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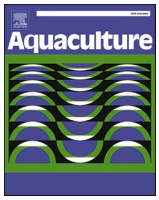

Differential performance of diploid, mated triploid, and induced triploid Pacific oysters under varied environmental conditions: Insights into impacts of temperature, dissolved oxygen, and pCO_2

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ABSTRACT

Pacific oysters (*Crassostrea gigas*) are an important aquaculture species due to their fast growth, high market demand, and adaptability. Triploid oysters, have an additional set of chromosomes relative to diploids, grow faster and are functionally sterile. Thus, triploids comprise a large proportion of oysters grown worldwide. Triploid oysters are reported to experience higher mortality than diploids. Growers must make decisions that balance the risks and rewards of growing triploids. Understanding how stressors affect oysters is essential to understanding the drivers of triploid mortality and to prepare for the impacts of climate change on individuals in aquaculture. Here, we examined impacts of temperature, dissolved oxygen (DO), and pCO_2 on genetically related juvenile diploid, chemically induced triploid, and mated triploid Pacific oysters. Diploid and induced triploid groups were full siblings, mated triploids were half-siblings. We measured whole organism physiological responses—growth, mortality and respiration — after a 4-week exposure to different environmental conditions. Survival was high in all groups across a broad range of temperature and DO levels. Survival of mated triploids was negatively impacted at lower (but higher than ambient) pCO_2 levels. Diploids and induced triploids had similar respiration across temperature and pCO_2 experiments. Diploids respired more across all dissolved oxygen treatments. Differing performance of mated triploids suggests that production method or genetic background may contribute to their resilience or susceptibility to stress. Considering the stressors that will be placed on individuals in commercial aquaculture when making ploidy selections is essential to ensure the resilience of aquaculture as the climate changes.

1. Introduction

Bivalve aquaculture is uniquely positioned to contribute to the food security of a growing human population (Costello et al., 2020) and can also support vital ecosystem functions (Filgueira et al., 2015; van der Schatte et al., 2020; Norrie et al., 2020). Most bivalve aquaculture takes place in the coastal ocean, which in addition to natural fluctuations in the environment, is particularly susceptible to negative anthropogenic impacts such as increased temperatures, ocean acidification, hypoxia, and eutrophication (Harley et al., 2006; Doney, 2010; He and Silliman, 2019). Understanding how environmental conditions affect technologies that enhance bivalve yields, such as polyploidy, is essential for planning, mitigating impacts, and ensuring the long-term sustainability of aquaculture.

Pacific oysters (*Crassostrea gigas*) are cultured globally and are valued at 1.2–1.4 billion USD annually (FAO, 2020). They are grown in diverse environments worldwide and are the most commercially important bivalve on the west coast of North America, where they are cultivated from Alaska to Baja California. Across this broad latitudinal gradient, Pacific oysters experience diverse environmental conditions where temperatures can range from 5 °C in the north to over 30 °C in the south (Brown and Hartwick, 1988; Ibarra et al., 2017). In addition,

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hypoxic and low pH events frequently occur in the estuaries where *C. gigas* is cultivated (Diaz and Rosenberg, 2008; Rabalais et al., 2010; Zhang et al., 2010; Cai et al., 2017) which can significantly impact oyster physiology and production. Laboratory studies, for example, have shown that, although oysters can tolerate short-term exposure to hypoxic conditions (DO <5.0 mg L^{-1}), longer-term exposure (> 2 weeks) can increase mortality (Coxe et al., 2023). Similarly, while some studies have suggested that oysters are able to tolerate a pH of as low as 6.6, physiological changes that reduce fitness have been observed at a pH of 7.7 (Bednaršek et al., 2022; Caillon et al., 2023).

Anthropogenic changes in the environment are likely to expand the range of conditions oysters face. Global scale processes such as chronic warming, marine heatwaves, ocean acidification, and decreases in availability of dissolved oxygen (Pörtner, 2010; Gattuso et al., 2015; Oliver et al., 2018, 2019) and local processes like eutrophication-driven hypoxia or acidification are likely to impact oyster habitats (Feely et al., 2010; Wallace et al., 2014; Cai et al., 2017; Wang et al., 2018). Understanding how oysters perform under current and future environmental conditions is crucial for identifying physiological limits and predicting their responses to climate change.

Triploid oysters (3n) possess an additional set of chromosomes relative to diploids (2n) and account for 30–50 % of farmed oysters worldwide (Stanley et al., 1981; Vignier et al., 2025). Triploids have two key advantages over diploids; 1) They are considered sterile and generally lack mature gonads during the summer, making them more marketable due to consumer preferences (Allen and Downing, 1986, 1991; Guo et al., 1996; Callam et al., 2016); 2) Under ideal conditions, their growth rates can exceed those of diploids, due to energy reallocation from gametogenesis to somatic growth, polyploid gigantism (Guo and Allen Jr, 1994; Callam et al., 2016; Wadsworth et al., 2019), or increased heterozygosity (Stanley et al., 2015). Although several methods exist, there are two primary techniques that are used to produce triploid oysters 1) chemical induction and 2) tetraploid crossbreeding. Chemical induction most commonly blocks the release of polar body II during meiosis using chemicals such as cytochalasin B (CB) or 6-dimethylaminopurine (6-DMAP) (Yang et al., 2018). Tetraploid crossbreeding involves mating tetraploid males with diploid females (Allen and Downing, 1986; Guo et al., 1996). Tetraploid males are produced by inhibiting polar body I in eggs from the few reproductive triploids fertilized with haploid sperm (Guo and Allen Jr, 1994). In aquaculture, tetraploid crossbreeding is the most common method for producing triploids, as it yields nearly 100 % triploids compared to an average of 88 % in induced triploids, while also improving larval survival rates (Wadsworth et al., 2019).

Despite the advantages of triploids, higher mortality is often reported compared to diploids (Wadsworth et al., 2019; Houssin et al., 2019; Matt et al., 2020). Triploid mortality is a major challenge for oyster growers, restricting potential yield gains. The mechanisms driving triploid mortality remain unclear, though summer mortality events, coincide with peak environmental stress, suggesting that stressors interact with physiological processes such as gametogenesis, to drive these negative effects (Huvet et al., 2010; Houssin et al., 2019). Although triploids are considered sterile, older triploids may still invest some energy in gonadal development, particularly under certain environmental conditions (Guo et al., 1996; Normand et al., 2009; Matt and Allen, 2021). Understanding physiological responses without the energy investment in gametogenesis helps isolate the effects of environmental stressors, providing clearer insights into how triploid oysters manage stress and their overall resilience.

The triploid production method can affect oyster performance. Mated triploids, for example, exhibit higher growth and larval survival rates than induced triploids (Wadsworth et al., 2019). Faster growth in mated triploids is often attributed to increased heterozygosity. However, other studies found increased heterozygosity does not always correlate with increased triploid bivalve growth (Allen et al., 1982; Jiang et al., 1993; Beaumont et al., 1995). Understanding how genetic contributions from each parent impact individual performance may also help to understand potential mechanisms driving performance differences between production methods. Induced triploids receive two sets of chromosomes from the egg, while crossbred triploids receive two sets from sperm. The larger amount of genetic material from one parent could mean a stronger influence on the offspring by that parent.

Given the challenges triploid oysters face in aquaculture, namely their increased mortality under environmental stress, this study examined their physiological responses—growth, mortality, and oxygen consumption – under a range of temperature, DO, and pCO_2 conditions. Experiments were conducted in the laboratory on diploid, crossbred triploid, and induced triploid Pacific oysters. We selected our physiological metrics as growth and mortality are essential metrics used to calculate yields on farms and oxygen consumption provides insight into the metabolic state of individuals. By examining two different groups of triploids, we were able to disentangle the impacts of triploid production method from ploidy effects. This approach allows us to address the following specific questions: 1) How do triploid and diploid oysters differ in their resilience or susceptibility to environmental stress? 2) To what extent does the triploid production method impact their stress responses?

2. Methods

2.1. Spawning

Diploid, chemically induced triploid (hereafter simply "induced triploids"), and tetraploid crossbred triploid (hereafter "mated triploids") Pacific oysters were produced using a standard strip spawning approach at the Taylor Shellfish hatchery in Quilcene, Washington, USA in April 2022. To reduce the impacts of genetic variation between groups, the same females were used to produce all groups (Fig. 1). The same males were used to produce diploids and induced triploids; however, a different tetraploid male was used to produce mated triploids. Apart from the tetraploid males, all broodstock was wild collected from Willapa Bay, WA, USA (+46.532568, −123.954032) and conditioned at the hatchery using standard practices (Helm et al., 2004) prior to spawning.

To produce the diploid and induced triploid groups 5 males and 5 females were individually pair mated (Fig. 1). The induced triploid group was created by blocking the release of polar body II. This was achieved by exposing a subset of embryos to a 300 micromolar solution of 6-dimethyalaminopurine (6-DMAP) at a temperature of 25 °C 17 min after fertilisation, for a duration of 16 min. 6-DMAP was chosen over cytochalasin B (CB) as CB is carcinogenic presents and safety concerns for hatchery staff. After fertilisation the embryos from all matings in each group ploidy were pooled and reared in the same conditions, with each ploidy group maintained separately. To produce the mated triploids additional eggs from the five females used to produce the diploids and induced triploids were combined and fertilized using the sperm from one tetraploid male. This resulted in diploids and induced triploids being full siblings and mated triploids being half-siblings of the other two groups. A subset of the triploid groups was examined using flow cytometry to confirm triploidy – 100 % of tested oysters (n = 16 per group) were confirmed as triploids (A. Romersa, Pacific Hybreed, unpublished data). Individuals were set on fine mesh screens and held in the hatchery until they reached a size of 3 mm. Individuals were then transferred to a floating upwelling system (FLUPSY) on Bainbridge Island, Washington where they were held for approximately ten weeks. Oysters were then transferred to an intertidal shellfish farm in Hood Canal, Washington where they were held in standard oyster grow bags (Intermas™, Barcelona, Spain) until experiments began.

2.2. Experimental manipulations

Three separate experiments investigating the impact of temperature,

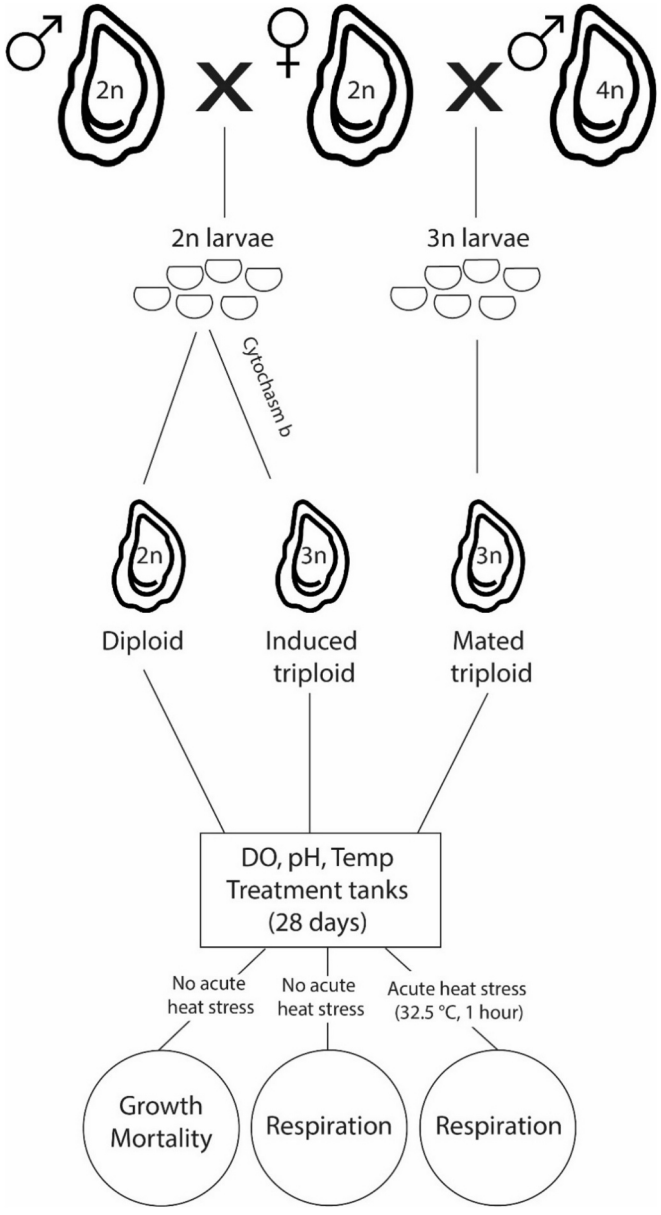

Fig. 1. Experimental design showing crossing, treatment and measured responses in the three experiments (pH, dissolved oxygen, and temperature). Note that each experiment was conducted independently using different individuals from the same cohort. Crossing of the 2n and 4n groups occurred in separate tanks.

dissolved oxygen (DO), or pCO_2 conditions (Fig. 1) were conducted on different individuals from the same cohort at different times. The temperature experiments were conducted in October 2022 (5-month-old oysters) the DO experiments were conducted in March 2023 (11-month-old oysters), and the pCO_2 experiments were conducted in October 2023 (16-month-old oysters). We used individuals of these ages, and at these times of year to reduce potential confounding effects of gametogenesis on our results. Using juveniles ensured that energy was directed toward growth and stress responses rather than reproductive processes, allowing for a more straightforward comparison of physiological performance across ploidy groups. Gametogenesis in the 16-month old oysters was possible, but we did not observe visible gametes during dissection, and October falls well outside the typical summer spawning period for *C. gigas* (Dumbauld et al., 2023).

Individuals were transferred from the oyster farm to the University of Washington, Seattle, USA and randomly split between 15 90 L mesocosm aquarium tanks at 30 individuals per ploidy per tank (i.e. total 90 individuals per tank) for temperature and dissolved oxygen experiments and 15 per ploidy for the pCO_2 experiment (i.e. total 45 individuals per tank). We used fewer individuals in the pCO_2 experiment as the increased biomass from larger individuals would be beyond the filtration capabilities of the experimental system. Water in each tank was collected from Elliot Bay (Seattle, WA, USA) and passed through a sand and UV filters before being introduced into experimental tanks. Within experimental tanks water was recirculated between water changes and passed through 100 μm sock filters and biofilters within each tank. 100 % water changes were conducted three times a week in the temperature and DO experiments and every 2 days in the pCO_2 experiments. In the temperature and pCO_2 experiments, oxygen levels were maintained by continually bubbling air through the tanks. Each tank was assigned to a treatment condition (Table 1; $n = 3$ tanks per treatment). We selected these treatment conditions to reflect the broad range of environmental conditions to which oysters may be realistically exposed on the West coast of the USA while remaining within the technical bounds of our experimental system.

Temperature was maintained in each mesocosm aquarium with Aqualogic (Monroe, North Carolina, USA) small heat exchanger packages (± 0.5 °C). Oxygen, pCO_2 and temperature manipulations were controlled in each individual tank by a web-based microprocessor (Apex, Neptune Systems, Morgan Hill, California, USA) attached to each tank. In the pH and dissolved oxygen experiments the temperature was maintained at a constant 17.5 °C. In the DO experiments oxygen levels were manipulated by bubbling nitrogen gas directly into each tank until the desired concentrations were obtained. Oxygen concentrations were monitored using Neptune Apex PM3 dissolved oxygen probes, and when oxygen concentrations exceeded set points, a solenoid valve opened to allow nitrogen to enter tanks. pCO_2 was manipulated by adding CO_2 directly to each tank. pH was monitored in each tank using a Neptune Apex PM1 pH/ORP module. When the pH exceeded setpoints, a solenoid valve opened to add CO_2 to each tank. We did not manipulate the carbonate conditions in control tanks during the pCO_2 experiment; thus, these values were based on the parameters of the water as it arrived in the laboratory. pH levels were changed after each water change due to fluctuations in salinity and alkalinity to maintain a constant pCO_2. New setpoints were established by measuring alkalinity and salinity in the seawater reservoir immediately prior to filling the tanks using the methods described in section 2.4. Throughout the course of the experiment, individuals were fed 0.5 mL shellfish diet 1800 (Reed Mariculture, Campbell, California: 40 % *Isochrysis galbana*, 25 % *Tetraselmis*, 20 % *Thalassiosira pseudonana*, and 15 % *Pavlova lutheri*) per gram of biomass daily according to producer instructions and generally accepted practices (Helm et al., 2004). Due to the alkalinity of the shellfish diet mix, the food volume was reduced by 25 % for the pCO_2 experiments.

2.3. Physiological measurements

Oyster shell height from the hinge to the most distal ventral edge was recorded to the nearest 1 mm, and individual mass of was recorded to the nearest 0.1 g at the start and end of the experiment. At the end of each experiment oxygen consumption (respiration) was also assessed (respirometry section 2.2.3). Prior to oxygen consumption

Table 1
Target treatment conditions (rows) used in the three stress experiments (columns).

Temperature (°C)	Dissolved oxygen (mg L^{-1})	pCO_2 (μatm)
7.5	1.91	~800 (ambient)
12.5	3.83	1100
17.5	5.74	1450
22.5	7.66	1700
27.5		2000

measurements, a subset of individuals ($n = 4$ from each treatment tank, $n = 12$ per ploidy per treatment) was subjected to additional acute heat stress by placing them in seawater at 32.5 °C for one hour (Fig. 1). This approach allowed us to examine the effects of acute stress on physiology under more extreme conditions. We selected 32.5 °C for acute heat exposure to assess controlled stress responses rather than the severe physiological breakdown that can occur at higher temperatures (George et al., 2023; Welford, 2023). Previous studies have shown that exposing bivalves to heat for one hour is sufficient to trigger a molecular heat shock response (Li et al., 2007; Zhang and Zhang, 2012; Yang et al., 2016). We used heat shock exposure to gauge the potential metabolic scope of individuals. Typically, we would expect an increase in oxygen consumption at higher temperatures due to the elevated rate of temperature-dependent reactions and the energy required to produce heat shock proteins, which are energetically costly.

2.3.1. Oxygen consumption measurements

Oxygen consumption was measured after the 28 day exposure using closed chamber respirometry based on the system described in Alma et al. (2024). We measured oxygen consumption on 3–4 individuals from each ploidy from each treatment tank. Respirometry was run at the temperature at which individuals were held for the chronic stress exposure treatments and at 32.5 °C for the individuals exposed to acute thermal stress. To remove the potential impact of digestion on metabolism, individuals were starved for 24 h prior to respiration analyses. Oysters were individually placed into 650 mL acrylic chambers equipped with a stir bar and positioned on a custom-made submersible stir table. The movement of the water by the stir bar ensured homogenous temperature and oxygen levels within each chamber. Oxygen consumption and temperature were monitored in each individual chamber with a fibre-optic oxygen probe (Presens dipping probes [DP-PSt7–10-L2.5-ST10-YOP], Regensburg, Germany) and a temperature probe (Pt1000) connected to an OXY-10 SMA G2 meter box, which was then connected to a computer with software PreSens Measurement Studio 2 (version 2.0.0.28). Temperature was maintained (± 0.1 °C) by a water bath controlled by an Aqualogic (Monroe, North Carolina, USA) heat pump (DSHP-4). The oxygen consumption of six oysters was measured simultaneously, and one empty chamber was used as a seawater control to determine background oxygen consumption. Prior to trials, the system was calibrated using a two-point oxygen calibration. 100 % oxygen was achieved by leaving a beaker of fresh seawater overnight to equilibrate with the atmosphere. 0 % oxygen was achieved by combining 1 g of sodium sulphite (Na_2SO3) (Sigma-Aldrich) with 50 μL of cobalt standard for ICP ($Co(NO_3)_2$) (Sigma-Aldrich) and 100 mL of deionised (1 MΩ cm^{-1}) water.

Oxygen consumption data was processed in R (v 4.4.0, R core team) using the respR package (Harianto et al., 2019). The 30-min period of each one-hour respirometry run that displayed the most linear decrease in oxygen was selected as the oxygen consumption rate. Rates were background corrected by subtracting oxygen changes from the control chamber in each run. Oxygen consumption for each individual was standardised to μmol h^{-1} g (dry tissue)$^{-1}$. Dry mass of oyster tissue was obtained by shucking each oyster and drying the tissue at 60 °C for 48 h in a drying oven. When the oxygen consumption values of oysters were lower than background respiration, it was assumed that no respiration occurred in this trial. As this lack of respiration was likely driven by a behavioural response, i.e. valve closure, rather than a physiological response these individuals were removed from the analyses.

2.4. Water carbonate chemistry

During the pCO_2 experiments we continuously monitored pH to maintain setpoints and took discrete water samples twice a week to adhere to best practices in ocean acidification research (Dickson et al., 2007, Riebesell et al., 2011). Alkalinity in these samples was calculated through volumetric titration using a Mettler Toledo T5 autotitrator coupled to a Rondolino autosampler. Instrument accuracy and precision (<1 %) was measured at regular intervals using Certified Reference Materials (CRMs), consisting of filtered and UV irradiated seawater supplied by the Dickson Lab (SIO). pH was measured spectrophotometrically using an Ocean Insight flame spectrometer (Ocean optics, Orlando, Florida, USA). Salinity was measured using a 2-electrode cell salinity probe (Thermo Scientific™ Orion™). Salinity probe calibration took place at the start of each experiment. Calculations to parameterise the carbonate system were undertaken in R (v 4.4.0, R core team) using the seacarb package (Gattuso et al., 2024). The default dissociation constants from seacarb were used, which are based on those presented in Lueker et al. (2000). Water carbonate chemistry information is presented in Table 2.

2.5. Statistical analyses

All data analysis was conducted using the R statistical programming language (v 4.4.0, R core team). To examine how oxygen consumption and growth varied between tanks, we used a linear mixed modelling (LMM) approach with each replicate tank included as a random factor (Norrie et al., 2018) using the nlme package in R (v 3.1.162 - Pinheiro and Bates, 2024). We compared the total mean mass of individuals (including shell) at the start and the end of the experiments within each of the ploidy groups across treatments to assess growth.

We assessed the oxygen consumption of individuals that were not exposed to acute heat stress and those that were using two separate LMMs. We compared oxygen consumption across ploidies and between individuals that were exposed to acute heat stress and those that were not with one LMM for each treatment level. Due to the high mortality in the pCO_2 experiments, we did not compare individual-specific growth rates across treatments. Where significant main effects or interaction were observed in the linear mixed models, we conducted pairwise comparisons with Tukey's adjustments using the emmeans package (v1.10.3 - Lenth et al., 2024). Prior to conducting these analyses, we inspected the data for normality and homogeneity of variance and, where appropriate, square root transformed the data which improved normality.

To compare survival rates across ploidies and treatments we compared hazard ratios. We first generated Kaplan-Meyer survival curves for each pCO_2, DO, temperature, and ploidy combination using the survival package in R (v3.5.5 - Therneau, 2024). To compare hazard ratios between treatments and ploidies, we fit a Cox proportional hazards model (Cox, 1972) using the coxme package in R (v 2.2–20 - Therneau, 2024). A Cox proportional hazards model examines how different factors influence the risk of mortality over time, without assuming a specific pattern for the baseline risk. We included tank as a random term in this model to account for variation between tanks. To understand how mortality varied across treatments and ploidies we performed post-hoc pairwise comparisons using the emmeans package (v1.10.3, Lenth et al., 2024) with Holms correction for multiple comparisons.

3. Results

3.1. Growth

3.1.1. Initial mass

Variations in growth rates before the laboratory experiments began led to significant differences in the mean initial mass of each ploidy group in the stress experiments (Fig. 2). At the start of the temperature experiment, diploids were significantly smaller than induced triploids, which were significantly smaller than mated triploids (Fig. 2a-e; Table S1). At the start of both the dissolved oxygen and pCO_2 experiments, diploids and induced triploids were significantly smaller than mated triploids and no significant differences in mass were observed between diploids and induced triploids (Fig. 2f-n; Table S1).

Table 2
Carbonate parameters for each of the target treatments for the pCO_2 experiment (Mean ± standard error - SEM). Abbreviations used in column headings: Temp – Temperature (°C), Sal = Salinity (psu), pH = pH (total scale), TA = total alkalinity (μmol kg^{-1}), DIC - Dissolved inorganic carbon (μmol kg^{-1}), pCO_2 = Partial pressure of dissolved CO_2 (μatm), Ω_C = calcite saturation state, Ω_A - aragonite saturation state.

Target pCO_2 (μatm)	Sal	Temp	pH	DIC	pCO_2	TA	Ω_C	Ω_A
800	30.86 (± 0.02)	17.61 (± 0.05)	7.81 (± 0.02)	2192.94 (± 46.88)	773.18 (± 24.12)	2309.27 (± 52.18)	1.58 (± 0.08)	2.47 (± 0.13)
1100	30.84 (± 0.06)	17.7 (± 0.11)	7.66 (± 0.01)	2372.67 (± 100.49)	1181.1 (± 43.56)	2441.07 (± 104.43)	1.23 (± 0.08)	1.92 (± 0.12)
1450	30.86 (± 0.03)	17.56 (± 0.06)	7.55 (± 0.02)	2337.13 (± 75.52)	1507.95 (± 55.2)	2369.16 (± 83.73)	0.95 (± 0.1)	1.48 (± 0.15)
1700	30.59 (± 0.28)	17.62 (± 0.06)	7.5 (± 0.01)	2434.23 (± 66.32)	1758.42 (± 29.27)	2449.05 (± 69.99)	0.87 (± 0.05)	1.35 (± 0.08)
2000	30.87 (± 0.09)	17.6 (± 0.09)	7.44 (± 0.03)	2507.75 (± 138.82)	2081.68 (± 71.59)	2503.84 (± 147.44)	0.79 (± 0.1)	1.23 (± 0.16)

3.1.2. Temperature

In the temperature experiment, we observed an increase in the mass of individuals from all ploidies over the course of the experiment (Fig. 2). However, no differences in mass were observed across treatments for any ploidy (Fig. 2a-e). These results are supported by the results of the linear mixed models (Table 3), which indicated significant main effects of time existed, but no main effect of treatment or interaction effect.

3.1.3. – Dissolved oxygen

The only increase in mass we observed between the start and the end of the experiment was observed in mated triploids, which were heavier at the end of the experiment (Fig. 2f-i; Table S1). This finding was supported by the results of the linear mixed models (Table 3) which showed a significant main effect of time in mated triploids but no significant time effects for the other two groups. No interactions were observed in the linear mixed models (Table 3).

3.1.4. pCO_2

We did not observe any changes in mean mass of any ploidy group over the course of the pCO_2 experiment (Fig. 2j-n; Table S1). This is shown by the lack of significant main effects of time, treatment or interactions in any of the linear mixed models (Table 4).

3.2. Mortality

Overall survival in the temperature and dissolved oxygen experiments was high. Only 18 individuals died over all treatments in both experiments (i.e. 1.3 % mortality; Fig. 3 a-i; Table S1). Due to the low mortality in these experiments we were unable to generate stable parameter estimates in the cox proportional hazards models. A minimum of 10 events per predictor variable is preferable to generate these stable estimates (Peduzzi et al., 1996). In the pCO_2 experiment, however, 250 individuals died across all treatments (37 % mortality; Fig. 3j-n; Table S1). Mated triploids were more sensitive to increased pCO_2 than the other ploidies, as shown by their higher mortality rate at lower pCO_2 levels (Table S1). The increased mortality in mated triploids became apparent at 1450 μatm. In the highest pCO_2 treatment (2000 μatm), high mortality was observed across all ploidies. The analysis of the deviance table from the mixed effects Cox proportional hazards model revealed a significant interaction between ploidy and pCO_2 levels ($\chi^2 = 23.94$, df = 8, $p = 0.002$).

Pairwise comparisons of the hazard ratios indicated that within ploidies but across treatments, mortality of diploids was significantly higher at 2000 μatm than 1450 μatm ($p = 0.046$). While there was a trend of increasing mortality with increasing pCO_2 within treatments but across ploidies in diploids and mated triploids, neither of these groups displayed any statistically significant pairwise differences in survival across pCO_2 levels. Across ploidies but within treatments, pairwise comparisons showed that at 1450 μatm and 1750 μatm differences in mortality existed between all groups. Mated triploids exhibited the highest mortality, followed by induced triploids, and diploids displayed the lowest mortality (Table 6). At 2000 μatm, there were no significant pairwise differences in mortality between ploidy types, as mortality was high across all groups.

3.3. Oxygen consumption

3.3.1. Temperature

In individuals not exposed to acute heat stress, oxygen consumption generally increased with rising temperature across all ploidy groups (Fig. 4a). Mated triploids consistently showed the lowest respiration rates across treatments. The results of the linear mixed model (Table S1) revealed significant main effects of ploidy and treatment, with no significant interaction effects Post hoc pairwise comparisons across ploidies showed that induced triploids had significantly higher oxygen consumption than mated triploids ($t_{86} = 3.375$, $p = 0.003$). Although diploids had higher respiration than mated triploids, this difference was only marginally statistically significant ($t_{86} = 2.314$, $p = 0.059$). Comparisons across temperature treatments revealed that oxygen consumption was significantly higher at 27.5 °C than at 7.5 °C ($t_{10} = 3.346$, $p = 0.0459$), and marginally higher at 22.5 °C compared to 7.5 °C ($t_{10} = 3.164$, $p = 0.061$).

Individuals exposed to acute heat stress did not show differences in oxygen consumption across ploidy groups or temperature treatments (Fig. 4b). This was supported by the lack of statistically significant effects of ploidy, treatment, or interactions in the linear mixed model (Table S1). Linear mixed models examining differences in respiration within temperature treatments, but across ploidies and acute heat exposure, showed no significant effects of heat exposure or ploidy at any temperature (Table 6).

3.3.2. Dissolved oxygen

In individuals not exposed to acute heat stress during the dissolved oxygen experiments, respiration was highest in diploids (Fig. 4c). Oxygen consumption was slightly higher in individuals from the 3.83 mg L^{-1} and 5.74 mg L^{-1} treatments, although these differences were not statistically significant. The linear mixed model showed effects of ploidy and treatment, but no interaction effects (Table 5). Post hoc tests showed that mated triploids had significantly higher respiration than diploids ($t_{77} = 3.350$, $p = 0.004$), while induced triploids had only marginally higher oxygen consumption than mated triploids ($t_{77} = 2.234$, $p = 0.072$).

Comparisons across acute heat treatments within each DO level showed that, across all DO levels, oxygen consumption was higher in heat-shocked individuals (Table 6), as indicated by the significant main effects of heat exposure. However, individuals from the 7.766 mg L^{-1} treatment also showed a significant ploidy effect. Triploids had significantly higher respiration rates compared to diploids ($t_{86} = 2.314$, $p = 0.0592$), while induced triploids showed no significant differences from diploids ($t_{86} = -0.969$, $p = 0.5985$). Furthermore, induced triploids exhibited significantly lower oxygen consumption compared to mated triploids ($t_{86} = 3.375$, $p = 0.0032$).

3.3.3. pCO_2

There was no impact of ploidy or pCO_2 level on respiration in individuals without acute thermal stress (Fig. 4e. Table 5). In diploids and induced triploids exposed to acute thermal stress, there was a trend of

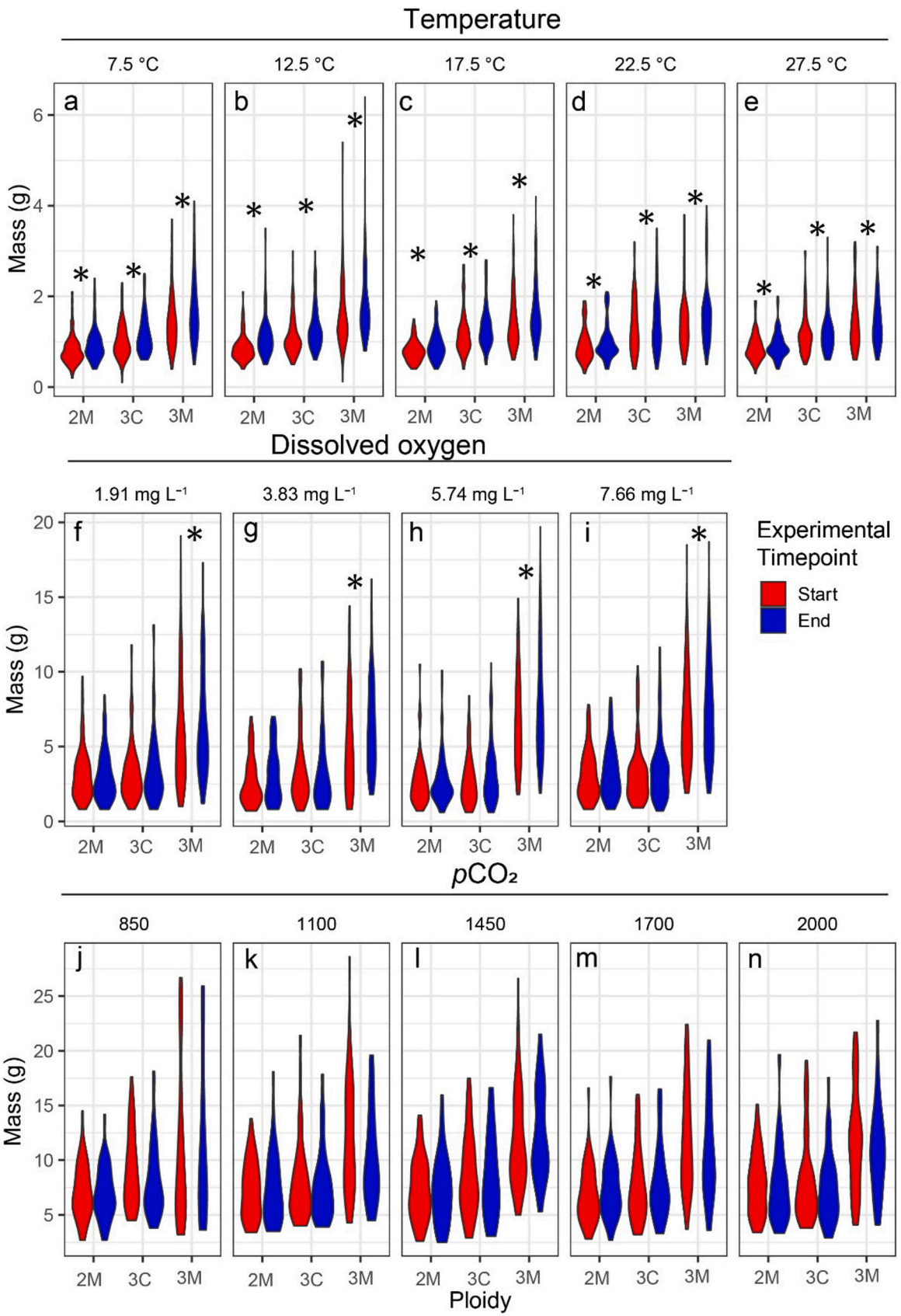

Fig. 2. The size distribution of diploid (2 M), induced triploid (3C), and mated triploids (3 M) at the start and end of the temperature (a-e), dissolved oxygen (f-i) and pCO_2 experiments (j-n). An asterisk indicates significant differences ($p < 0.05$) in size between the two time points for each ploidy-treatment combination.

Table 3
Summary of results from the linear mixed models examining the differences in size at the start and the end of each experiment. F and p values are shown. Asterix (*) and bold indicates significant effects.

Experiment	Ploidy	Main Effect: Time	Main Effect: Treatment	Time*Treatment Interaction
Temperature	Diploid	$F_{1, 831}$ = 29.469, **p < 0.005***	$F_{4, 832}$ = 0.816, p = 0.515	$F_{4, 831}$ = 1.766, p = 0.134
	Induced Triploid	$F_{1, 840}$ = 15.120, **p < 0.005***	$F_{4, 10}$ = 2.661, p = 0.095	$F_{4, 840}$ = 0.931, p = 0.445
	Mated Triploid	$F_{1, 862}$ = 17.325, **p < 0.005***	$F_{4, 10}$ = 1.683, p = 0.230	$F_{4, 862}$ = 1.124, p = 0.335
Dissolved Oxygen	Diploid	$F_{1, 619}$ = 0.366, p = 0.550	$F_{3, 619}$ = 0.848, p = 0.468	$F_{3, 619}$ = 0.760, p = 0.517
	Induced Triploid	$F_{1, 675}$ = 2.099, p = 0.148	$F_{3, 675}$ = 1.473, p = 0.221	$F_{3, 675}$ = 0.020, p = 0.996
	Mated Triploid	$F_{1, 742}$ = 17.168, **p < 0.005***	$F_{3, 742}$ = 0.335, p = 0.800	$F_{3, 742}$ = 1.538, p = 0.203
pCO_2	Diploid	$F_{1, 378}$ < 0.001, p = 0.999	$F_{4, 10}$ = 0.166, p = 0.951	$F_{4, 378}$ = 0.252, p = 0.908
	Induced Triploid	$F_{1, 367}$ = 1.144, p = 0.285	$F_{4, 10}$ = 0.711, p = 0.603	$F_{4, 367}$ = 0.30, p = 0.877
	Mated Triploid	$F_{1, 367}$ = 1.086, p = 0.298	$F_{4, 10}$ = 2.220, p = 0.140	$F_{4, 326}$ = 0.656, p = 0.623

Table 4
p-values from pairwise comparisons of survival across ploidies from the pCO_2 experiment. 2 M – diploids, 3 M – mated triploids, 3C – induced triploids.

	pCO_2				
Comparison	850	1100	1450	1700	2000
2 M - 3C	0.147	0.718	**<0.001**	**<0.001**	0.960
3 M - 3C	0.638	1	**0.047**	**0.055**	0.960
2 M - 3 M	0.242	1	**<0.001**	**0.002**	0.821

increasing respiration rates with rising pCO_2 levels (Fig. 4f). Conversely, mated triploids under the same thermal stress showed a decrease in respiration as pCO_2 levels increased. Although these groups exhibited opposing directional trends, no statistically significant pairwise differences in respiration were detected between mated and induced triploids at any individual pCO_2 level. Diploids had significantly higher oxygen consumption relative to mated triploids ($t_{73} = 2.516$, $p = 0.037$), while no significant pairwise difference existed between diploids and induced triploids or between mated and induced triploids.

When comparing individuals exposed to heat stress with those not exposed, no significant differences in oxygen consumption were observed at the 850 μatm or 1400 μatm treatments. Specifically, the linear mixed model results (Table 6) showed no significant effects for ploidy or treatment at these levels, nor were any interactions significant. However, at the 2000 μatm treatment, significant treatment effects were observed, indicating that heat stress impacted oxygen consumption at this level. Post hoc tests revealed that the difference was driven by lower oxygen consumption in mated triploids compared to both diploids ($t_{57} = 3.530$, $p = 0.003$) and induced triploids ($t_{57} = -2.401$, $p = 0.050$), but no significant difference was found between diploids and induced triploids ($t_{57} = -1.144$, $p = 0.491$).

4. Discussion

Understanding how environmental conditions affect diploid and triploid oysters is key to developing management strategies that maximize triploid yield benefits while mitigating mortality risks. This study provides the first explicit comparison of full- and half-sibling diploid, chemically induced triploid, and mated triploid oysters under three key climate-related stressors: temperature, dissolved oxygen (DO), and pCO_2. By examining responses within each experiment, we clarify how triploid production method and ploidy-associated physiological changes influence resilience or susceptibility to climate change stressors. These findings lay the groundwork for understanding the mechanisms shaping farmed oyster tolerance under current and future climate conditions. This knowledge will help anticipate environmental impacts and inform management decisions, such as selecting the appropriate ploidy for specific sites, ultimately enhancing oyster aquaculture resilience and productivity.

Our results indicate that triploid production method influences performance under pCO_2 and temperature stress. Mated triploids were more sensitive to these stressors than both diploids and induced triploids, exhibiting (1) higher mortality at lower pCO_2 levels, (2) lower oxygen consumption than induced triploids and diploids at higher temperatures, and (3) reduced oxygen consumption under a broad range of pCO_2 levels. When interpreting our results, it is important to highlight that comparisons cannot be made between experiments as we performed experiments at different times, meaning that ontogenetic changes (i.e. age and size) or differences in environmental history may have impacted physiological responses to stressors. We therefore focus our discussion on differences in performance between ploidies, within each experiment.

4.1. Survival

The high survival of all ploidy groups across temperature and dissolved oxygen levels is unsurprising, given that Pacific oysters have high phenotypic plasticity and are cultivated along the US West Coast in marine and estuarine habitats with substantial temperature and oxygen variability (Brown and Hartwick, 1988; Diaz and Rosenberg, 2008; Rabalais et al., 2010). Their ability to tolerate hypoxia, which is common in estuarine environments, suggests mechanisms such as valve closure and anaerobic respiration that allow them to persist regardless of ploidy (Le Moullac et al., 2007; Porter and Breitburg, 2016). Although no studies have directly compared hypoxia tolerance between diploid and triploid *C. gigas*, findings from Coxe et al. (2023) on eastern oysters (*Crassostrea virginica*) support ours, showing minimal survival differences across ploidies under hypoxia when no additional stressors are present.

In our study, we did not observe differences in mortality across temperatures or ploidies. However, previous studies have reported lower survival rates in both adult (Pernet et al., 2010; George et al., 2023; Bodenstein et al., 2023) and larval stages (Li et al., 2007; Qin et al., 2022) of Pacific oysters under temperature stress in both field and laboratory settings. Laboratory studies, like ours, offer more controlled conditions, isolating specific stressors, while field studies are subject to a broader range of environmental variables that can interact to influence survival rates. The use of juvenile oysters (<10 g mean mass) may have contributed to their resilience to DO and temperature stress. Their higher surface area-to-volume ratio improves oxygen delivery compared to larger individuals (Pörtner, 2010; Eymann et al., 2020), potentially helping buffer stress by directing energy toward heat shock protein or antioxidant enzyme production (Brokordt et al., 2015, Yang et al., 2016 201, Delorme et al., 2020). It is also possible that the periodic oxygenation of water in each tank, achieved by adding fresh water multiple times a week, further supported juveniles' resilience by providing necessary oxygen during brief intervals, although DO levels returned to treatment levels within 1 h of each water change.

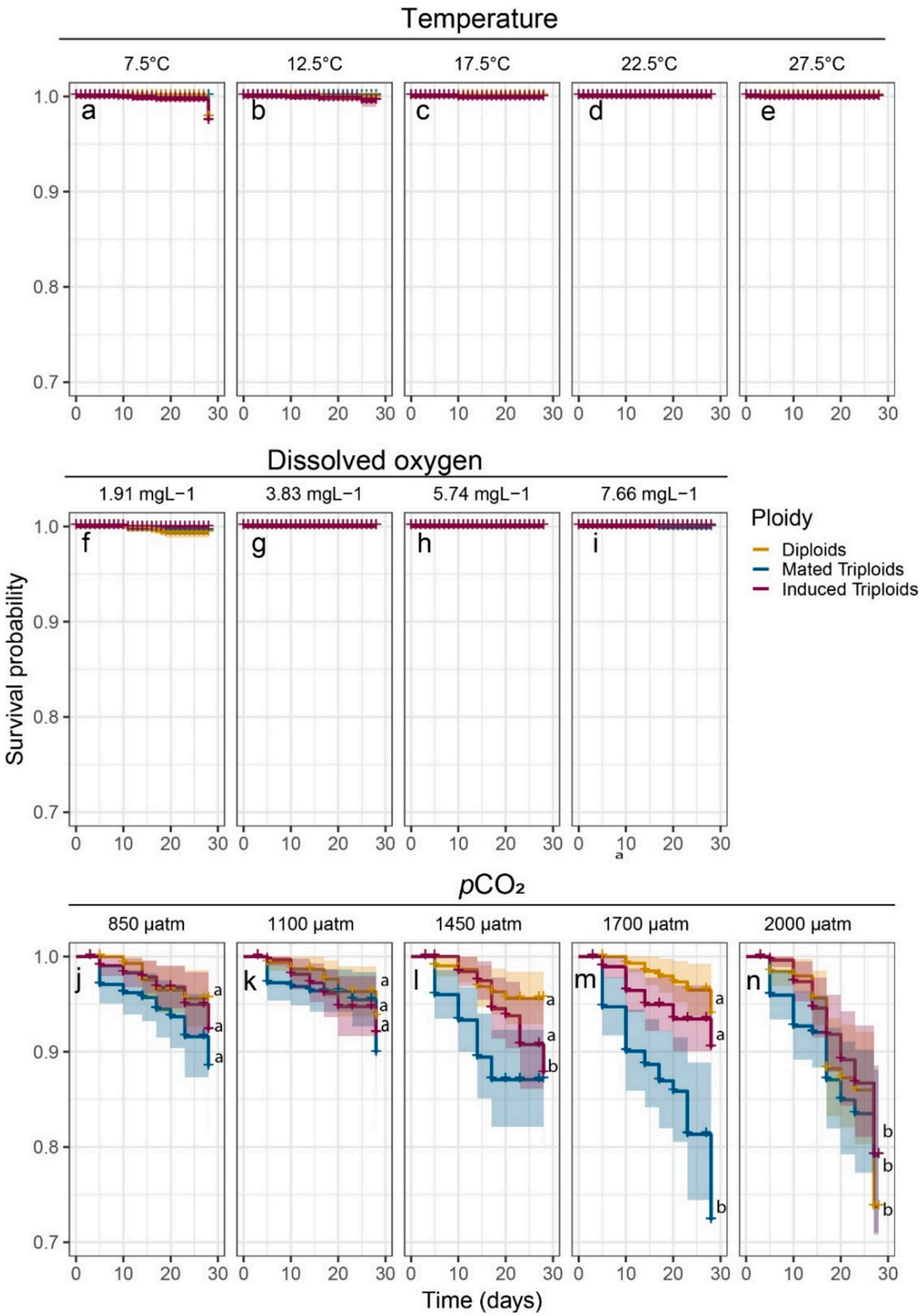

Fig. 3. Kaplan-Meier survival plots indicating the probability of survival (± 95 % CI in shaded area) of individuals from each of the temperature (a-e), dissolved oxygen (f-i), and pCO_2 experiments (j-n). Letters represent statistical groupings.

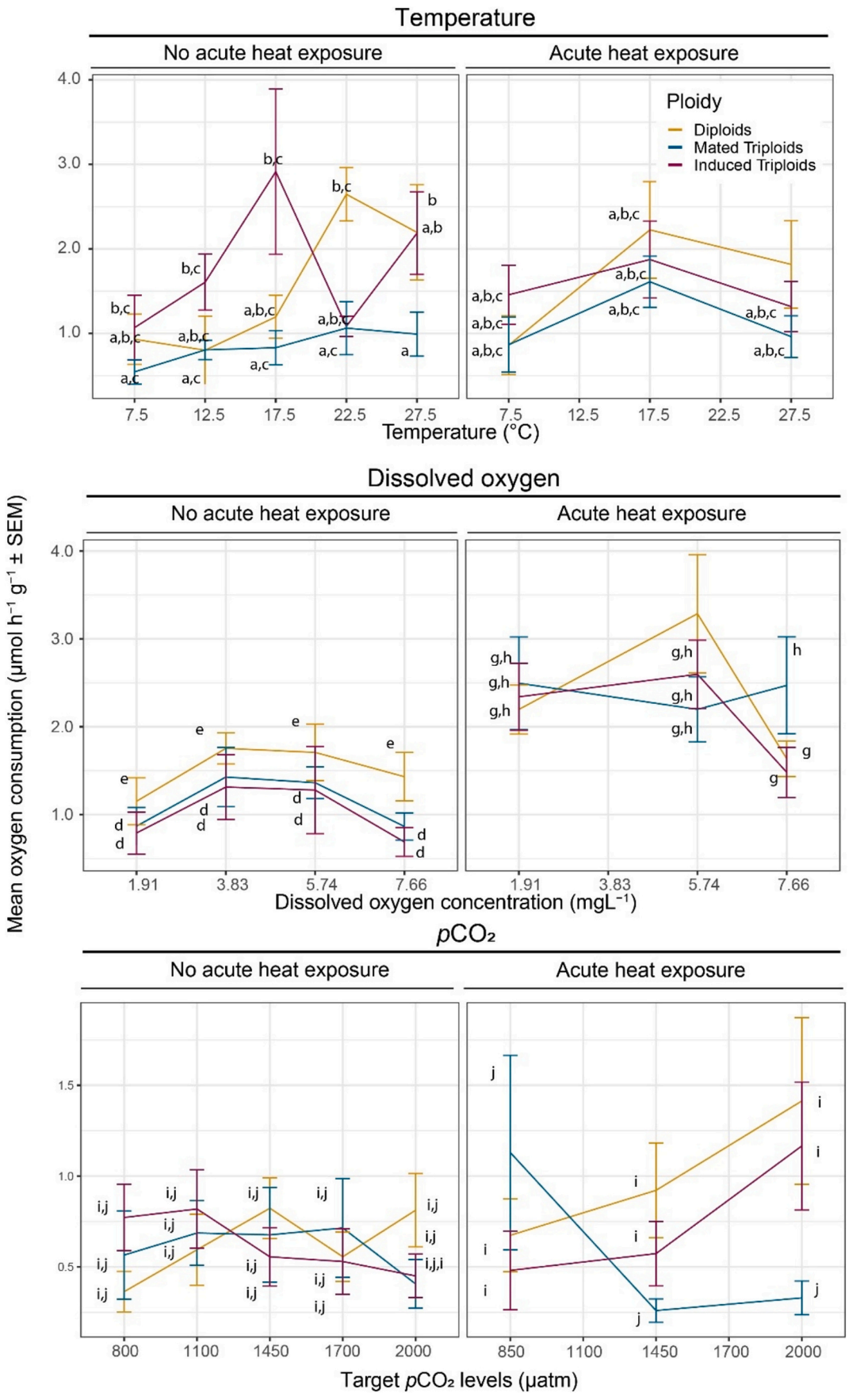
Temperature
No acute heat exposure
Acute heat exposure
Ploidy
Diploids
Mated Triploids
Induced Triploids
Temperature (°C)
Dissolved oxygen
Mean oxygen consumption (µmol h⁻¹ g⁻¹ ± SEM)
Dissolved oxygen concentration (mgL⁻¹)
pCO₂
Target pCO₂ levels (µatm)

Fig. 4. Physiological performance curves showing mean oxygen consumption (± SEM) in individuals across temperatures (a,b), dissolved oxygen levels (c,d), and pCO_2 levels (e,f). Oxygen consumption was measured at holding temperatures (a,c,e) and 32.5 °C (b,d,f). Letters indicate statistical groupings within each experiment.

Table 5
Summary of results from linear mixed models investigating differences in oxygen consumption of individuals. F and *p* values are shown. Asterix (*) and bold indicates significant effects.

Experiment	Heat stress treatment	Main Effect: Ploidy	Main Effect: Treatment	Interaction Effect
Temperature	No Heat Stress	**$F_{(2, 86)} = 7.509$, $p = 0.001$***	**$F_{(4, 10)} = 4.031$, $p = 0.03$***	$F_{(8, 86)} = 1.892$, $p = 0.072$
	Acute Heat Stress	$F_{(2, 44)} = 0.784$, $p = 0.776$	$F_{(2, 6)} = 3.661$, $p = 0.091$	$F_{(4, 44)} = 1.129$, $p = 0.355$
Dissolved Oxygen	No Heat Stress	**$F_{(2, 77)} = 6.793$, $p = 0.002$***	**$F_{(3, 8)} = 4.152$, $p = 0.048$***	$F_{(6, 77)} = 0.170$, $p = 0.0.984$
	Acute Heat Stress	$F_{(2, 56)} = 0.128$, $p = 0.880$	$F_{(2, 6)} = 1.038$, $p = 0.410$	$F_{(4, 56)} = 0.621$, $p = 0.649$
pCO_2	No Heat Stress	$F_{(2, 126)} = 0.265$, $p = 0.768$	$F_{(4, 10)} = 0.290$, $p = 0.878$	$F_{(8, 126)} = 0.723$, $p = 0.671$
	Acute Heat Stress	**$F_{(2, 73)} = 3.443$, $p = 0.037$***	$F_{(2, 6)} = 1.733$, $p = 0.255$	$F_{(4, 73)} = 1.844$, $p = 0.129$

4.2. Oxygen consumption

Respiration rate provides insights into sublethal metabolic changes that affect an organism's performance and ability to tolerate environmental perturbations. Interpreting changes in respiration under stress is complex, as physiological responses can lead to either an increase or decrease in oxygen consumption (Sokolova et al., 2012; Schulte, 2015; Lesser, 2016). An increase may indicate an active stress response, such as the production of molecular chaperones or antioxidants to mitigate cellular damage (Sokolova et al., 2012; Masanja et al., 2023). Conversely, a decrease may suggest metabolic depression, a strategy to conserve energy, limit oxygen depletion, and reduce reactive oxygen species accumulation (Abele et al., 2002, Storey & Storey 2004, Lesser, 2016). Metabolic depression however can reduce the amount of energy available to mount stress responses and meet basal metabolic demands (Sokolova et al., 2012).

4.2.1. Respiration under different temperature levels

Although only diploids showed a statistically significant increase in oxygen consumption across temperatures, trends indicated that oxygen consumption in both diploids and induced triploids increased with rising temperatures. Regardless of temperature treatment, mated triploids consistently consumed less oxygen than the other groups. As temperatures rise, metabolism is expected to increase due to faster temperature-dependent biochemical reactions and, once physiological thresholds are crossed, due to the production of heat shock proteins or antioxidants (Clarke and Fraser, 2004; Ferreira-Rodríguez et al., 2018; Masanja et al., 2023). The absence of mortality in the temperature experiment suggests that these physiological thresholds were not exceeded. However, the lower overall oxygen consumption and minimal increases in mated triploids at higher temperatures suggest that these individuals may struggle to mobilize energy for stress responses if thresholds are crossed.

A reduced ability to mount stress responses is supported by recent work by Vignier et al. (2025), which found that mated triploids had lower survival than closely related induced triploids and diploids. Lower oxygen and energy availability may also explain the findings of Li et al. (2022), who reported that mated triploids had reduced transcriptomic responses of genes related to thermal stress, inflammation, and apoptosis (e.g., HSP, IAP) compared to diploids, although induced triploids were not included in that study. Future studies should apply more extreme stress conditions to determine whether mated triploid respiration and gene expression patterns remain consistent across ploidy types. This could clarify whether mated triploids increase respiration at stress levels more likely to cause mortality.

Table 6
Summary of results from linear mixed models examining differences in oxygen consumption between individuals that were exposed to acute heat stress and those that were not in each of the experiments. F and *p* values are shown. Asterix (*) and bold indicates significant effects.

Experiment	Treatment Condition	Main Effect: Acute heat exposure (F-value, *p*-value)	Main Effect: Ploidy (F-value, *p*-value)	Interaction Effect (F-value, *p*-value)
Temperature	7.5 °C	$F_{(1, 27)} = 0.323$, $p = 0.137$	$F_{(2, 27)} = 1.266$, $p = 0.298$	$F_{(2, 27)} = 0.839$, $p = 0.443$
	17.5 °C	$F_{(1,32)} = 2.329$, $p = 0.137$	$F_{(2,32)} = 1.127$, $p = 0.337$	$F_{(2, 32)} = 1.771$, $p = 0.186$
	27.5 °C	$F_{(2, 40)} = 1.132$, $p = 0.294$	$F_{(1,40)} = 2.985$, $p = 0.062$	$F_{(2,40)} = 0.397$, $p = 0.675$
Dissolved Oxygen	7.66 mg L^{-1} DO	**$F_{(1, 40)} = 20.243$, $p = 0.001$***	**$F_{(2, 40)} = 5.534$, $p = 0.007$***	$F_{(2, 40)} = 2.930$, $p = 0.065$
	5.74 mg L^{-1} DO	**$F_{(1,33)} = 14.658$, $p = 0.001$***	$F_{(2,33)} = 1.901$, $p = 0.165$	$F_{(2, 33)} = 0.375$, $p = 0.690$
	1.91 mg L^{-1} DO	**$F_{(1, 46)} = 9.225$, $p = 0.004$***	$F_{(2, 40)} = 0.784$, $p = 0.460$	$F_{(2, 40)} = 0.234$, $p = 0.791$
pCO_2	850 µatm	$F_{(1, 47)} = 0.042$, $p = 0.873$	$F_{(2, 47)} = 0.257$, $p = 0.774$	$F_{(2, 47)} = 1.922$, $p = 0.157$
	1400 µatm	$F_{(1, 51)} = 0.696$, $p = 0.402$	$F_{(2, 51)} = 1.43$, $p = 0.247$	$F_{(2, 51)} = 0.201$, $p = 0.818$
	2000 µatm	$F_{(1, 53)} = 1.874$, $p = 0.177$	**$F_{(2, 53)} = 5.905$, $p = 0.005$***	$F_{(2, 53)} = 1.418$, $p = 0.251$

4.2.2. Respiration under different DO levels

Oysters maintained consistent respiration rates across different DO levels, likely reflecting successful efforts to maintain sufficient oxygen through ventilatory behaviours (Tran et al., 2000; Le Moullac et al., 2007; Li et al., 2019). While this suggests resilience to hypoxia, our lowest DO level was 1.91 mg L^{-1}, just below the accepted hypoxia threshold of 2 mg L^{-1}. However, thresholds vary between species (Vaquer-Sunyer and Duarte, 2008), and in some bivalves, hypoxia effects are not evident until levels drop below 1 mg L^{-1} (e.g. Le et al., 2016; Cheng et al., 2024). Although this study aimed to quantify physiological responses across a broad range of environmental conditions, future research should investigate lower DO levels to better understand the effects of hypoxia and anoxia in oysters.

It is also important to consider that adding nitrogen to manipulate seawater DO levels may have altered carbonate chemistry across tanks.

In addition to displacing oxygen, nitrogen may have affected CO_2 levels. Although we did not extensively monitor carbonate chemistry during the DO experiments, the lack of performance differences across treatment levels suggests that any potential pCO_2 variation had minimal impact on individual performance.

4.2.3. Respiration after an acute thermal stress

Physiological performance metrics after acute thermal stress are important for understanding metabolic differences between ploidy groups and predicting their response to short-term thermal stress events such as heat waves (Oliver et al., 2019; Smith et al., 2023). In our study, only individuals from the DO experiment increased their metabolic rates after an acute thermal stress (Fig. 3b), suggesting they had sufficient metabolic scope to increase their metabolism and mobilize energy reserves for synthesizing heat shock proteins and antioxidant enzymes (Brokordt et al., 2015; Yang et al., 2016; Delorme et al., 2020). The low respiration rates in individuals from the temperature experiment that were subject to additional acute heat stress suggest they were already near their physiological limits and unable to increase metabolic activity further in response to stress.

In individuals exposed to acute heat stress in the pCO_2 experiment, respiration tended to increase in diploids and induced triploids, while mated triploids showed a decrease with increasing pCO_2 levels. Although these trends were observed across treatments, statistical significance was only detected at 2000 μatm. This suggests that mated triploids are more sensitive to the combined effects of temperature and pCO_2. The mechanisms underlying this increased sensitivity remain unclear, but previous studies have shown that triploids have lower haemolymph pH than diploids (Casas et al., 2024), which may increase their susceptibility to seawater carbonate chemistry changes. Larger triploid cell size may also slow intracellular ion transport, making pH regulation more difficult (Miettinen et al., 2017; Hermaniuk et al., 2021; Casas et al., 2024).

4.3. Role of genetic background

If triploidy alone determined performance differences, induced and mated triploids should respond similarly. However, diploids and induced triploids performed more similarly than mated triploids, particularly in terms of mortality under pCO_2 stress and oxygen consumption in the temperature (without acute heat stress) and pCO_2 (with acute heat stress) experiments. Separating the effects of genetic background from the triploid production method is difficult, as few studies compare closely related triploids from different methods. However, since both triploid groups in our study were produced from the same females, our results offer insight into how parental genetic contributions shape performance. The greater sensitivity of mated triploids suggests that genetic background (i.e. parentage and the origin of the majority of the genetic material) and diversity may play a key role in susceptibility to climate stressors.

Although direct comparisons between the three experiments are not possible due to differences in age, mated triploids appeared to be the most sensitive across the pCO_2 and temperature experiments. This sensitivity was evident through higher mortality at lower pCO_2 levels, lower respiration rates at high temperatures, and reduced respiration after acute thermal stress under elevated pCO_2. While diploids and induced triploids in this study were full siblings, mated triploids were half-siblings and shared less genetic material. Additionally, mated triploids were produced using sperm from a single tetraploid male, reducing genetic diversity within this group. Moreover, mated triploids inherited two sets of chromosomes from the tetraploid sperm, while induced triploids inherited two sets from the egg, resulting in two-thirds of their genetic material differing. This aligns with findings by Vignier et al. (2025), who compared the growth of two groups of mated triploids, each produced from different tetraploid sperm but same diploid eggs, with closely related induced triploids and diploids. Their results indicated that performance was more strongly influenced by the tetraploid father than by triploidy alone.

In general, tetraploid males used to produce mated triploids come from a limited number of established lines, restricting genetic diversity and increasing the risk of inbreeding (Brianik and Allam, 2023). This is critical, as bivalves have strong potential for local adaptation, which may be lost when genetic diversity is limited (Burford et al., 2014; Bible and Sanford, 2016; Brianik and Allam, 2023). In contrast, diploids and induced triploids may have retained genetic traits associated with local adaptation, contributing to their resilience. Matt et al. (2020) also showed that mated triploid performance varies across genetic lines and locations. Since mated triploid production achieves nearly 100 % triploidy, compared to 88 % from induction methods (Guo & Allen 1994, Wadsworth et al., 2019), developing locally adapted tetraploid lines could enhance resilience, increase genetic diversity, and reduce triploid mortality. This strategy has significant potential to improve production yields.

4.4. Caveats and future directions

The size and age of the individuals in this work may have contributed to the subtle differences in responses observed between experiments and treatments. Using juvenile oysters, which were not reproductive, eliminated the impact of gonadal development on physiological performance. Smaller juveniles may also experience less limitation than adults in oxygen delivery to tissues due to a larger body surface area to volume ratio (Portner et al. 2010; Eymann et al., 2020), potentially explaining why we did not observe performance differences seen in previous studies with diploids and triploids. As the experiments were conducted at different times, we could not account for ontogenetic effects or directly compare individual responses across stressors. This limitation prevented us from examining how single stressors impact individuals throughout their life stages. Understanding these impacts is essential for predicting how aquaculture production will be impacted by climate change and for determining optimal outplanting and harvesting times.

While our study examined a broad range of stressors, some conditions may not reflect long-term commercial aquaculture environments or may have been too extreme to yield informative physiological insights. For example, the maximum pCO_2 targets used in this study were 2000 μatm, which is extreme and is reflected in the low survival of all ploidy groups. This was partially because the water used in these experiments was sourced from the Puget Sound in Washington State, USA, which is an inland sea with a naturally high pCO_2 of ~800 μatm (Murray et al., 2015). Indeed, the level at which we began to observe differences in oxygen consumption and survival of mated triploids was well beyond future predictions of maximum ocean pCO_2 levels under the most extreme of emissions scenarios (RPC 8.5 - Jiang et al., 2023). Future research should focus on a narrower range of conditions relevant to commercial aquaculture and on extreme environmental stress levels predicted to trigger physiological responses, to better understand the mechanisms driving these responses.

Finally, understanding the impact of multiple interacting stressors, as well as how this interacts with partial or full gametogenesis, is essential for developing management strategies to improve survival in aquaculture. Climate change stressors often interact in additive, antagonistic, or synergistic ways (Gobler et al., 2014; Steckbauer et al., 2015; Gissi et al., 2021), influencing organismal responses. For instance, while survival under low oxygen conditions was high, combining low oxygen with high temperatures could significantly impact oyster survival (Pörtner, 2010; Marshall and McQuaid, 2020; Coxe et al., 2023). Additionally, understanding interactions with biological changes, such as gonadal development, is vital for identifying mechanisms driving differences in the resistance or sensitivity of individuals to climate change stress. These interactions will become increasingly relevant as marine heatwaves and upwelling events intensify (Wang et al., 2015; Oliver et al., 2018). Upwelling can bring low-DO, low-pH water to

surface aquaculture sites, while heatwaves may trigger extreme hypoxia (Li et al., 2024). As all combinations of multiple stressors cannot be examined at once, using a scenario-based approach to test the responses of diploid and triploid individuals to these short-term episodic events will be an important step to future proofing the aquaculture industry.

5. Conclusions

Overall, this work showed that juvenile diploid, mated triploid, and induced triploid Pacific oysters exhibit relative resilience to temperature and dissolved oxygen stress. Mated triploids had the lowest survival rates under elevated pCO_2 compared to induced triploids and diploids, highlighting the sensitivity of juvenile Pacific oysters to seawater carbonate chemistry. The similar performance of diploids and induced triploids indicates that the production method may influence stress sensitivity more than ploidy status. Therefore, carefully considering the environmental conditions that will be experienced now and into the future, along with the production method used to produce triploid individuals, is essential to assuring the successful future of the triploid aquaculture industry in a changing climate.

CRediT authorship contribution statement

Craig Roger Norrie: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **D. Shallin Busch:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jonathan Davis:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Paul McElhany:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jacqueline L. Padilla Gamiño:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Craig Norrie reports financial support was provided by NOAA Seagrant and NOAA Ocean Acidification Program. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742866.

Data availability

Data will be made available on request.

References

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205, 1831–1841.
Allen, S.K., Downing, S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol. 102, 197–208.
Allen, S.K., Downing, S.L., 1991. Consumers and experts alike prefer the taste of sterile triploid over gravid diploid Pacific oysters (*Crassostrea gigas*, Thunberg, 1793). J. Shellfish Res. 10, 19–22.
Allen, S.K., Gagnon, P.S., Hidu, H., 1982. Induced triploidy in the soft-shell clam: cytogenetic and allozymic confirmation. J. Hered. 73, 421–428.
Alma, L., McElhany, P., Crim, R.N., Newton, J.A., Maher, M., Mickett, J.B., Padilla-Gamiño, J.L., 2024. Phenotypic plasticity and carryover effects in an ecologically important bivalve in response to changing environments. Front. Mar. Sci. 11, 1178507.
Beaumont, A.R., Fairbrother, J.E., Hoare, K., 1995. Multilocus heterozygosity and size: a test of hypotheses using triploid *Mytilus edulis*. Heredity 75, 256–266.
Bednaršek, N., Beck, M.W., Pelletier, G., Applebaum, S.L., Feely, R.A., Butler, R., Byrne, M., Peabody, B., Davis, J., Štrus, J., 2022. Natural analogues in pH variability and predictability across the coastal pacific estuaries: extrapolation of the increased oyster dissolution under increased pH amplitude and low predictability related to ocean acidification. Environ. Sci. Technol. 56, 9015–9028.
Bible, J.M., Sanford, E., 2016. Local adaptation in an estuarine foundation species: implications for restoration. Biol. Conserv. 193, 95–102.
Bodenstein, S., Callam, B.R., Walton, W.C., Rikard, F.S., Tiersch, T.R., La Peyre, J.F., 2023. Survival and growth of triploid eastern oysters, *Crassostrea virginica*, produced from wild diploids collected from low-salinity areas. Aquaculture 564, 739032.
Brianik, C.J., Allam, B., 2023. The need for more information on the resistance to biological and environmental stressors in triploid oysters. Aquaculture 577, 739913.
Brokordt, K., Pérez, H., Herrera, C., Gallardo, A., 2015. Reproduction reduces HSP70 expression capacity in *Argopecten purpuratus* scallops subject to hypoxia and heat stress. Aquat. Biol. 23, 265–274.
Brown, J.R., Hartwick, E.B., 1988. Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*. Aquaculture 70, 231–251.
Burford, M.O., Scarpa, J., Cook, B.J., Hare, M.P., 2014. Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 505, 161–175.
Cai, W.-J., Huang, W.-J., Luther, G.W., Pierrot, D., Li, M., Testa, J., Xue, M., Joesoef, A., Mann, R., Brodeur, J., Xu, Y.-Y., Chen, B., Hussain, N., Waldbusser, G.G., Cornwell, J., Kemp, W.M., 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. Nat. Commun. 8, 369.
Caillon, C., Pernet, F., Lutier, M., Poi, C.D., 2023. Differential reaction norms to ocean acidification in two oyster species from contrasting habitats. J. Exp. Biol. 226, jeb246432.
Callam, B.R., Allen, S.K., Frank-Lawale, A., 2016. Genetic and environmental influence on triploid *Crassostrea virginica* grown in Chesapeake Bay: growth. Aquaculture 452, 97–106.
Casas, S.M., Comba, D., La Peyre, M.K., Rikard, S., La Peyre, J.F., 2024. Rates of osmoconformation in triploid eastern oysters, and comparison to their diploid half-siblings. Aquaculture 580, 740326.
Cheng, M.C.F., Zamora, L.N., Ragg, N.L.C., Hickey, A.J.R., Dunphy, B.J., 2024. Responses of the New Zealand green-lipped mussel, *Perna canaliculus*, to acute environmental changes designed to depress metabolism. Aquaculture 580, 740332.
Clarke, A., Fraser, K.P.P., 2004. Why does metabolism scale with temperature? Funct. Ecol. 18, 243–251.
Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M.Á., Free, C.M., Froehlich, H.E., Golden, C.D., Ishimura, G., Maier, J., Macadam-Somer, I., Mangin, T., Melnychuk, M.C., Miyahara, M., de Moor, C.L., Naylor, R., Nøstbakken, L., Ojea, E., O'Reilly, E., Parma, A.M., Plantinga, A.J., Thilsted, S.H., Lubchenco, J., 2020. The future of food from the sea. Nature 588, 95–100.
Cox, D.R., 1972. Regression models and life-tables. J. R. Stat. Soc. B. Methodol. 34, 187–202.
Coxe, N., Mize, G., Casas, S., Peyre, M.K.L., Lavaud, R., Callam, B., Rikard, S., Peyre, J.L., 2023. Hypoxia and anoxia tolerance in diploid and triploid eastern oysters at high temperature. Shellfish Res. 42, 29–43.
Delorme, N.J., Biessy, L., South, P.M., Zamora, L.N., Ragg, N.L.C., Burritt, D.J., 2020. Stress-on-stress responses of a marine mussel, *Perna canaliculus*: food limitation reduces the ability to cope with heat stress in juveniles. Mar. Ecol. Prog. Ser. 644, 105–117.
Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science 321, 926–929.
Dickson, A.G., Sabine, C.L., Christian, J.R., Bargeron, C.P., North Pacific Marine Science Organization (Eds.), 2007. Guide to Best Practices for Ocean CO_2 Measurements. North Pacific Marine Science Organization, Sidney, BC.
Doney, S.C., 2010. The growing human footprint on coastal and open-ocean biogeochemistry. Science 328, 1512–1516.

Dumbauld, B.R., Du, X., Hunsicker, M., Forster, Z., 2023. Multi-decade changes in the condition index of adult Pacific oysters (*Crassostrea gigas*) in response to climate in a US west coast estuary. J. Sea Res. 193, 102383. https://doi.org/10.1016/j.seares.2023.102383.
Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A.H., Lannig, G., Sokolova, I.M., Aberhan, M., Pörtner, H.O., 2020. Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758) — explaining ecological findings under climate change. Mar. Biol. 167, 17.
FAO (Ed.), 2020. The State of World Fisheries and Aquaculture. Sustainability in action, Rome.
Feely, R.A., Alin, S.R., Newton, J., Sabine, C.L., Warner, M., Devol, A., Krembs, C., Maloy, C., 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuar. Coast. Shelf Sci. 88, 442–449.
Ferreira-Rodríguez, N., Fernández, I., Cancela, M.L., Pardo, I., 2018. Multibiomarker response shows how native and non-native freshwater bivalves differentially cope with heat-wave events. Aquat. Conserv. Mar. Freshwat. Ecosyst. 28, 934–943.
Filgueira, R., Byron, C.J., Comeau, L.A., Costa-Pierce, B., Cranford, P.J., Ferreira, J.G., Grant, J., Guyondet, T., Jansen, H.M., Landry, T., McKindsey, C.W., Petersen, J.K., Reid, G.K., Robinson, S.M.C., Smaal, A., Sonier, R., Strand, Ø., Strohmeier, T., 2015. An integrated ecosystem approach for assessing the potential role of cultivated bivalve shells as part of the carbon trading system. Mar. Ecol. Prog. Ser. 518, 281–287.
Gattuso, J., Epitalon, J., Lavigne, H., Orr, J., 2024. _seacarb: Seawater Carbonate Chemistry_. R package version 3.3.0.
Gattuso, J.P., Magnan, A., Billé, R., Cheung, W.W.L., Howes, E.L., Joos, F., Allemand, D., Bopp, L., Cooley, S.R., Eakin, C.M., Hoegh-Guldberg, O., Kelly, R.P., Pörtner, H.-O., Rogers, A.D., Baxter, J.M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U.R., Treyer, S., Turley, C., 2015. Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science 349, aac4722.
George, M.N., Cattau, O., Middleton, M.A., Lawson, D., Vadopalas, B., Gavery, M., Roberts, S.B., 2023. Triploid Pacific oysters exhibit stress response dysregulation and elevated mortality following heatwaves. Glob. Chang. Biol. 29, 6969–6987.
Gissi, E., Manea, E., Mazaris, A.D., Fraschetti, S., Almpanidou, V., Bevilacqua, S., Coll, M., Guarnieri, G., Lloret-Lloret, E., Pascual, M., Petza, D., Rilov, G., Schonwald, M., Stelzenmüller, V., Katsanevakis, S., 2021. A review of the combined effects of climate change and other local human stressors on the marine environment. Sci. Total Environ. 755, 142564.
Gobler, C.J., DePasquale, E.L., Griffith, A.W., Baumann, H., 2014. Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. PLoS One 9, e83648.
Guo, X., Allen Jr., S.K., 1994. Sex determination and polyploid gigantism in the dwarf surfclam (*Mulinia lateralis* say). Genetics 138, 1199–1206.
Guo, X., DeBrosse, G.A., Allen, S.K., 1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142, 149–161.
Harianto, J., Carey, N., Byrne, M., 2019. respR—an R package for the manipulation and analysis of respirometry data. Methods Ecol. Evol. 10, 912–920.
Harley, C.D.G., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. Ecol. Lett. 9, 228–241.
He, Q., Silliman, B.R., 2019. Climate change, human impacts, and coastal ecosystems in the Anthropocene. Curr. Biol. 29, R1021–R1035.
Helm, M.M., Bourne, N., Lovatelli, A., Bourne, N. (Eds.), 2004. Hatchery Culture of Bivalves: A Practical Manual. Food and Agriculture Organization of the United Nations, Rome.
Hermaniuk, A., van de Pol, I.L.E., Verberk, W.C.E.P., 2021. Are acute and acclimated thermal effects on metabolic rate modulated by cell size? A comparison between diploid and triploid zebrafish larvae. J. Exp. Biol. 224, jeb227124.
Houssin, M., Trancart, S., Denechere, L., Oden, E., Adeline, B., Lepoitevin, M., Pitel, P.H., 2019. Abnormal mortality of triploid adult Pacific oysters: is there a correlation with high gametogenesis in Normandy, France? Aquaculture 505, 63–71.
Huvet, A., Normand, J., Fleury, E., Quillien, V., Fabioux, C., Boudry, P., 2010. Reproductive effort of Pacific oysters: a trait associated with susceptibility to summer mortality. Aquaculture 304, 95–99.
Ibarra, A.M., Ascencio-Michel, R., Ramírez, J.L., Manzano-Sarabia, M., Rodríguez-Jaramillo, C., 2017. Performance of diploid and triploid *Crassostrea gigas* (Thunberg, 1793) grown in tropical versus temperate natural environmental conditions. Shellfish Res. 36, 119–139.
Jiang, L.-Q., Dunne, J., Carter, B.R., Tjiputra, J.F., Terhaar, J., Sharp, J.D., Olsen, A., Alin, S., Bakker, D.C.E., Feely, R.A., Gattuso, J.P., Hogan, P., Ilyina, T., Lange, N., Lauvset, S.K., Lewis, E.R., Lovato, T., Palmieri, J., Santana-Falcón, Y., Schwinger, J., Séférian, R., Strand, G., Swart, N., Tanhua, T., Tsujino, H., Wanninkhof, R., Watanabe, M., Yamamoto, A., Ziehn, T., 2023. Global surface ocean acidification indicators from 1750 to 2100. J. Adv. Model. Earth Syst. 15 e2022MS003563.
Jiang, W., Li, G., Xu, G., Lin, Y., Qing, N., 1993. Growth of the induced triploid pearl oyster, *Pinctada martensii* (D.). Aquaculture 111, 245–253.
Le, D.V., Alfaro, A.C., Ragg, N.L.C., Hilton, Z., King, N., 2016. Aerobic scope and oxygen regulation of New Zealand geoduck (*Panopea zelandica*) in response to progressive hypoxia. Aquaculture 463, 28–36.
Le Moullac, G., Quéau, I., Le Souchu, P., Pouvreau, S., Moal, J., René Le Coz, J., François Samain, J., 2007. Metabolic adjustments in the oyster *Crassostrea gigas* according to oxygen level and temperature. Mar. Biol. Res. 3, 357–366.
Lenth, R.V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Piaskowski, J., Riebl, H., Singmann, H., 2024. Emmeans: Estimated Marginal Means, aka Least-Squares Means.
Lesser, M.P., 2016. Climate change stressors cause metabolic depression in the blue mussel, *Mytilus edulis*, from the Gulf of Maine. Limnol. Oceanogr. 61, 1705–1717.
Li, C., Huang, J., Liu, X., Ding, L., He, Y., Xie, Y., 2024. The ocean losing its breath under the heatwaves. Nat. Commun. 15, 6840.
Li, Q., Sun, S., Zhang, F., Wang, M., Li, M., 2019. Effects of hypoxia on survival, behavior, metabolism and cellular damage of Manila clam (*Ruditapes philippinarum*). PLoS One 14, e0215158.
Li, Y., Qin, J.G., Abbott, C.A., Li, X., Benkendorff, K., 2007. Synergistic impacts of heat shock and spawning on the physiology and immune health of *Crassostrea gigas*: an explanation for summer mortality in Pacific oysters. Am. J. Phys. Regul. Integr. Comp. Phys. 293, R2353–R2362.
Li, Y., Xu, C., Li, Q., 2022. Physiological and gene expression responses of diploid and triploid Pacific oyster (*Crassostrea gigas*) to heat acclimation. Aquac. Res. 53, 6641–6650.
Lueker, T.J., Dickson, A.G., Keeling, C.D., 2000. Ocean *p*CO2 calculated from dissolved inorganic carbon, alkalinity, and equations for *K*1 and *K*2: validation based on laboratory measurements of CO2 in gas and seawater at equilibrium. Mar. Chem. 70, 105–119.
Marshall, D.J., McQuaid, C.D., 2020. Metabolic regulation, oxygen limitation and heat tolerance in a subtidal marine gastropod reveal the complexity of predicting climate change vulnerability. Front. Physiol. 11.
Masanja, F., Yang, K., Xu, Y., He, G., Liu, X., Xu, X., Xiaoyan, J., Xin, L., Mkuye, R., Deng, Y., Zhao, L., 2023. Impacts of marine heat extremes on bivalves. Frontiers in marine. Science 10.
Matt, J.L., Allen, S.K., 2021. A classification system for gonad development in triploid *Crassostrea virginica*. Aquaculture 532, 735994.
Matt, J.L., Guévélou, E., Small, J.M., Allen, S.K., 2020. A field test investigating the influence of brood stock origin and ploidy on the susceptibility of *Crassostrea virginica* to "triploid mortality" in the Chesapeake Bay. Aquaculture 526, 735375.
Miettinen, T.P., Caldez, M.J., Kaldis, P., Björklund, M., 2017. Cell size control – a mechanism for maintaining fitness and function. BioEssays 39, 1700058.
Murray, J.W., Roberts, E., Howard, E., O'Donnell, M., Bantam, C., Carrington, E., Foy, M., Paul, B., Fay, A., 2015. An inland sea high nitrate-low chlorophyll (HNLC) region with naturally high pCO2. Limnol. Oceanogr. 60, 957–966.
Normand, J., Ernande, B., Haure, J., McCombie, H., Boudry, P., 2009. Reproductive effort and growth in *Crassostrea gigas*: comparison of young diploid and triploid oysters issued from natural crosses or chemical induction. Aquat. Biol. 7, 229–241.
Norrie, C.R., Dunphy, B.J., Ragg, N.L.C., Lundquist, C.J., 2018. Ocean acidification can interact with ontogeny to determine the trace element composition of bivalve shell. Limnology and Oceanography Letters 3, 393–400.
Norrie, C., Dunphy, B., Roughan, M., Weppe, S., Lundquist, C., 2020. Spill-over from aquaculture may provide a larval subsidy for the restoration of mussel reefs. Aquac. Environ. Interact. 12, 231–249.
Oliver, E.C.J., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V., Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Straub, S.C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9, 1324.
Oliver, E.C.J., Burrows, M.T., Donat, M.G., Sen Gupta, A., Alexander, L.V., Perkins-Kirkpatrick, S.E., Benthuysen, J.A., Hobday, A.J., Holbrook, N.J., Moore, P.J., Thomsen, M.S., Wernberg, T., Smale, D.A., 2019. Projected marine heatwaves in the 21st century and the potential for ecological impact. Front. Mar. Sci. 6, 734.
Peduzzi, P., Concato, J., Kemper, E., Holford, T.R., Feinstein, A.R., 1996. A simulation study of the number of events per variable in logistic regression analysis. J. Clin. Epidemiol. 49, 1373–1379.
Pernet, F., Barret, J., Marty, C., Moal, J., Gall, P.L., Boudry, P., 2010. Environmental anomalies, energetic reserves and fatty acid modifications in oysters coincide with an exceptional mortality event. Mar. Ecol. Prog. Ser. 401, 129–146.
Pinheiro, J., Bates, D., R Core Team, 2024. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-168. https://CRAN.R-project.org/package=nlme.
Porter, E.T., Breitburg, D.L., 2016. Eastern oyster, *Crassostrea virginica*, valve gape behavior under diel-cycling hypoxia. Mar. Biol. 163, 218.
Pörtner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–893.
Qin, Y., Zhang, Y., Yu, Z., 2022. Aquaculture performance comparison of reciprocal triploid *C. Gigas* produced by mating tetraploids and diploids in China. Aquaculture 552, 738044.
Rabalais, N.N., Díaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7, 585–619.
van der Schatte, Olivier A., Jones, L., Vay, L.L., Christie, M., Wilson, J., Malham, S.K., 2020. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 12, 3–25.
Riebesell, U., Fabry, V., Hansson, L., Gattuso, J-P., 2011. Guide to best practices for ocean acidification research and data reporting. Publications Office, LU.
Schulte, P.M., 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J. Exp. Biol. 218, 1856–1866.
Smith, K.E., Burrows, M.T., Hobday, A.J., King, N.G., Moore, P.J., Gupta, A.S., Thomsen, M.S., Wernberg, T., Smale, D.A., 2023. Biological impacts of marine heatwaves. Annu. Rev. Mar. Sci. 15, 119–145.
Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15.
Stanley, J.G., Allen, S.K., Hidu, H., 1981. Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23, 1–10.

Stanley, R.R.E., Bradbury, I.R., DiBacco, C., Snelgrove, P.V.R., Thorrold, S.R., Killen, S.S., 2015. Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). ICES J. Mar. Sci. 72, 2350–2363.

Steckbauer, A., Ramajo, L., Hendriks, I.E., Fernandez, M., Lagos, N., Prado, L., Duarte, C. M., 2015. Synergistic effects of hypoxia and increasing CO_2 on benthic invertebrates of the central Chilean coast. Front. Mar. Sci. 2.

Therneau, T.M., 2024. Coxme: Mixed Effects Cox Models.

Tran, D., Boudou, A., Massabuau, J.C., 2000. Mechanism for maintaining oxygen consumption under varying oxygenation levels in the freshwater clam *Corbicula fluminea*. Can. J. Zool. 78, 2027–2036.

Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proc. Natl. Acad. Sci. 105, 15452–15457.

Vignier, J., Reardon, M., Exton, M., Delisle, L., Rolton, A., Malpot, E., Scholtens, M., Welford, M., Zamora, L., Delorme, N., Dunphy, B., Adams, S., 2025. Comparative performance of selected triploid oysters *Crassostrea (Magallana) gigas*, produced by chemical induction and mated triploid techniques, to their diploid counterparts. Aquaculture 596, 741894.

Wadsworth, P., Wilson, A.E., Walton, W.C., 2019. A meta-analysis of growth rate in diploid and triploid oysters. Aquaculture 499, 9–16.

Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal Ocean acidification: the other eutrophication problem. Estuar. Coast. Shelf Sci. 148, 1–13.

Wang, B., Hu, J., Li, S., Yu, L., Huang, J., 2018. Impacts of anthropogenic inputs on hypoxia and oxygen dynamics in the Pearl River estuary. Biogeosciences 15, 6105–6125.

Wang, D., Gouhier, T.C., Menge, B.A., Ganguly, A.R., 2015. Intensification and spatial homogenization of coastal upwelling under climate change. Nature 518, 390–394.

Welford, M., 2023. Ecophysiological Assessment of Climate Change Resilience in Commercial Polyploid Pacific Oysters (*Magallana Gigas*). MSc Thesis. University of Auckland, Auckland, New Zealand.

Yang, C.Y., Sierp, M.T., Abbott, C.A., Li, Y., Qin, J.G., 2016. Responses to thermal and salinity stress in wild and farmed Pacific oysters *Crassostrea gigas*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 201, 22–29.

Yang, H., Simon, N., Sturmer, L., 2018. Production and Performance of Triploid Oysters for Aquaculture. University of Florida Extension.

Zhang, J., Gilbert, D., Gooday, A.J., Levin, L., Naqvi, S.W.A., Middelburg, J.J., Scranton, M., Ekau, W., Peña, A., Dewitte, B., Oguz, T., Monteiro, P.M.S., Urban, E., Rabalais, N.N., Ittekkot, V., Kemp, W.M., Ulloa, O., Elmgren, R., Escobar-Briones, E., Van der Plas, A.K., 2010. Natural and human-induced hypoxia and consequences for coastal areas: synthesis and future development. Biogeosciences 7, 1443–1467.

Zhang, Z., Zhang, Q., 2012. Molecular cloning, characterization and expression of heat shock protein 70 gene from the oyster *Crassostrea hongkongensis* responding to thermal stress and exposure of Cu^{2+} and malachite green. Gene 497, 172–180.