UROP Project

Salinity tolerances of eastern oysters bred from native Louisiana broodstock

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Abstract

The objective of this project was to compare the tolerance of eastern oysters from different populations and of different ploidies to low salinity events that are increasing in frequency in Louisiana estuaries. Oysters from three different stocks, collected from three different sites in Louisiana, as well as diploid and triploid oysters for each stock, for a total of 6 groups, were placed in tanks with salinities of 2, 4, 20, and 36. The oysters' cumulative mortality, condition index, and osmolality was tracked over a 52-day period. Mortality was significantly higher in the tank with a salinity of 2 but there were no significant differences observed between groups within that tank. Oyster health, evaluated by measuring condition index and osmolality, was also not affected by stock or ploidy in most of the salinity treatments. The lack of difference between stocks for cumulative mortality indicates that adaptive evolution due to different salinity conditions among sites did not have a large enough effect to impact mortality at set salinities. The physiological differences between diploids and triploids additionally did not have a large enough effect to impact mortality at set salinities. Future experiments could examine if these observations would hold true with rapid changes in salinity or with consistent salinity changes.

Introduction

The eastern oyster, *Crassostrea virginica*, is an important species in Louisiana due to its commercial value in oyster farming as well as its role in coastal ecosystems. The culture of oysters is a sustainable farming practice (Shumway, et al. 2003). Stock and ploidy are two factors which have been shown to impact oyster survival in field studies under different environmental conditions. Specifically in Louisiana, diploid oyster stocks differ in their salinity tolerances (Leonhardt et al. 2017, Sehlinger et al., 2019). Triploid oysters, oysters with 3 sets of chromosomes instead of the normal 2 sets, have also displayed higher mortalities than diploid oysters in previous field studies (Callam et al 2016; Wadsworth et al 2019) but they are preferred by oyster farmers due to having more meat and better condition indexes. As periods of low salinity become more frequent in Louisiana estuaries such as Barataria Bay and Breton Sound, it is important to find oyster populations that are capable of surviving low salinity.

Wild broodstock from Calcasieu Lake, Vermillion Bay, and Sister Lake were used to produce diploid oysters from each estuary as well as triploid oysters by crossing wild diploid female oysters from each estuary with generic tetraploid male oysters. The salinity tolerance of the progenies were compared after acclimation to multiple salinities. The objectives of the experiment were to determine whether stock and ploidy affected oyster mortality, condition index and plasma osmolality of oysters acclimated to a range of salinities. This information could help oyster farmers improve their selection criteria for which oysters to farm in terms of which will survive better and have the highest condition index.

Methods

Wild broodstock oysters were collected from three different Louisiana estuaries between November 2018 and February 2019: Calcasieu Lake (CL), Vermillion Bay (VB), and Sister Lake (SL). The three estuaries displayed different salinity profiles; CL had an average salinity of 12.5 ppt, VB had an average salinity of 11.2 ppt, and SL had an average salinity of 5.5 ppt (USGS monitoring stations 2019). The oysters were placed in bags suspended on long lines in Grand Isle until Summer 2019 when they were spawned using standard to produce 3 diploid stocks (CL2n, VB2n, SL2n). Wild oysters from each estuary were also crossed with tetraploid oysters in order to produce 3 triploid stocks (CL3n, VB3n, SL3n). After growing them out on long lines in Grand Isle, 320 oysters of each group were collected in September 2020 and placed in 8 tanks at 25°C and approximately 20 ppt (40 oysters of each group per tank). Two tanks stayed at 20 ppt while 2 were adjusted to 2 ppt, 2 were adjusted to 4 ppt, and 2 were adjusted to 36 ppt. The tanks were adjusted by 3 ppt every other day in order to reach the desired salinity. Once target salinities were reached, mortality was checked every Monday, Wednesday, and Friday of each week. Oysters were considered dead when they were unable to effect shell closure when squeezed at least 5 times. Cumulative mortalities were calculated by dividing the number of oysters that had died with the number of live oysters at the start of the study. Condition index (CI) was measured at the start and end of the study. The formula used for CI was (Dry Tissue Weight/Shell Cavity Weight)*100. The CI measured at the end of the experiment was then subtracted from the initial CI to determine the change in CI. Plasma osmolality data was also measured at the end of the experiment to see how the oysters of different stocks and ploidies adjusted to the salinities. Results of mortality, condition index and osmolality were analyzed using two-way ANOVA tests in R.

Results

Cumulative Mortality: The highest cumulative mortality was seen in a salinity of 2 for all stocks and ploidies. Since salinity was shown to have a large impact on oyster mortality (ANOVA, $p < 0.01$), the effect of each ploidy and stocks was analyzed at each salinity level. However, no significant differences of cumulative mortality were found at any of the salinities for ploidy (ANOVA, $p \geq 0.07$ for all cases) or stock (ANOVA, $p \geq 0.08$ for all cases) (Fig 1).

Change in Condition Index (CI): A 2-way ANOVA found that there was no interaction between salinity and group (stock and ploidy combined; e.g. Sister Lake Diploid) (ANOVA, $p = 0.99$). However, the ANOVA found there to be a significant difference between groups (ANOVA, p <= .01) and salinities (ANOVA, p <= .01) in terms of change in CI. After running a post-hoc Tukey test, differences between the 2-36 salinity tanks were observed (ANOVA, $p = .01$) as well as the 4-36 salinity tanks (ANOVA, $p = .01$). For the group, there were significant differences observed between CL3N and CL2N (ANOVA, p <= .01), SL3N and CL2N (ANOVA, $p = .04$), VB2N and CL3N (ANOVA, $p = .01$), VB3N and CL3N (ANOVA, $p = .03$), and VB3N and SL2N (ANOVA, $p = .05$) (Figure 2) (Table 1).

Osmolality: Between ploidies, there were no significant differences observed in any of the tanks for osmolality (ANOVA, p >= 0.07 for all cases) (Table 2). The only significant difference observed for stock was in the 36 salinity tank (ANOVA, $p \geq 0.01$ for all cases). In this tank, Calcasieu Lake had the highest osmolality by a value of 15 mOsm/kg. None of the salinities displayed any interactions between stock and ploidy (p >= 0.10 for all cases).

Discussion

For cumulative mortality, change in condition index, and osmolality, salinity is known to have a significant impact. Since our studies also found this to be the case, ploidy and stock were analyzed for each salinity to determine their effects on cumulative mortality and osmolality. While it was expected that ploidy would have an effect on cumulative mortality (Callam et al 2016, Wadsworth et al 2019), there were no significant differences observed between diploid and triploid mortalities at any salinity level. Though triploids in the tank with a salinity of 4 tended to have higher mortality than diploids, this difference was not significant (0.07). Additionally, since a salinity of 2 applied the most stress to the oysters, that tank would have theoretically been the tank where the largest difference was observed between the ploidies if there had been any difference in their abilities to survive in harsher conditions. Stock also was shown to have no significant effect on cumulative mortality (closest p-value = .08). While it was possible that the different populations of oysters had evolved different salinity tolerances due to the different salinity profiles of CL, VB, and SL, this potential effect was not strong enough to have a significant impact on their respective mortality rates in this experiment.

Significant differences in condition index were observed between groups in a 2-way ANOVA that was analyzing the interaction between salinity and group. Since group is a combination of the ploidy and stock factors, it’s possible that these factors combined to produce a significant effect. CL oysters were involved in many of the significant differences between groups that were observed in the post-hoc Tukey test. CL2N had consistently less change in CI than other groups while CL3N had consistently more change in CI than other groups. The two VB groups both displayed small changes in CI, indicating that the VB oysters’ CI is not very affected by lower salinity conditions. The triploid oysters appeared to have larger changes in CI on average than diploid oysters, meaning that adverse conditions most likely impacted triploid oysters more than diploids in terms of CI. In terms of salinity, the only significant differences between tanks were observed between the 2-36 salinity tanks and the 4-36 salinity tanks. Both the 2 and the 4 salinity tanks caused a larger change in CI in their respective oysters than did the 36 salinity tank, indicating that the lesser salinity has a larger negative impact on oyster CI than higher salinity.

Osmolality measured how the oysters physiologically adjusted to each salinity. The only significant result was that the CL stock was measured to have the highest osmolality by 15.25 Osm/kg in the 36 salinity tank. Although this result does indicate that these CL oysters had pretty different osmolality values from the other oysters in this tank, it does not seem to correlate with a particular change in mortality.

Overall, stock and ploidy appear to have little to do with oyster mortality at these set salinities. Since other studies (Leonhardt et al, 2017, Callam et al 2016, Wadsworth et al 2019) have demonstrated that ploidy and stock do play a role in oyster mortality, it is important to build upon this study. Future experiments could be conducted that measure the effect of rapid salinity change instead of a slow change was used in our study, on diploid and triploid oysters as well as the effects of continuous salinity change. Since the oysters in this experiment were acclimated to very consistent conditions, these future experiments would serve to better mimic the actual environments oysters would actually be living in.

References

Callam, Brian R., Standish K. Allen Jr, and Anu Frank-Lawale. "Genetic and environmental influence on triploid Crassostrea virginica grown in Chesapeake Bay: growth." Aquaculture 452 (2016): 97-106.

Megan K. La Peyre, James Geaghan, Gary Decossas, Jerome F. La Peyre; Analysis of Environmental Factors Influencing Salinity Patterns, Oyster Growth, and Mortality in Lower Breton Sound Estuary, Louisiana, Using 20 Years of Data. Journal of Coastal Research 1 May 2016; 32 (3): 519–530.

Sehlinger T., Lowe M. R., La Peyre M. K., & Soniat T. M. (2019). Differential Effects of Temperature and Salinity on Growth and Mortality of Oysters (Crassostrea virginica) in Barataria Bay and Breton Sound, Louisiana. Journal of Shellfish Research, 38(2), 317-326. doi:10.2983/35.038.0212

Shumway S E, Davis C, Downey R, Karney R, Kraeuter J, Parsons J, Rheault R, Wikfors G (2003) Shellfish aquaculture - In praise of sustainable economies and environments. J World Aquacult Soc 34

Pandora Wadsworth, Sandra Casas, Jerome La Peyre, William Walton; Elevated mortalities of triploid eastern oysters cultured off-bottom in northern Gulf of Mexico. Aquaculture, Volume 505, 2019, Pages 363-373.

Tables

Table 1: Shell height (mm), initial CI, final CI, and change in CI percent by group (mean +/- SD, n=20). Change in CI was calculated by doing the ((Final CI – Initial CI)/Initial CI) x 100.

Shell Height		Initial CI		Final CI			
Group	Height	Group	CI	Salinity	Group	CI	Change in CI(%)
CL2N	67.03 +/- 9.23	CL2N	4.95 +/- 1.50	2	CL2N	2.80 +/- 1.83	-39.42 +/- 28.7
CL3N	69.46 +/- 5.63	CL3N	8.24 +/- 2.12	2	CL3N	5.03 +/- 1.78	-34.25 +/- 31.55
VB2N	67.98 +/- 6.37	VB2N	4.70 +/- .65	2	VB2N	2.53 +/- 0.81	-44.85 +/- 19.84
VB3N	68.42 +/- 8.80	VB3N	6.50 +/- 3.54	2	VB3N	3.96 +/- 1.26	-53.94 +/- 76.97
SL2N	68.71 +/- 7.03	SL2N	5.03 +/- 1.32	2	SL2N	2.59 +/- 1.02	-42.95 +/- 34.5
SL3N	68.82 +/- 9.62	SL3N	8.36 +/- 1.83	2	SL3N	5.00 +/- 1.42	-36.5 +/- 24.61
				4	CL2N	2.67 +/- 0.81	-38.51 +/- 33.86
				4	CL3N	4.57 +/- 1.75	-39.94 +/- 29.26
				4	VB2N	2.73 +/- 0.62	-41.08 +/- 15.53
				4	VB3N	4.31 +/- 3.31	-8.36 +/- 64.55
				4	SL2N	2.79 +/- 0.59	-41.22 +/- 18.34
				4	SL3N	5.47 +/- 1.88	-30.33 +/- 36.91
				20	CL2N	3.47 +/- .74	-25.12 +/- 21.99
				20	CL3N	5.57 +/- 1.74	-27.63 +/- 30.79
				20	VB2N	3.07 +/- 1.09	-53.18 +/- 112.41
				20	VB3N	5.15 +/- 2.27	-10.74 +/- 90.3
				20	SL2N	2.89 +/- 1.82	-35.12 +/- 45.31
				20	SL3N	5.44 +/- 1.56	-31.7 +/- 23.94
				36	CL2N	4.28 +/- 1.27	-4.86 +/- 39.45
				36	CL3N	5.67 +/- 2.77	-22.92 +/- 43.31
				36	VB2N	3.80 +/- 0.50	-18.18 +/- 13.32
				36	VB3N	4.92 +/- 1.93	-24.42 +/- 82.29
				36	SL2N	3.94 +/- 1.19	-16.57 +/- 30.47
				36	SL3N	5.98 +/- 1.91	-23.09 +/- 26.90

Table 2: Osmolality (mOsm/kg) of oysters of all groups (mean +/- SD, n=4)

Tank salinity (osmolality)				
	2 (70)	4 (120)	20 (581)	36 (1079)
Group	Osmolality +/- SD			
CL2N	70.5 +/- 4.72	118 +/- 5.73	587.5 +/- 15.92	1088.5 +/- 6.56
CL3N	75.25 +/- 9.28	127.5 +/- 1.73	60.5 +/- 10.37	1095.25 +/- 3.68
VB2N	72.25 +/- 3.2	135.5 +/- 18.55	599 +/- 3.37	1080.25 +/- 14.5
VB3N	70 +/- 2.45	128.25 +/- 6.84	597 +/- 6.21	1073 +/- 15.98
SL2N	70 +/- 1.41	126.75 +/- 6.65	587 +/- 1.41	1072.25 +/- 8.96
SL3N	81.75 +/- 9.91	129.25 +/- 2.63	593.75 +/- 1.25	1081 +/- 6.68

Figures

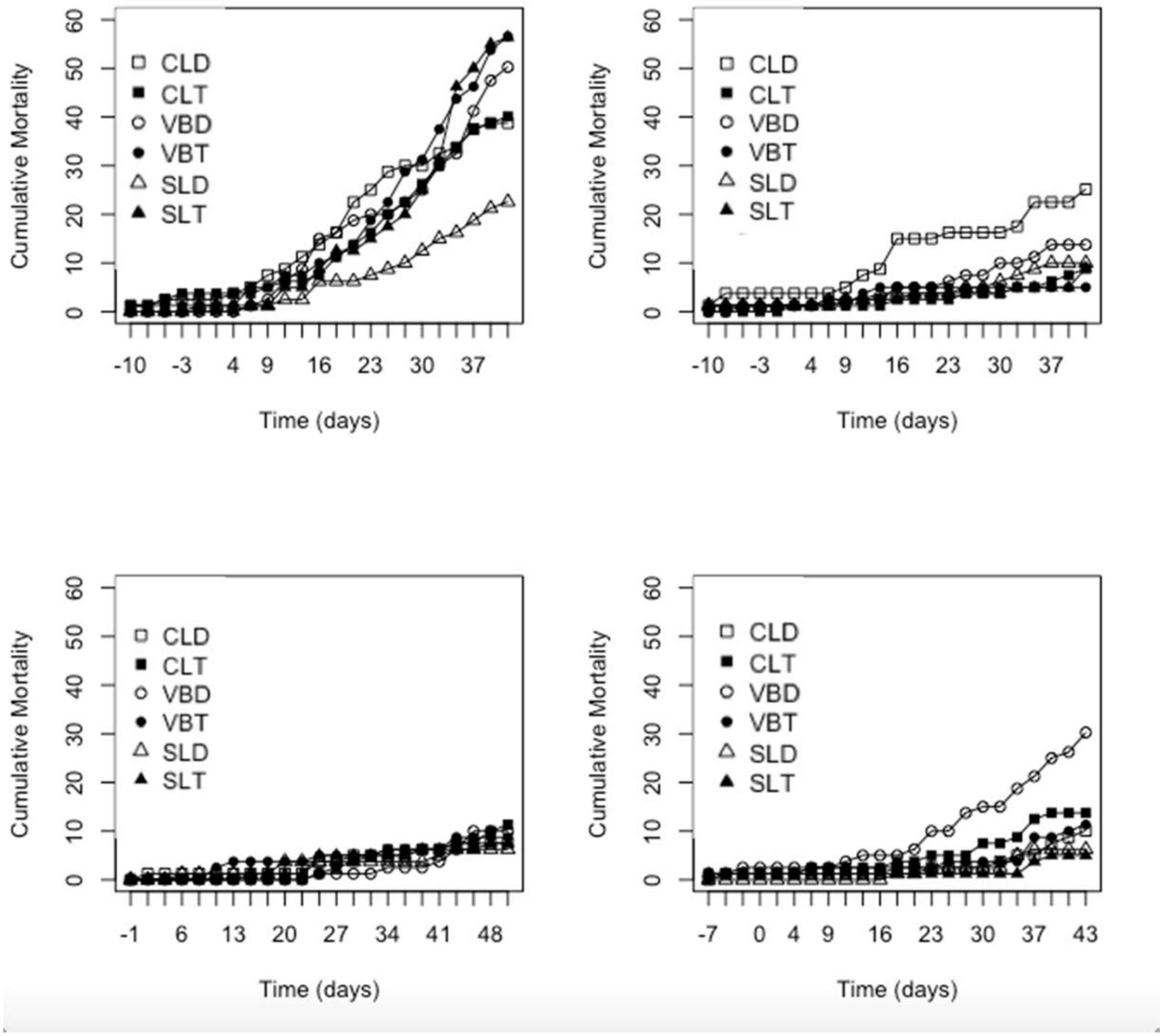

Figure 1: Cumulative Mortality for each group by salinity. The 2 salinity tank is top left, 4 is top right, 20 is bottom left, 36 is bottom right. The numbers on the x-axis represent the day in relation to when the desired salinity was reached with "0" representing that day.

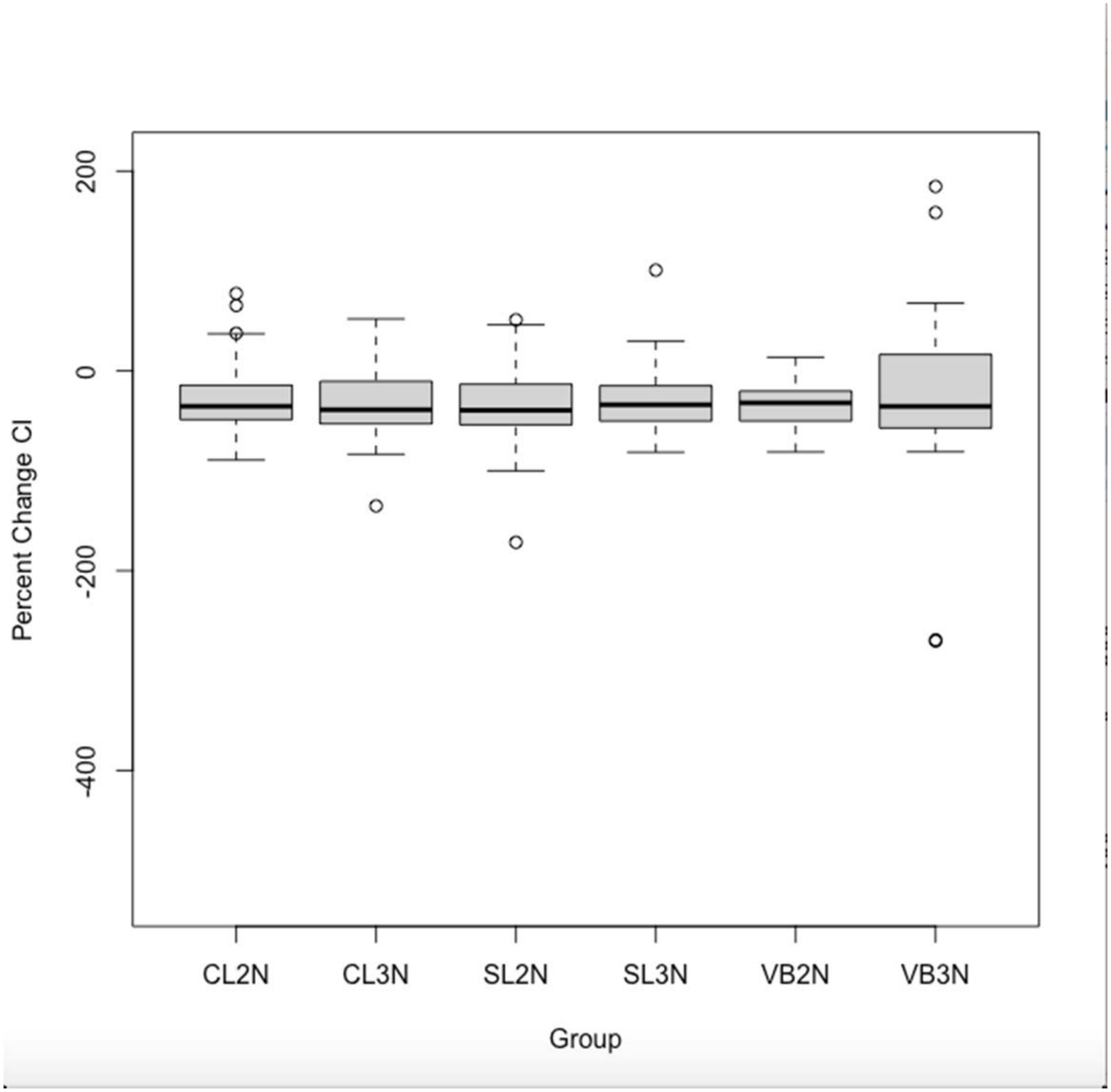

Figure 2: Change in CI Percentage by group across all salinities. A negative percentage indicates a decrease in CI.