Genetic diversity and population structure of oysters in Apalachicola Bay, Florida

Matthew F. Lawrance*[1], Kathryn P. Mercier*[1], Joshua A. Solomon[1], Linda J. Walters[1], and Christopher L. Parkinson[1]

[1]Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816

Introduction

The eastern oyster *Crassostrea virginica* is a keystone species because of the ecosystem services it provides (Pollack et al. 2013, Kellogg et al., 2013, Manis et al. 2014, Barber et al. 2010). Additionally, *C. virginica* is economically important, serving as a food resource for both humans and numerous commercially important shellfish species (e.g. stone crabs, blue crabs) (Boudreaux et al. 2006). This sessile bivalve mollusk has a broad geographic range that extends from the Gulf of St. Lawrence in Canada to the southern Gulf of Mexico (Buroker 1983).

Crassostrea virginica is a facultative hermaphrodite that reproduces via broadcast spawning, and larvae must survive for two or more weeks navigating prevailing currents before recruiting to available hard substrates (Dame 1972, North et al. 2008). Despite this, *C. virginica* populations can become genetically distinct. For example, in Texas genetic structuring occurs where genetically distinct populations overlap in Corpus Christi/Aransas Bay estuarine complex, despite shared tidal currents (Anderson et al. 2014). On smaller spatial scales, patterns of isolation by distance have been found within the Chesapeake Bay (Rose et al. 2006).

The Apalachicola National Estuarine Research Reserve (ANERR) covers 998.6 square km and is located in Liberty, Gulf, and Franklin Counties in the panhandle region of Florida (Edminston 2008). Historically, oyster harvests in Apalachicola Bay approached 1200 bushels per acre and accounted for 10% of the total United States harvest (Livingston 1984, Edminston 2008, Zu Ermgassen et al. 2012). However, this once-abundant oyster population has recently undergone a drastic collapse, motivating investigations into the underlying causes (Petes et al. 2012, Camp et al. 2015).

Rapid constriction of population size has serious consequences for population health and survival: with a reduction in population size, decreases in genetic diversity may also occur (Bouzat et al. 1998, Frankham et al. 2014). Loss of genetic diversity may impair a population's ability to adapt to a changing environment

---

*These authors contributed equally, and order was determined by coin-flip.
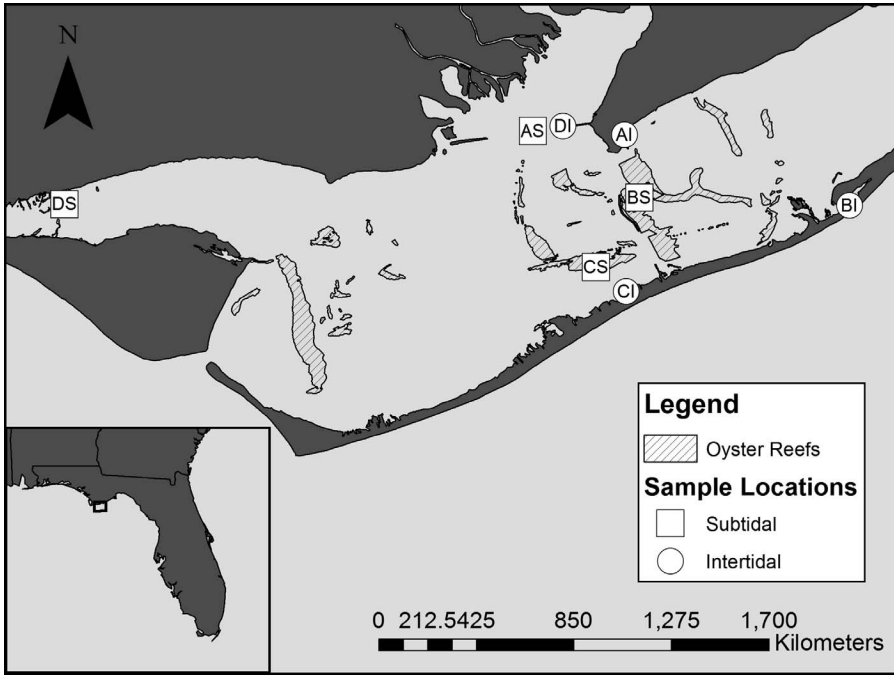Corresponding author: Christopher Parkinson, Parkinson@ucf.edu

Figure 1. Sampling locations in Apalachicola Bay, Florida.

(Frankham et al. 2002, Pauls et al. 2013), and lead to reduced survival, reproduction, and local extinction (Bouzat et al. 1998, Hostetler et al. 2010). Because knowledge of genetic diversity of oysters in Apalachicola Bay is limited either before or after the collapse, it is unknown whether the population harbors low genetic diversity and may therefore be at risk. Our objective was to assess levels of genetic diversity within this *C. virginica* population, in order to determine what, if any, genetic consequences have resulted from this bottleneck.

Materials and Methods

Four subtidal and four intertidal reefs in ANERR were sampled between 2 and 5 April 2014 (Figure 1). At each site, 40 individuals were collected for genotyping. All samples were no less than 44.5 mm in shell length and therefore sexually mature according to specifications set forth by Hayes and Menzel (1981). Approximately 500 mg of adductor tissue was removed and stored in 100% ethanol. Extraction was performed with Serapure beads following Faircloth and Glenn (2014). Sixteen samples representing two individuals from each of the eight sample sites were subjected to polymerase chain reaction (PCR) for a candidate set of 29 microsatellite markers (Wang and Guo 2007). A subset of 11 microsatellite loci were selected based on their successful amplification and variation in size between loci in order to facilitate PCR multiplexing (Supplemental Table 1).

Reaction mixtures were comprised of: 1x PCR Buffer, 1.5mM $MgCl_2$, 0.2mM of each dNTP, 1% final DMSO, 0.1μM M13 dye, 0.5μM of forward primer, 1μM of reverse primer, and 0.75 units of Taq Polymerase. The PCR protocol was comprised of an initial denaturing step at 95°C for 2 minutes, followed by 30 cycles of 95°C for 30 seconds, an annealing step for 1 minute at locus specific

Table 1. Number of individuals from each site included in the study (N). Pairwise F_{ST} values below the diagonal and associated p-values above the diagonal.

	N	AI	BI	CI	DI	AS	BS	CS	DS
AI	40	0.000	0.729	0.050	0.213	0.361	0.898	0.838	0.412
BI	40	0.007	0.000	0.987	0.508	0.425	0.700	0.952	0.490
CI	40	0.011	0.005	0.000	0.429	0.117	0.138	0.798	0.120
DI	39	0.009	0.008	0.009	0.000	0.156	0.452	0.342	0.161
AS	36	0.008	0.009	0.011	0.010	0.000	0.336	0.863	0.269
BS	38	0.006	0.007	0.010	0.008	0.009	0.000	0.514	0.667
CS	30	0.007	0.006	0.007	0.009	0.007	0.009	0.000	0.171
DS	40	0.007	0.007	0.009	0.009	0.009	0.007	0.010	0.000

temperatures (Supplemental Table 1), and an extension step at 72°C for 2 minutes, followed by a final 10 minute extension step. PCR products were visualized on agarose gel.

Genotyping of all individuals was performed at Arizona University Genetics Core, Tucson, AZ then microsatellites were sized and categorized using the program GeneMarker v2.6.3 (SoftGenetics, State College, PA). We first performed a genotyping error analysis, which is an important consideration given that incorrect genotyping may significantly bias results (Guichoux et al. 2011). We compared 200 allele calls of repeated genotyping for a set of 24 individuals. Additionally MicroChecker (van Oosterhout et al. 2004) was used to check for scoring error due to stuttering, large allelic dropout, and null alleles.

The program GenAlEx v6.5 (Peakall and Smouse 2012) was used to assess Hardy-Weinberg equilibrium then to estimate pairwise F_{ST} between sample sites. STRUCTURE v2.3.4 (Pritchard et al. 2000) was utilized to assign individuals to populations. We tested for k one through nine across ten iterations, with 1,000,000 MCMC samples with the first 10,000 discarded as burn in. STRUCTURE analysis was performed under the admixture model with correlated allelic frequencies. An analysis of molecular variation (AMOVA) was performed in GenAlEx utilizing genetic distance as measured by F_{ST} of our sample groups (999 permutations).

We then tested for patterns of isolation by distance between sampling sites in GenAlEx using pairwise F_{ST} values. Additionally, we calculated the number of alleles (N_a), effective number of alleles (N_e), observed (H_o) and expected heterozygosity (H_e) for each locus in GenAlEx. We calculated allelic richness for each locus in FSTAT v2.9.3.2 (Goudet 2002). We used sign, Wilcoxon, and mode-shift tests implemented in the program BOTTLENECK v1.2.03 with an infinite allele model to determine if there was evidence for a historical bottleneck (Piry et al. 1999).

Results

Extraction failed for 17 of the 320 sampled individuals, leading to a variable number of individuals at each site (Table 1). Repeated genotyping across 100 comparisons showed few instances where genotype calls were different after repeated genotyping. Zero errors were found in nine of the eleven loci; two loci, RUCV61 and RUCV06, showed unacceptable error rates and were removed from the study. RUCV01 showed evidence for null alleles and was also removed from further analyses.

All of the eight remaining loci were in Hardy-Weinberg equilibrium. Pairwise F_{ST} values were not significantly different from zero at the p=0.05 level, indicating no genetic differentiation between sites (Table 1) (Wright 1965). Bayesian analysis found no evidence of distinct populations within the samples. Although the number of populations, K=2, had the highest log probability of the data, it was not

Table 2. AMOVA partitioning of variation within our samples after 999 permutations.

Source	DF	SS	MS	EST. VAR.	%
Among Regions	1	15.733	15.733	0.029	1%
Among Sites	6	41.468	6.911	0.042	1%
Among Individuals	295	1102.011	3.736	0.494	15%
Within Individuals	303	832.500	2.748	2.748	83%
Total	605	1991.713		3.313	100%

significantly more supported than K=1 (Supplemental Table 2). Further supporting the conclusion of population admixture across the study range, the proportion of each site assigned to presumed clusters was approximately 1/K regardless of the K value. Increasing K resulted in greater and greater admixture of clusters across our sites. Genetic variation was not partitioned between subtidal and intertidal sites (AMOVA; Table 2). The variation in our data was almost wholly (83%) contained within individuals rather than sites. Therefore, we assumed a single population for the population frequency analyses and bottleneck analyses. Additionally, the standard metric for H_e was used instead of an unbiased metric recommended for small sample sizes, because the population size was sufficiently large.

We found no pattern of isolation by distance among sample sites (p=0.26). Six of the eight microsatellites loci were highly variable, with >11 recovered alleles as well as high observed and expected heterozygosity across all loci, averaging 0.797 and 0.817 respectively (Supplemental Table 3). This indicates high levels of intra-individual genetic diversity. We found evidence of a historical bottleneck in Apalachicola Bay oysters in two out of three analyses. Ten loci demonstrated heterozygosity excess versus the expected six loci (Sign test, p = 0.01655). The Wilcoxon test showed similar results for heterozygote excess (p=0.00195, one-tailed test). The mode shift test showed a normal-L shaped distribution, which does not support the hypothesis of a bottleneck (Cornuet and Luikart, 1997).

Discussion

The goal of this study was to characterize the genetic diversity of *C. virginica* in Apalachicola Bay, FL. The pairwise F_{ST} values, model-based clustering, and AMOVA, indicate that *C. virginica* in Apalachicola Bay are one panmictic population. Oysters are broadcast spawners, and their larvae move with currents for two weeks or longer weeks before settling (Buroker 1983). There life-history traits were sufficient to produce a pattern of panmixia in Apalachicola Bay.

Populations bottlenecked by rapid decreases in population tend to demonstrate reduced evolutionary potential due to a loss of rare alleles and overall population homogenization (Nei et al. 1975). Consequently, population adaptability to changing environmental pressures is reduced, inbreeding depression may occur, and the population may enter the 'extinction vortex' (Corti et al., 2011). Variability at microsatellite loci have been shown to be generally reflective of overall variation across the genome, and thereby are representative of genetic health (Markett et al. 2011). *C. virginica* in this study demonstrated an excess of homozygotes at a

greater than expected number of microsatellite loci. While these genetic data are in agreement with previous observational reports of population collapse, oysters in Apalachicola Bay retain relatively high heterozygosity overall. Given the current genetic diversity of this population, prognosis for population recovery is good.

References

Anderson JD, Karel WJ, Mace CE, Bartram BL, Hare MP. 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(9):1671–1685.

Barber A, Walters L, Birch A. 2010. Potential for restoring biodiversity of macroflora and macrofauna on oyster reefs in Mosquito Lagoon, Florida. Biological Sciences 73:47–63.

Boudreaux ML, Stiner JL, Walters LJ. 2006. Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. Journal of Shellfish Restoration 25(3):1079-1089.

Bouzat JL, Paige KN, Lewin HA. 1998. The Ghost of Genetic Diversity Past: Historical DNA Analysis of the Greater Prairie Chicken. The American Naturalist 152(1):1-6

Buroker NE. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. Marine Biology 75:99–112.

Camp EV, Pine WE, Havens K, Kane AS, Walters CJ, Irani T, Lindsey AB, Morris JG. 2015. Collapse of a historic oyster fishery: diagnosing causes and identifying paths toward increased resilience. Ecology and Society 20(3):45.

Corti P, Shafer AB, Coltman DW, Festa-Bianchet M. 2011. Past bottlenecks and current population fragmentation of endangered huemul deer (*Hippocamelus bisulcus*): implications for preservation of genetic diversity. Conservation Genetics 12(1):119-128.

Cournuet JM, Luikart G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144(4):2001-14.

Dame RF. 1972. The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Marine Biology 17:243–250.

Edminston HL. 2008. "A river meets the bay: The Apalachicola Estuarine System." Apalachicola National Estuarine Research Reserve, Florida Department of Environmental Protection: Apalachicola, FL, USA.

Faircloth BC, Glenn TC. 2014. Protocol: Preparation of an AMPure XP substitute (AKA Serapure). Web document doi: 10.6079/J9MW2F26.

Frankham R, Bradshaw CJ, Brook BW. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170: 56–63.

Frankham R, Briscoe DA, Ballou JD. 2002. Introduction to conservation genetics. Cambridge University Press.

Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices.

Guichoux E, Lagache L, Wagner S, Chaumeil P, Leger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ. 2011. Current trends in microsatellite genotyping. Molecular Ecology Resources 11:591–611.

Hayes PF, Menzel RW. 1981. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico, and its implications for population recruitment. Biological Bulletin 160(1):80-88.

Hostetler JA, Onorato DP, Nichols JD, Johnson WE, Roelke ME, O'Brien SJ, Jansen D, Oli MK. 2010. Genetic introgression and the survival of Florida panther kittens. Biological conservation 143(11):2789-96.

Kellogg ML, Cornwell JC, Owens MS, Paynter KT. 2013. Denitrification and nutrient assimilation on a restored oyster reef. Marine Ecology Progress Series 480:1–19.

Livingston RJ. 1984. The relationship of physical factors and biological response in coastal seagrass meadows. Estuaries 7(4):377-390.

Manis JE, Garvis SK, Jachec SM, Walters LJ. 2014. Wave attenuation experiments over living shorelines over time: a wave tank study to assess recreational boating pressures. Journal of Coastal Conservation 19:1-11.

Markett S, Montag C, Walter NT, Plieger T, Reuter M. 2011. On the molecular genetics of flexibility: the case of task-switching, inhibitory control and genetic variants. Cognitive, Affective, & Behavioral Neuroscience 11(4):644-651.

Nei M, Maruyama T, Chakraborty R. 1975. The bottleneck effect and genetic variability in populations. Evolution 1:1-10.

North EW, Schlag Z, Hood RR, Li M, Zhong L, Gross T, Kennedy VS. 2008. Vertical swimming behavior influences the dispersal of simulated oyster larvae in a coupled particle-tracking and hydrodynamic model of Chesapeake Bay. Marine Ecology Progress Series 359:99-115.

Pauls SU, Nowak C, Bálint M, Pfenninger M. 2013. The impact of global climate change on genetic diversity within populations and species. Molecular Ecology 22(4):925-946.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Petes LE, Brown AJ, Knight CR. 2012. Impacts of upstream drought and water withdrawals on the health and survival of downstream estuarine oyster populations. Ecology and Evolution 2(7):1712-1724.

Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. Journal of Heredity 90:502-503.

Pollack JB, Yoskowitz D, Kim H, Montagna PA. 2013. Role and value of nitrogen regulation provided by oysters (*Crassostrea virginica*) in the Mission-Aransas Estuary, Texas, USA. PLoS ONE 8(6).

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945-959.

Rose CG, Paynter KT, Hare MP. 2006. Isolation by distance in the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay. Journal of Heredity 97(2): 158–170.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

Wang Y, Guo X. 2007. Development and characterization of EST-SSR markers in the eastern oyster *Crassostrea virginica*. Marine Biotechnology 9:500-51.

Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19(3):395-420.

Zu Ermgassen PSE, Spalding MD, Blake B, Coen LD, Dumbauld B, Geiger S, Grabowski JH, Grizzle R, Luckenbach M, McGraw K, Rodney W, Ruesink JL, Powers SP, Brumbaugh R. 2012. Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. Proceedings of the Royal Society B 279:3393-3400.