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Morphological Assessment of the Eastern Oyster *Crassostrea virginica* throughout the Gulf of Mexico

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Abstract

The eastern oyster *Crassostrea virginica* is a commercially and ecologically important organism found throughout the western North Atlantic and Gulf of Mexico. Morphological differences in shell shape of eastern oysters are known to arise from environmental, genetic, and husbandry-related factors. Here, live eastern oysters were collected from 17 sites along the U.S. Gulf of Mexico coast from Texas to Florida to examine morphological differences among geographic samples. Six morphological metrics were recorded for each individual, and four different composite ratios commonly used to describe oyster shape (fan, cup, volume ratio, and weight ratio) were calculated. Principal component analysis was used to demonstrate geographic differences in ordinated shape ratios, correlating roughly with eastern (Florida), northern (Alabama, Louisiana, north Texas), and western (south Texas) samples. In Texas, differences in shape were correlated with previously described genetic population boundaries, indicating that populations north versus south of Aransas Bay had different overall shell shapes. On a broader scale, shell shape variation correlated roughly with previously described genetic population boundaries throughout the Gulf of Mexico as well as tide depth (intertidal versus subtidal reefs). Among the various factors that might act as drivers of shell shape, individual variation is important, but population structure and tide height are also significant predictor variables of shape in this species.

Estuaries are highly productive ecosystems with key habitats, including salt marshes, seagrasses, mangroves, and shellfish reefs, that support spawning, nursery, and feeding functions for recreational and commercial fishery

species (USEPA 1999). Shellfish reefs in the western Atlantic Ocean and Gulf of Mexico are primarily constructed by the eastern oyster *Crassostrea virginica*, which is a foundation species that ranges from the Gulf of St.

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Lawrence to Venezuela (Stanley and Stellers 1986). Eastern oysters provide critical habitat for other species as well as ecosystem services, including increased landscape diversity (Puckett and Eggleston 2012), water filtration (Newell 2004; zu Ermgassen et al. 2013), carbon sequestration (Fodrie et al. 2017), biomass production (Shepard et al. 2018), enrichment of fisheries resources (Galtsoff 1964; Stunz et al. 2010; Humphries et al. 2011), nutrient regulation (Bricker et al. 2008; Beseres Pollack et al. 2013), and habitat stabilization (Scyphers et al. 2011). Eastern oysters also provide significant economic benefit to coastal communities. Over 9.5 million kg of eastern oysters were harvested in the USA in 2018, with Texas producing the second largest harvest of 1.7 million kg and generating US\$23 million (NOAA Fisheries 2018).

In the Gulf of Mexico, there exists the potential for at least three genetic populations of eastern oysters: (1) an eastern population that spans from the southern Atlantic coast of Florida to Cedar Key and Apalachicola in the Florida Panhandle (Buroker 1983; Reeb and Avise 1990; Karl and Avise 1992; Hare and Avise 1996; Hoover and Gaffney 2005; Varney et al. 2009), (2) a northern population that extends from Apalachicola to Aransas Bay, Texas (King et al. 1994; Hoover and Gaffney 2005; Varney et al. 2009; Anderson et al. 2014; Thongda et al. 2018), and (3) a western population that spans from Aransas Bay to estuaries further south in the Gulf of Mexico (Groue and Lester 1982; Buroker 1983; King et al. 1994; Hoover and Gaffney 2005; Varney et al. 2009; Anderson et al. 2014; Thongda et al. 2018). There may be additional unobserved population structure through this range, but the three-population model of genetic structure in the Gulf of Mexico is the most accepted model based on all previous studies.

Although there are ample data describing genetic divergence among populations of the eastern oysters in the

Gulf of Mexico, much less is known about whether there are also differences in biological characteristics, such as gross morphology. Eastern oysters are constantly growing in all directions (Galtsoff 1964), with abiotic and biotic factors influencing growth (Stanley and Stellers 1986; Robinson et al. 2014; Mizuta and Wikfors 2019). The shell of an eastern oyster is characterized by a great deal of individual variation in shape but can generally be described as elongated on one axis, rounded, and asymmetrical. The left valves are thicker and more deeply cupped than the right valves, which are generally flat (Figure 1). Young eastern oysters (<0.85 cm) tend to have a circular shell shape, while older eastern oysters start to be elliptical and elongated and have growth shifted to one side (Galtsoff 1964). Given the great potential for interindividual differences in shape, it is not unreasonable to assume that there may also be shape variables that are correlated with either environment or genetics (Mizuta and Wikfors 2019), and in either case it is possible that these variables could be used as correlates with known population structure. Observing changes in morphology over a broad geographic scale is the first step in determining the drivers of morphological variation at the estuary scale and above.

In this study, eastern oysters were collected in estuaries along the U.S. Gulf of Mexico coast, from Texas to Florida, in an effort to elicit patterns in shell shape above the level of mere random individual variation. Four composite ratios were calculated from raw morphological measurements made from each individual, and differences in these ratios were assessed statistically to evaluate the null hypothesis that shape variation is not correlated with the presence of regional population structure. The alternative hypothesis was evaluated by assessing morphology in the context of known population structure from previous studies as well as general characteristics of reefs from which

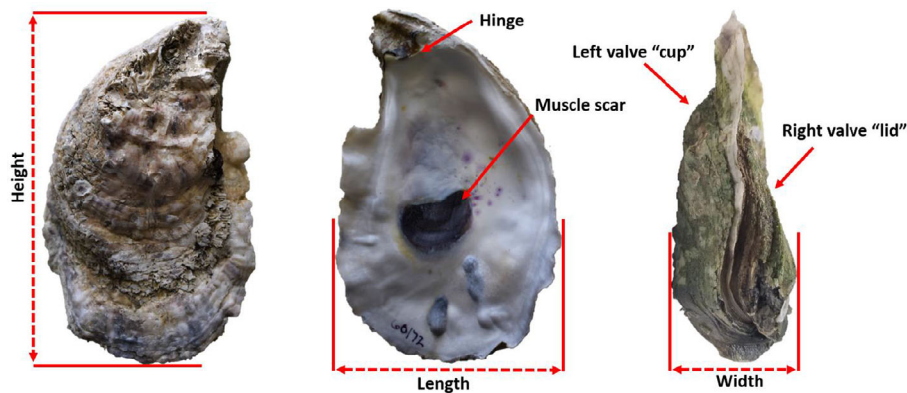


FIGURE 1. Images of an eastern oyster illustrating the height, length, and width measurements used in this study and as previously described in Galtsoff (1964).

samples were taken (subtidal versus intertidal). This is the first attempt to correlate gross morphology in the eastern oyster with known genetic populations in the Gulf of Mexico. These data will be useful as a first step in examining the link between regional morphology and population genetics in this important estuarine species; such a linkage may imply additional regional variation in growth, physiology, reproduction, disease resistance, or other important biological parameters that might have one or more underlying genetic drivers.

METHODS

Study area and sampling.—Eastern oysters were collected in 17 estuaries from throughout the Gulf of Mexico, including sampling locations in Texas (Port Mansfield, Upper Laguna Madre, Corpus Christi Bay, Redfish Bay, Aransas Bay, Copano Bay, Mesquite Bay, San Antonio Bay, Matagorda Bay, Galveston Bay, and Sabine Lake), Louisiana (Calcasieu Lake and Sister Lake), Alabama (Mississippi Sound), and Florida (Apalachicola, Tampa Bay, and Caloosahatchee River; Figure 2). Live eastern oysters were collected from randomly selected reefs between January and February 2020 in each bay system. For sampling outside of Texas, eastern oysters were collected from reefs by oyster dredges by the Florida Fish and Wildlife Conservation Commission, Alabama Department of Conservation and Natural Resources, or Louisiana Department of Wildlife and Fisheries. For sampling inside of Texas, eastern oysters were collected by hand or dredged from known oyster reefs during routine monthly oyster reef monitoring by Texas Parks Wildlife Department. A total of 30 eastern oysters were randomly collected from each bay, in each state, except for the Mission–Aransas Bay system in Texas, an area where two

highly divergent genetic populations of eastern oysters coincide (Anderson et al. 2014). The Mission–Aransas estuary is composed of Mesquite, Copano, Aransas, and Redfish bays; 15 eastern oysters were collected from each of these areas. At every sample location, it was noted whether eastern oysters were collected from intertidal (occasionally exposed to air) or subtidal (never exposed) reefs.

Laboratory methods.—All collected eastern oysters were separated from each other and fouling organisms carefully by the use of a hammer and chisel. Any loosely attached fouling organisms and sediment were hosed off. Height, length, and width were measured as in Galtsoff (1964). Height was measured using calipers as the longest straight-line distance from the umbo to the lip of the eastern oyster on the left valve (nearest mm). Length was recorded as the longest straight-line measurement across the eastern oyster in the axis roughly perpendicular to height on the left valve (mm). Width was the thickest measurement of the two valves closed together (mm; Figure 1). Whole eastern oyster volume was measured via water displacement in a graduated cylinder (mL). Whole eastern oyster (wet) weight (mg) was recorded with a calibrated scale, after which the oyster was shucked and wet tissue weight (mg) was weighed separate from the shell. Wet weight was used rather than dry weight because fresh tissue samples were needed for a concurrent project, and tissues were excised immediately following weight measurements.

Four composite ratios (fan, cup, volume, weight) were calculated from the six raw variables. The first three ratios were calculated by dividing each original variable by height (size-corrected ratios): fan was calculated as length divided by height, cup was calculated as width divided by the height (Bellaaj-Zouari et al. 2012; Mizuta and Wikfors



FIGURE 2. Eastern oyster samples were obtained from 17 sampling locations in the Gulf of Mexico between January and February 2020. The color of the dots represents the placement of samples within three assumed regional populations described in literature cited in the text: green = eastern population, red = northern population, blue = western population.

2019), and volume ratio was calculated as whole eastern oyster volume divided by height. Lastly, the weight ratio was calculated as wet tissue weight divided by whole organism weight. These ratios were used in downstream analyses in lieu of the raw data. Since the ratios were all weighted by other gross size measurements (height and whole weight), it was expected that multivariate analyses using these four composite ratios were not overly biased by the gross size (height) differences of individual eastern oysters sampled on different reefs.

Statistical methods.—To account for the varied growth allometry that is expected to be associated with different stages of ontogeny, eastern oysters were grouped into five different 20-mm size-classes based on the distribution of the variable height (<50, 50–69, 70–89, 90–109, and ≥110 mm). Principal component analysis (PCA) was used to calculate ordinated, multivariate combinations of the four composite ratios within each size-class. Qualitative observation of biplots of the five separate PCAs suggested that similar patterns of shape among Gulf of Mexico regions persisted regardless of size, with the exception of the smallest size-class (<50 mm; see Results). As such, the final PCA included all individuals >50 mm in a single analysis. Individual loadings on each of the first two PCA axes (hereafter “PC1” and “PC2,” respectively) were plotted using a scatterplot to distinguish differences in multivariate morphology among eastern oysters sampled from different areas. The relative importance of each of the four composite ratios on PC1 and PC2 was assessed using the individual loading scores.

To assess statistically the differences in shape indicated by the more exploratory PCA, we also evaluated the relationship of height against length, width, and volume using three stepwise regression analyses and all sampled individuals (eastern oysters <50 mm were included). In each case, tidal zone (intertidal versus subtidal) and Gulf of Mexico region were included as random effects, allowing the regression intercept to vary. Variables were entered and removed using forward stepwise regression, and variable retention was based on Akaike information criterion minimization (hereafter, “AIC”). The importance of each model variable was assessed by examining effect test *F*-ratios, and statistical significance of variables in the model was assessed by estimating the frequency of larger values of *F* in each case.

Four ANOVA analyses were conducted using the four composite ratios (fan, cup, volume, weight) as univariate dependent variables, with each sample site as independent nominal explanatory variables ($n = 17$ sites). Fan and cup were the two composite ratios that loaded most significantly onto PC1 (cup) and PC2 (fan; see Results), and sample means of these two ratios were plotted against the overall studywide mean using stem-and-leaf plots. Tukey’s post hoc tests were used to assess significance between

pairwise site comparisons. Fan and cup have been used to assess meat potential in the commercial oyster industry; differences in these ratios among areas might point to differences in product quality associated with observed shell shape differences.

RESULTS

Eastern oysters from the northern population (upper Texas, Louisiana, and Alabama) on average had taller, wider, thicker, and heavier shells than those from the western (lower Texas) and eastern (Florida) populations, although there was a great deal of variation even within populations (Table 1). Qualitative observation of the PCA biplots suggested that regional signal of general shape patterns persisted throughout all size-classes (Supplementary Figures S1–S5 available separately online), with the possible exception of the smallest oysters (<50 mm). Regardless of the size-class examined, cup, fan, and volume tended to load positively on the first PCA axis. Also, regardless of size, weight ratio loaded positively on the second PCA axis; loading of weight ratio on the first PCA axis was variable but generally loaded negatively. Exceptions to this last finding were in the <50-mm size-class and the >110-mm size-class; in each case, weight ratio was not significantly correlated with the first PCA axis. With the exception of the smallest size-class, individuals from regional Gulf of Mexico populations (western, northern, eastern) tended to load similarly regardless of size. For this reason, the smallest size-class was removed for the final PCA with all sizes combined.

The first and second principal components (PC1 and PC2) of the combined PCA explained 57.3% and 22.7% of the variation within the data set, respectively (80.0% total; Figure 3). The first axis of ordination PC1 was highly correlated with all four composite ratios used to build the PCA (Table 2). Thus, PC1 represented a trade-off between eastern oysters with large gross shell metrics versus those with high ratios of tissue to weight and was the most explanatory axis in eliciting differences among individuals. At the population level, oysters from the northern population tended to have a strong positive correlation with PC1 (group mean 0.862), whereas oysters from both the western and eastern populations tended to be negatively correlated with PC1 (group mean of –0.912 and –1.665, respectively). At the scale of tidal zone, intertidal oysters were negatively correlated with PC1, whereas subtidal oysters were positively correlated with PC1. Fan and weight ratio were positively correlated with PC2, although this second ordinated variable was less explanatory at the population scale.

Stepwise regression analysis suggested that univariate size-based metrics varied significantly by Gulf of Mexico region as well as by tidal zone (Figure 4). Based on AIC, the best model of regression for length (overall $F = 190.9$, $r^2 = 0.63$, $P < 0.0001$), width ($F = 245.3$, $r^2 = 0.63$, $P <$

TABLE 1. Six eastern oyster shell shape variables (mean \pm SD) measured from January to February 2020 for bays throughout the Gulf of Mexico. Tidal zone is noted in parentheses for each bay: I = intertidal, S = subtidal, and M = mixture of both zones. State abbreviations are as follows: TX = Texas, LA = Louisiana, AL = Alabama, and FL = Florida.

Gulf population	Bay	<i>n</i>	Height (mm)	Length (mm)	Width (mm)	Volume (mL)	Tissue weight (mg)	Whole weight (mg)
Western	Port Mansfield, TX (I)	30	82.8 \pm 14.9	49.4 \pm 6.4	25.2 \pm 6.0	44.3 \pm 11.6	61.49 \pm 17.20	71.32 \pm 18.98
	Upper Laguna Madre, TX (I)	30	91.5 \pm 32.2	50.3 \pm 12.1	24.1 \pm 8.9	63.6 \pm 37.1	90.87 \pm 52.21	100.06 \pm 56.89
	Corpus Christi Bay, TX (I)	30	66.1 \pm 8.4	45.6 \pm 6.1	20.3 \pm 4.5	22.6 \pm 7.2	32.59 \pm 10.37	37.07 \pm 11.61
	Redfish Bay, TX (I)	30	74.3 \pm 11.1	49.6 \pm 8.9	24.8 \pm 4.7	37.1 \pm 19.3	61.87 \pm 34.19	68.98 \pm 37.85
Northern	Aransas Bay, TX (S)	15	78.1 \pm 9.5	58.9 \pm 5.1	32.7 \pm 3.7	58.9 \pm 14.9	112.55 \pm 26.37	121.61 \pm 28.79
	Copano Bay, TX (S)	15	76.4 \pm 11.2	55.1 \pm 5.7	34.4 \pm 4.4	55.0 \pm 16.8	99.50 \pm 28.19	106.01 \pm 30.08
	Mesquite Bay, TX (S)	15	76.0 \pm 31.3	62.3 \pm 23.1	29.5 \pm 15.2	70.7 \pm 51.9	119.47 \pm 87.87	129.27 \pm 94.69
	San Antonio Bay, TX (S)	30	77.3 \pm 28.5	56.2 \pm 19.4	32.3 \pm 11.6	66.5 \pm 41.9	123.32 \pm 76.17	132.57 \pm 81.39
	Matagorda Bay, TX (I)	30	87.2 \pm 16.8	54.1 \pm 7.9	27.7 \pm 7.2	51.0 \pm 23.4	82.36 \pm 40.51	90.65 \pm 42.60
	Galveston Bay, TX (S)	30	79.0 \pm 23.3	59.2 \pm 15.0	34.5 \pm 13.5	74.71 \pm 55.3	133.43 \pm 83.40	146.63 \pm 90.94
	Sabine Lake, TX (S)	30	69.8 \pm 19.2	50.8 \pm 12.3	33.4 \pm 11.0	56.2 \pm 30.1	112.04 \pm 68.78	121.33 \pm 72.42
	Calcasieu Lake, LA (S)	30	84.8 \pm 14.2	66.3 \pm 10.0	36.0 \pm 7.3	87.8 \pm 36.9	158.58 \pm 64.39	173.79 \pm 69.92
	Sister Lake, LA (S)	30	71.6 \pm 11.3	55.6 \pm 7.9	31.1 \pm 6.3	55.9 \pm 20.2	103.74 \pm 37.54	113.67 \pm 40.96
	Mississippi Sound, AL (S)	30	94.0 \pm 10.7	68.3 \pm 8.0	36.7 \pm 6.1	89.3 \pm 20.1	154.32 \pm 34.38	171.14 \pm 36.80
Eastern	Apalachicola, FL (S)	30	66.2 \pm 17.3	47.2 \pm 10.7	23.2 \pm 7.3	26.6 \pm 17.9	42.88 \pm 32.34	50.63 \pm 36.64
	Tampa Bay, FL (I)	30	63.0 \pm 11.7	36.3 \pm 7.0	17.5 \pm 3.9	15.8 \pm 8.9	21.33 \pm 12.26	25.36 \pm 14.69
	Caloosahatchee River, FL (M)	30	69.1 \pm 17.4	37.0 \pm 6.7	18.2 \pm 3.5	17.3 \pm 9.1	25.79 \pm 14.26	28.57 \pm 15.40

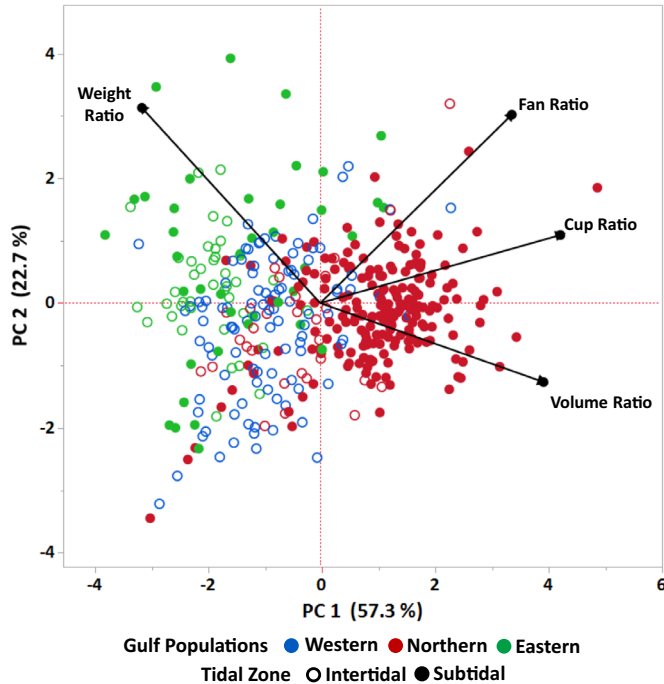


FIGURE 3. Biplot of loadings scores from the principal component analysis of composite morphological ratios of eastern oysters. Dot color represents regional population structure, and general reef depth is indicated as intertidal (open circles) versus subtidal (solid circles). The vectors emanating from the center of the plot represent loading scores of each composite ratio superimposed onto the first two axes of ordination (PC1 and PC2).

TABLE 2. Principal component (PC) loading scores of the composite ratios used to generate the PCA and group mean loadings of Gulf of Mexico populations, Gulf of Mexico regions, and tidal zone.

Grouping	Variable	n	PC1	PC2
Ratio for PCA	Fan (length/height)	420	0.691	0.618
	Cup (weight/height)	409	0.866	0.223
	Volume (volume/height)	396	0.806	-0.259
	Weight (tissue/shell)	397	-0.643	0.641
Gulf population	Western	101	-0.912	-0.338
	Northern	239	0.862	-0.182
	Eastern	80	-1.665	0.474
Gulf region	Lower Texas	101	-0.912	-0.340
	Upper Texas	150	0.689	-0.308
	Louisiana	59	1.307	0.054
	Alabama	30	0.856	-0.013
	Florida	80	-1.665	0.472
Tidal zone	Intertidal	171	-1.067	-0.209
	Subtidal	249	0.656	0.017

0.0001), and volume ($F=318.2$, $r^2 = 0.75$, $P < 0.0001$) all were inclusive of height, gulf region, and tidal zone. While height was the most reliable predictor in each case, tidal

zone was a slightly more reliable predictor than gulf region based on effect tests (Table 3). All three explanatory variables were significant predictors of shape metrics in each case.

There were statistically significant differences among single sample sites in eastern oyster fan ($F=12.0952$, $P < 0.001$), cup ($F=21.8147$, $P < 0.001$), volume ratio ($F=32.7720$, $P < 0.001$), and weight ratio ($F=43.6668$, $P < 0.001$), with bays located south of Aransas Bay in Texas and southeast of Apalachicola in Florida having lower composite fan, cup, and volume ratios but higher weight ratios. Although broad regional differences accounted for the greatest variability in shell shape, there was some localized variation embedded in the broader trends. With regard to fan and cup specifically, there were significant differences among some sample sites within regions (Figure 5).

DISCUSSION

The main finding of this study is that variability in eastern oyster morphology seems to rise beyond the level of random individual variation, with shell shape patterns indicating variation in morphology among regional populations in the Gulf of Mexico. This finding was supported by multivariate ordination of shape ratios as well as differences in the univariate ratios themselves, which suggested that oysters from northern gulf areas (northern Texas, Louisiana, and Alabama) were on average taller, wider, and thicker and had lower tissue to shell weight ratios than those from in southern Texas and Florida. Regional differences in shell morphology are expected across a broad range due to the expectation that either environmental influences, population genetics, or both may impose localized or regional impacts on growth parameters (Mizuta and Wikfors 2019). One exception to this pattern was in smaller (<50 mm) oysters; although small juvenile oysters seemed to exhibit regional shape differentiation, the pattern of differentiation was qualitatively inconsistent with what was observed in larger individuals. This could be due either to actual biological processes (driven by ontological changes in the drivers of gross shape; Galtsoff 1964) or sampling error. The number of individuals falling into this size-class was small ($n=30$), and thus outliers may have had a heavier impact on PCA in this size range relative to larger oysters. Given this latter point, it was decided that including these smaller oysters in the final multivariate analysis was inappropriate. However, inclusion of these individuals did not change the general results of the study (data not shown).

In addition to the geographic correlation with morphology, there was also an environment-driven pattern as reef depth seemed to play a role in driving multivariate shell shape. Intertidal reefs were generally negatively correlated with PC1, while subtidal reefs had positive correlations.

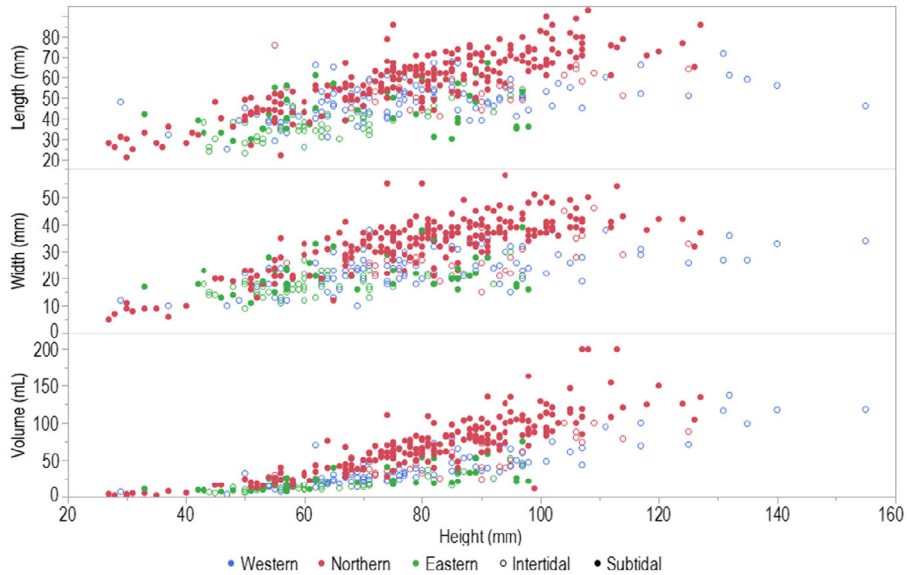


FIGURE 4. The relationship between three variables (length, width, and volume) versus eastern oyster height from three Gulf of Mexico regions (western = blue, northern = red, eastern = green). Intertidal and subtidal samples are overlaid as open and closed dots, respectively.

TABLE 3. Effect tests of variables included in stepwise regression models for shell length, width, and volume. In each case, variables were entered into forward stepwise regression and retention was based on minimization of AIC. Gulf region and tidal zone were recoded as nominal explanatory variables, and the regression intercept was allowed to vary.

Variable	df	Sum of squares	F-ratio	Prob > F
Response: length				
Height (mm)	1	28,305.2	389.0	<0.0001
Tidal zone	1	2,413.5	33.2	<0.0001
Gulf region	2	4,800.3	33.0	<0.0001
Response: width				
Height (mm)	1	12,140.4	325.7	<0.0001
Tidal zone	1	1,414.5	37.9	<0.0001
Gulf region	2	2,130.2	28.6	<0.0001
Response: volume				
Height (mm)	1	251,646.2	750.4	<0.0001
Tidal zone	1	11,352.6	33.9	<0.0001
Gulf region	2	21,836.8	32.6	<0.0001

Additionally, tide height was a significant predictor of length, width, and shell volume in univariate analyses. Intertidal eastern oysters tended to have smaller fan, cup, and volume ratios and higher weight ratios than their subtidal counterparts. It should be noted that tidal zone was a variable that was incompletely crossed within regional population structure, and thus this study is not well equipped to parse the impacts of these interrelated spatial variables because a majority of reefs sampled in the northern population were subtidal. An exception to this pattern was for oysters in Matagorda Bay, which had fan and cup

ratios that were similar to southern Texas bays, despite being located north of Aransas Bay on the middle Texas coast, speaking to the interactive quality of this variable within the broader regional context. The relationship between depth and shape in this study indicates that environmental parameters associated with reef placement may play at least as important a role as regional population genetic structure in driving shell shape, if not more so. Other such environmental factors, such as water energy and tide strength (Orton 1936; Mehrubeoglu et al. 2013), water quality (Manzi et al. 1977), biofouling (Marshall and Dunham 2013), and predation (Robinson et al. 2014) very likely play a role, although these variables were not assessed in this study. Additional research into the impact of other abiotic and community-level processes on shape represents a logical “next step” in parsing the drivers of shell shape in Gulf of Mexico oyster populations.

This study was designed to assess shell shape variation in the context of previously described genetic population structure in eastern oysters in the Gulf of Mexico (Hoover and Gaffney 2005; Varney et al. 2009), and results indicate that variation in oyster shell morphology roughly correlates with the putative boundaries of those populations. Genetic data indicate there is an oyster population transition zone in the northeastern Gulf of Mexico (Hoover and Gaffney 2005; Varney et al. 2009), although the transition between these populations may be “clinal” in nature, rather than a hard geographic break between divergent groups (Reeb and Avise 1990; Karl and Avise 1992; Hare and Avise 1996). In either event, the current data indicate that oyster populations southeast of Apalachicola (i.e.,

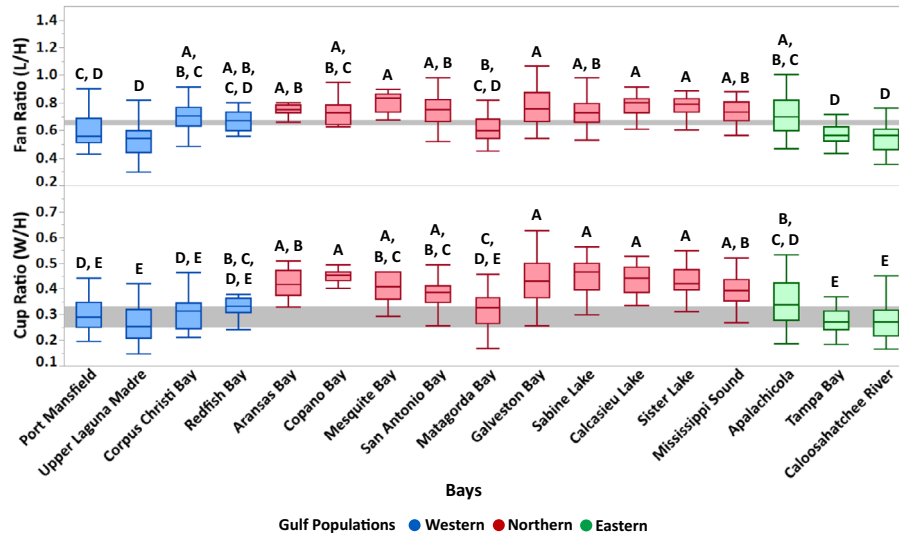


FIGURE 5. Eastern oyster fan and cup ratios for all sampled bays, embedded within Gulf of Mexico populations (blue = western, red = northern, green = eastern). Common letters above the boxes represent statistical similarity as defined by Tukey's post hoc test among bays. For the box plots, the horizontal line in each box indicates the median, the box dimensions show the interquartile range, and the whiskers are 1.5 times the interquartile range. For reference, the shaded gray band represents the minimum range for an acceptable shell ratio in commercial oyster aquaculture operations (fan = 0.63–0.67 and cup = 0.25–0.33; Blake et al. 2003).

Tampa Bay, Caloosahatchee River) are morphologically divergent from samples further west (i.e., Mississippi Sound), roughly coinciding with the genetic data. Apalachicola appears to harbor oysters with intermediate morphology with fan and cup ratios that more closely resemble northern sample sites. This is consistent with studies indicating that the northeastern Gulf of Mexico is a transition zone between taxonomically different races and species groups (Dahlberg 1970; McClure and McEachran 1992; Portnoy and Gold 2012) and indicates that Apalachicola may indeed represent a taxonomic boundary between divergent oyster populations, harboring both general types of shell morphology (northern and eastern).

In Texas, previous work has elicited significant genetic structure centered around Aransas Bay and a well-defined transition zone between divergent “northern” and “western” Texas populations based on studies with a gulfwide focus (Hoover and Gaffney 2005; Varney et al. 2009) as well as a Texas-specific focus (King et al. 1994; Anderson et al. 2014). Genetic divergence between eastern oysters from the Lower Laguna Madre (Texas) and elsewhere in Texas was first observed by Groue and Lester (1982), who also reported that Laguna Madre oysters were significantly smaller and lighter than oysters from other Gulf of Mexico locations. The current data support this finding and the similarity in pattern with that previous study indicates that variation in shape is stable across generations. Strong genetic subdivision occurring between northern and southern oyster populations in Texas is thought to be

due to historical geographic subdivision, with differing spawning seasons potentially acting as a premating isolation mechanism (Anderson et al. 2014). While this might stimulate hypotheses surrounding genetic drivers of shell shape, it is equally likely that the extreme environments that caused geographical subdivision and genetic drift between oyster populations may have also influenced shell morphologies by divergent environmental interactions. That is, shell shape could be a phenotypically plastic response to different environmental inputs over time, although it is known to have an underlying genetic component (Ward et al. 2005) and can potentially be manipulated via artificial or natural selection (Toro and Newkirk 1991). Either way, the magnitude of interindividual variability in oyster shape, even on single reefs, indicates enough morphological variation is present that natural selection need not be invoked as the primary driver.

A final point should be made with regard to the potential for variation in eastern oyster shape to be driven by individual response to harvest pressure. Mizuta and Wikfors (2019) suggested that morphological differences are most likely to arise from genetic, environmental, and husbandry-related drivers. When undersized oysters are harvested, they are culled and returned to the reef as single oysters. These individuals may be affected by water motion from waves and currents, tumbling, and other dynamic processes. Oysters subjected to tumbling in aquaculture operations produce thicker, more cupped shells due to repeated breakage and repair of shell extremities

(Robert et al. 1993; Mizuta and Wikfors 2019). It is not well understood whether these processes similarly impact oyster shape on natural reefs; if so, persistent harvest might directly impact shape at the individual level and also may drive adaptive traits related to shell morphology at the population level. In this study, thicker, rounder shells were characteristic of subtidal (harvested) versus intertidal (unharvested) oysters, and thus the impacts of depth and wave energy (environmental effects) versus harvest pressure (husbandry effects) may be impossible to disentangle. However, it is noteworthy that oysters from southern Texas and Florida clustered together more closely than either of them did with the adjacent northern population. Oyster harvest is generally centered in the northern Gulf of Mexico; neither the eastern nor the western regional oyster populations are heavily fished (GSMFC 2012). The clustering of these groups in multivariate ordination of shape suggests that frequency and intensity of harvest could indeed impact shell shape.

Eastern oyster shell shape is likely to be significantly influenced by a number of factors, including those discussed here (i.e., genetics, environment, harvest). We have demonstrated that there are regional groupings of similar oyster shell shape based on simple morphometrics. While these data cannot be used to directly test hypothesis surrounding the mechanics of shell shape variation, we believe these data can be used as a baseline to formulate such hypotheses. Additionally, the current data set can be used to make a number of general inferences about oyster shell shape in the Gulf of Mexico. First, there were differences among oysters sampled in subtidal versus intertidal areas, suggesting that environmental factors play a predictable role in shell shape. Second, regional groupings (eastern, northern, and western gulf) based on previous population genetic studies appear to harbor oysters that have different multivariate shell shapes, although these differences cannot be reliably attributed to underlying genetic drivers versus regional differences in environmental inputs. Third, oysters from areas that receive heavy harvest pressure (northern gulf) harbored oysters that were more similar to one another in shell shape than they were to oysters from areas of low harvest pressure. The impacts of population structure, genetics, environment, and harvest on shell shape all seem to be intertwined, and as such future studies should focus on a design that is meant to disentangle these various drivers of morphology in eastern oysters.

Taken on its face, the fact that there are broad-scale, regional differences in eastern oyster shape in the Gulf of Mexico presents limited direct opportunity or insight for management. While shape may be an important aesthetic quality for the commercial industry, from the standpoint of maintaining healthy eastern oyster populations it may be of only academic interest. However, the fact that observed variation in shape is coupled strongly with known genetic

population boundaries suggests the potential for local adaptation on the population scale. This potential indirectly invokes important implications for long-term management. For instance, if differences in multivariate shape can be significantly tied to genomic variation, it would not be unreasonable to assume that there might also be regional adaptive phenotypes tied to growth, physiology, reproduction, disease resistance, or other important biological processes. Therefore, the correlation between genetic boundaries and multivariate shape in eastern oysters speaks to the necessity of managing regional populations in such a way as to maintain genetic variability of this species in the Gulf of Mexico. Conservation of unique regional populations in the gulf and elsewhere should be a priority for management of the eastern oyster.

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REFERENCES

- Anderson, J. D., W. J. Karel, C. E. Mace, B. L. Bartram, and M. P. Hare. 2014. Spatial genetic features of eastern oysters (*Crassostrea virginica* Gmelin) in the Gulf of Mexico: northward movement of a secondary contact zone. *Ecology and Evolution* 4:1671–1685.

- Bellaaj-Zouari, A., S. Dkhili, R. Gharsalli, A. Derbali, and N. Aloui-Bejaoui. 2012. Shell morphology and relative growth variability of the invasive pearl oyster *Pinctada radiata* in coastal Tunisia. *Journal of the Marine Biological Association of the United Kingdom* 92:553–563.
- Beseres Pollack, J., D. Yoskowitz, H.-C. Kim, and P. A. Montagna. 2013. Role and value of nitrogen regulation provided by oysters (*Crassostrea virginica*) in the Mission–Aransas estuary, Texas, USA. *PLoS ONE* [online serial] 8(6):e65314.
- Brake, J., F. Evans, and C. Langdon. 2003. Is the beauty in the eye of the beholder? Development of a simple method to describe shell shape for the Pacific oyster industry. *Journal of Shellfish Research* 22:767–771.
- Bricker, S. B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae* 8:21–32.
- Buroker, N. E. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. *Marine Biology* 75:99–112.
- Dahlberg, M. D. 1970. Atlantic and Gulf of Mexico menhadens, genus *Brevoortia* (Pisces:Clupeidae). *Bulletin of the Florida State Museum* 15:91–162.
- Fodrie, F. J., A. B. Rodriguez, R. K. Gittman, J. H. Grabowski, N. L. Lindquist, C. H. Peterson, M. F. Piehler, and J. T. Ridge. 2017. Oyster reefs as carbon sources and sinks. *Proceedings of the Royal Society B: Biological Sciences* 284(1859):20170891
- Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. U.S. Fish and Wildlife Service Fishery Bulletin 64.
- Groue, K. J., and L. T. Lester. 1982. A morphological and genetic analysis of geographical variation among oysters in the Gulf of Mexico. *Veliger* 24:331–335.
- GSMFC (Gulf States Marine Fisheries Commission). 2012. The oyster fishery of the Gulf of Mexico United States: a regional management plan. GSMFC, Fisheries Management Plan, Oyster Technical Task Force, Ocean Springs, Mississippi.
- Hare, M. P., and J. C. Avise. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution* 50:2305–2315.
- Hoover, C. A., and P. M. Gaffney. 2005. Geographic variation in nuclear genes of the eastern oyster, *Crassostrea virginica* Gmelin. *Journal of Shellfish Research* 24:103–112.
- Humphries, A. T., M. K. La Peyre, and G. A. Decossas. 2011. The effect of structural complexity, prey density, and “predator-free space” on prey survivorship at created oyster reef mesocosms. *PLoS ONE* [online serial] 6(12):e28339.
- Karl, S., and J. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* 256:100–102.
- King, T. L., R. Ward, and E. G. Zimmerman. 1994. Population structure of eastern oysters (*Crassostrea virginica*) inhabiting the Laguna Madre, Texas, and adjacent bay systems. *Canadian Journal of Fisheries and Aquatic Sciences* 51:215–222.
- Manzi, J. J., V. G. Burrell, and W. Z. Carson. 1977. A comparison of growth and survival of subtidal *Crassostrea virginica* (Gmelin) in South Carolina salt marsh impoundments. *Aquaculture* 12:293–310.
- Marshall, R. D., and A. Dunham. 2013. Effects of culture media and stocking density on biofouling, shell shape, growth, and survival of the Pacific oyster (*Crassostrea gigas*) and the Manila clam (*Venerupis philippinarum*) in the suspended culture. *Aquaculture* 406–407:68–78.
- McClure, M. R., and J. D. McEachran. 1992. Hybridization between *Prionotus alatus* and *P. paralatus* in the northern Gulf of Mexico (Pisces: Triglidae). *Copeia* 1992:1039–1046.
- Mehrubeoglu, M., D. K. Smith, D. W. Smith, and D. L. Smee. 2013. Investigating oyster shell thickness using three imaging modalities: hyperspectral imaging, thermal imaging and digital photography. *Proceedings of SPIE (Imaging Spectrometry XVIII)* 8870:887000.
- Mizuta, D. D., and G. H. Wikfors. 2019. Seeking the perfect oyster shell: a brief review of current knowledge. *Reviews in Aquaculture* 11:586–602.
- Newell, R. I. E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research* 23:51–61.
- NOAA Fisheries (National Oceanic and Atmospheric Administration Fisheries). 2018. Office of Science and Technology, annual commercial landing statistics. Available: http://www.st.nmfs.noaa.gov/stl/commercial/landings/annual_landings.html. (March 2020).
- Orton, J. H. 1936. Habitat and shell shape in the Portuguese oyster, *Ostrea angulata*. *Nature* 138:466–467.
- Portnoy, D. S., and J. R. Gold. 2012. Evidence of multiple vicariance in a marine suture-zone in the Gulf of Mexico. *Journal of Biogeography* 39:1499–1507.
- Puckett, B. J., and D. B. Eggleston. 2012. Oyster demographics in a network of no-take reserves: recruitment, growth, survival, and density dependence. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* [online serial] 4:605–627.
- Reeb, C. A., and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics* 124:397–406.
- Robert, R., G. Trut, M. Borel, and D. Maurer. 1993. Growth, fatness and gross biochemical composition of the Japanese oyster, *Crassostrea gigas*, in Stanway cylinders in the Bay of Arcachon, France. *Aquaculture* 110:249–261.
- Robinson, E. M., J. Lunt, C. D. Marshall, and D. L. Smee. 2014. Eastern oysters *Crassostrea virginica* deter crab predators by altering their morphology in response to crab cues. *Aquatic Biology* 20:111–118.
- Scyphers, S. B., S. P. Powers, K. L. Heck Jr., and D. Byron. 2011. Oyster reefs as natural breakwaters mitigate shoreline loss and facilitate fisheries. *PLoS ONE* [online serial] 6(8):e22396.
- Shepard, C., J. Brenner, K. L. Goodin, K. W. Ames. 2018. Ecological resilience indicators for oyster ecosystems. Pages 209–242 in K. L. Goodin, editor. *Ecological resilience indicators for five northern Gulf of Mexico ecosystems*. NatureServe, Arlington, Virginia.
- Stanley, J. C., and M. A. Sellers. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico)—American oyster. U.S. Fish and Wildlife Service, Biological Report 82(11.64) and U.S. Army Corps of Engineers, TR EL-82-4, Vicksburg, Mississippi.
- Stunz, G. W., T. J. Minello, and L. P. Rozas. 2010. Relative value of oyster reef as habitat for estuarine nekton in Galveston Bay, Texas. *Marine Ecology Progress Series* 406:147–159.
- Thongda, W., H. Zhao, D. Zhang, L. N. Jescovitch, M. Liu, X. Guo, M. Schrandt, S. P. Powers, and E. Peatman. 2018. Development of SNP panels as a new tool to assess the genetic diversity, population structure, and parentage analysis of the eastern oyster (*Crassostrea virginica*). *Marine Biotechnology* 20:385–395.
- Toro, J. E., and G. F. Newkirk. 1991. Response to artificial selection and realized heritability estimate for shell height in the Chilean oyster *Ostrea chilensis*. *Aquatic Living Resources* 4:101–108.
- USEPA (U.S. Environmental Protection Agency). 1999. Ecological condition of estuaries in the Gulf of Mexico. USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, EPA 620-R-98004, Gulf Breeze, Florida.
- Varney, R. L., C. E. Galindo-Sánchez, P. Cruz, and P. M. Gaffney. 2009. Population genetics of the eastern oyster *Crassostrea virginica* (Gmelin, 1791) in the Gulf of Mexico. *Journal of Shellfish Research* 28:855–864.
- Ward, R. D., P. A. Thompson, S. A. Appleyard, A. A. Swan, and P. D. Kube. 2005. Sustainable genetic improvement of Pacific oysters in Tasmania and South Australia. Fisheries Research and Development, Project 2000/206, Final Report to Commonwealth Scientific and

Industrial Research Organisation, Marine and Atmospheric Research, Hobart, Australia.

zu Ermgassen, P. S. E., M. D. Spalding, R. E. Grizzle, and R. D. Brumbaugh. 2013. Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. *Estuaries and Coasts* 36:36–43.

SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.