was the rapid decrease in the concentration of hemocytes circulating in hemolymph in diploid and triploid oysters. The decrease in hemocyte concentration could be due to cells dying or moving within tissues via diapedesis. Hemocyte mortality has been shown to increase in other bivalve species when exposed to low DO compared with normoxia (Pampanin et al. 2002, Chen & Chen 2007, Wang et al. 2012). In addition, cellular and mitochondrial vacuolization along with the collapse of mitochondrial cristae were recently observed in foot muscle cells of Manila clams (*Ruditapes philippinarum*) exposed to low DO (Li et al. 2019). With this cellular damage, cells would not be able to maintain their energetic status under the combined stress effects of hypoxia and elevated temperature, leading to oyster death (Ivanina et al. 2012). The loss of cellular function in hemocytes would also result in a compromised immune system and lead to bacterial proliferation contributing as a cause of mortality under hypoxia and elevated temperature (Boyd & Burnett 1999, Macey et al. 2008, Fogelson et al. 2011, Nogueira et al. 2017, Coffin et al. 2021).

There were also differences in the number of hemocytes, which tended to be greater in diploid than triploid oysters and the percentage of granulocytes, which in contrast was greater in triploid than diploid oysters. These results may be linked to the condition or health of the oysters and the greater mortality of diploid oysters compared with triploid oysters in one of the control tanks under normoxia. Hemocyte concentration typically increases, and the percentage of granulocytes typically decreases in oysters under some stress conditions, such as high temperature or with the progression of dermo disease (Chu & La Peyre 1993, Anderson et al. 1995). In an earlier study, triploid oysters had lower mortality rates than did diploid oysters after exposure to *Perkinsus marinus* (Meyers et al. 1991), but other studies have found no conclusive differences (Chu et al. 1996, Dégremont et al. 2012). Although the generally higher condition (i.e., reserve) of triploid oysters, especially in summer, would be expected to contribute to greater disease resistance because of greater energetic reserve, the typically greater shell height of triploids, in contrast, would result in higher acquisition of parasites because of higher filtration rate from their larger size. Many other endogenous and exogenous factors acting together, or in opposition, can contribute to the susceptibility of diploid and triploid oysters to dermo disease illustrating the general complexity of host-parasite interaction.

In conclusion, exposure of market-sized and seed diploid and triploid eastern oysters similar in size range but different in genetic background to hypoxia and anoxia indicated that ploidy had minimal effects on their tolerance and response to low DO, despite the added stress of high temperature. It remains unclear, however, how other factors or stressors acting alone or in combination, such as condition, season, and infection intensity, impact oyster tolerance to low DO. Reports of high triploid mortality compared with diploids under certain conditions (Dégremont et al. 2012, Guévélou et al. 2017, Wadsworth et al. 2019b, Bodenstein et al. 2023) suggest that some physiological differences between diploid and triploid oysters exist but do not appear to differentially impact oyster tolerance to low DO. As climate change continues to impact water quality and other environmental regimes, the effect these changes will have on oysters in the wild as well as in aquaculture remains unclear. With the oyster aquaculture industry expanding across the GoM, understanding how diploids and triploids compare in their performance under potentially shifting environmental regimes will be imperative. Future laboratory and field investigations can further elucidate diploid and triploid physiology and resilience under various combinations of environmental stressors.

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APPENDIX TABLE 1.

Results of ratio test comparing median lethal time (LT_{50}) of market-sized oysters exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) in Study 1 (market-size oysters) and Study 2 (seed oysters).

Study 1	Estimate	SE	*z* ratio	*P* value
Normoxia–Hypoxia	0.809	0.138	5.877	<0.001
Normoxia–Anoxia	−1.562	0.160	−9.738	<0.001
Hypoxia–Anoxia	−0.753	0.117	−6.429	<0.001
Study 2	**Estimate**	**SE**	***z* ratio**	***P* value**
Normoxia–Hypoxia	1.870	0.168	11.159	<0.001
Normoxia–Anoxia	−3.100	0.190	−16.303	<0.001
Hypoxia–Anoxia	−1.230	0.084	−14.548	<0.001

Diploid and triploid oyster numbers in replicate tanks were pooled to compare mortalities among the three DO levels.

APPENDIX TABLE 2.

Results of ratio test comparing median lethal time (LT_{50}) between diploid and triploid market-sized oysters when exposed to continuous normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) in Study 1 (market-size oysters) and Study 2 (seed oysters).

Study 1				
Treatment	**Rep**	**SE**	**Test stat**	***P* value**
Normoxia	1	1.03	1.01	0.312
	2	1.58	5.91	<0.001
Hypoxia	1	0.32	1.68	0.093
	2	0.33	4.25	<0.001
Anoxia	1	0.23	0.65	0.515
	2	0.19	0.39	0.694
Study 2				
Treatment	**Rep**	**SE**	**Test stat**	***P* value**
Normoxia	1	<0.01	<0.01	1.000
	2	<0.01	<0.01	1.000
Hypoxia	1	0.06	0.64	0.515
	2	0.06	1.30	0.198
Anoxia	1	0.09	3.02	0.002
	2	0.07	2.37	0.023

Comparisons were made between diploids and triploids in each replicate (Rep) tank.

APPENDIX TABLE 3.

Mean ± SD percentage of time the valves of individual diploid (2N) and triploid (3N) market-sized oysters were open (% Open), number of times opened (# Times Opened), average amount of time (h) of each opening (Average Time Open, h), and average gape angle (°) during normoxia (DO > 5.0 mg L^{-1}), hypoxia (DO < 2.0 mg L^{-1}), and anoxia (DO < 0.5 mg L^{-1}) exposure in Study 1.

	Normoxia—Tank 1				Hypoxia—Tank 1			
Oyster	% of time Open	# of times opened	Average time open (h)	Gape angle (°)	% open	Times opened	Average time open (h)	Gape angle (°)
2N 1	43.5	22	1.43 ± 0.93	1.97 ± 0.32	47.3	7	4.86 ± 1.73	2.56 ± 0.28
2N 2	37.4	10	2.70 ± 1.21	2.37 ± 0.27	35.5	5	5.11 ± 5.77	2.84 ± 0.29
2N 3	12.4	4	2.23 ± 1.40	2.70 ± 0.28	29.8	2	10.75 ± 3.91	2.84 ± 0.38
2N 4	41.5	11	2.72 ± 0.86	2.05 ± 0.21	43.1	9	3.44 ± 2.31	2.25 ± 0.47
3N 1	26	9	2.08 ± 0.78	2.20 ± 0.34	100.0	1	71.99 ± 0.00	1.36 ± 0.74
3N 2	55.5	16	2.50 ± 3.03	2.23 ± 0.30	13.4	3	3.22 ± 2.12	2.46 ± 0.59
3N 3	35.7	6	4.29 ± 3.53	2.19 ± 0.39	33.7	2	12.13 ± 3.73	2.56 ± 0.28
3N 4	13.1	2	4.70 ± 0.21	2.21 ± 0.25	27.4	4	4.93 ± 1.26	2.03 ± 0.80
	Normoxia—Tank 2				Anoxia—Tank 2			
2N 5	54.8	21	1.88 ± 0.90	2.93 ± 0.41	34.6	9	2.78 ± 3.88	1.31 ± 0.89
2N 6	27.7	7	2.85 ± 1.66	2.80 ± 0.39	21.7	7	2.23 ± 3.24	2.00 ± 0.65
2N 7	45.9	20	1.65 ± 0.16	1.70 ± 0.32	32.8	7	3.38 ± 3.40	0.74 ± 0.37
2N 8	47.1	12	2.83 ± 0.39	1.99 ± 0.22	57.5	21	1.97 ± 6.63	0.95 ± 0.43
3N 5	45	5	6.48 ± 1.52	2.17 ± 0.26	57.9*	4*	10.43 ± 16.04*	1.58 ± 0.86*
3N 6	21.6	3	5.18 ± 1.18	2.39 ± 0.32	39.4	26	1.09 ± 0.84	1.56 ± 0.71
3N 7	42.3	5	6.09 ± 2.44	2.12 ± 0.23	21.1	1	15.16 ± 0.00	0.83 ± 0.46
3N 8	39	7	4.02 ± 1.01	2.79 ± 0.30	38.6	15	1.86 ± 2.45	1.86 ± 0.95

Individuals were exposed to 3 days of normoxia (Day −4 to −1; Figs. 1 and 2), followed by 3 days of either hypoxia or anoxia (Day 0–3; Figs. 1 and 2). Values were calculated using data from the last 3 days of normoxia exposure and the first 3 days of hypoxia or anoxia exposure.
The numbers 1–4 denote the oysters exposed to hypoxia, and 5–8 denote the oysters exposed to anoxia as in Figs. 1 and 2.
* Indicates the oyster died during exposure (Figs. 1 and 2).