Effects of Thermal Stress Events and Correlated Response on Thermal Tolerance of the Eastern Oyster (*Crassostrea virginica*)

by

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Abstract

The eastern oyster (*Crassostrea virginica*) is an economically and ecologically important species. There is interest in improving the heat tolerance of this species for both aquaculture and restoration purposes. I examined the effects of induced acquired thermal tolerance (ATT) and genomic selection for Dermo disease resistance on the heat tolerance of this species. In the induced ATT study, oysters were exposed to sublethal heat stress at pre- and post- metamorphosis life stages. After recovery, animals were exposed for 30 days to 25 °C, 36 °C, 38 °C, and 40 °C. In the genomic selection study, genomically selected, phenotypically selected, genomic control, and wild control oyster lines were exposed for 20 days to 23 °C, 28 °C, 36 °C, and 38 °C. We did not see an impact of induced ATT on heat tolerance. However, the genomically selected oysters survived significantly longer at 38 °C than the other lines, indicating a positive relationship between disease tolerance and heat tolerance.

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ABSTRACT

The eastern oyster (*Crassostrea virginica*) is an ecologically and economically important species. Cultured oysters are experiencing unexplained mortality events which may be, in part, due to increasing water temperatures. To combat these events, a tool called acquired thermal tolerance (ATT) can be used to raise an individual's thermal tolerance temporarily. To induce ATT, the animal is exposed to sublethal heat stress followed by an extended recovery period. Previous studies on bivalves have reported ATT lasting up to 21 days after exposure to sublethal heat stress, but these have focused on ATT induction at the adult stage. Induced ATT has not been shown to have a strong effect in adult eastern oysters, and no published studies have investigated multiple life stages. The absence of an ATT response in eastern oysters may depend on tolerance to environmental stressors. This suggests that the ability to induce ATT may vary by life stage in eastern oysters. To test this, I exposed eastern oysters at pre- (veliger) and post- (spat) metamorphosis stages to sublethal heat stress. Veligers were exposed to 32 °C for four days. As the ambient water temperature of the Gulf of Mexico (GOM) was 32 °C during the spat event, spat were exposed to 34 °C for four days. A thermal tolerance assay was conducted two months post spat thermal stress exposure and three months post veliger thermal stress exposure. During the final thermal tolerance assay, the animals were exposed to 25 °C, 36 °C, 38 °C, and 40 °C for 30 days. Neither veliger nor spat from ATT groups had a significant advantage over control groups based on survival probabilities and lethal time at any temperature of the thermal tolerance assay. The methods used in this study did not induce ATT at either the veliger or the spat stage for time period tested.

INTRODUCTION

The eastern oyster (*Crassostrea virginica*) is a species of economic and ecological importance with a native range from the Caribbean and Gulf of Mexico (GOM) to the St. Lawrence River estuary in eastern Canada (Kennedy et al. 1996). Eastern oysters are ecosystem engineers and can create reefs that reduce erosion and provide shelter for many micro- and macro-organisms (Lenihan et al. 1998). A single adult eastern oyster can filter 40 gallons of water daily in controlled laboratory conditions and can filter up to 25 gallons of water a day in wild conditions (Gray et al. 2022; Donnelly et al. 2023). As they filter, the oysters can remove 23% of 1.0 to 2.0 µm particles and 56% of 3.0 to 4.0 µm particles (Haven and Morales-Alamo 1970). Additionally, oysters remove nitrogen from the water through filtering algae, sediment, and other suspended solids and incorporate it into their shells and soft tissues (Donnelly et al. 2023). Eastern oysters are also increasingly vital to the economy of many GOM coastal areas. In 2021, Alabama produced 4.7 million farmed oysters with a selling price ranging from \$0.10–\$0.50 per oyster (Petrolia and Caffey 2024).

Starting in the 1960s, farming of eastern oysters shifted from the sea bottom (i.e. on-bottom aquaculture) to floating cages/rafts or long-line systems (i.e. off-bottom aquaculture) (Shaw 1969). On-bottom aquaculture could only be conducted in limited areas due to increased susceptibility to predation and sedimentation (Shaw 1969). Today, with off-bottom aquaculture, farmers are able to grow their eastern oysters in food-rich surface waters across a wider area (Walton et al. 2013).

As recently as 2009, there were no off-bottom oyster farms found between Texas and Florida (Grice 2018). However, due to an increase in the demand for oysters in the culinary field and the decrease in wild oyster reefs, the number of oyster farms in the northern GOM increased to over

200 oyster farms as of 2020 (Sturmer et al. 2020). Because of this demand, the eastern oyster has created a livelihood for many in North America (Kaiser et al. 2011; Soniat et al. 2014). Wild eastern oysters have been harvested in the GOM for centuries, but eastern oyster cultivation in the GOM started in the 1990's (Athanas 1996).

Eastern oysters on farms and wild reefs face challenges related to environmental stressors including decreases in salinities and increases in marine heatwaves due to hurricanes such as Hurricane Michael in 2018 (Sturmer et al. 2020). Previous studies have shown that eastern oysters at both veliger and spat stages have an optimal temperature range of 20 °C to 30 °C with 32 °C being considered physiologically stressful (Stanley and Sellers M.A. 1986; Boudreaux et al. 2023). During thermal stress, oysters may experience protein denaturation, reduced feeding rates, impaired metabolic function, and oxidative stress, even if they appear to recover quickly (Zhao et al. 2021; Joyner et al. 2022). Repeated or prolonged exposure to sublethal temperatures can reduce growth and reproductive output, creating vulnerability over time (Fisher et al 2019). Average water temperatures in GOM increased by 1.0 °C from 1950 to 2021 with maximum temperatures reaching or exceeding 34 °C in recent years (Wang et al. 2023; AUSL 2024). Because of this, there is interest in developing and testing various approaches to increase the thermal tolerance of eastern oysters.

Acquired thermal tolerance (ATT) can be induced by exposing individuals to a sublethal thermal stress event followed by a recovery period, after which individuals exhibit a temporary increase in upper thermal tolerance (Song et al. 2024). This strategy has been effective in some bivalve species but not others (Brun et al. 2009; Jackson et al. 2011; Song et al. 2024). For example, adult Fujian oysters (*Crassostrea angulata*), when exposed to 40 °C for one hour with a recovery of 20 °C for three days, were able to obtain at ATT for up to 21 days after exposure

(Song et al. 2024). Additionally, when exposed to a sublethal thermal stress event (37 °C for 1 h) at reproductive age, Pacific oysters (*Crassostrea gigas*) developed an ATT that lasted up to 21 days after exposure and eastern oysters exhibited an ATT that lasted 14 days after the same exposure (Jackson et al. 2011). Increases in tolerances to hypoxia and heat stress have been seen to last up to one year after exposure in eastern oysters (Donelan et al. 2022). This gives us reason to believe sublethal exposures in eastern oysters can trigger a mechanism (heat shock proteins, etc.) that can improve tolerances to environmental stressors in this species. Although ATT has been induced in oysters at the adult stage, inducing ATT in larval and juvenile oysters for aquaculture and restoration would be more practical. Once they are adults and held in off-bottom cages or on-bottom reefs, manipulation of conditions inducing ATT is nearly impossible. However, induction of ATT is more feasible while they are held under controlled conditions in hatcheries and nurseries. Tolerances to environmental stressors have been shown to differ between life stages of the eastern oysters (McFarland et al. 2022; Boudreaux et al. 2023), leading to questions as to whether strength and duration of ATT might vary among life-stages.

For ATT induction in hatchery-produced oysters to be useful for grow-out farms and restoration, the duration of the ATT effect should be at least three months post-larval stage at which point they have been removed from the controlled conditions of the nursery and transferred to a field site for further grow out (Supan 2002). Field sites have uncontrolled temperatures compared to hatcheries (Wallace 2001), so generating oysters with ATT may help the animals cope with the stressful conditions that can be experienced in the field (Song et al. 2024).

In this study, we exposed eastern oysters to a sublethal thermal stress event at the veliger stage, approximately 48 hours after fertilization of spawned eggs (pre-metamorphosis), and 10 days post-metamorphosis at the stage commonly known as spat. The objectives were to determine 1) if a sublethal thermal stress event at either the veliger or spat stage can induce an ATT lasting for ≥ 2–3 months, and 2) if ATT is induced, does the magnitude vary between pre- and post-metamorphosis exposure.

METHODS

Spawn

A spawn of the diploid AUBIE genetic line was conducted at the Auburn University Shellfish Lab (AUSL, Dauphin Island, AL) on July 18th, 2024 (Day 0). The AUBIE genetic line originated from wild eastern oyster founder population collected from Cedar Point in 2007, Heron Bay in 2009, Perdido Pass in 2009 and 2011, and Alonzo Landing in 2012 in Alabama. The line has been phenotypically selected since 2012 for desirable shell characteristics including size, shape (broad fan and deep cup), and a relatively flat hinge bend (selected out downturned and upturned hinges) for aesthetic and shucking. The spawn used 48 adult oysters from AUBIE 2021 and 2022 cohorts. Prior to the spawn, sperm was extracted from 10 additional male oysters and heated to eliminate viability and was introduced to the spawning system as a spawning inducement cue without prematurely fertilizing eggs (Wallace et al. 2008).

Before spawning, oysters were scrubbed and soaked in freshwater to eliminate debris, barnacles, mussels, anemones, and shell-burrowing annelids. The oysters were then placed into individual 3L flow-through tanks positioned in a rack system. Inflow and outflow of each tank were kept separate to isolate oysters when spawning initiated. Source water was pumped from the GOM into two aerated 22,500-L reservoirs and allowed to settle for at least 24 h to reduce turbidity. Settled water was then pumped through a filtration system comprised of two 20 micron cartridge filters (Pentair Aquatic Ecosystems; Cary, NC, USA), two 10-µm bag filters (PRM

Filtration, Butner, NC, USA), and two 1-µm bag filters (PRM Filtration, Butner, NC, USA) (hereon referred to as FSW (filtered sea water)) and held in two 4,000-L tanks, one of which was held at 30 °C (ambient temperature) and the other heated to 35 °C.

To initiate spawning, each 4,000-L source-water tank was dosed with 10 mL of Shellfish Diet 1800 (Reed Mariculture Inc; Campbell, CA, USA). The water source for each individual flow-through tank was cycled between the 30 °C and 35 °C source-water tanks every 30 minutes to provide spawning cues associated with food quantity and temperature (Wallace et al. 2008). Salinity was held at a consistent 18 PSU. Each oyster was exposed to immobilized sperm (dispensed in increments of ~5 mL) over the course of the spawn. As each individual oyster spawned, their water source was turned off and they were identified as male or female by observing gamete characteristics. Spawned eggs have a granular appearance whereas sperm has a cloudy appearance when broadcast. Out of the 50 total oysters exposed to spawning cues, 29 females and 14 males were observed to actively spawn. Eggs were collected within 45 minutes of spawning to optimize viability. Eggs from all 29 females were filtered through a 125-µm screen to eliminate debris, collected onto concentrated by collection of eggs on a 15 -µm screen to concentrate them, and then resuspended into a single bucket containing 14 L FSW. Approximately 40 mL of sperm from all 14 males was likewise pooled in a 1-L beaker.

To fertilize the eggs, 50 to 100 mL of pooled sperm was added to the 14-L bucket of pooled eggs. Eggs were examined using brightfield settings under a compound microscope (Nikon; Melville, New York, USA) every 5-10 minutes for the presence of polar bodies to confirm fertilization success (Wallace et al. 2008). Once fertilization was confirmed, the total number of fertilized eggs was volumetrically calculated by gently mixing the number of fertilized eggs and taking two 100 µL samples that were counted using a Sedgwick-Rafter chamber (Hausser Scientific; Horsham, PA, USA). Samples were placed under a compound microscope (Nikon; Melville, New York, USA), and the number of fertilized eggs was multiplied by 10 to get the number per ml and then by 14,000 ml in the bucket to get the total number of fertilized eggs. Based on this estimate, a subsample of eggs was distributed into each of six 1,000-L tanks (Figure 1.1A), filled with FSW at ~20 practical salinity unit (PSU), to achieve a concentration of 10 larvae/ml (10 million larvae per tank). Tanks were held in a covered outdoor hatchery facility. For the first 48 h, each tank was subjected to ambient photoperiod and temperature (27 °C to 30 °C; Fig. 1.3).

Thermal stress event at the veliger stage

On Day 2, approximately 48 h after fertilization, all six 1,000-L tanks were drained and through a 20-µm bags to collect larvae (Dynamic Aqua Supply; Surrey, BC, Canada) and distributed into 14 L of ~20 PSU FSW. Size via reticle within a compound microscope (Nikon; Melville, New York, USA) and number of larvae was then calculated for each tank. Larval abundance was determined by taking three 100-µL samples with a 1000-µL pipetter (VWR International; Radnor, PA, USA), placed on a 1-mL Sedgwick-Rafter chamber (Hausser Scientific; Horsham, PA, USA). Larvae were immobilized by the addition of ~50 µL of 5% formalin (CH_2O) to be counted and measured under a light microscopy (Nikon; Melville, New York, USA). The average count of the three samples was then multiplied by 10 to estimate the number of larvae/mL. Next, this number was multiplied by 14,000 to get the total number of larvae from the tank. While the larvae were being counted and measured, each 1,000-L tank was replenished with FSW (~20 PSU; 27-30 °C). Once larvae were measured and counted, they were restocked at a reduced concentration of 5 larvae/ml (5 million larvae per 1000-L tank). Larval concentration was reduced to account for increases in larval size. After the larvae were put back in their tanks, they were fed Shellfish Diet 1800 with an addition of ClorAm-X (2.5 mL Shellfish Diet 1800 per day per million larvae; 0.12 g of ClorAm-X (AquaScience Research Group, Inc.; Kansas City, MO, USA) per 1 mL of Shellfish Diet 1800 ® (Reed Mariculture Inc; Campbell, CA, USA ; Rikard and Walton 2012).

On Day 2, after all the tanks were fed, three of the tanks were randomly chosen to be subjected to a four-day thermal stress event while the other three remained at ambient temperature (27–30 °C; Figure 1.3). Each thermal stress tank was equipped with a submersible heater bar (800-Watt, JSK Merchandising Inc; Chicago, IL, USA) and a temperature controller (INKBIRD Tech; C.L, Shenzhen, China; Figure 1.1B). Water temperature in the thermal stress tanks was increased at a rate of 1℃ per hour until reaching 32 °C (Adapted from Boudreaux et al. 2023). Temperature in the thermal stress tanks was then held at a constant 32 °C for four days which has been previously shown to be stressful but not cause 100% mortality (Boudreaux et al. 2023). During this time, all six tanks were drained every other day through a 40-µm bag to larvae collected (Dynamic Aqua Supply; Surrey, BC, Canada) and transferred to a 14-L bucket for counts and measurements. After counting and measuring were completed, larvae from thermal stress tanks were transferred to three 1,000-L tanks filled with ~20 PSU water preheated to 32 °C. Control larvae were placed in their original tanks after a 100% water change and kept at ambient temperature.

On Day 7, the temperature of thermal stress tanks was allowed to decline to ambient conditions (27–30 °C). From Day 7 to Day 14, larvae in all tanks were drained down every other day and larvae were counted, measured, fed recommended feed rates (Rikard and Walton 2012), and monitored for abnormal shell shape while being counted and measured. Once the larvae had reached the pediveliger stage (commonly referred to as eyed-larvae; Days 10 to 15) as indicated by eye spots and a length of 290 µm, tanks were drawn down daily and pediveliger larvae removed for transfer to setting tanks by screening on a 200 µm sieve to (Wallace et al. 2008).

Thermal stress event at the spat stage

During daily drain-downs on days 10 to 15, larvae were graded on a 200-µm sieve. Larvae retained on the sieve were volumetrically enumerated and transferred to 180-µm mesh downweller silos held within a recirculating system. Larvae that passed through the sieve were counted, measured, and returned to their original larval tank for further development. Daily draining and sieving continued until Day 15 when each downweller silo reached maximum stocking density (300,000 pediveligers). Each of the three larval thermal stress treatment tanks were used to stock one downweller silo whereas each of the three control larval tanks were used to stock two downweller silos to account for spat needed for the thermal stress event at the spat stage (nine silos total). The bottom of each downweller silo was covered with 150 mL of 250-300 µm cultch (finely ground oyster shell) as a substrate for the pediveligers to adhere themselves to. The recirculating system holding the silos underwent a water change every 48 h with FSW (~20 PSU). Larvae that set on undesirable surfaces (silo mesh or inner sides of the silo) were removed daily via a beveled edge caulk scraping tool (Lowe's Companies, Inc.; Mooresville, NC, USA) and rinsing screens rinsing with low pressure ~20 PSU FSW. Pediverligers were fed twice a day with Shellfish Diet 1800 (Reed Mariculture Inc; Campbell, CA, USA) such that algal concentration was maintained at ~200,000 cells/mL estimated using a double Neubauer counting chamber (Hausser Scientific, Horsham, PA, USA) (Wallace et al. 2008).

Once a given silo had been stocked to an estimated density of 300,000 pediveligers/silo, the pediveligers were given an additional four days to allow sufficient time for attachment to the cultch. When a given silo reached four days post-stocking (Days 16 to 18), newly metamorphosed spat were graded on a 300-µm mesh to remove excess cultch material. The spat that retained on the mesh were placed back into the silo. Once all silos had been graded, they were transferred to a flow-through upweller system (Day 18 to 29). Water to this tank was pumped directly from the GOM passing through an intake head (19 mm holes) and a strainer basket (3 mm holes) to remove large debris before going through a 200-µm bag at the in-flow point of the tank. Flow-through tanks were drained daily for cleaning of the oysters and removal of accumulated sediment on the oysters and the bottom of the tank. Flow was then resumed after cleaning. Spat were left to grow in the flow-through system until Day 27, at which time spat from each silo were graded on a 710-µm sieve and the retained spat were transferred to six 300-L tanks. The thermal stress treatment spat were distributed among three 500-µm screen tray and placed in three tanks (Figure 1.2). Spat from the spat control, larval stress, and larval control treatments were distributed among nine 500-µm screen tray (3 per treatment). One replicate from each treatment was placed in a tank, resulting in 3 tanks total (adapted from Boudreaux et al. 2023). Each replicate had ~26,700 spat.

On Day 29, each thermal stress tank was raised from ambient temperature (30 °C) to 34 °C at a ramp of 1 °C per hour (Adapted from Waite and Sorte 2022 & Boudreaux et al. 2023). Once at 34 °C, they were held for 4 days after which heaters were turned off and water temperatures were allowed to gradually cool back down to room temperature (~28 °C) over a 12h period. A water change was performed every 48 hours to keep water quality optimal (Boudreaux et al. 2023). On Day 33, when temperatures were ~29 °C, spat were transferred to

500 µm mesh upweller silos in the flow-through tank where they were held. All animals were monitored daily for mortalities or abnormal phenotypic traits such as abnormal shell growth. On day 41, all silos were graded on a 1.5-mm sieve. On Day 47, each silo was graded on a 2-mm sieve. This grading system was used to allow oysters room to grow in each upweller and to prevent a stratification in size classes in any given upweller. The seed that passed through the sieve was discarded. On Day 50, animals that retained on a 2-mm sieve were placed into 1.5-mm flat mesh bags (Ketcham Supply Co. Inc.; New Bedford, MA, USA) at 15,000 animals per bag. These bags were placed in two floating cages at Grand Bay Oyster Park (Grand Bay, AL) and held until Day 57 before being transferred into 2-mm flat mesh bags (Ketcham Supply Co. Inc.; New Bedford, MA, USA) and reduced to approximately 7,500 oysters per bag. Once in the 2-mm mesh bags, stocking density in the bags was reduced every 2 weeks to maintain bags at 50% capacity, resulting in a final density of ~1,500 oysters per bag. At Day 83, all oysters were brought to AUSL, graded on a 12-mm sieve, counted, and height, width, and length was measured via digital calipers (Mitutoyo Inc.; Kawasaki, Kanagawato) to prepare them for the thermal tolerance assay.

Thermal tolerance assay

Twelve 300-L tanks of the same design as the spat thermal stress tanks (Figure 1.2) were used for the thermal tolerance assay. Each tank held a replicate bag from each thermal stress group (larval thermal stress, spat thermal stress) and their respective controls (total of four bags per tank). Each bag held 100 animals, resulting in 400 animals per tank. To provide additional insulation, a foam board was placed over each tank. Each tank was fed 15 mL of Shellfish Diet 1800 twice a day (Reed Mariculture Inc; Campbell, CA, USA) supplemented with 1.8 g of ClorAm-X (AquaScience Research Group, Inc.; Kansas City, MO, USA) to maintain 100,000 cells/mL estimated using a double Neubauer counting chamber (Hausser Scientific, Horsham, PA, USA). Each tank was equipped with a submersible heater bar (800-Watt, JSK Merchandising Inc; Chicago, IL, USA) and a temperature controller (INKBIRD Tech, C.L; Shenzhen, China). Once larvae were distributed throughout the tanks, three of the twelve tanks were randomly chosen to serve as controls and were held at 25 °C for the duration of the assay. The other nine tanks served as treatment tanks and were each brought up to one of three experimental temperatures (36 °C, 38 °C, or 40 °C) at a rate of 2 °C per day, three tanks per temperature. The initiation of each temperature ramp was staggered such that all tanks reached their treatment temperature on the same day. Once tanks were brought up to temperature, they were held at their target temperature for 30 days (adapted from Marshall et al. 2021). During the 30 days, temperature, salinity, dissolved oxygen, pH, ammonia, nitrite, nitrate, and alkalinity were monitored daily. Nitrate, nitrite, and alkalinity were measured using Tetra EasyStrips 6-in-1 aquarium test strips and ammonia was measured using Tetra EasyStrips ammonia test strips (Tetra Spectrum Brands Pet, LLC; Blacksburg, VA, USA). All water quality parameters were tested daily. Ammonia, nitrite, and nitrate were kept within optimal ranges via 50% water changes as necessary. Alkalinity was maintained by using Alkalinity Up (Leslie's Swimming Pool Supplies; Columbus, GA, USA), pH was maintained using sodium bicarbonate, and salinity was maintained by adding deionized water. Mortalities were checked at 9 am, 1 pm, 5 pm, and 9 pm for the first 15 days (adapted from Marshall et al. 2021). For the remaining 15 days, mortality checks were reduced down to 9 am, 1 pm, and 5 pm because the tanks at highest risk for mortality (38 °C and 40 °C) had 100% mortality. Once the last experiment day was reached (Day 30), all surviving animals were counted.

Data Analysis

To analyze differences in morphometric measurements, an ANOVA and Tukey post hoc test was conducted in Microsoft Excel (Microsoft Office, 2021). For all exposure temperatures (23 °C, 36 °C, 38 °C, and 40 °C), data were analyzed using a log rank survival analysis in SigmaPlot 15 (Systat Software©, Inc. 2022). This analysis identifies significant differences between survival probabilities of each treatment among all temperatures. To calculate LT_{25}, LT_{50}, and LT_{75} (lethal time that causes 25%, 50%, and 75% mortality, respectively), the 25th, 50th, and 75th percentile's survival times and 95% confidence intervals were calculated from the report generated by the log rank survival analysis. These values were used to generate a scatterplots with 95% confidence intervals. Values with non-overlapping confidence intervals were considered significantly different from each other.

RESULTS

Larval and spat thermal stress event survival

For the larval thermal stress event, each treatment started with 5M larvae and after the 4-day event, the mean number (± *SE*) of survivors for the larval stress (LS) treatment was 2.3M ± 348,010 which was not significantly different from the larval control (LC) treatment (2.53M ± 548,735; $p = 0.67$). Additionally, for the spat thermal stress event, each replicate started with 26.7K spat and after the event, the mean number of survivors for the spat stress (SS) treatment was 25.3K ± 1,144, which was not significantly different than the spat control (SC) treatment (22.69K ± 1,200; $p = 0.19$).

Morphometrics prior to the thermal tolerance assay

Prior to the thermal tolerance assay, the average of each treatment's shell height for the LS, LC, SS, and SC treatment groups were not significantly different among treatments (p =

0.091; Table 1.1). The shell lengths also did not significantly differ among treatments ($p = 0.087$; Table 1.1). However, the LC treatment's average shell width was 7.05 ± 0.17, which was significantly higher than the SS treatment (6.23 ± 1.57; $p = 0.0012$) and the SC treatment (6.33 ± 2.05; $p = 0.01$). All height, length, and width are expressed as mean measurement (mm) ± *SE*.

Whole wet (g) of the LC treatment (5.29 ± 0.31) did not significantly differ from the SS treatment (5.41 ± 0.47; $p = 0.90$; Table 1.1) or the SC treatment (5.4 ± 0.39; $p = 0.90$). Additionally, the whole wet weights of the SC and SS treatments did not significantly differ from one another ($p = 0.90$). However, the LS treatment had a mean weight of 6.41 g (± 0.29) which was significantly heavier than the LC ($p < 0.01$), SS ($p < 0.01$), and SC ($p < 0.01$) treatments. All weights are expressed as weight (g) ± *SE*.

Water quality during thermal tolerance assay

The temperature in all control tanks was maintained at 25.58 °C ± 0.01. Temperatures in all thermal tolerance assay tanks were maintained at 36.12 °C ± 0.00, 38.20 °C ± 0.01, or 39.98 °C ± 0.02. All temperatures are expressed as mean temperature °C ± standard error (*SE*). Other water quality parameters were kept within optimal ranges including salinity 1530 PSU, dissolved oxygen (DO) ≥ 6.0 mg/L, ammonia < 3.0 mg/L, nitrite < 3.0 mg/L, nitrate < 80 mg/L, pH 7.4–8.2, and alkalinity 140–220 mg/L (Epifanio and Srna 1975; Gobler and Talmadge 2014; Gomez et al. 2021; Marshall et al. 2021).

Thermal tolerance assay results

All the following survival probabilities, LT_{25}'s, LT_{50}'s, and LT_{75}'s are expressed as mean ± *SE*. At 25 °C, survival probabilities in days for the LS, LC, SS, and SC were 29.25 ± 0.071, 29.19 ± 0.09, 29.27 ± 0.06, and 29.15 ± 0.11. Survival probabilities did not differ significantly among treatments ($X^2 = 2.47$; $p = 0.48$; Figure 1.4).

At 36 °C, the survival probability of the LS treatment (19.42 ± 0.45,) was significantly lower than the LC (20.94 ± 0.49; $p = 0.03$) and the SS (21.13 ± 0.48; $X^2 = 10.12$; $p = 0.04$) treatments (Figure 1.5A). Additionally, the LT_{25}'s of the LS, LC, SS, and SC treatment were 12.33 ± 1.00, 13.17 ± 1.00, 13.00 ± 1.29, and 13.00 ± 1.00, respectively, which did not significantly differ from one another (Figure 1.5B). The LT_{50} of the LS treatment (20.17 ± 0.94) was significantly lower than the SS treatment (24.33 ± 0.68) as indicated by non-overlapping confidence intervals (Figure 1.5C).

At 38 °C, the survival probabilities of the LS, LC, SS, and SC treatments were 4.79 ± 0.12, 4.83 ± 0.13, 4.85 ± 0.13, and 4.56 ± 0.12, respectively, which did not significantly differ from one another ($X^2 = 3.88$; $p = 0.28$; Figure 1.6A). The LT_{25}'s for the LS, LC, SS, and SC treatments were 3.33 ± 0.09, 3.17 ± 0.08, 3.33 ± 0.05, and 3.17 ± 0.08, respectively, which did not significantly differ from one another (Figure 1.6B). The LT_{50}'s of the LS, LC, SS, and SC treatments were 4.00 ± 0.07, 4.00 ± 0.08, 4.00 ± 0.06, and 4.00 ± 0.07, respectively, which did not significantly differ from one another (Figure 1.6C). Lastly, the LT_{75}'s of the LS, LC, SS, and SC treatments were 6.00 ± 0.59, 6.00 ± 0.08, 5.17 ± 0.06, and 5.00 ± 0.07, respectively, which did not significantly differ from one another (Figure 1.6D).

At 40 °C, the survival probability of SS treatment was 1.27 (± 0.03) which was significantly lower than the LS (1.71 ± 0.04; $X^2 = 92.05$; $p < 0.0001$), the LC (1.70 ± 0.05; $X^2 = 92.05$; $p = < 0.0001$), and the SC (1.58 ± 0.04; $X^2 = 92.05$; $p < 0.0001$) treatments (Figure 1.7A). The LT_{25}'s of the LS, LC, SS, and SC treatments were 1.17 ± 0.05, 1.17 ± 0.07, 1.00 ± 0.06, and 1.00 ± 0.08, respectively, which were not significantly different from one another (Figure 1.7B). However, the SS treatment (1.17 ± 0.03) had a significantly lower LT_{50} than the LS (2.00 ± 0.045), LC (2.00 ± 0.04), and SC (1.50 ± 0.03) treatments (Figure 1.7C). Lastly, the SS treatment

(1.50 ± 0.06) had a significantly lower LT_{75} than the LS (2.00 ± 0.05), LC (2.00 ± 0.04), and SC (2.0 ± 0.05) treatment as indicated by non-overlapping confidence intervals (Figure 1.7D).

DISCUSSION

Eastern oysters in the GOM have a higher tolerance to stressful temperatures (32 °C) than populations on the east coast (Stanley and Sellers 1986). However, they are experiencing stressful temperatures (>32 °C) more frequently and for longer durations with increasing impacts of climate change (Stanley and Sellers 1986; Marshall et al. 2021). With this increase in temperature, eastern oysters are experiencing increasingly detrimental mortality events and decreases in recruitment for wild reefs (Marshall et al. 2021; Plumlee et al. 2024). In places like Mobile Bay, researchers have found that long periods of high temperatures can make it harder for early life stage oysters to survive and grow and can hinder adult oysters' ability to reproduce (Plumlee et al. 2024). Previous studies have shown that inducing an ATT may be useful in combating mass mortality events related to thermal stress in other oyster species (Song et al. 2024). However, the few studies attempting to induce ATT in the eastern oyster have shown results lasting as long as other oyster species (e.g. Jackson et al. 2011; Song et al. 2024). An additional challenge relates to the duration of ATT. If ATT is induced in the hatchery, effects must be of sufficient duration to increase survival of maturing oysters after months in the field, when summer temperatures are peaking.

The control temperature (25 °C) in the chronic thermal challenge conducted on 83-day old juveniles confirmed that experimental animals were healthy. We saw nearly 100% survivorship over 30 days when juveniles were held within the previously identified optimal temperature range of 20–30 °C (Stanley and Sellers 1986; Marshall et al. 2021; Boudreaux et al. 2023). However, similar to previous studies (e.g. Marshall et al. 2021), temperatures ≥ 36 °C were lethal. Larval control and spat control treatments exhibited LT_{50}'s of 22–24 days, 4 days, and 1–2 days at 36, 38, and 40 °C, respectively (Figure 1.5C, Figure 1,6C, and Figure 1.7C). The temperature threshold of 36 °C appeared to be particularly significant as LT_{50} values decreased fivefold when temperature increased by just 2 °C. In recent years, surface temperatures in the northern GOM have fluctuated between 32 °C and 34 °C for weeks at a time in July and August and are predicted to increase with impacts of climate change (Wang et al. 2023; AUSL 2024). Thus, techniques to increase thermal tolerance are expected to be of increasing value to oyster aquaculture and conservation.

In this study, we tested whether we could induce ATT effects lasting 50 to 80 days after exposure to a sublethal thermal stress event (~4°C above ambient temperatures for 4 days) during the veliger or spat stage. However, we found no evidence that exposure to short-term thermal stress at either life-history stage resulted in a long-term ATT. Rather than inducing thermal tolerance, we found limited evidence that short-term exposure to sublethal thermal stress reduced long-term thermal tolerance in *C. virginica*. At 36°C, larvae exposed to thermal stress had significantly reduced survival relative to controls. At 40 °C, juveniles exposed to thermal stress at the spat stage exhibited lower overall survival and reduced LT_{50} and LT_{75} values than controls.

Previous studies have found reduced performance of *C. virginica* a year after being exposed to temperatures that were 2.5 °C above ambient, although this reduced performance could be negated if oysters were concurrently exposed to hypoxia stress (Donelan et al. 2022). Thus, in *C. virginica*, long-term effects of early exposure to sublethal thermal stress may be detrimental or neutral, rather than positive. If this is a general rule for this species, the increased thermal tolerance of *C. virginica* in the GOM compared to *C. virginic*a along the eastern coast (Johnson et al. 2021) may be due to environmental selection of genotypes rather than ATT resulting from periodic high temperatures occurring in the GOM.

Exposure to environmental stress can have positive carry-over effects in *Crassostrea* spp. lasting at least 1 year from the initial exposure, but the characteristics of these effects and the specific protocols required to produce positive rather than negative or neutral acquired thermal tolerance are not fully understood (Donelan et al. 2022; Song et al. 2024). In the Fujian oyster, *Crassostrea angulata*, thermotolerance was improved for up to 21 days following exposure to short-term thermal stress. The strength and duration of this effect was maximized upon the priming temperature of 40 °C, and a subsequent recovery temperature of 20 °C and recovery duration of three days (Song et al. 2024). In our trial, we exposed oysters to relatively low thermal stress (32 or 34 °C) for a relatively long period (4 d) and subsequently cooled oysters to ambient temperatures (~29–32 °C). The recovery temperature (> 25 °C) used in my study could have been a possible reason why I did not see an induction of ATT in eastern oysters. In the Fujian oyster, thermotolerance was induced when they were exposed to relatively high temperatures (36–40 °C) for a relatively short period of time (1 h) and brought down to a controlled recovery temperature of < 25 °C for > 4h (Song et al. 2024). Future studies that manipulate priming temperatures, recovery temperatures, and recovery times, may be required to develop a successful protocol for inducing ATT in *C. virginica*.

Future studies aiming to observe significant results should maintain recovery systems at temperatures below 25 °C so the thermal stress event will have a higher chance of inducing an ATT (Song et al. 2024). The longest recorded ATT seen was up to 21 days after the exposure (Song et al. 2024), so a chronic thermal stress challenge at one month after the sublethal thermal stress event or at a different period of time where the ambient temperatures were lower may have produced different results than 3 months after the event. It would also be worth targeting a broader range in temperatures and durations during the priming and recovery process (Song et al. 2024). Although the Song et al. 2024 study was conducted on adult Portuguese oysters (*Crassostrea angulata*), it is worth testing if the methods transfer to other species. Developing an assay that induces ATT could benefit hatcheries and farmers to make their oysters more resistant to thermal stress once placed in the field for grow-out (Song et al. 2024). Inducing ATT at the adult stage in the field is not feasible but inducement in the hatchery or nursery setting where the conditions are more controlled is more feasible for growers. Lastly, this research and future studies can have management implications. Having a more thermally tolerant oyster could help make the oysters on restored reefs more resilient to temperature stress (La Peyre et al. 2014). This study expands on the research needed for increasing thermal tolerance of eastern oysters through ATT. Looking further into how long an ATT can be induced in eastern oysters can be beneficial for restoration, management, and farming practices.

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Table 1.1. Height (mm), length (mm), width (mm), and whole wet weight (g) of the larval stress (LS), larval control (LC), spat control (SC), and spat stress (SS) treatments. All measurements expressed as mean ± standard error (SE) (standard calculated by standard deviation divided by square root of sample size).

Size metric	Larval Stress (LS)	Larval Control (LC)	Spat Control (SC)	Spat Stress (SS)
Height (mm)	27.687 ± 0.756 (a)	28.403 ± 0.636 (a)	27.057 ± 0.641 (a)	26.078 ± 0.636 (a)
Length (mm)	18.936 ± 0.348 (a)	18.501 ± 0.308 (a)	18.137 ± 0.327 (a)	17.847 ± 0.281 (a)
Width (mm)	6.55 ± 0.135 (ab)	7.051 ± 0.170 (b)	6.231 ± 0.145 (a)	6.328 ± 0.165 (a)
Whole wet weight (g)	6.413 ± 0.139 (a)	5.293 ± 0.145 (b)	5.413 ± 0.176 (b)	5.400 ± 0.162 (b)

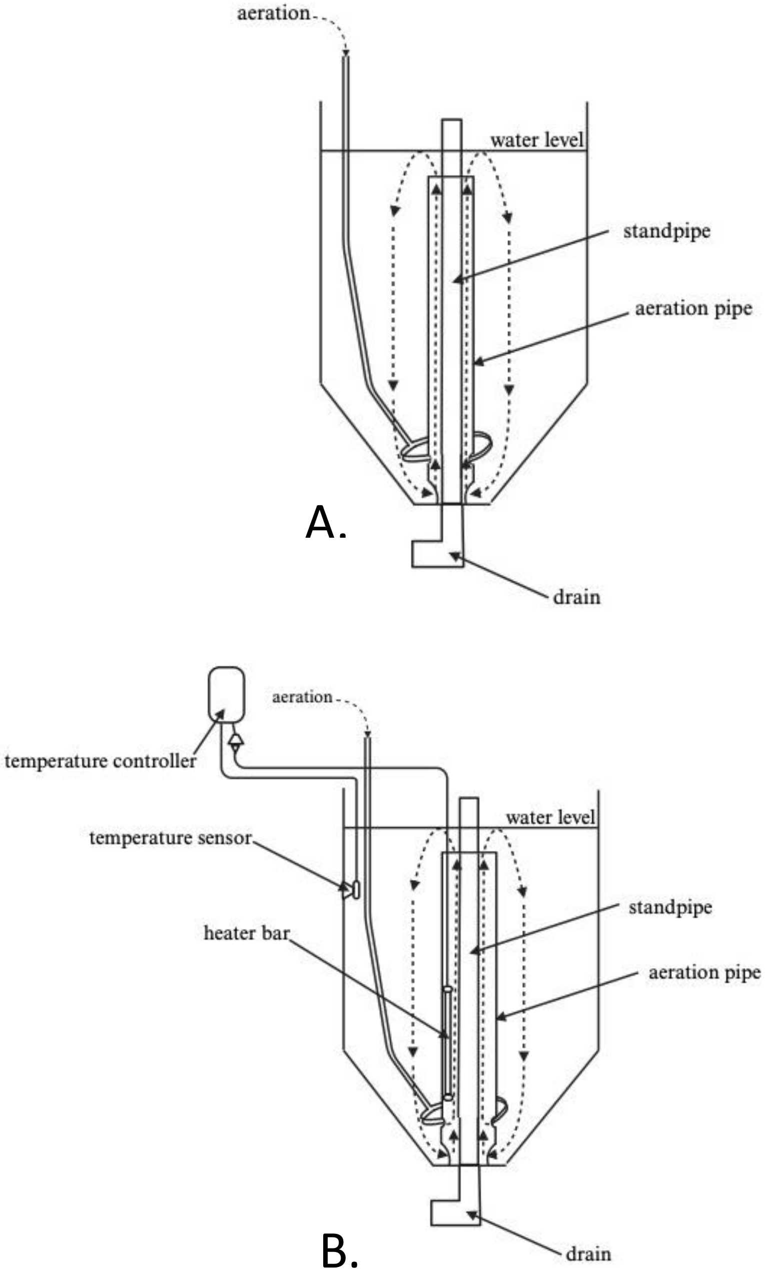

Figure 1.1. Larval thermal stress event tank set up. A) The set-up of each individual larval control (LC) treatment tank. B) The set-up of each individual larval stress (LS) treatment tank. Created in BioRender.

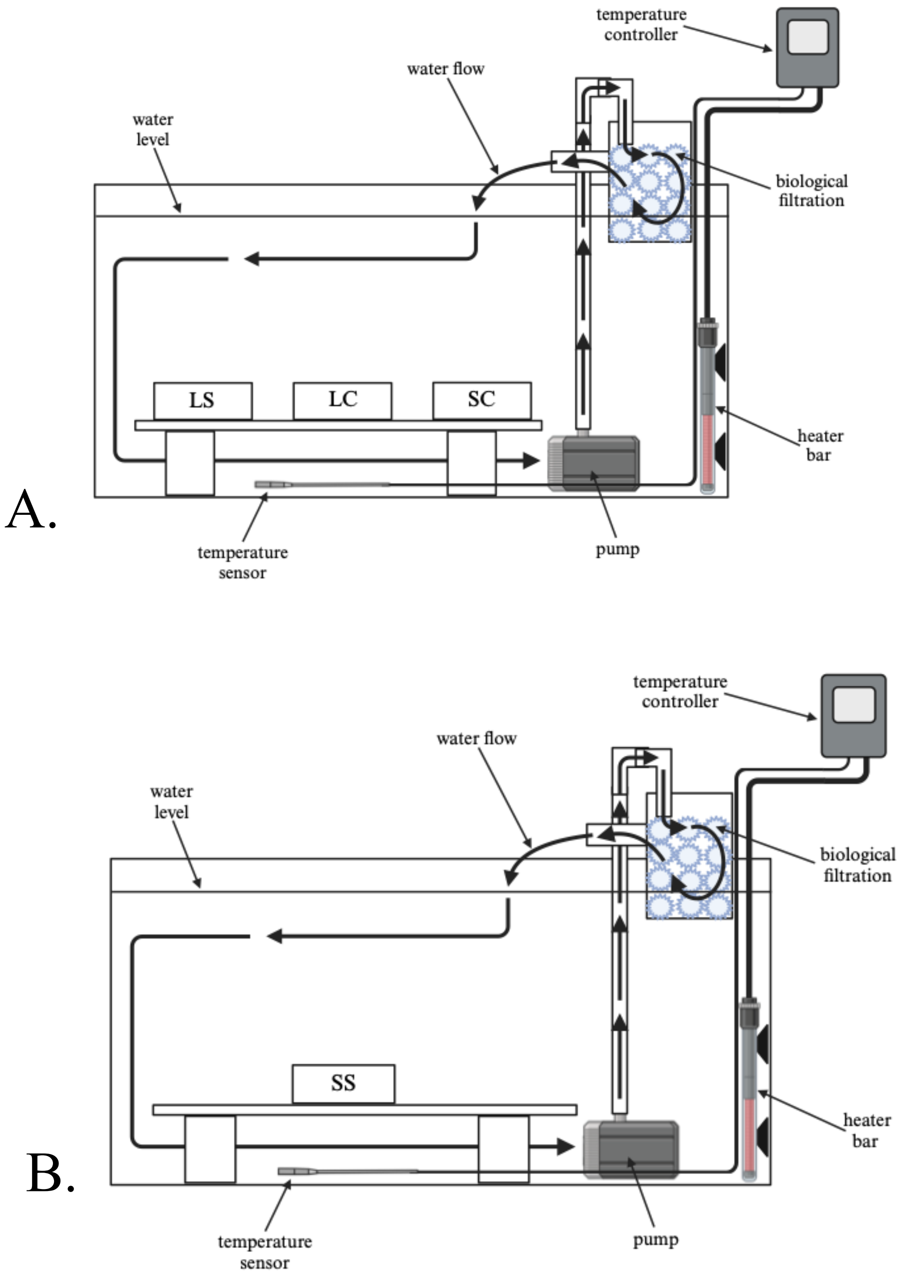

Figure 1.2. Spat thermal stress event tank set up. A) The set-up of the tanks containing spat control (SC), larval control (LC), and larval stress (LS) treatments (control tanks). B) The set-up of the tanks containing spat stress (SS) treatments (thermal stress tanks). Created in BioRender.

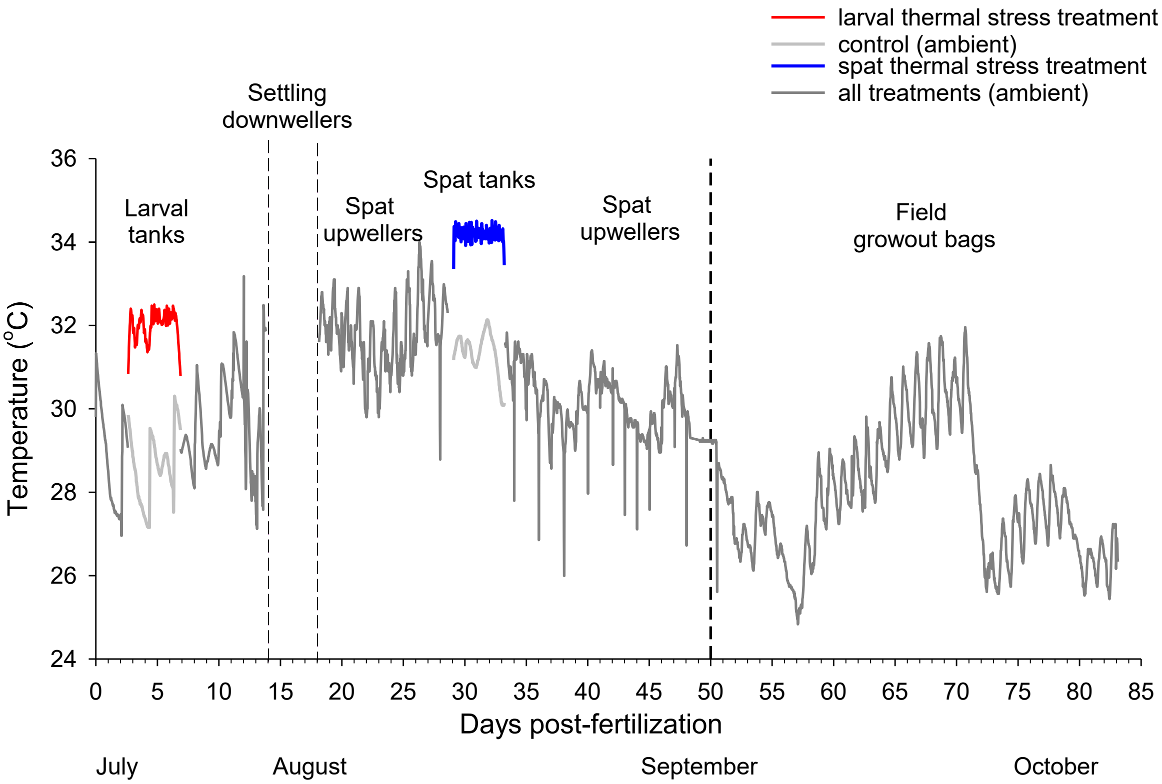

Figure 1.3. Temperatures each treatment was exposed to from spawn (Day 0) to being taken out of the field site for thermal challenge (Day 83). The light blue portion of the graph represents the optimal temperature range of eastern oysters (Stanley and Sellers 1986). No temperatures were available for settling downwellers due to recorder malfunction.

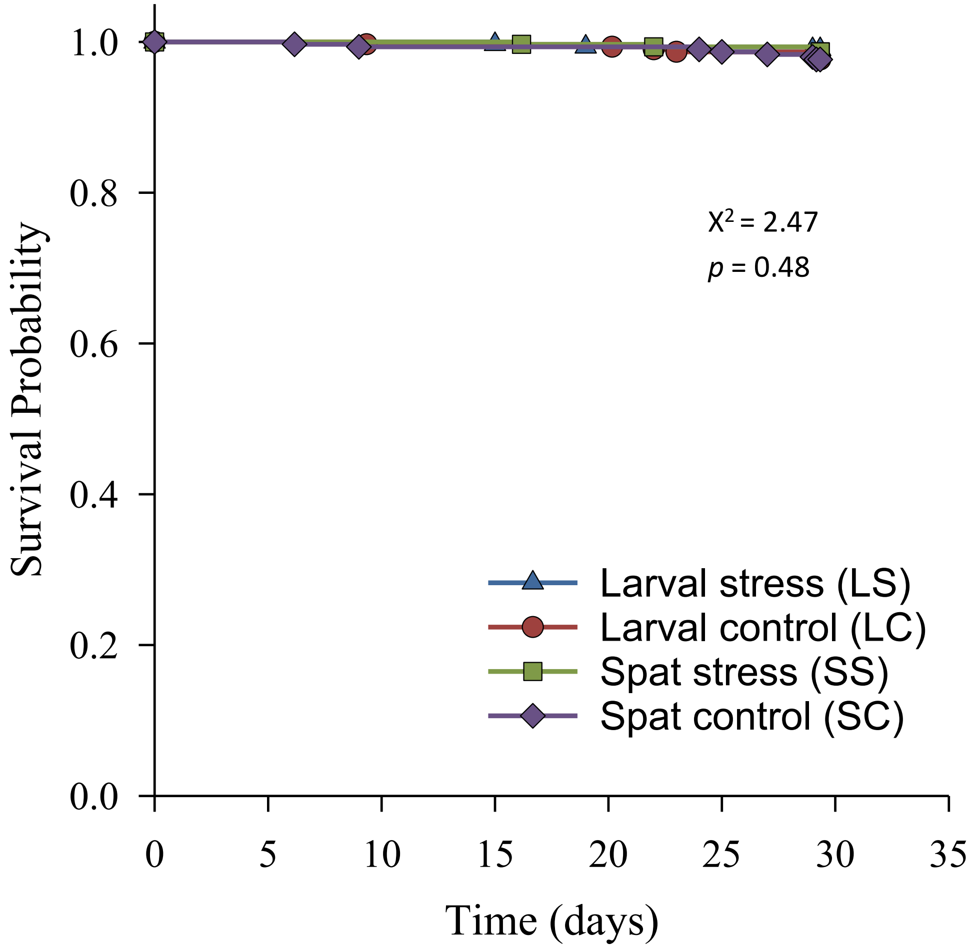

Figure 1.4. Kaplan-Meier survival curves at 25 °C for four treatments: Larval Stress (LS), Larval Control (LC), Spat Stress (SS), and Spat Control (SC). No significant differences in survival were observed among treatments.

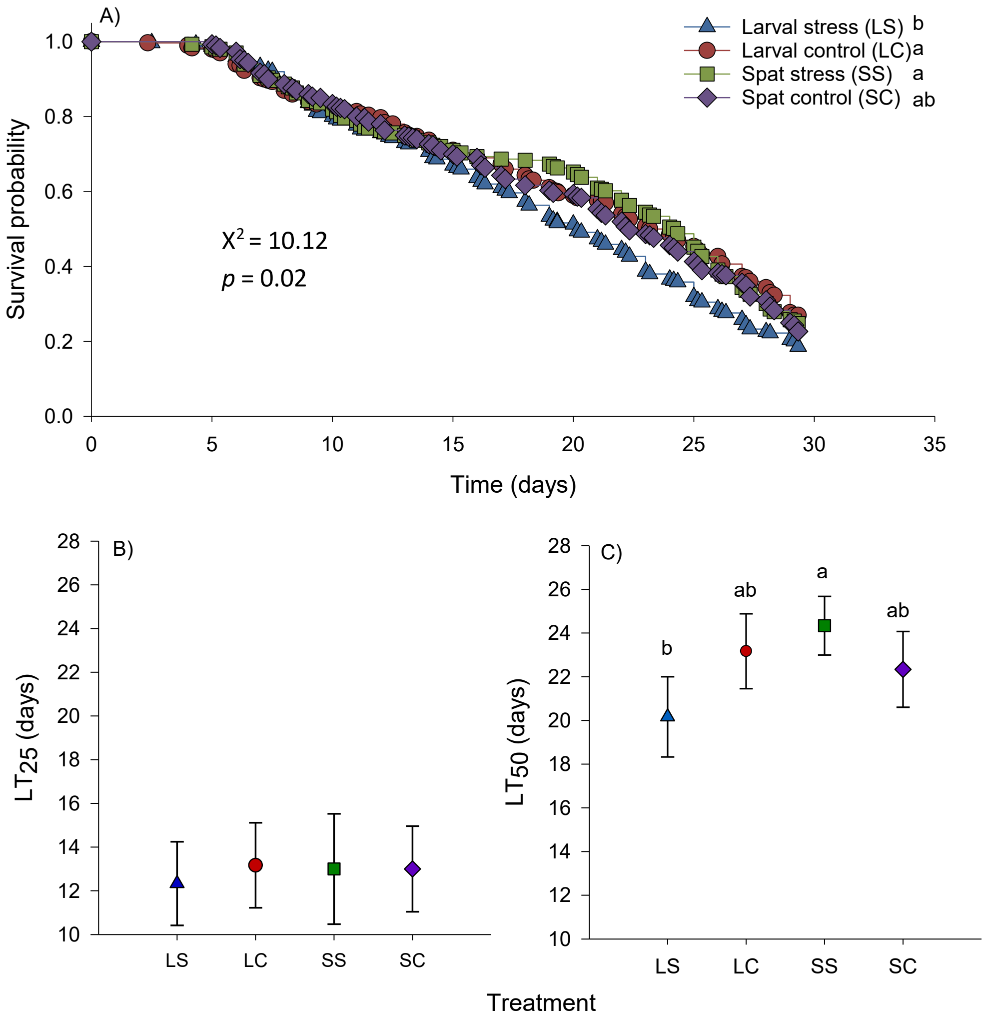

Figure 1.5. A) The relationship between survival probability and time (days) at 36 °C. Each line represents one of four treatments: larval stress (LS), larval control (LC), spat stress (SS), or spat control (SC). B) LT_{25} (lethal time that causes 25% mortality) of each treatment. C) LT_{50} (lethal time that causes 50% mortality) of each treatment. Error bars represent 95% confident intervals. Each letter (a, b, and ab) represents statistical significance.

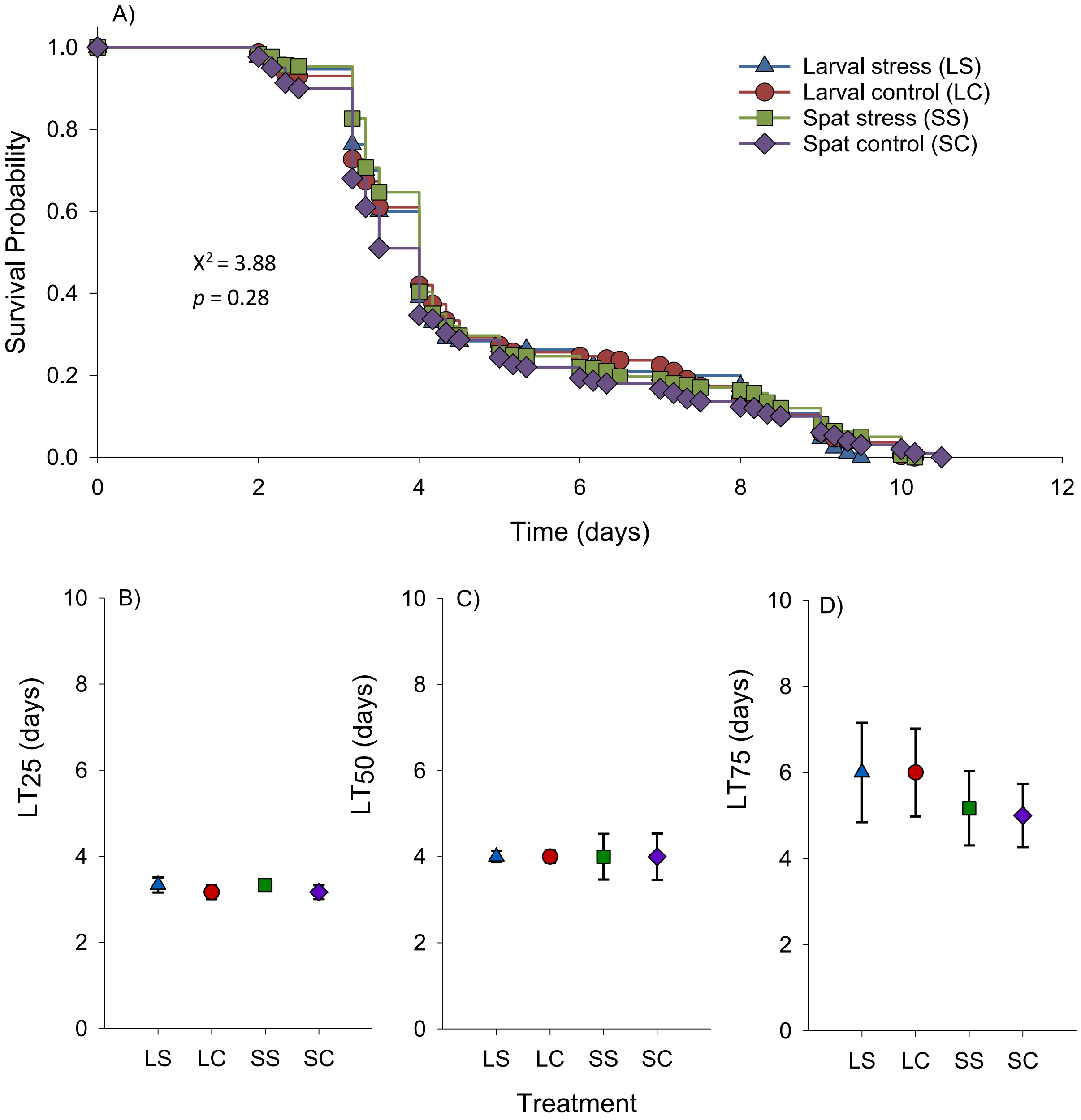

Figure 1.6. A) The relationship between survival probability and time (days) at 38 °C. Each line represents one of four treatments: larval stress (LS), larval control (LC), spat stress (SS), or spat control (SC). B) LT_{25} (lethal time that causes 25% mortality) of each treatment. C) LT_{50} (lethal time that causes 50% mortality) of each treatment. D) LT_{75} (lethal time that causes 75% mortality) of each treatment. Error bars represent 95% confident intervals.

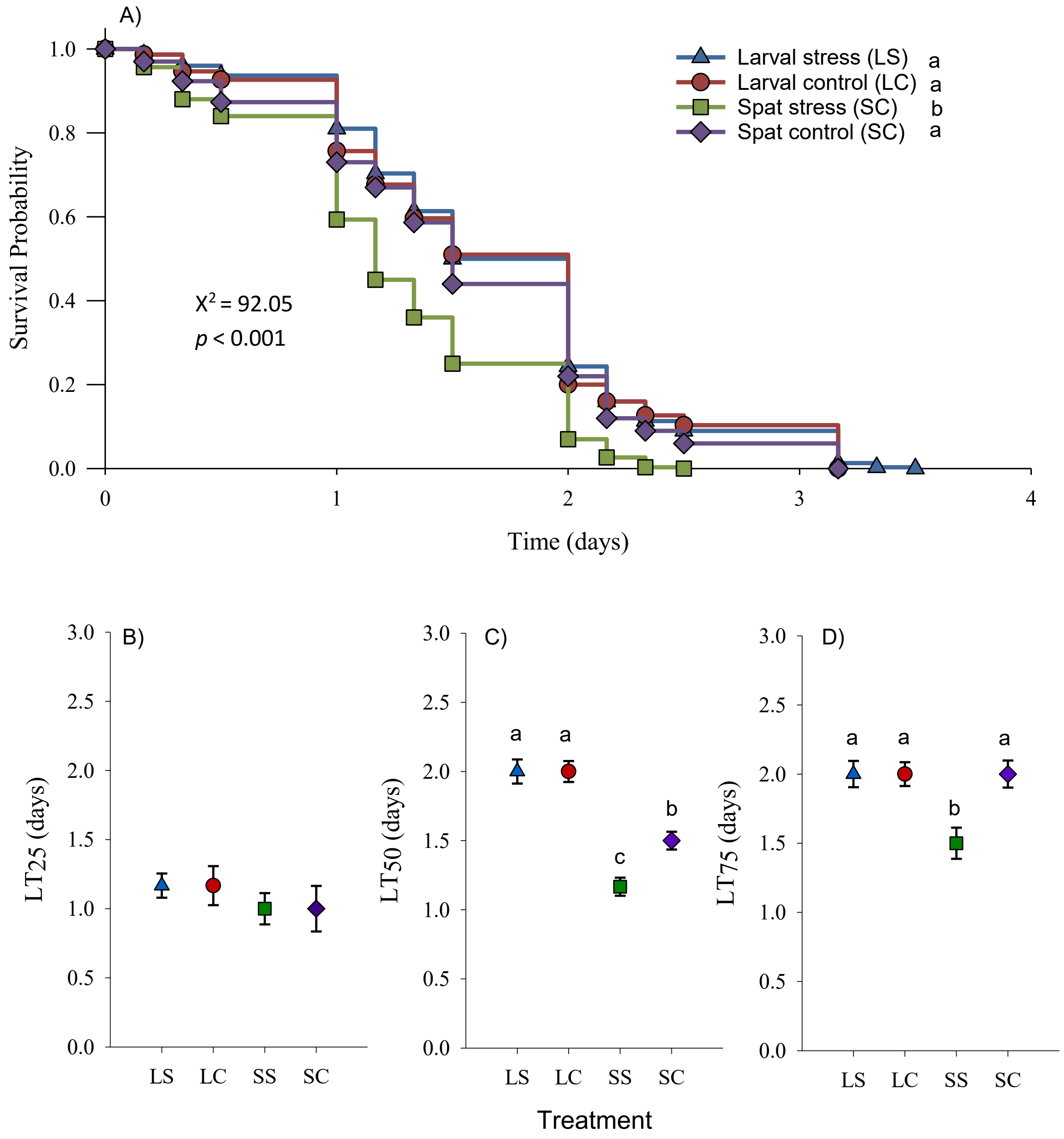

Figure 1.1. A) The relationship between survival probability and time at 40 °C (days). Each line represents a treatment. B) LT$_{25}$ (lethal time that causes 25% mortality) of each treatment. B) LT$_{50}$ (lethal time that causes 50% mortality) of each treatment. D) LT$_{75}$ (lethal time that causes 75% mortality) of each treatment. Error bars represent 95% confidence intervals.

Chapter 2: Thermal tolerance of oysters (*Crassostrea virginica*) selected for increased growth and resistance to Dermo disease

ABSTRACT

The eastern oyster (*Crassostrea virginica*) is a species that holds strong ecological and economic importance. This dual importance has made eastern oysters a focal point for both conservation and aquaculture efforts that are increasingly challenged by emerging threats, such as Dermo disease. Dermo disease, caused by the protozoan parasite *Perkinsus marinus*, is a prominent cause of mortality in the eastern oyster, and is predicted to increase in prevalence with climate change. Thus, there is interest in using genomic and phenotypic selection to increase resistance to Dermo for oysters produced for restoration efforts. However, selection for some traits may result in an unintentional correlated response that impacts other traits. To that end, the purpose of this study was to determine if selection for growth and Dermo resistance impacts thermal tolerance in the eastern oyster. The oyster lines used in this study included: 1) an unselected wild control, WC; 2) a genomic control mated from oysters with average genomic estimated breeding values (GEBVs) for Dermo resistance and whole oyster wet weight, GC; 3) phenotypically selected oysters based on survival from a laboratory Dermo challenge, PS; and 4) genomically selected oysters based on high GEBVs for Dermo resistance and whole oyster wet weight, GS. A chronic LT_{50} (lethal time that causes 50% mortality rate) assay was conducted, with each line of oysters exposed to 23 °C, 28 °C, 36 °C, and 38 °C for 20 days. For each temperature, three replicate recirculating aquaculture systems held 50 animals from each line. Mortality exceeding 50% only occurred in the 38 °C trial. Analysis shows that the LT_{50} of the GS line was significantly higher than that of the WC, GC, PS lines. There was no evidence that selection for Dermo resistance and increased wet weight had an adverse effect on thermal tolerance. In fact, there was evidence that selection for these traits induced a positive correlated

response in thermal tolerance at 28 °C and 38 °C. This correlated response was induced by genomic but not phenotypic selection.

INTRODUCTION

The eastern oyster (*Crassostrea virginica*) is a species of ecological importance that is considered a keystone species - helping to maintain water quality and providing habitat for many micro- and macro-organisms (Kennedy et al. 1996; Lenihan et al. 1998; Donnelly et al. 2023; Petrolia and Caffey 2024). They are also considered a species that provides livelihoods for farmers throughout north America (Walton et al. 2013). Dermo disease, caused by the protozoan parasite *Perkinsus marinus*, is a prominent cause of mortality for 1 year old adult eastern oysters, and is predicted to increase in prevalence with climate change (Mackin 1961; Ewart and Ford 1993; Gignoux-Wolfsohn et al. 2021). When first discovered in the late 1940s, the disease caused an estimated 30% annual mortality in populations from the Gulf of Mexico (GOM) to the Chesapeake Bay (Andrews 1988; Cook et al. 1998). Since the mid-1980s, the impacts of Dermo have increased, causing an average mortality rate of about 70% in affected oyster populations (Cook et al. 1998). Although Dermo is a major concern for bivalve species, the protozoan is not harmful to humans (Silvy et al. 2020).

Oysters are exposed to Dermo through suspension feeding (Mackin 1961). The parasite zoospores infect the mantle tissue and are most prevalent in the area where pseudofeces are discharged (Allam et al. 2013; Silvy et al. 2020). Once inside the host tissues, the zoospores become trophozoites which multiply and cause lysis of the oyster tissue (Ben- Horin et al. 2015). Mortality typically does not occur until after oysters reach sexual maturity at which point the parasite has completed its life cycle and lyses the oysters mantle tissue (Andrews and Hewatt 1957; Powell et al. 2008; Silvy et al. 2020). Thus, larger, older oysters are more susceptible to mortality from Dermo disease than younger, smaller oysters. Cultured oysters in the GOM are typically harvested within the first year, which is typically before the point where mortality would occur from Dermo (Ben-Horin et al. 2018). When an infected oyster dies, Dermo zoospores are released into the water column, infecting additional oysters and repeating the cycle (Silvy et al. 2020). Many ectoparasitic snail species feeding on infected oysters have also been seen to be a cause of the spreading of the parasite (Chu et al. 2002). Due to the high impact that Dermo disease has on eastern oyster wild populations, there is strong interest in phenotypic and genomic selection of oyster stocks to increase resistance to Dermo for restoration.

Genomic selection uses genetic markers that can accurately predict genomic estimated breeding values (Goddard and Hayes 2007). In selection for resistance to Dermo disease, genomic selection has been seen to be successful (Wang et al. 2025). More specifically, Wang et al. (2025) tested genomic selection for Dermo resistance in eastern oysters by first injecting wild Florida oysters with *Perkinsus marinus* and identifying survivors as resistant. These oysters were genotyped using a 66k single-nucleotide polymorphism (SNP) array, and predictive models were used to estimate the genomic breeding values. Based on estimates, high-GEBV oysters were selected to breed the genomic-selected group, while others formed control groups. Additionally, a phenotypically selected group was made by choosing wild oysters that survived a lab injected with *Perkinsus marinus*, assuming their survival indicated resistance. These resistant oystsers were then bred to produce offspring with presumed phenotypic resistance, forming the phenotypically selected group. The genomically and phenotypically selected groups and their controls then underwent a Dermo challenge where it was found that genomic selection was more successful in selecting Dermo resistance.

Although phenotypic and genomic selection for resistance to Dermo disease have been successful (Wang et al. 2025), selection for a specific trait may result in changes in additional traits (Gromko 1995). This phenomenon is called a correlated selection response and has been seen in catarina scallops and Hongkong oysters (Ibarra et al. 1999; Jiang et al. 2025). Within the bivalve correlated response literature, few studies have looked at how genomic selection for increased wet weight and increased resistance to Dermo disease and phenotypic selection for Dermo resistance may trigger a correlated response that affects tolerance to other environmental stressors (temperature, salinity, etc.). Selection for increased growth performance (i.e. increased wet weight) has been shown to increase thermal tolerance in Hongkong oysters (*Crassostrea hongkongensis*; Jiang et al. 2025). Because temperature regimes impact eastern oyster's susceptibility to *Perkinsus marinus* (Chu and La Peyre 1993; Encomio and Chu 2007; Malek and Byers 2018) it is possible that sensitivity to thermal stress is related to susceptibility to Dermo infections. With the strong relationship between increased growth and thermal tolerance shown in previous studies (Jiang et al. 2025) and the strong correlation between Dermo disease infection and water temperature (Chu and La Peyre 1993; Encomio and Chu 2007; Malek and Byers 2018), investigating whether selecting for enhanced Dermo resistance and greater wet weight leads to correlated changes in thermal tolerance could be valuable, given the potential ecological and breeding implications.

In this study, I test for correlated selection responses between Dermo resistance using the offspring of the animals used in Wang et al. (2025). Specifically, I asked: 1) Does genomic and phenotypic selection for increased wet weight and Dermo resistance trigger a correlated response that impacts thermal tolerance in *C. virginica*? and 2) If so, does the strength of the correlated response differ between genomic and phenotypic selection?

METHODS

Spawn, grow-out, and collection

Four eastern oyster lines were produced at the Auburn University Shellfish Laboratory (AUSL). Broodstock for each of the lines was from an F1 generation in a previous study conducted at AUSL, Louisiana State University Agriculture Center, and Haskin Shellfish Research Laboratory (Wang et al. 2025). For production of the F2 generation used in this study, one spawn was allocated for each line as follows: 1) June 29th, 2023, genomically selected (GS) oysters based on GEBVs for Dermo resistance and whole oyster wet weight; 2) July 5th, 2023, phenotypically selected (PS) oysters based on survival from Dermo challenge in the laboratory; 3) July 7th, 2023, a genomic control (GC) mated from oysters with average GEBVs for Dermo resistance and whole oyster wet weight; and 4) July 11th, 2023, unselected wild control (WC). After spawning, larvae were reared in static tanks with water changes every other day for the first 10 days until reaching pediveliger stage and switched to every day drain downs until day 15, then set on 250 µm culch in downweller systems for 4 days (Wallace et al. 2008). After setting, spat were moved to upweller flow-through systems to grow-out for 4-5 weeks and then transported to the Grand Bay Park (GBOP; Grand Bay, AL) field site for additional grow-out until reaching the experiment date. On April 9th, 2024, oysters were collected from GBOP (23 °C, 14 PSU).

Chronic Thermal Stress assay

After collection, oysters were immediately transported to the lab, cleaned, and distributed among 12 300L recirculating tank systems (Figure 2.1) set at a temperature of 23 °C and salinity of 14 PSU, similar to the collection site. Each tank received 50 individuals from each of the four lines, resulting in 200 animals per tank. Each line was held separately in trays within each tank.

Height (hinge to bill), length (perpendicular to height dorsal to ventral), and width (cup depth) measurements were obtained for all 2,400 animals using digital calipers (Mitutoyo American Corporation; Aurora, IL, USA). Once the oysters were in their respective tanks, they were held at 23 °C for one week to acclimate oysters to laboratory conditions prior to conducting chronic thermal stress assay.

After the one-week laboratory acclimation period, three of the twelve tanks were randomly chosen to serve as controls and were held at 23 °C for the duration of the assay. The other nine tanks served as treatment tanks and were each brought up to one of three experimental temperatures (28 °C, 36 °C, or 38 °C) at a rate of 2 °C per day, three tanks per temperature. The initiation of each temperature ramp was staggered such that all tanks reached their treatment temperature on the same day. Treatment temperatures were then maintained for the duration of the experiment (20 days). Mortalities were checked four times a day. Oysters were considered dead if they were gaping with no response to probing.

During the acclimation and LT_{50} assay periods, oysters in each tank were fed 55 mL/day/tank of Shellfish Diet 1800 (Reed Mariculture, Inc; Campbell, CA, USA), supplemented with 0.12 g of ClorAm-X (Reed Mariculture Inc; Campbell, CA, USA) per 1 mL of shellfish diet. Feeding rate was subsequently recalculated with changes in oyster abundance due to mortality. Feed was diluted into 10 L of ~20 PSU saltwater and distributed in 1-hour increments using an automated dosing system (DP-4, ~150 mL/hour, Jebao Co., LTD.; Zhongshan, China) to maintain an approximate density of 100,000 algal cells/mL using a double Neubauer counting chamber (Hausser Scientific, Horsham, PA, USA). Feed was kept suspended by using a 5W submersible pump (Purelake Group Co., LTD; Guangzhou, China).

During acclimation and subsequent LT_{50} assays, the target temperature in each tank was maintained using a 500-Watt submersible heater (JSK Merchandising Inc; Chicago, IL, USA) connected to a temperature controller (INKBIRD Tech, C.L; Shenzhen, China). Salinity, temperature, and pH were monitored using a YSI Pro 10 (YSI Inc.; Yellow Springs, OH, USA). Nitrate, nitrite, and alkalinity were measured using Tetra EasyStrips 6-in-1 aquarium test strips and ammonia was measured using Tetra EasyStrips ammonia test strips (Tetra Spectrum Brands Pet, LLC; Blacksburg, VA, USA). All water quality parameters were tested daily (Table 2.1). Ammonia, nitrite, and nitrate kept below harmful levels with 50% water changes as needed. Alkalinity was maintained by using Alkalinity Up (Leslie's Swimming Pool Supplies; Columbus, GA, USA), pH was maintained using sodium bicarbonate, and salinity was maintained by adding deionized water. On day 20, oysters were collected from each tank, counted, and discarded.

Data analysis

For all treatment temperatures (23 °C, 28 °C, 36 °C, and 38 °C), data were analyzed using a log-rank survival analysis in SigmaPlot 15 (Systat Software©, Inc. 2022). Similarly to chapter 1, when calculating LT_{25}, LT_{50}, and LT_{75} (lethal time that causes 25%, 50%, and 75% mortality, respectively), the 25th, 50th, and 75th percentiles survival times and 95% confidence intervals (95% CI) were calculated using the log rank survival analysis. To calculate an LT_{10}, the macros function in SigmaPlot 15 (Systat Software©, Inc. 2022) was used to generate a Kaplan Meier survival curve for each line (WC, GC, GS, and PS). This analysis generated the survival time and standard error (*SE*). Furthermore, 95% CI for cumulative probability were calculated (*SE* x 1.96). The upper and lower confidence limits for cumulative probability were calculated by adding and subtracting cumulative probability and the 95% CI. Once the upper and lower confidence limits were calculated, they were graphed into a scatterplot in Excel (Microsoft

Software©, Inc. 2025) along with cumulative probability. Using this scatterplot, the day at which 0.9 cumulative probability occurred and the days at which the upper and lower confidence limits occurred were recorded and used to identify survival time (days) and 95% CI.

RESULTS

Similar to chapter 1, all mortphometrics expressed as mean ± *SE.* Average shell height of the WC line was 45.09 ± 0.30 which was significantly lower than the PS line (46.38 ± 0.26; p = 0.02). Additionally, the GS line (47.56 ± 0.32) had a significantly higher average shell height than WC ($p < 0.01$), GC (45.53 ± 0.39; $p = 0.009$), and PS ($p = 0.02$) lines. Lastly, there were no significant differences between average shell heights of the GC line and the WC or PS lines.

Average shell length of the WC line was 31.78 ± 0.15 which was significantly smaller than the PS (33.02 ± 0.15; $p = 0.008$) and the GC (32.42 ± 0.14; $p = 0.007$) lines and not significantly different from the GS (31. 85 ± 0.15; $p = 0.09$) line. Additionally, the PS line (33.02 ± 0.15) had a significantly higher shell length than the GC ($p = 0.04$) and GS ($p = 0.009$) lines. Lastly, the GC line had a significantly higher shell length than the GS line ($p = 0.03$).

For average shell width, there were no significant differences between the WC line (10.89 ± 0.66) and the GC (10.78 ± 0.06), PS (11.07 ± 0.07), GS (11.04 ± 0.07) lines ($p = 0.09$). Additionally, there were no significant differences in shell width between the PS and GS lines ($p = 0.08$). However, the PS line had a shell width that was significantly higher than the GC line ($p = 0.04$) and the GS line had a shell width that was significantly higher than the GC line ($p = 0.03$).

Similarly to chapter 1, all the following survival probabilities, LT_{10}'s, LT_{25}'s, LT_{50}'s, and LT_{75}'s are expressed as mean ± *SE*. At 23 °C (control), all lines exhibited > 90% survival over 20 days (Figure 2.2A). There were no significant differences in survival probabilities among WC (19.05 ± 0.16), GC (19.18 ± 0.13), GS (18.96 ± 0.37), and PS (19.28 ± *SE* not able to be calculated) lines ($X^2 = 4.77$; $p = 0.19$). At 28 °C, survival to day 20 ranged from 79% to 92% among the lines (Figure 2.2B). The WC (18.06 ± 0.25) had a significantly lower survival probability through time than the GC (18.91 ± 0.17; $p = 0.01$) and the GS line (18.89 ± 0.18; $X^2 = 15.33$; $p = 0.01$; Figure 2.2B). Additionally, the PS line survival probability (18.22 ± 0.26) was not significantly different from the GC ($p = 0.20$), the GS ($X^2 = 15.33$; $p = 0.34$), or the WC lines ($X^2 = 15.33$; $p = 0.34$; Figure 2.2B). Lastly, the survival probability of the GC line was not significantly different from the GS line ($X^2 = 15.33$; $p = 0.69$).

At 36 °C, survival to day 20 ranged from 78% to 85% with no significant differences in overall survival probabilities through time among the WC (17.11 ± 0.41), GC (18.04 ± 0.28), GS (17.72 ± 0.37), and PS (17.76 ± 0.36) lines ($X^2 = 3.72$; $p = 0.35$; Figure 2.3A). Similarly, there were no significant differences in LT_{10} between the WC, GC, GS, and PS selected lines (Figure 2.3B).

At 38 °C, all lines experienced 100% mortality by day 12, with no significant differences in overall survival probabilities through time among lines ($X^2 = 6.83$; $p = 0.08$; Figure 2.4A). However, there were significant differences in time to reach specific mortality benchmarks. There was no significant difference in LT_{25} between the WC (6.17 ± 0.15), GC (6.17 ± 0.32), GS (6.33 ± 0.04), and PS (6.17 ± 0.04) lines (Figure 2.4B). The LT_{50} of the GS (7.00 ± 0.10) line was significantly higher than the WC (6.33 ± SE), GC (6.33 ± 0.03), and PS (6.33 ± 0.11) lines (Figure 2.4C). Conversely, there were no significant differences in LT_{75} among lines (Figure 2.4D).

DISCUSSION

Dermo disease, caused by a protozoan parasite *Perkinsus marinus*, causes mortality in 50% to 90% of adult oysters in affected populations (Burreson and Calvo 1996; Ford and Tripp 1996; Bushek et al. 2012; La Peyre et al. 2018). In a previous study, animals were selected for increased resistance to Dermo disease through genomic and phenotypic selection (Wang et al. 2025). However, selection for desirable traits can sometimes trigger a correlated response in other, non-targeted traits (Cobb and Bartholomé 2022). These correlated responses may be beneficial, neutral, or harmful (Gromko 1995). For example, selecting for faster growth in species like rainbow trout and Atlantic salmon has sometimes led to unintended changes in disease susceptibility-either improving resistance due to overall vigor or increasing vulnerability due to trade-offs in immune investment (Cobb and Bartholomé 2022).

Not only has Dermo disease been a major cause of mortality in eastern oysters but increases in water temperatures due to climate change have been a major cause of mortality as well (Marshall et al. 2021; Plumlee et al. 2024). For example, marine heatwaves due to hurricanes like Hurricane Michael in 2018 have been a major cause of eastern oyster mortality (Sturmer et al. 2020). In this study, I looked to see if genomic or phenotypic selection can trigger a correlated response for thermal tolerance. I found evidence that a correlated response occurred due to genomically but not phenotypically selected oysters. Selection for Dermo resistance and wet weight did not have an adverse effect on thermal tolerance. Rather, genomic selection for Dermo resistance and wet weight induced a positive correlated response in thermal tolerance at 38 °C. This supports the result of the GS line having a significantly higher shell height than all other lines and previous studies that showed increased wet weight having a direct correlated response that impacted thermal tolerance (Jiang et al. 2025).

Oysters that were exposed to 36 °C remained above 0.70 survival probability for the entire 20 days of exposure. These results did not line up with previous studies that showed eastern oysters having below a 0.30 survival probability at day 20 of exposure to 36 °C (Marshall et al. 2021). This difference in results could be due to multiple factors. One of which being that the use of artificial sea water, which was used in Marshall et al. (2021), has been seen to be worse for oysters and other invertebrate culture than natural sea water, which was used in my study (USEPA 1996; Pechenik et al. 2019). It is likely that the GOM will see 34 °C and above more frequently and for longer durations in coming years with increasing water temperatures due to climate change, so understanding how these ecologically and economically important animals will be able to withstand temperatures ≥ 34 °C that will likely become relevant in coming years will be crucial for management and farming practices (Bodenstein et al. 2021; Wang et al. 2023; AUSL 2024).

At 38 °C, oysters that were GS survived up to 17 hours longer than the GC, PS, and WC lines at 38 °C. Genomic selection, in aquaculture, when compared to phenotypic selection, has been seen to be more successful in enhancing traits (Cobb and Bartholomé 2022) like disease resistance to nodavirus in the giant fresh water prawn (Robinson et al. 2023) and disease resistance to Dermo disease in eastern oysters (Wang et al. 2025). The genomic selection could have led to an increase in overall fitness that raised the line’s ability to withstand higher temperatures. Since the GS line was also selected for increased wet weight and the phenotypically selected line did not, this could be a reason for this increase in thermal tolerance. Selection for increases in wet weight has been seen to raise thermal tolerance in *Crassostrea hongkongensis* (Jiang et al. 2025). These selectively bred oysters can offset energy budget inflation caused by marine heatwaves by upregulating feeding and respiration, allowing for more effective reallocation of energetic resources (Jiang et al. 2025).

This slight rise in thermal tolerance could be beneficial for both restoration and aquaculture purposes. Temperatures in the GOM have been seen to fluctuate up to 3 °C between night and day going from optimal (30 °C; Marhsall et al. 2021) to stressful (> 32 °C; AUSL 2024). Given that the GS line lived around 17 hours longer than the other lines when exposed to thermal stress, this gives us reason to believe that these animals will be better fit for the stressful periods that occur during the three to four hours each day of peak temperatures in marine heatwaves. Many natural oyster reefs have dwindled over the years due to increased temperature and overharvesting (Plumlee et al. 2024; La Peyre et al. 2014). These reefs face extreme heatwaves, increases in disease infection (Bushek et al. 2012), and freshwater influx events that completely wipe out a population (La Peyre et al 2014; Plumlee et al. 2024). The results of this study combined with future studies on salinity tolerances in these lines, could prove to be greatly beneficial for further efforts looking into selection impacting thermal tolerance.

Not only would expanding research on eastern oyster thermal tolerance be valuable but investigating how *Perkinsus marinus* responds to the warming temperatures increasingly observed in the GOM would also be beneficial. Prior studies have assessed infection rates across various thermal conditions (Chu and La Peyre 1993; Encomio and Chu 2007; Malek and Byers 2018) and have found that the parasite prefers water temperatures of 25 °C to 30 °C (La Peyre et al. 2008), but there has been little focus on the dynamics of the parasite's full life cycle under thermal stress. Evidence suggests parasite life cycles can deteriorate at certain thermal thresholds (Santos and Ebert 2022), implying there may be a temperature tipping point at which reproduction ceases altogether (OIE 2019). Identifying this breakdown point and comparing it to oyster thermal tolerance could illuminate whether parasite prevalence accelerates oyster mortality more than temperature stress alone.

This study helps bridge the literature gap on how selection for disease resistance in restoration can impact thermal tolerance. Although other studies have looked at how selection for increased growth performance can increase thermal tolerance in other bivalve species (Jiang et al. 2025), there are no other studies looking at how selection for Dermo disease may impact thermal tolerance in eastern oysters. Due to this study being the first of its kind, adding literature on this topic is beneficial not only for oyster research, but for research being conducted in other bivalve species that may use oyster research as a reference.

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Table 2.2. Preferred Water Quality Parameters. Ammonia, nitrite, and nitrate thresholds are based on sublethal effects on oyster clearance rates (Epifanio and Srna 1975). Alkalinity thresholds are based on average alkalinity in the northern Gulf of Mexico as there is no specific study looking at eastern oyster alkalinity tolerance (Gomez et al. 2021). Salinity and pH thresholds are based on previous studies that have looked at tolerances to each of these parameters (Gobler and Talmadge 2015; Marshall et al. 2021; Schwaner et al. 2023).

Ammonia	Nitrite	Nitrate	Alkalinity	pH	Salinity
< 3.0 mg/L	< 3.0 mg/L	< 80 mg/L	110–120 mg/L	7.5–8.0	18.0–22.0 PSU

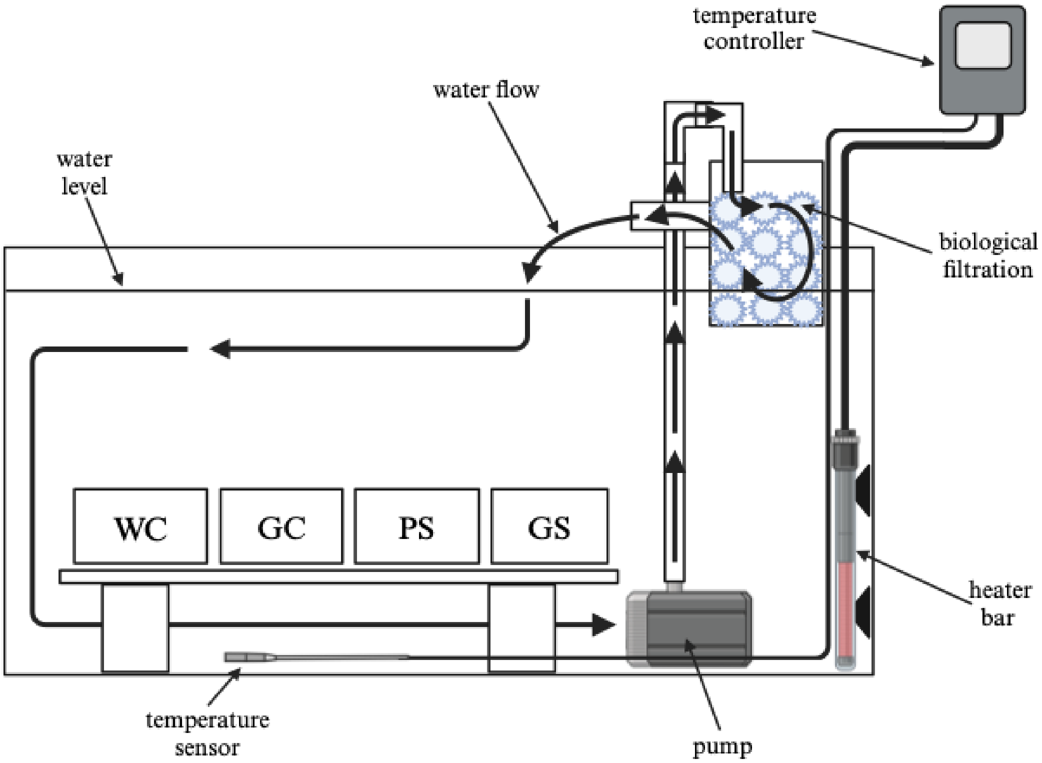

Figure 2.1. 300L recirculating aquaculture system setup. This system holds wild control (WC), genomic control (GC), phenotypically selected (PS), and genomically selected (GS) oyster lines.

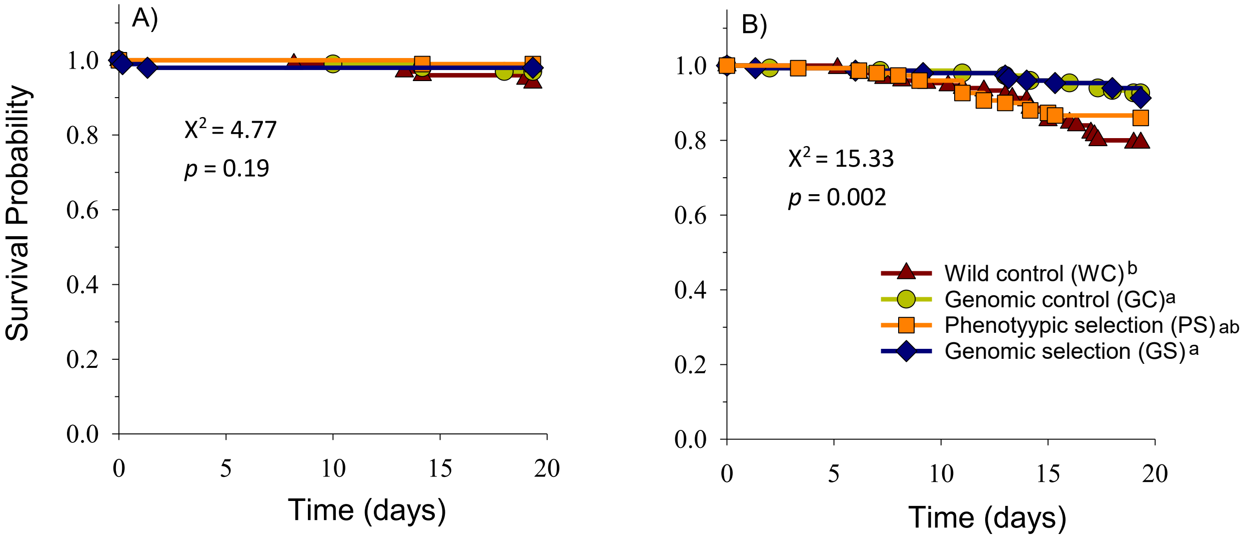

Figure 2.2. The relationship between survival probability and time (days) at 23 °C (A) and 28 °C (B). Each line represents one of the four oyster lines (WC, GC, GS, PS). Significant differences among lines are indicated by lower case letters.

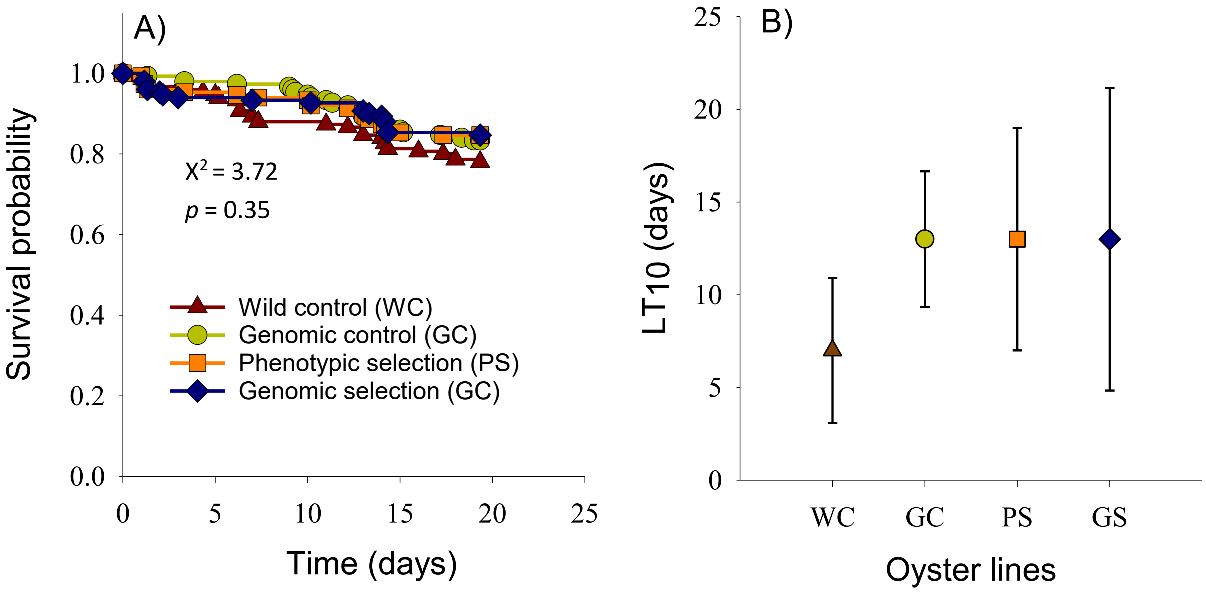

Figure 2.3. A) The relationship between survival probability and time (days) at 36 °C. Each line represents one of the four oyster lines (WC, GC, GS, PS). B) Lethal time that caused 10% mortality (LT10) in the wild control (WC), genomic control (GC), phenotypically selected (PS), and genomically selected (GS) lines. Error bars represent 95% confidence intervals.

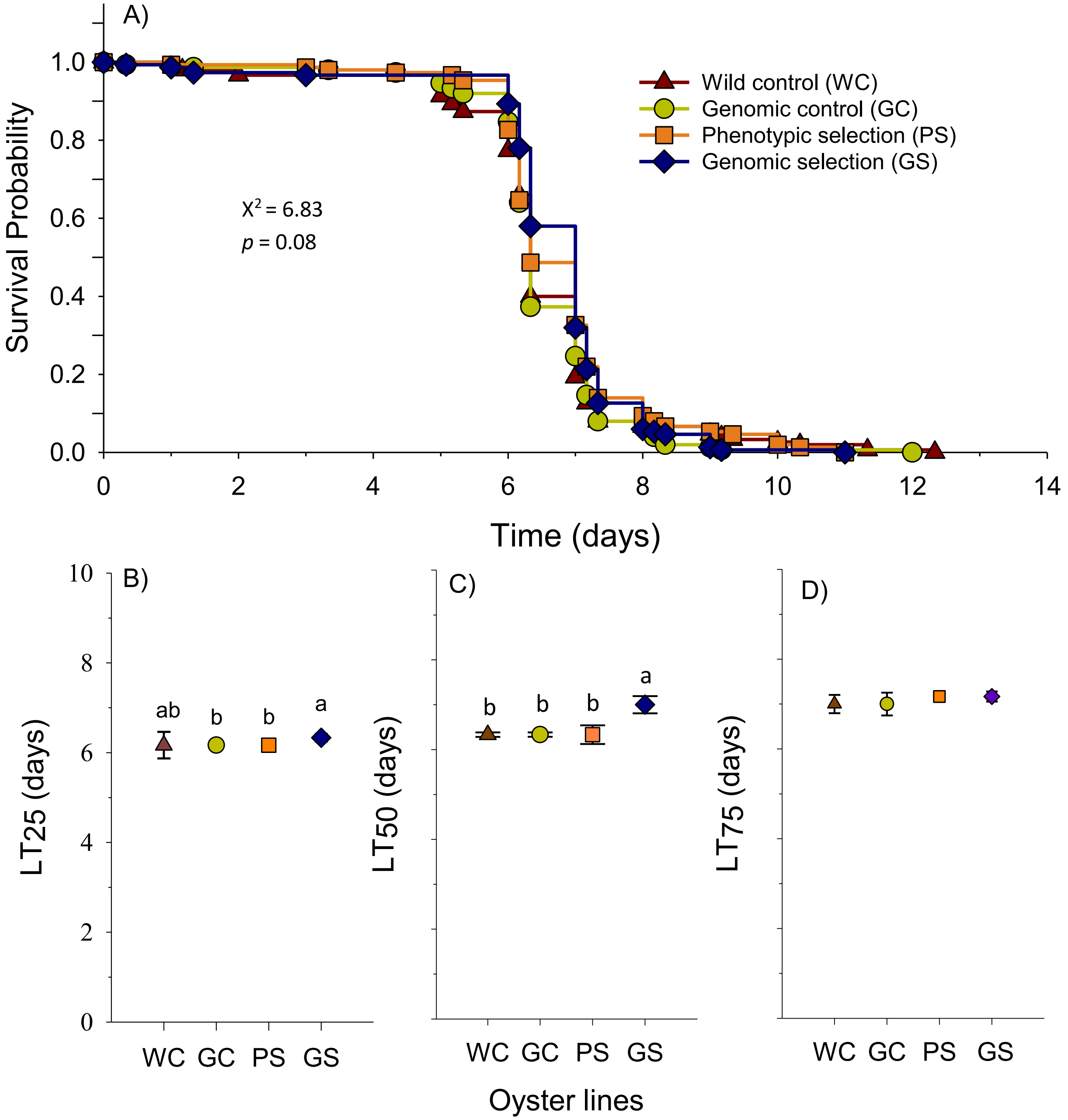

Figure 2.3. A) The relationship between survival probability and time (days) at 38 °C. Each line represents one of the four oyster lines (WC, GC, GS, PS). B) The LT_{25} (lethal time that caused 25% mortality) of each line. C) The LT_{50} (lethal time that caused 50% mortality) of each line. D) The LT_{75} (lethal time that caused 75% mortality) of each line. Error bars represent 95% confidence intervals. Significant differences among lines are indicated by lower case letters.