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Effect of ploidy on salinity and temperature tolerance in early life stages of the eastern oyster (*Crassostrea virginica*)

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ABSTRACT

The U.S. Gulf of Mexico contains the largest remaining wild oyster fishery in the world, but populations have declined in recent decades. A growing interest in off-bottom aquaculture that relies on triploid eastern oysters (*Crassostrea virginica*) has emerged in the Gulf region, yet these faster growing oysters suffer high mortality as adults during low salinity (<5) events in warmer summer months. The combined effects of low salinity and high temperature stress on early life stages of triploid oysters are unknown. Early life stages are particularly crucial to understand because triploid oysters do not occur naturally and must be reared in hatchery settings, requiring appropriate water conditions to yield the greatest survival and growth. Thus, we tested the effects of different temperatures (28 °C and 32 °C) and salinities (5, 10, and 15) on diploid and triploid oysters at three critical production stages: veliger, pediveliger, and spat. Veliger survival was significantly lower for triploids relative to diploid oysters at all experimental conditions, but triploid veligers had faster growth than diploids at 32 °C and salinity of 15. Pediveliger settlement was not affected by ploidy type and was reduced only at high temperature (32 °C) and the lowest salinity (5). Diploid spat showed highest survival at 28 °C and 15 salinity, while triploids survived best at 32 °C and 15 salinity. Triploid spat attained greater shell height compared to diploids in our 6-day exposures, but growth decreased for both ploidies at lower salinities. At the salinity and temperature levels examined, diploid early life stages performed best at 28 °C and 15 salinity, whereas triploids were more successful at 32 °C and 15 salinity. A broader understanding of the combined effects of environmental stressors will improve the success of hatchery production yields and the resulting economic and environmental benefits of the oyster industry.

1. Introduction

The eastern oyster (*Crassostrea virginica*) is an ecologically and economically important species that provides invaluable services for coastal communities. In 2019, U.S. commercial oyster landings and aquaculture production were valued at over $470 million, with more than half of the oyster volume supplied by the Gulf states (National Marine Fisheries Service, 2021). Beyond food provision, oysters provide other ecosystem services in the form of water quality improvement, habitat provision, and shoreline protection (Grabowski et al., 2012; van der Schatte Olivier et al., 2020). Historically, *C. virginica* production in the northern Gulf of Mexico (nGoM) has come from fishing public reefs or transplanting wild spat or seed oysters to private leases for harvest once legal market size is reached (Leard et al., 1999; Vanderkooy, 2012). The nGoM contains the largest remaining wild oyster fishery in the world (Beck et al., 2011), but populations have declined in recent years due to the Deepwater Horizon oil spill, hurricanes, and alterations in estuarine salinity regimes (Camp et al., 2015; Gledhill et al., 2020; Grabowski et al., 2017). Climate change threatens to further increase the frequency and intensity of stressful salinity and temperature conditions experienced by wild and in situ aquaculture populations (Collins et al., 2019; Reid et al., 2019).

Hatchery oyster production has become increasingly popular to improve harvest yields and provides a potential solution to enhance

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declining wild fisheries and benefit oyster reef restoration efforts (Botta et al., 2020). In the nGoM, off-bottom aquaculture that relies on hatchery seed production has seen recent rapid growth since it began commercially in 2009 (Walton and Swann, 2021). Successful demonstration of faster growth rates and potential disease tolerance in triploid *C. virginica* grown in the Chesapeake Bay (Dégremont et al., 2012) inspired the use of triploids for this species in nGoM oyster aquaculture (Walton et al., 2013). Triploid oysters are known to have reduced fertility and may even be sterile and thus able to channel extra energy from reproduction to faster growth, resulting in a larger, more flavorful oyster available for year-round consumption (Guo et al., 2008; Wadsworth et al., 2019b). However, elevated mortality rates relative to diploids have been observed for triploid oysters under low salinity (< 5) conditions during late spring and summer months in both the Chesapeake Bay (Callam et al., 2016) and nGoM (Bodenstein et al., 2023; Wadsworth et al., 2019a). The physiological or genetic causes for these triploid die-offs remain unresolved (Guévélou et al., 2019; Mizuta et al., 2021), but mortality is a major concern for the farming industry because many growers rely heavily on triploids (Callam et al., 2016; Wadsworth et al., 2019a).

Due to the inability of triploid oysters to reproduce naturally, triploid aquaculture relies entirely on successful larval development, settlement, and post-settlement survival under cultured settings. Salinity and temperature are important abiotic conditions that influence oyster physiology, development, and settlement (Shumway, 1996), but the combined effects of salinity and temperature on early life stages of triploid *C. virginica* have not been reported previously. Salinity in nGoM estuaries is heavily influenced by river discharges and large fluctuations can coincide with warmer summer water temperatures (Dzwonkowski et al., 2018; La Peyre et al., 2016). Hatcheries depend on local water inputs to supply tanks and production can be hindered or completely collapse during low salinity events (Gray et al., 2022). In 2019–2020, hatchery oyster seed production was insufficient to supply the growing number of farms and increasing demand in the region (S. Rikard, personal communication), thus understanding stage-specific acute stressor responses, which can vary between early life stages (Pruett et al., 2022), is important for optimizing eastern oyster hatchery production in the nGoM.

This study compares the salinity and temperature tolerances of early life stage diploid and triploid eastern oysters. In controlled laboratory exposures, we tested the effects of early and late summer spawning temperatures (28 ºC and 32 ºC) along a gradient of salinities (5, 10, and 15) on diploid and triploid performance at key developmental stages: veliger larvae, pediveliger larvae, and post-settlement spat. We hypothesized that early life stage triploids will have higher growth rates at optimal salinity and temperature conditions but may be more vulnerable than diploids to low salinity and high temperature conditions due to lower tolerances in adults. The results from this study provide valuable information on larval and early spat performance under variable culture conditions to help enhance *C. virginica* hatchery production.

2. Material and methods

2.1. Oysters

Oyster larvae utilized in the experiments were obtained from the Auburn University Shellfish Laboratory (AUSL; Dauphin Island, AL, USA). Diploid larvae were produced using eggs from 46 diploid females fertilized by sperm from 26 diploid males. Diploid broodstock was a genetic line from AUSL with original lineage from wild oysters collected at multiple sites in Alabama. Triploid larvae were produced using eggs from the same 46 diploid females as the diploid spawn but fertilized by sperm from 8 tetraploid males. Tetraploid broodstock was a genetic line (4nGNL) from Louisiana Sea Grant's Oyster Research Laboratory at Grand Isle, LA. The diploid and triploid spawns both occurred on 17 May 2022. Embryos were shipped overnight to the University of Mississippi (UM) and used in the veliger larvae experiment. Eyed-pediveliger larvae reared from the same paired diploid-triploid spawn were shipped overnight fourteen days later and used in the pediveliger and spat experiments. The average hatchery salinity and temperature conditions, with range in parentheses, during larval culture were salinity of 21.0 (16.8–24.7) and 26.1 ºC (24.0–27.4). Upon arrival, oysters were placed in aerated artificial seawater prepared by mixing deionized water with Crystal Sea® Bioassay Mixture (Pentair, Minneapolis, MN, USA) to reach a salinity of 15. Water temperature was maintained between 26 and 28 ºC. Larvae were fed Shellfish Diet 1800® (Reed Mariculture, Campbell, CA, USA) with added ClorAm-X® (0.12 g mL^{-1} Shellfish Diet; AquaScience, Richmond, MO, USA) to prevent ammonia buildup (Rikard and Walton, 2012). Veliger and pediveliger larvae experiments were performed 24 h after each set of larvae was received. Larval survival during the acclimation period was assessed immediately prior to the start of experiments and was greater than 95 % for both veliger and pediveligers. Spat were obtained by allowing pediveliger larvae to settle on the surface of high-density polyethylene buckets for 48 h after arrival at UM. Settled spat were then carefully removed from the plastic surface and used four days later in the spat experiment.

2.2. Experimental design

Three salinity levels (5, 10, and 15) and two temperatures (28 and 32 ºC) were tested to assess the salinity and temperature tolerances of early life stage triploid oysters relative to diploids. These salinity and temperature levels were selected to evaluate triploid tolerance to low salinities (5 and 10) at an early summer spawning temperature (May/June: 28 ºC) compared to a late summer spawning temperature (July/August: 32 ºC) based on water quality conditions observed in nGoM estuaries (Pruett et al., 2022). All experiments were performed in glass Mason jars (473 mL) and consisted of 12 treatments with five replicates for the veliger and spat experiments and six replicates for the pediveliger experiment. Experimental methods have previously been described (Pruett et al., 2022). Briefly, lower salinity treatments were obtained by adding deionized water to artificial seawater (15 salinity) prepared using the same methods as holding tank water (see above) to reach salinities of 5 and 10. Initial water conditions in salinity treatments were measured with a YSI Professional Plus Multiparameter Meter (Yellow Springs, OH, USA) and 400 mL of salinity treatment water was added to experimental jars with oysters. Jars were placed in one of two incubators, set to 28 ºC and 32 ºC, to maintain temperature treatment levels. Salinity and temperature were measured in jars before each water change and at the end of the experiment to confirm salinity and temperature treatment levels were maintained. At the end of experiments, oysters from each jar were preserved in 75 % ethanol to measure growth or settlement and final survival.

2.2.1. Veliger larval experiment

At the start of the veliger larval experiment, approximately 3750 D-stage diploid or triploid veliger larvae (~48-hr old) were pipetted into experimental jars (15 mL of 250 larvae mL^{-1} stock) to create an initial stocking density of 9–10 larvae mL^{-1}. Salinity treatment water mixed with Shellfish Diet 1800® with added ClorAm-X® (16 μL jar^{-1}; ~4 ng $larvae^{-1}$ as recommended in Rikard and Walton, 2012) was added and jars were then capped and placed in an incubator set at 28 ºC or 32 ºC. Water changes were performed every 24 h by pouring the jar contents across a 25 μm mesh sieve and adding new salinity treatment water with Shellfish Diet. Larvae were exposed for 96 h overall. At the end of the experiment, veliger larvae were immediately preserved by pouring jar contents across a 25 μm mesh sieve and concentrating larvae into 20 mL of 75 % ethanol. Final survival was determined by counting the number of live (with tissue) and dead (without tissue) larvae in three 1 mL subsamples of preserved sample using a compound microscope equipped with a digital camera (Pruett et al., 2022). Ten photos were taken of larvae in each subsample, for a total of 30 photos per jar, to measure

final shell length (maximum anterior-interior measurement) using ImageJ software (version 1.52, National Institutes of Health). Additionally, 30 photos of both diploid and triploid veliger stock larvae preserved at the start of the experiment were photographed to measure initial shell length for each ploidy. Shell length increase was calculated as the difference between average shell length in each jar at the end of the experiment and average initial shell length of stock larvae. There was no difference in initial shell length size between ploidies ($t = 1.31$, $P = 0.19$), so the average diploid and triploid initial larvae shell length was used (67.2 ± 0.5 μm; mean ± SE).

2.2.2. Pediveliger larval experiment

Approximately 200 diploid or triploid eyed-pediveliger larvae were pipetted into experimental jars (15 mL of 10–15 larvae mL^{-1} stock) to create an initial stocking density of < 1 larva mL^{-1}. Each jar contained one sun-bleached adult oyster shell (~10 cm^2 surface area) as a settlement substrate. Salinity treatment water with no food was added, and jars were then capped. Jars were held in an incubator set at 28 °C or 32 °C to maintain respective temperature treatments. Larvae were exposed for 48 h with no water change. At the end of the experiment, the number of larvae attached to the oyster shell or jar walls was immediately counted using a dissecting microscope. Unattached larvae were preserved in 75 % ethanol to assess the number of live and dead larvae that did not settle. Percent settlement was calculated as the number of attached larvae (on shell and jar wall) divided by the sum of attached and unattached larvae per jar.

2.2.3. Spat experiment

At the start of the spat experiment, 15–20 spat that had metamorphosed 4–5 days prior were added to experimental jars. Salinity treatment water with Shellfish Diet 1800® with ClorAm-X® was added (2 μL jar^{-1}), and the jars were capped. Jars were held in an incubator set at 28 °C or 32 °C to maintain respective temperature treatment. Water changes were performed every 48 h by pouring contents of jars across a 180 μm mesh sieve and adding new salinity treatment water with Shellfish Diet, as previously described (Pruett et al., 2022). Spat were exposed for 6 days overall. At the end of the experiment, spat were immediately preserved in 75 % ethanol and final survival was determined by counting the number of live and dead spat per jar at the time of preservation using a dissecting microscope equipped with a digital camera. Photographs were taken of each live spat to measure final shell height (maximum umbo-tip measurement) using ImageJ software.

2.3. Data analysis

All statistical analyses were performed in R 4.0.3 (R Core Team, 2020). Generalized linear models (GLM) with binomial and logit link were used to assess the effect of ploidy, salinity, and temperature treatments on veliger and spat survival. Pediveliger survival was high for both diploid and triploid oysters, regardless of salinity and temperature treatment (~94–99 %), so statistical analysis was not performed. Three-way ANOVAs were used to analyze ploidy, salinity, and temperature treatment effects on veliger shell growth, pediveliger settlement, and spat final shell height. Data met the assumptions of homoscedasticity and normality for each parametric statistical test performed. Pairwise comparisons between salinity and temperature treatments were assessed separately for diploid and triploid oysters at each life stage using Tukey's post hoc tests. Significance was determined at an alpha level of $P < 0.05$.

3. Results

3.1. Veliger survival and growth

Overall, diploid veliger survival was higher than triploid larvae survival, but dependent on salinity and temperature conditions (Table 1,

Table 1
Statistical results of the main effects and interactions of ploidy, salinity, and temperature treatments on veliger survival and growth. Analysis of deviance results fit with logistic regression analysis (generalized linear model with binomial distribution and logit link) shown for survival. Analysis of variance results shown for growth. Significant *P*-values (< 0.05) highlighted in bold.

		Survival		Growth	
Source	*df*	LR $\chi 2$	*P*-value	*F*-value	*P*-value
Ploidy	1	6334.2	**<0.001**	15.4	**<0.001**
Salinity	2	352.7	**<0.001**	39.4	**<0.001**
Temperature	1	574.4	**<0.001**	8.10	**0.006**
Ploidy* Salinity	2	56.7	**<0.001**	1.11	0.34
Ploidy* Temperature	1	162.6	**<0.001**	9.07	**0.004**
Salinity* Temperature	2	138.1	**<0.001**	1.33	0.28
Ploidy* Salinity* Temperature	2	0.7	0.70	0.14	0.87

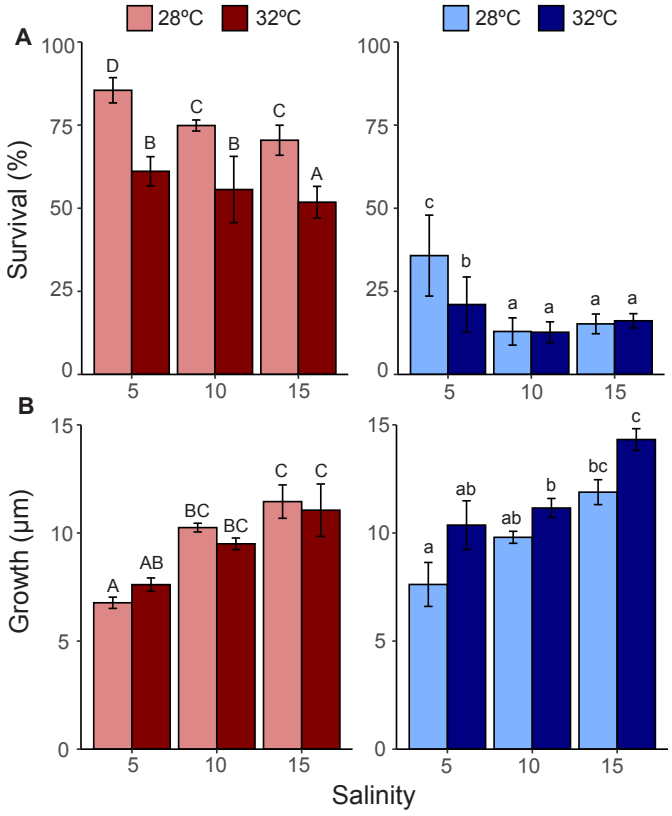

Fig. 1. Diploid (red) and triploid (blue) veliger larvae (A) percent survival (mean ± SE) and (B) growth (mean ± SE), measured as shell length increase, after 96-h exposure to different salinity and temperature conditions. Different letters denote significant differences based on Tukey's post hoc tests ($P \leq 0.05$).

Fig. 1a). At 15 salinity and 28 °C, diploid survival (70.4 ± 4.5 %; mean ± SE) was more than 3.5 times greater than triploid survival (15.2 ± 3.0 %) after 96 h of exposure. For diploid veligers, survival was significantly lower at 32 °C relative to 28 °C for all salinity levels (Fig. 1a). Survival was highest at 5 salinity, with 85.5 ± 3.8 % and 61.1 ± 4.4 % survival at 28 °C and 32 °C, respectively (Fig. 1a). Triploid veliger survival was also highest at 5 salinity, the only salinity level with differential survival between temperature treatments (35.7 ± 12.1 % at 28 °C and 21.0 ± 8.3 % at 32 °C) (Fig. 1a).

Veliger growth, measured as change in shell length, was faster for

triploid larvae, but only at 32 ºC (Table 1). Salinity treatment also significantly affected growth (Table 1), which was reduced for both diploid and triploid veligers at 5 salinity compared to 15 salinity (Fig. 1b). Shell growth for diploid veligers was similar between temperature treatments and fastest at 15 salinity and 28 ºC (11.4 ± 0.77 μm) and slowest at 5 salinity and 28ºC (6.77 ± 0.26 μm) (Fig. 1b). Triploid shell length increases were greatest at 15 salinity and 32ºC (14.3 ± 0.50 μm) and lowest at 5 salinity and 28 ºC (7.62 ± 1.02 μm) (Fig. 1b).

3.2. Pediveliger settlement

There was no effect of ploidy on the percentage of pediveliger larvae that settled after 48 h (Table 2). However, salinity and temperature treatments had an interactive effect, in which settlement was significantly lower at 5 salinity and 32 ºC compared to 15 salinity and 28 ºC for both diploid and triploid pediveligers (Table 2, Fig. 2). Settlement for diploid pediveligers was 59.3 ± 6.1 % at 15 salinity and 28 ºC relative to 36.5 ± 5.1 % at 5 salinity and 32 ºC (Fig. 2). For triploid pediveligers, settlement was 55.5 ± 7.8 % at 15 salinity and 28 ºC compared to 32.6 ± 2.2 % at 5 salinity and 32 ºC (Fig. 2).

3.3. Spat survival and growth

The interactive effect of salinity and temperature on spat survival was dependent on ploidy type (Table 3). Diploid spat survival was highest at 15 salinity and 28 ºC (94.3 ± 2.9 %), and lowest at 15 salinity and 32 ºC (74.3 ± 3.7 %) (Fig. 3a). Survival at lower salinity conditions was similar between temperature treatments for diploid spat and averaged 78.0 ± 7.6 % at 5 salinity and 80.0 ± 7.4 % at 10 salinity, irrespective of temperature (Fig. 3a). Triploid spat survival at 15 salinity was higher at 32 ºC (93.5 ± 3.1 %) compared to 28 ºC (76.9 ± 6.0 %) (Fig. 3a). Survival was significantly reduced at lower salinities for triploid spat at 32 ºC but was similar among salinities at 28 ºC (Fig. 3a).

After 6 days, triploid spat were larger than diploids, regardless of salinity or temperature conditions (Table 3, Fig. 3b). However, for both ploidies, final shell height was reduced at lower salinities, but not affected by temperature (Table 3). For diploid spat, final shell height averaged 0.61 ± 0.02 mm between both temperature treatments at 15 salinity compared to 0.52 ± 0.01 mm at 5 salinity (Fig. 3b). Final shell height of triploid spat averaged between temperature treatments 0.71 ± 0.03 mm at 15 salinity and 0.60 ± 0.02 mm at 5 salinity (Fig. 3b).

4. Discussion

Several comparative studies have suggested differences in salinity and temperature tolerances between adult diploid and triploid eastern oysters (Bodenstein et al., 2023; Callam et al., 2016; Wadsworth et al., 2019a), but none have reported the tolerances of triploid early life stages to salinity or temperature stress. Our results show that independent of salinity and temperature conditions, triploid veliger larvae had much lower survival than diploids. Across early life stages, triploid performance was not more vulnerable to low salinity stress compared to diploids, and both ploidy types demonstrated reduced growth and settlement at the lowest salinity level tested. In response to temperature treatments, at the highest salinity tested (15), diploids generally performed better at 28 ºC, whereas triploid performance was improved at 32 ºC.

Table 2
Analysis of variance results of the main effects and interactions of ploidy, salinity, and temperature treatments on pediveliger settlement. Significant P-values (< 0.05) highlighted in bold.

		Settlement	
Source	*df*	*F*-value	*P*-value
Ploidy	1	0.01	0.91
Salinity	2	9.54	**0.02**
Temperature	1	5.32	**0.02**
Ploidy* Salinity	2	0.07	0.93
Ploidy* Temperature	1	0.16	0.69
Salinity* Temperature	2	4.68	**0.01**
Ploidy*Salinity* Temperature	2	0.78	0.46

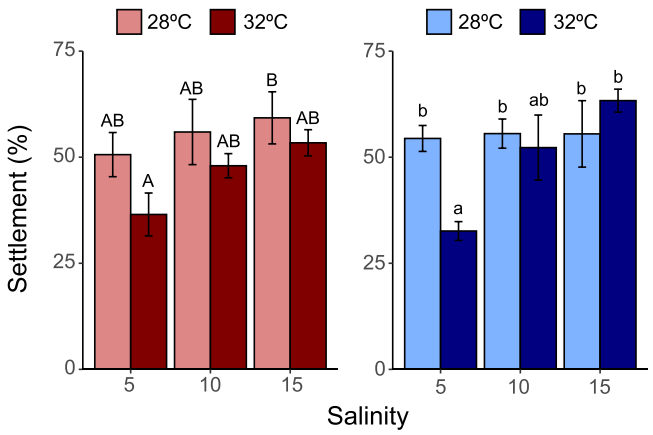

Fig. 2. Diploid (red) and triploid (blue) pediveliger larvae percent settlement (mean ± SE) after 48-h exposure to different salinity and temperature conditions. Different letters denote significant differences based on Tukey's post hoc tests ($P \leq 0.05$).

Table 3
Statistical results of the main effects and interactions of ploidy, salinity, and temperature treatments on spat survival and growth. Analysis of deviance results fit with logistic regression analysis (generalized linear model with binomial distribution and logit link) shown for survival. Analysis of variance results shown for growth. Significant P-values (< 0.05) highlighted in bold.

		Survival		Growth	
Source	*df*	LR χ2	*P*-value	*F*-value	*P*-value
Ploidy	1	0.24	0.62	59.5	**<0.001**
Salinity	2	4.17	0.13	25.4	**<0.001**
Temperature	1	6.03	**0.01**	2.4	0.13
Ploidy* Salinity	2	1.33	0.51	0.58	0.56
Ploidy* Temperature	1	0.22	0.64	1.08	0.30
Salinity* Temperature	2	2.14	0.33	0.07	0.93
Ploidy* Salinity* Temperature	2	30.7	**<0.001**	0.18	0.84

4.1. Veliger survival and growth

The largest difference in performance between diploids and triploids was observed at the veliger stage, in which diploid veliger larvae had substantially higher survival (3.5-fold) than their triploid counterparts, regardless of exposure conditions. High triploid larval mortality has been documented as a limitation of triploids produced using chemical induction (Piferrer et al., 2009), but few studies have reported larval survival rates for triploids produced by mating tetraploids with diploids (Wadsworth et al., 2019b). Matt and Allen (2014) reported 25 % survival in day-4 triploid *C. virginica* larvae produced by crossing tetraploid males with diploid females, which falls within the 12–36 % range of triploid veliger survival observed in this study. There was lower survival relative to diploid controls in *C. gigas* triploid larvae produced using the

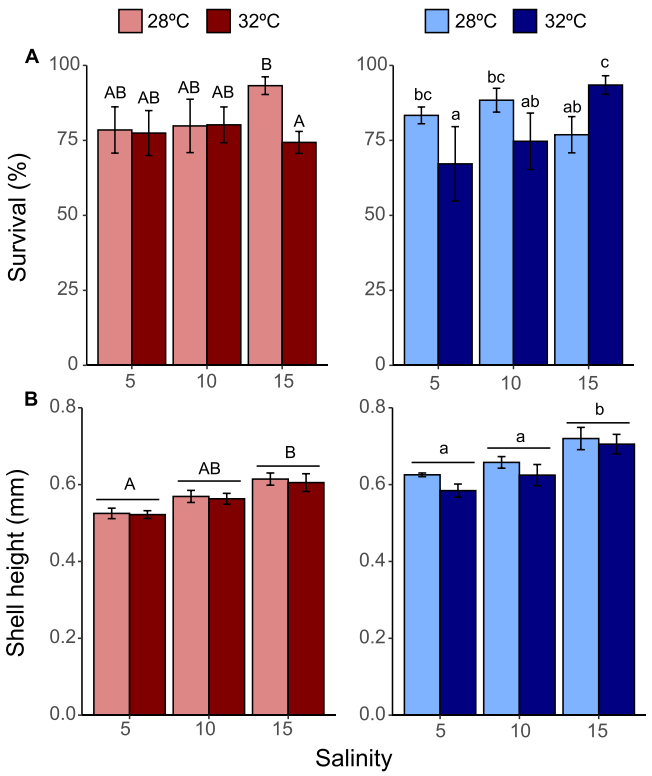

Fig. 3. Diploid (red) and triploid (blue) post-settlement spat (A) percent survival (mean ± SE) and (B) final shell height (mean ± SE) after 6-d exposure to different salinity and temperature conditions. Different letters denote significant differences based on Tukey's post hoc tests ($P \leq 0.05$).

mated tetraploid method (Li and Li, 2022). Differential survival between diploid and triploid early veliger larvae may be due to a higher likelihood of chromosome loss (i.e., aneuploidy) during early cell division in triploids (de Sousa et al., 2016), which could have detrimental impacts on cellular function and development (Comai, 2005), but requires further investigation. Although both diploid and triploid larvae were generated from the same female parental stock, ploidy differences in early veliger survival could also be due, at least in part, to genetic differences between the male diploid and tetraploid broodstock (Nascimento-Schulze et al., 2021).

Hatchery production efficiency is improved by higher larval survival coupled with faster growth rates (Gray et al., 2022). Previous works (Calabrese and Davis, 1970; Lough, 1975) have established that the optimal salinity and temperature conditions for diploid *C. virginica* D-stage larval growth and survival are between salinities of 10–30 and temperatures of 27.5 °C to 32.5 °C, but tolerance ranges can vary based on broodstock genetics and conditioning environment (Davis, 1958; Eierman and Hare, 2013; Nascimento-Schulze et al., 2021). Our study examined the effect of low salinity stress on diploid and triploid veliger larvae produced from mesohaline broodstock and found that for both ploidy types survival was highest at the lowest salinity level tested (5), but growth rates were fastest at the highest salinity (15). Higher temperature (32 °C) reduced diploid veliger survival at all salinity levels tested, but only at the lowest salinity for triploids. Oysters reduce metabolic rates in response to osmotic stress in order to tolerate acute exposures to low salinities, but the cost is reduced rates of growth and development (Parker et al., 2017; Pourmozaffar et al., 2020; Shumway, 1996). The additional metabolic demands of higher temperatures can narrow the range of larval salinity tolerances (Davis and Calabrese, 1964; McFarland et al., 2022). We observed that triploid larval growth was similar to that of diploids, despite the overall lower survival, and growth was significantly faster at 15 salinity and 32 °C. The highest larval shell increases in our study (11.4 ± 0.77 μm for diploid, 14.3 ± 0.50 μm for triploid) were slower than eastern oyster larval development after 4 days under standard hatchery protocols when fed live algal diets (35 μm; Wallace et al., 2008), but comparable to larvae reared successfully on microalgae concentrates (20 μm; Rikard and Walton, 2012).

4.2. Pediveliger settlement

Spat production is limited by the number of pediveliger larvae that are competent to settle and successfully undergo metamorphosis into juveniles under hatchery conditions or in remote-setting facilities (Gosling, 2015), but studies on salinity and temperature tolerances at the settlement stage are severely lacking. Ploidy type had no effect on settlement rates, and only at 5 salinity and 32 °C was settlement significantly reduced relative to 15 salinity and 28 °C. The effect of decreased salinity on the reduction of pediveliger settlement has been previously observed, but *C. virginica* settlement can occur in salinities as low as 5–6 (Priester, 2016; Pruett et al., 2022; Prytherch, 1934). Oyster settlement rates are highly variable even between controlled genetic and environmental backgrounds, but an optimal salinity range between 11 and 30 at 28 °C was found in repeated experiments using pediveligers from a mesohaline *C. virginica* broodstock (Priester, 2016). Rapid increases in water temperature from 24 to 29 °C in laboratory settings can trigger pediveliger settlement (Lutz et al., 1970), and eastern oyster settlement for field populations occurs in the nGoM between 28 and 32 °C (Lee, 1979; Morgan and Rakocinski, 2022). Salinity and temperature have interactive effects on optimal oyster settlement conditions for other commercially produced oyster species (Devakie and Ali, 2000; Dove and O'Connor, 2007; Henderson, 1983), but the optimal salinity-temperature combinations have not been reported for *C. virginica*.

4.3. Spat survival and growth

Metamorphosis is an energetically costly process and recently settled spat can experience high mortality rates in suboptimal conditions (Baker and Mann, 1994; García-Esquivel et al., 2001; Pruett et al., 2022). The combined effect of salinity and temperature treatments on spat survival differed between ploidy types in this study. Diploid spat survival was greater at 28 °C compared to 32 °C in the highest salinity treatment (15), but intermediate between these treatments at the other salinity and temperature combinations. In contrast, triploid spat survival was highest at 32 °C and 15, but significantly lower at 32 °C and 5. Low salinities and high temperatures have negative interactive effects in acute short-term exposures on larger (10–35 mm) juvenile *C. virginica* survival (McFarland et al., 2022; Rybovich et al., 2016; Southworth et al., 2017). Elevated temperatures and osmotic stress increase metabolic rates, which when coupled with reduced feeding rates at lower salinities, can result in energy imbalances that lead to mortality (Heilmayer et al., 2008; Sokolova et al., 2012).

Triploid spat growth was higher than diploid growth overall, but both ploidies had slower growth at lower salinity. The triploid growth advantage has been demonstrated at the juvenile stage for *C. virginica* (Dégremont et al., 2012; Stone et al., 2013), as well as other species of *Crassostrea* (Li and Li, 2022; Zhang et al., 2017). Adult triploids grow faster than diploids due to the diversion of energy from gonadal to somatic growth, whereas at the non-reproductive juvenile stages, triploid growth benefits are hypothesized to result from larger cell size due to a higher volume of chromosomes (i.e., polyploid gigantism) and higher genomic heterozygosity in triploids (Guo et al., 1996; Piferrer et al., 2009). Our results demonstrate that triploidy can already produce growth benefits less than two-weeks post-settlement. However, final spat size decreased at the lowest salinity for both ploidies, regardless of temperature conditions, likely due to reduced feeding rates that occur in salinities outside the optimal range (Casas et al., 2018; McFarland et al.,

2013).

5. Conclusions

Triploid oyster aquaculture in the nGoM shows promise to help boost oyster production and alleviate fishing pressure on declining wild stocks. Our findings demonstrated that triploid pediveligers and spat were relatively tolerant to extreme conditions of low salinity and high temperature that frequently occur during the spawning season in nGoM estuaries. Yet, triploid veliger survival was much lower than for diploids, indicating that the early larval stages present an important bottleneck for hatchery triploid production yields. However, future investigations to determine the impact of triploidization on hatching rate and survival throughout larval development under hatchery settings are necessary to help inform selective breeding and rearing processes to improve triploid early life stage performance. Tetraploid broodstock improvements may be able to enhance triploid larval viability, as well as potentially select for low salinity tolerance in older, grow-out stages (Callam et al., 2016; Li et al., 2022). Diploid oysters should also be better utilized in aquaculture production due to the genetic adaptation of natural populations to variable salinity regimes (Leonhardt et al., 2017), importance in Gulf-wide restoration efforts (La Peyre et al., 2022), and the inherent risks of complete reliance on triploids prone to massive die-off events in adverse environmental conditions (Wadsworth et al., 2019a). Improving approaches to optimize hatchery production of both diploid and triploid oysters will enhance food production and restoration aquaculture to benefit declining wild populations increasingly threatened by climate change.

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CRediT authorship contribution statement

Christian Boudreaux: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Jessica L. Pruett:** Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Alexz Carpenter:** Conceptualization, Investigation, Writing – review & editing. **Kristine L. Willett:** Conceptualization, Writing – review & editing, Funding acquisition. **Deborah J. Gochfeld:** Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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