



Tolerance of northern Gulf of Mexico eastern oysters to chronic warming at extreme salinities

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ABSTRACT

The eastern oyster, *Crassostrea virginica*, provides critical ecosystem services and supports valuable fishery and aquaculture industries in northern Gulf of Mexico (nGoM) subtropical estuaries where it is grown subtidally. Its upper critical thermal limit is not well defined, especially when combined with extreme salinities. The cumulative mortalities of the progenies of wild *C. virginica* from four nGoM estuaries differing in mean annual salinity, acclimated to low (4.0), moderate (20.0), and high (36.0) salinities at 28.9 °C (84 °F) and exposed to increasing target temperatures of 33.3 °C (92 °F), 35.6 °C (96 °F) or 37.8 °C (100 °F), were measured over a three-week period. Oysters of all stocks were the most sensitive to increasing temperatures at low salinity, dying quicker (i.e., lower median lethal time, LT₅₀) than at the moderate and high salinities and resulting in high cumulative mortalities at all target temperatures. Oysters of all stocks at moderate salinity died the slowest with high cumulative mortalities only at the two highest temperatures. The F1 oysters from the more southern and hypersaline Upper Laguna Madre estuary were generally more tolerant to prolonged higher temperatures (higher LT₅₀) than stocks originating from lower salinity estuaries, most notably at the highest salinity. Using the measured temperatures oysters were exposed to, 3-day median lethal Celsius degrees (LD₅₀) were estimated for each stock at each salinity. The lowest 3-day LD₅₀ (35.1–36.0 °C) for all stocks was calculated at a salinity of 4.0, while the highest 3-day LD₅₀ (40.1–44.0 °C) was calculated at a salinity of 20.0.

1. Introduction

Eastern oysters (*Crassostrea virginica*) grow and reproduce in a wide range of environmental conditions, from the Gulf of St. Lawrence (latitude ~48 °N) to the Gulf of Mexico (latitude ~26 °N) (Shumway 1996; Lazoski et al., 2011). With minimal empirical data, the upper critical thermal limit, defined as a temperature beyond which only short-term survival is possible, for subtidal adult *C. virginica* has been reported to be 30 °C (Quick, 1971; Shumway, 1996) with 36 °C being acutely lethal (reported by Galtsoff 1964). However, mean monthly water temperature in northern Gulf of Mexico (nGoM) estuaries where *C. virginica* is grown subtidally can exceed 30 °C during several months of the year

(July–September) while monthly summer mortalities are generally not excessive (<5%) unless associated with low salinity (<5) or heavy *Perkinsus marinus* infection intensity (La Peyre et al. 2013, 2018, 2019; Wadsworth et al., 2019). A better understanding of the effects of prolonged exposure to elevated (30–40 °C), but not extreme (>40 °C), temperatures on subtidal *C. virginica* and the impact of extreme salinity (<5, >35) on the upper critical thermal limit of this foundation species is needed and timely considering global warming.

Fodrie et al. (2010) have already reported increases of air and sea surface temperature (>3 °C) over the past 30 years in nGoM concomitant with an increase in the percentage of tropical and subtropical species in seagrass-associated fish assemblages. Warming is expected to

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continue at even higher rates in summer (Allard et al., 2016). Along the nGoM (TX, LA, MS, AL), coastal surface temperature is projected to increase by 2–4 °C towards the end of the 21st century, paralleling changes in global temperatures in response to greenhouse gases (Biasutti et al., 2012; Rhein et al., 2013). Record high temperatures and duration of heat waves are also anticipated to increase (Biasutti et al., 2012), with the number of days with temperatures above 32 °C rising from the current level of 77 days–115 days (plus or minus 16 days) per year (Keim et al., 2008). Along with general warming trends, the frequency and intensity of rainfall are expected to increase across the southeastern United States with the associated effects of decreasing estuarine salinity, and, to decrease across the southern great plains with the associated effects of increasing estuarine salinity in southwestern Texas estuaries (Powell and Keim 2015; Kloesel et al., 2018; Carter et al., 2018).

While *C. virginica* is well adapted to estuarine conditions and can tolerate a wide range of water temperatures and salinities, there are limits to how long oysters can cope with marginal temperature and salinity conditions, especially when those conditions occur simultaneously. We therefore assessed the upper critical thermal limits of *C. virginica* in the southern part of its geographic range where they grow subtidally, and how those limits are impacted by extreme salinity. The cumulative mortalities of the progenies of wild *C. virginica* from four nGoM estuaries differing in mean salinity, acclimated to low (4.0), moderate (20.0), and high (36.0) salinities at 28.9 °C (84 °F) and exposed to increasing temperatures of 33.3 °C (92 °F), 35.6 °C (96 °F) or 37.8 °C (100 °F) were measured and median lethal times (LT₅₀) and median lethal Celsius degrees (LD₅₀) were determined.

2. Material and methods

2.1. Oysters

Oysters used in this study were the progeny of wild oysters collected from four estuarine sites along the nGoM differing in mean annual salinity (Fig. 1, Table 1). The progeny were produced in August 2018 at the Auburn University Shellfish Laboratory in Dauphin Island, Alabama, as described in Marshall et al. (2021). The F1 oysters were grown in bags on an adjustable long line system (ALS, BST Oyster Co., Cowell, South Australia) in Bayou Sullivan (30° 21' 52" N, 88° 12' 57" W), Alabama, before being moved in March 2019 to the Grand Bay Oyster Park (GBOP), AL, 30° 22' 15" N, 88° 19' 0" W) for further grow-out.

2.2. Experimental design

F1 oysters from each stock were transported to the Louisiana State University Agricultural Center Animal and Food Sciences laboratory building (AFL) in Baton Rouge, Louisiana, for testing thermal tolerance at salinities of 4.0, 36.0, and 20.0 consecutively, beginning in August and ending in November 2019, because of the limited number of tanks available. Each time, the shell height of 20 oysters from each stock was measured using a digital caliper (Mitutoyo USA, Aurora, Illinois), and mean shell height calculated (Table 1).

In August, 45 oysters from each stock were placed in eight 400-L tanks with bio-filters and filled with vigorously aerated artificial seawater (Crystal Sea Marinemix, Marine Enterprises International, Baltimore, Maryland, USA) adjusted to a salinity of 20.0 and temperature of 28.9 °C (84 °F), similar to conditions at GBOP at the time of collection. Temperature was controlled using submersible heaters with thermostat setting in °F (Cobalt Aquatics Flat Neo-Therm 300 W, Hygger Saltwater Tank Titanium Tube Submersible 500 W). Water salinity and temperature were checked using a YSI-Pro30 handheld multimeter (YSI Incorporated, Yellow Springs, Ohio). Salinity of all tanks was gradually adjusted at a rate of 3.0 every 2–3 days until the target salinity of 4.0 was reached. After a week of acclimation at salinity of 4.0, the number of oysters of each stock in each tank was adjusted to 40 prior to raising temperatures. Only 6 out of 360 oysters died during the period when oysters were acclimating to adjusted salinities. Temperatures in replicate tanks (2 tanks per temperature) were then gradually raised at a rate of 2.2 °C (4 °F) every 2–3 days until target temperatures of 33.3 °C (92 °F), 35.6 °C (96 °F) or 37.8 °C (100 °F) were reached while two control tanks were kept at 28.9 °C (84 °F). Temperature and salinity of each tank were measured daily and adjusted as needed to maintain target salinity and temperature. Oysters were fed daily with Shellfish Diet 1800® (Reed Mariculture Inc, Campbell, CA, USA), with 15 mL added to each tank. Once the desired salinity and temperature combinations were reached, the numbers of live and dead oysters in each tank were counted daily and any dead oysters removed over a three-week period, or until all oysters had died (whichever occurred first). Water quality (ammonia, nitrite, nitrate, pH) in the tanks was checked weekly using test strips (Life-guard Aquatics 5-way Test Strips and Ammonia Test Strips, Santa Fe Springs, California). The experiment was repeated at a target salinity of 36 (October) and 20 (November).

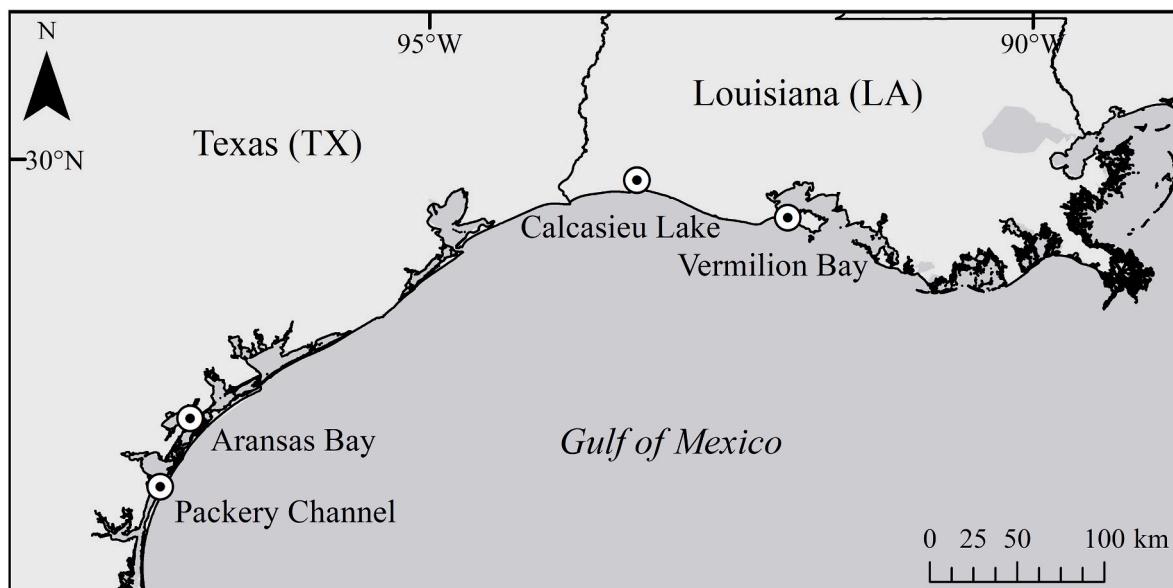


Fig. 1. Map of the broodstock collection sites: Packery Channel (PC), Aransas Bay (AB), Calcasieu Lake (CL) and Vermilion Bay (VB).

Table 1

Geographic coordinates, annual mean salinity and temperature ($^{\circ}\text{C}$) $\pm$ standard deviation (SD; 2009–2018), of sites where oyster broodstocks (*Crassostrea virginica*) from Packery Channel (PC) and Aransas Bay (AB) in Texas, and from Calcasieu Lake (CL) and Vermilion Bay (VB) in Louisiana, were collected and experimental mean shell height (SH, mm) $\pm$ SD of their progenies (F1) exposed to increasing temperatures at three salinities in September, October or November. Salinity and temperature data for the 4 sites were obtained from the Texas Parks and Wildlife Department fisheries-independent monitoring program (tpwd.texas.gov) and the Louisiana Department of Wildlife and Fisheries oyster program (wlf.louisiana.gov). Only stations within ~ 4 km of the oyster Texas collection site were used. Because these data are not collected consistently at the same day/time each month, some months were not included in the means.

Stock	Latitude, Longitude	Salinity $\pm$ SD	Temperature ($^{\circ}\text{C}$) $\pm$ SD	Salinity 4.0 September SH (mm) $\pm$ SD	Salinity 36.0 October SH (mm) $\pm$ SD	Salinity 20.0 November SH (mm) $\pm$ SD
PC	27 $^{\circ}$ 37' 38" N, 97 $^{\circ}$ 13' 59" W	35.5 $\pm$ 5.1	26.1 $\pm$ 1.7	54.6 $\pm$ 3.9	59.4 $\pm$ 4.8	66.7 $\pm$ 7.8
AB	28 $^{\circ}$ 7' 38" N, 96 $^{\circ}$ 59' 8" W	23.0 $\pm$ 6.9	22.9 $\pm$ 1.1	51.1 $\pm$ 3.9	55.7 $\pm$ 4.8	65.3 $\pm$ 4.9
CL	29 $^{\circ}$ 50' 58" N, 93 $^{\circ}$ 17' 1" W	16.2 $\pm$ 2.8	21.7 $\pm$ 1.8	53.8 $\pm$ 5.3	58.7 $\pm$ 8.0	63.1 $\pm$ 5.4
VB	29 $^{\circ}$ 34' 47" N, 92 $^{\circ}$ 2' 4" W	7.4 $\pm$ 1.6	22.0 $\pm$ 1.4	53.0 $\pm$ 3.2	58.9 $\pm$ 6.0	62.5 $\pm$ 4.5

2.3. Statistical analyses

Mortality data from each replicate tank were compared among stocks and treatments using probit analysis (R package 'ecotox'; [Wheeler et al., 2006](#)). For all stocks with $>15\%$ mortality at each salinity-temperature treatment, median lethal times (LT_{50}) with 95% confidence intervals (95% CI) were determined. Using the mean of daily measured temperatures ($^{\circ}\text{C}$) from Day 0 (defined as the day that target temperatures were reached), median lethal Celsius degrees (LD_{50}) on Day 3 were calculated. LT_{50} or LD_{50} are considered statistically different when confidence intervals do not overlap ([Wheeler et al., 2006](#)). All analyses were performed using R 3.6.3 (R Foundation for Statistical Computing, 2018). While some stocks experienced mortality during the acclimation period to target temperatures, all probit analyses used data collected from the day target temperatures were reached for each treatment to the end of each trial. For graphical representation, cumulative mortality of each stock at each temperature and salinity was calculated by dividing the total number of oysters that had died over the course of the study by the total number of oysters at the start of the study in replicate tanks.

3. Results

3.1. Water quality

During each experiment, the measured tank salinity remained at the desired levels. The measured tank temperatures, however, differed from the target temperatures. All replicate tanks were within 1°C of target temperatures, and mean temperatures in replicate tanks were within 0.5°C of each other (Table 2). Water quality parameters were maintained within non-harmful ranges for oysters (ammonia and nitrite < 3 mg/L, nitrate < 80 mg/L, pH > 7.5) throughout the study ([Epifanio and Srna 1975](#)).

3.2. Mortality and median lethal time (LT_{50}) at target temperatures

At a salinity of 20.0, cumulative mortalities of all stocks were low ($<15\%$) at the control (28.9°C) and lowest experimental temperature (33.3°C) but increased significantly at the higher temperatures reaching 60.5–66.0% at 35.6°C and 100% at 37.8°C (Fig. 2). Oysters died significantly faster at 37.8°C (LT_{50} : 3.7–5.0 days) than at 35.6°C (LT_{50} : 14.4–21.6 days) with little differences among stocks (Table 2).

At the lowest salinity (4.0), all stocks experienced high ($>75\%$) cumulative mortality above the control temperature (28.9°C) with oysters dying more rapidly (lower LT_{50}) at the higher the temperature (Fig. 2, Table 2). Significant differences in LT_{50} were found among stocks at salinity 4.0 at all experimental target temperatures with PC oysters generally taking longer to die (higher LT_{50}) than VB oysters (Table 2).

Similar to the experiment at salinity of 20.0, cumulative mortality at a salinity of 36.0 was low ($\leq 15\%$) at 28.9 and 33.3°C , and moderate to high (50–100%) at the higher temperatures, though oysters died more rapidly (lower LT_{50}) at a salinity of 36.0 compared to 20.0 (Fig. 2, Table 2). Significant differences in LT_{50} were also found among stocks at

all experimental target temperatures with PC oysters taking longer to die (higher LT_{50}) than oysters from all other stocks (Table 2).

3.3. Three-day median lethal Celsius degree (LD_{50})

Median lethal Celsius degrees (LD_{50}) on Day 3 were calculated but few confidence intervals were obtained because of the limited number of temperature treatments tested which caused significant mortalities. Nevertheless, the 3-day LD_{50} was generally lowest (i.e., lower temperature) at a salinity of 4.0, intermediate at a salinity of 36.0 and highest at a salinity of 20.0 (Table 3). Overall, the PC stock tended to have a higher 3-day LD_{50} than all other stocks at both salinity extremes.

4. Discussion

At a favorable salinity of 20.0, calculated LT_{50} indicated that half of the oysters were already dead by day 3.7–5.0 at 37.8°C while it took about four times longer (14.4–21.6 days) to kill the same number at 35.6°C , depending on stock and replicate tank. The highest temperature used is slightly above the 36°C reported to be acutely lethal to *C. virginica* by [Galtsoff \(1964\)](#). In [Galtsoff \(1964\)](#) foundational review, details about when and where oysters were collected, their sizes or weights, the rate and magnitude of the temperature increase and the duration of exposure to 36°C which all influence thermal tolerance were not disclosed. In our November experiment, oysters were acclimated to 28.9°C prior to temperatures being increased at a slow rate of 2.2°C (4°F) every 2–3 days to reach target temperatures. This rate of increase is well within the range observed in open nGoM estuarine waters ([Leonhardt et al., 2017](#); [La Peyre et al., 2019](#)). A number of studies indicate that *C. virginica* physiological functions generally decline as temperature rises above 30°C . Early on, [Loosanoff \(1958\)](#) measured pumping rates of *C. virginica* from Long Island Sound (northeastern Atlantic coast) and observed the highest pumping rates between 30°C and 32°C followed by an abrupt decrease above 34°C . Later, [Feng and Van Winkle \(1975\)](#) observed a decrease in heart rate at 35 and 40°C from a maximum at 30°C in *C. virginica* from Connecticut (northeastern Atlantic coast) at mid-salinity (14, 19). Temperature also exerted a major effect on *C. virginica* oxygen consumption rate which increased markedly from 10°C to 30°C and showed little evidence of suppression of metabolic energy demands following acclimation to summer temperatures ([Shumway and Koehn 1982](#); [Casas et al., 2018](#)). It is clear from these studies that prolonged exposure to temperature above the thermal optimum negatively impacts *C. virginica* physiology. According to the Oxygen and Capacity Limited Thermal Tolerance (OCLTT) concept, as temperature continues to increase above the thermal optimum, oxygen supply to tissues becomes increasingly limiting resulting in a decrease in tissue oxygen and increasing transition from aerobic to anaerobic metabolism, ultimately resulting in death ([Pörtner 2010](#); [Pörtner et al., 2017](#); [Eymann et al., 2020](#)). When oysters die rapidly ($\text{LT}_{50} < 5$ days), it is also likely that the integrity of cells, the functioning of organelles, and enzyme activities are negatively impacted by the high temperatures, especially when combined with extremes in salt concentrations ([Zhang](#)

Table 2

Median lethal time (LT₅₀) of F1 oysters (*Crassostrea virginica*) exposed to increasing temperatures after acclimation to salinity of 4.0, 20.0 or 36.0 at 28.9 °C. Values are LT₅₀ with 95% confidence interval (95% CI) results of the probit analysis for each salinity-temperature treatment above the control temperature (28.9 °C; 84 °F). Stocks with cumulative mortality <15% are not included (–) as LT₅₀ could not be calculated by the probit analysis. No mortalities were recorded in the control tanks (28.9 °C; 84 °F) at each salinity. Daily mean, minimum, and maximum measured temperatures from replicate (Rep) tanks are included. LT₅₀ are considered statistically different when their confidence intervals do not overlap. Oysters used were the progenies of broodstocks from Packery Channel (PC) and Aransas Bay (AB), Texas, and from Calcasieu Lake (CL) and Vermilion Bay (VB), Louisiana.

Target temperature (°C)	Rep	Stock	Salinity 4.0			Salinity 20.0			Salinity 36.0		
			Mean ± SD, min, max measured temp (°C)	LT ₅₀ (days)	95% CI	Mean ± SD, min, max measured temp (°C)	LT ₅₀ (days)	95% CI	Mean ± SD, min, max measured temp (°C)	LT ₅₀ (days)	95% CI
33.3 (92 °F)	A	PC	33.9 ± 0.1, 33.6, 34.1	16.1	(15.2, 17.2)	33.4 ± 0.2, 33.1, 33.8	–	–	33.9 ± 0.2, 33.4, 34.1	–	–
		AB		17.0	(15.8, 18.7)						
		CL		14.7	(13.8, 15.9)						
		VB		13.8	(12.9, 14.8)						
	B	PC	33.8 ± 0.1, 33.6, 33.9	8.7	(7.9, 9.7)	33.9 ± 0.3, 32.9, 34.2	–	–	33.8 ± 0.2, 33.4, 34.2	–	–
		AB		9.6	(9.0, 10.4)						
		CL		7.0	(6.1, 8.0)						
		VB		8.1	(7.5, 8.8)						
35.6 (96 °F)	A	PC	36.3 ± 0.4, 35.5, 36.7	3.4	(3.1, 3.8)	36.0 ± 0.3, 35.6, 36.3	14.4	(13.4, 15.7)	36.4 ± 0.2, 36.1, 36.7	18.3	(16.5, 23.2)
		AB		1.8	(1.5, 2.1)		17.4	(16.2, 18.9)		10.1	(9.5, 10.7)
		CL		2.0	(1.7, 2.4)		15.6	(14.6, 16.9)		12.1	(11.4, 13.1)
		VB		2.1			16.9	(15.5, 18.7)		6.8	(6.2, 7.5)
	B	PC	36.8 ± 0.3, 36.1, 37.0	2.9	(2.0, 5.1)	35.8 ± 0.9, 34.6, 36.6	20.7	(18.8, 23.5)	36.9 ± 0.2 36.6, 37.1	7.4	
		AB		2.0	(1.3, 3.2)		20.3	(19.1, 22.0)		4.7	
		CL		1.2	(0.8, 1.7)		21.6	(19.9, 24.0)		5.1	(4.2, 7.8)
		VB		1.4	(1.1, 1.7)		18.4	(16.8, 20.7)		4.9	(4.5, 5.3)
37.8 (100 °F)	A	PC	37.8 ± 0.2, 37.5, 38.1	2.1	(1.9, 2.3)	37.6 ± 0.3, 37.1, 37.9	4.4	(4.0, 4.9)	38.2 ± 0.3 37.7, 38.5	3.6	(3.3, 4.0)
		AB		1.7	(1.2, 2.7)		3.7	(3.4, 4.2)		2.5	(2.3, 2.7)
		CL		1.1	(0.9, 1.4)		4.2	(3.8, 4.9)		2.6	(2.4, 2.8)
		VB		1.6	(1.0, 2.5)		4.3	(3.9, 4.9)		2.2	(1.6, 3.1)
	B	PC	37.8 ± 0.3, 37.4, 38.1	1.5	(1.3, 1.8)	37.4 ± 0.1, 37.2, 37.6	5.0	(4.6, 5.6)	38.1 ± 0.4, 37.6, 38.5	4.2	(4.0, 4.6)
		AB		1.3	(1.0, 1.5)		4.4	(4.1, 4.7)		2.1	(1.6, 2.9)
		CL		1.1	(0.8, 1.3)		4.7	(4.5, 4.9)		2.7	(1.6, 3.9)
		VB		0.9	(0.7, 1.2)		4.4	(4.2, 4.7)		2.4	(2.1, 2.6)

et al., 2006; Cherkasov et al., 2010). In addition to the direct effects of temperature and salinity, pathogen proliferation within oysters under chronic warming and immunosuppression can also contribute substantially to mortality (Chu and La Peyre 1993; Green et al., 2019; Coffin et al., 2021).

Low salinity (4.0) significantly decreased the tolerance of oysters to all target temperatures compared to a salinity of 20.0, with LT₅₀ of only 0.9–2.1 days at 37.8 °C, 1.2–2.4 days at 35.6 °C and 7.0–17.0 days at 33.3 °C, depending on stock and replicate tank. In contrast, no mortality was observed at 28.9 °C in agreement with a recent laboratory study showing *C. virginica*, from the same nGoM estuaries used in this current study, can withstand a salinity of 4.0 at 28 °C for several months with limited mortality (<10%) (Marshall et al., 2021). At higher temperatures or below a salinity of 3, however, mortalities of experimental

oysters increase noticeably with some *C. virginica* populations dying more than others (La Peyre et al., 2013; Rybovich et al., 2016; McCarty et al., 2020; Marshall et al., 2021). Under field conditions, extended exposure (weeks) to low salinity events during hot summer months have long been associated with high *C. virginica* mortalities not only in the nGoM but also in mid-Atlantic estuaries (Butler 1952; Andrews et al., 1959; May, 1972; La Peyre et al., 2013). These low salinity events (or “freshets”) occur periodically from floodwaters, openings of freshwater diversion structures, or hurricanes. At low salinity, oysters’ valves remain closed much longer than at higher salinity, forcing anaerobic metabolism that results in the buildup of carbon dioxide in tissues, the accumulation of waste products, and subsequent death which is hastened at higher temperatures (de Zwaan and Wijsman, 1976; Lombardi et al., 2013; Casas et al., 2018). With the combination of increased

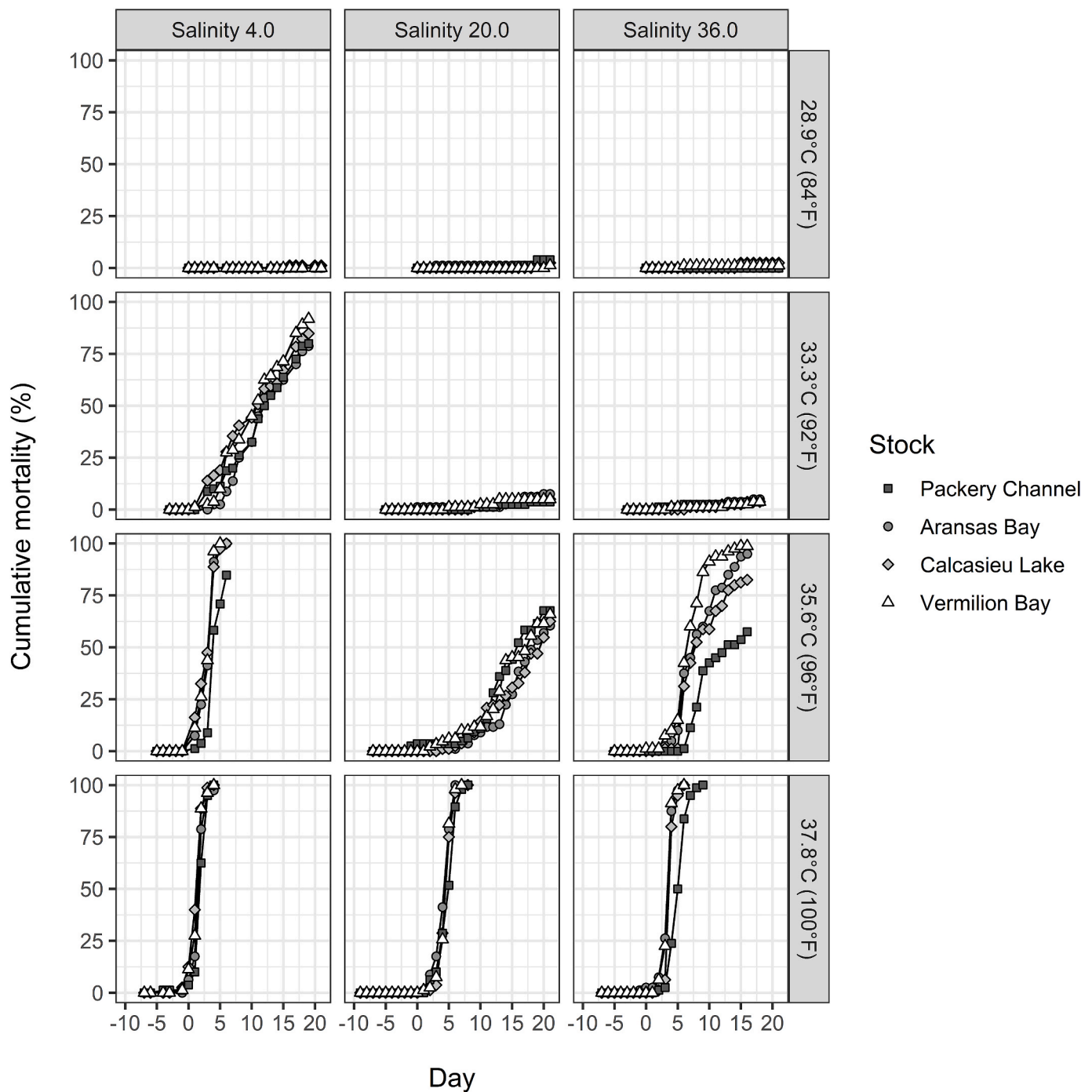


Fig. 2. Cumulative mortality of the progenies (F1) of Texas oysters from Packery Channel and Aransas Bay broodstocks, and of Louisiana oysters from Calcasieu Lake and Vermilion Bay broodstocks. Day 0 indicates the day target temperatures of 33.3 °C (92 °F), 35.6 °C (96 °F), or 37.8 °C (100 °F) were reached from the control temperature of 28.9 °C (84 °F). Negative days indicate period during which temperatures were being increased at a rate of 2.2 °C (4 °F) every 2–3 days. Data are for both replicate tanks at each salinity and temperature.

warming and extreme storm events along the nGoM expected to continue, field mortalities are likely to increase at low salinity in summer.

At a salinity of 36.0, no mortality was observed at 33.3 °C above that of 28.9 °C, and oysters died more slowly than at a salinity of 4.0 but faster than at a salinity of 20.0 at the two highest temperatures. Interestingly, differences in LT_{50} among stocks were more pronounced at this high salinity. Specifically, oysters from the PC stock took significantly longer to die than oysters from all the other stocks at the two highest temperatures. LD_{50} of the PC stock also tended to be higher than that of

the other stocks. These findings are not surprising because the PC broodstock oysters were collected from the more southern and hypersaline Upper Laguna Madre estuary and are likely more tolerant of chronic warming at high salinities than oysters from stocks originating from lower salinity estuaries. Divergence in salinity tolerance between the PC stock and the other nGoM stocks has recently been shown, although the physiological mechanisms have yet to be characterized (Marshall et al., 2021). Moreover, there is strong evidence of genetic differentiation between Laguna Madre and other nGoM oyster populations (Buroker 1983; Varney et al., 2009; Anderson et al., 2014). It

Table 3

Day 3 median lethal Celsius degree (LD₅₀) for F1 oysters (*Crassostrea virginica*) exposed to increasing temperatures after acclimation to salinity of 4.0, 20.0 or 36.0 at 28.9 °C. Values are 3-day LD₅₀ with 95% confidence interval (95% CI) results of the probit analysis for each salinity-stock combination. LD₅₀ are considered statistically different when their confidence intervals do not overlap. Oysters used were the progenies of broodstocks from Packery Channel (PC) and Aransas Bay (AB), Texas, and from Calcasieu Lake (CL) and Vermilion Bay (VB), Louisiana.

Salinity	Stock	Day 3 LD ₅₀ (°C)	95% CI
4.0	PC	36.0	(35.4, 36.6)
	AB	35.7	(34.7, 36.4)
	CL	35.1	(34.6, 35.8)
	VB	35.3	
20.0	PC	40.1	(38.7, 49.7)
	AB	44.0	
	CL	41.6	
	VB	41.9	(39.4, 66.2)
36.0	PC	39.5	
	AB	37.4	
	CL	37.6	(37.5, 37.7)
	VB	37.3	

will be interesting to determine in future studies whether the higher tolerance to high temperature at high salinity compared to low salinity might be related to higher concentration of the osmolyte taurine in osmoconforming *C. virginica*, which acts as a cytoprotectant (Gleason et al., 2017).

Salinity significantly modulated the thermotolerance of *C. virginica*. The combination of extreme temperature and salinity had a greater effect than either stressor experienced individually. It is important to note that environmental and endogenous factors or stressors other than those already mentioned (i.e., salinity, population) might also significantly affect *C. virginica* survival at elevated temperatures (Todgham and Stillman 2013). Those might include animal size, dissolved oxygen concentration, infection by pathogens, and gonadal stage, which directly affect *C. virginica* metabolism and its thermal tolerance as would be expected from the OCLTT concept (Bayne and Widdows 1978; Casas et al., 2018; Thomas and Bacher 2018; Götze et al., 2020). Noteworthy, in relation to our study, is the decreased resilience of larger animals to environmental stressors, including elevated temperature and salinity reported in oysters and other animals and attributed to size-related scaling effects on energetics (Sukhotin et al., 2003; Peck et al., 2009; Rybovich et al., 2016; Peralta-Maraver and Rezende 2020). In our study, oysters exposed to increasing temperatures at salinity of 20.0 were the largest used so that their LT₅₀ values were likely underestimated because these oysters would be expected to die more rapidly (lower LT₅₀) than smaller oysters. The differences in LT₅₀ among salinities would have been even more pronounced in our study had the exposure to the different salinities been conducted at the same time with the same batch of oysters.

The negative impact of rising temperatures on *C. virginica* performance and health in nGoM estuaries is likely to be significant and will be greatly exacerbated by extreme and fluctuating salinity conditions resulting from climate change. Moreover, as the hottest temperatures occur during the summer spawning season when adult oysters are already energetically vulnerable from reproductive processes, increased adult mortality may also have a significant impact on long-term oyster population sustainability. Increasing the length of exposure to elevated temperatures may lead to a reduction in *C. virginica* lifespan and shift age structure towards younger individuals in nGoM estuaries (Martínez et al. 2016; Peralta-Maraver and Rezende 2020). This is supported by the observation of smaller oysters and the lack of extensive oyster forming reefs in GoM estuaries further south of Texas (latitude < 26°N) (Collier 1954; Copeland and Hoese 1966). In contrast, *C. virginica* at the northern end of its range may benefit from increased temperature because the

period when conditions are optimal for growth and reproduction will be extended (Filgueira et al., 2016; Steeves et al., 2018). Overall, better characterization of the effect of prolonged exposure to elevated temperatures (30–40 °C) on the physiology and metabolism of *C. virginica* populations along its latitudinal range is paramount considering rising water temperatures and increased frequency of marine heat waves, salinity fluctuations, and extremes.

CRedit authorship contribution statement

Danielle A. Marshall: Investigation, Formal analysis, Visualization, Data curation, Writing – original draft. **Nicholas C. Coxe:** Investigation. **Megan K. La Peyre:** Conceptualization, Supervision, Writing – review & editing. **William C. Walton:** Resources, Writing – review & editing. **F. Scott Rikard:** Resources, Writing – review & editing. **Jennifer Beseres Pollack:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Morgan W. Kelly:** Conceptualization, Funding acquisition, Writing – review & editing. **Jerome F. La Peyre:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

None.

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