Adult Red Drum Genetic Diversity and Population Structure

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Introduction

Red drum (*Sciaenops ocellatus*) is an estuarine dependent finfish found from Northern Mexico to Southern Florida in Gulf of Mexico and from Southern Florida to the Chesapeake Bay in the Atlantic Ocean (Hildebrand and Schroeder 1928; Bigelow and Schroeder 1953; Simmons and Breuer 1962). Red drum supports a large recreational fishery, but significant range-wide declines in abundance, due to commercial exploitation in the 1980's, generated serious concern regarding the sustainability of the spawning stock (Goodyear 1989). As such, red drum is a priority for research and conservation, and substantial efforts have been directed toward assessing this species. However, most red drum research has centered on the estuarine-dependent subadult portion of the population, which is spatially segregated from the oceanic reproductive portion of the population (subadults remain in estuaries until they reach sexual maturity at approximately 3-5 years of age, after which they recruit to the offshore adult population; Wenner 2000). Focus on the subadults has led to a data deficiency regarding adult red drum, particularly in the Atlantic, and there is only limited genetic information available for adults in this region. The objective of this study was to address this deficiency through the evaluation of genetic diversity, structure, and movement of adult red drum along the southeastern coast of the U.S.

Sampling

For this study, we analyzed a total of 2893 samples collected from 2003-2009 from four states: Florida (FL), Georgia (GA), South Carolina (SC), and North Carolina (NC). Samples from FL, GA, and NC were collected in relatively the same locations, while SC samples were collected from three separate estuaries (Table 1, Figure 1). Thus, for the evaluation of genetic diversity and population structure, samples were divided into 6 sites: Florida (FL), Georgia (GA), North Carolina (NC), and 3 sites within South Carolina (Port Royal Sound (PRS), Charleston Harbor (CH), Winyah Bay (WB)). All samples from GA, SC, and NC were from adult red drum; FL samples included both adult and some subadult red drum, due to the unique life history of red drum in this location (juveniles and adults are often found together inshore) as well its lower sample size. Samples from FL were provided by the FL Fish and Wildlife Conservation Commission, samples from GA were provided by the GA Department of Natural Resources, samples from SC were provided by cooperating recreational anglers and the SC Department of Natural Resources, and samples from NC were provided by the NC Division of Marine Fisheries.

Table 1. Number of red drum samples collected from each site in 2003-2009.

	2003	2004	2005	2006	2007	2008	2009	Total
Indian River Lagoon, FL					17	48	39	104
Offshore GA					65			65
Port Royal Sound, SC	44		197	52	131	134	102	660
Charleston Harbor, SC	333	227	197	101	24	33	120	1035
Winyah Bay, SC	66			180	92	78	49	465
Pamlico Sound, NC					87	156	321	564
Total	443	227	394	333	416	449	631	2893

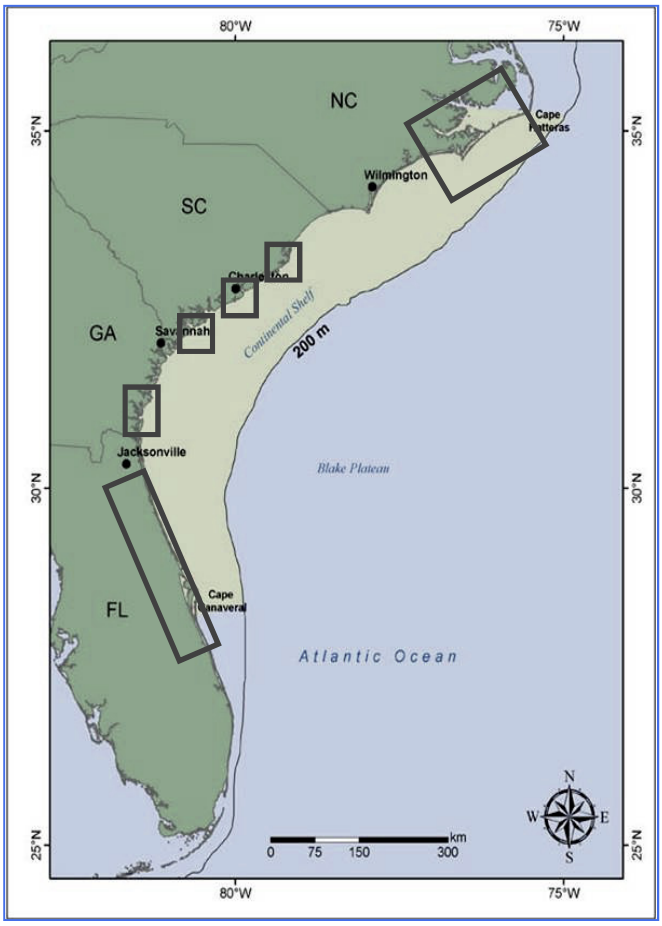

Figure 1. Sampling locations of red drum collected from 2003-2009. In order from north to south: Pamlico Sound (NC), Winyah Bay (SC), Charleston Harbor (SC), Port Royal Sound (SC), offshore GA, Indian River Lagoon (FL).

Laboratory protocols

All fin-clip samples were stored in a sarcosyl-urea solution (1% sarcosyl, 8M urea, 20mM sodium phosphate, 1mM EDTA), which serves to both lyse the cells and preserve the DNA. Genomic DNA was extracted from the fin clip samples using a metal beads isolation procedure (SERADYN; Indianapolis, IN). Red drum samples were genotyped across a suite of 8 microsatellite primers (Table 2) combined into three multiplexed panels containing Milli-Q H_2O (Millipore; Billerica, Massachusetts), 1X HotMaster PCR Buffer (5 Prime Inc.; Gaithersburg, MD), 1.0mM $MgCl_2$, 0.8mM dNTPs (0.2mM each), 0.3µm each of forward and reverse primers (for individual primer concentrations see Table 2), 0.03U/µl of HotMaster *Taq* (5 Prime Inc.), and 1µl of DNA (10-50ng/µl) for a total reaction volume of 11µl. Forward primers were labeled with a fluorescent Well-RED dye (SIGMA-Proligo; The Woodlands, TX). PCR amplifications were preformed on a BIORAD iCycler (Bio-Rad Laboratories; Hercules, CA) and commenced with an initial denaturation step of 2:00 mins at 94ºC, followed by an 18 cycle touchdown. The initial cycle of the touchdown was 94ºC for 30 secs, 55ºC for 30 secs, and 65ºC for 40 secs. The annealing temperature was than lowered by 1.5ºC every two cycles for a reduction from 55ºC to 43ºC over the 18 cycles. The touchdown was followed by 17 cycles of 94ºC for 30 secs, 43ºC for 30 secs, and 65ºC for 40 secs; and a final extension at 65ºC for 1 hr. Amplified fragments (1µl) were mixed with 40µl of a 1% solution of 400 base pair size standard in formamide, and were separated by capillary electrophoresis on a Beckman Coulter CEQ™ 8000 automated sequencer (Beckman Coulter Inc.; Brea, CA, USA). Resulting fragments were scored by two independent readers using Beckman Coulter Fragment Analysis Software (Beckman Coulter).

Table 2. Eight microsatellite loci used to genotype red drum from the southeastern coast of the U.S. Panel number, fluorescent label (dye), final PCR concentration (µM), repeat motif, and original reference are given for each locus.

Panel	Locus	Dye	Concentration(µM)	Repeat motif	Reference
1	Soc029	D3	0.081	$(GT)_{10}$	Chapman et al. 2002
1	Soc060	D2	0.161	$(AGG)_8$	Turner et al. 1998
1	Soc129	D4	0.058	$(TATC)_{11}$	Turner et al. 1998
2	Soc014	D3	0.225	$(GT)_{21}$	Chapman et al. 2002
2	Soc017	D4	0.075	$(GT)_{14}$	Chapman et al. 2002
3	Soc243	D2	0.124	$(CCT)_9$	Turner et al. 1998
3	Soc083	D3	0.124	$(TG)_{19}$	Turner et al. 1998
3	Cne612	D4	0.052	$(GT)_5n_{10}(GT)_{11}$	Chapman et al. 1999

Note: D2 = black, D3 = green, D4 = blue

Statistical Analysis

Marker validation

Once samples were genotyped, tests for Hardy-Weinberg Equilibrium (HWE), linkage disequilibrium, and null alleles were performed for all loci from each site, both within collection years and with all collection years combined. Examinations for HWE were conducted using

exact tests performed with Markov Chain randomization in the program Arlequin 3.11 (Excoffier et al. 2005). Chains had 100,000 steps with a 1,000 step burn-in. Tests for linkage equilibrium between all microsatellite pairs were executed in Arlequin using 10,000 permutations. Tests for the presence of null alleles were performed with the program Microchecker 2.2.3 (van Oosterhout et al. 2004). Significance levels for all simultaneous analyses were adjusted using a sequential Bonferroni correction (Rice 1989).

After correction for multiple testing, no consistent deviations from Hardy-Weinberg equilibrium or instances of linkage disequilibrium were found for any of the six sites, either within collection years or with all collection years combined. Null alleles were not found at any locus at any of the sites.

Temporal differentiation

To determine the validity of combining samples across collection years, an evaluation of temporal genetic variation was performed. Within each site, collection years were compared to one another to determine whether there was a significant difference in allele frequencies. An exact G-test with Markov Chain permutations, as implemented in GenePop 4.1 (Raymond and Rousset 2005), was used to test for pairwise differences in allelic frequency distributions among collection years. Markov chain parameters included 10,000 dememorizations, 100 batches, and 5000 iterations per batch. Also, pairwise comparisons of F_{ST} between collections years were calculated in Arlequin 3.11 using default parameters.

No significant temporal genetic differentiation was found between any of the collection years at any of the sites, using both the G-tests and F_{ST}. All of the collections years were combined for each of the six sites.

Sample partitioning (by season)

For some marine finfish species, spawning aggregations have been shown to be genetically distinct from "mixed" non-spawning assemblages. In order to determine the effects of season on population structure, samples from all 6 locations were partitioned into "Spawning" and "Non-spawning" groups based on the date of collection (Table 3). Spawning windows were identified using National Estuarine Research Reserve System (NERRS) water temperature data. All relevant NERRS station temperature data was analyzed to identify an accurate spawning time period as July 1st to October 31st for all locations and years, corresponding to a water temperature range of 25.5°C - 30.2°C at which red drum are reported to spawn (Renkas 2010). In addition, eight years of rotenone survey data (1986-1994) supplied by the SCDNR Inshore Fisheries group indicated the first settled fish are seen at the beginning of August extending until mid-October. As settlement occurs within 4-8 mm (Rooker and Holt 1998) or an estimated time of approximately 17 days (Peters and McMichael 1987), these data support the spawning window of July 1st to October 31st (Arnott 2009; Figure 2). In addition Rooker and Holt (1998) identified the overall hatch window from 29 August until 31 October for red drum, verifying the results of the temperature analysis. For each site, all samples which fell within the spawning window were placed in the "spawning" group, while all samples that fell outside this window were placed in the "non-spawning" group.

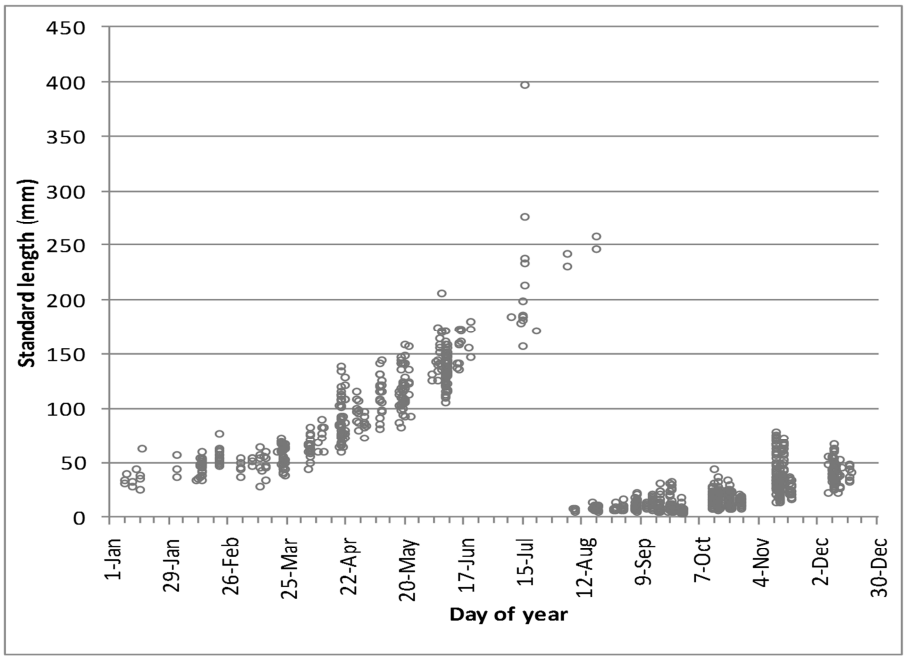

Figure 2. Rotenone survey showing combined red drum settlement data from 1986-1994 with individual fish length (SL) plotted against the calendar date (Arnott 2009).

Table 3. Partitioned sample sizes for red drum in spawning and non-spawning periods.

Sample Location	Non-spawn	Spawn	Total
Pamlico Sound, NC	13	551	564
Winyah Bay, SC	92	373	465
Charleston Harbor, SC	369	666	1035
Port Royal Sound, SC	308	352	660
Georgia	25	40	65
Indian River Lagoon, FL	62	42	104
Total	869	2024	2893

Spatial Differentiation/Genetic structure

During both the spawning and non-spawning seasons, the six sites were compared to one another to test for spatial differentiation (i.e. genetic structure). Examinations for spatial differentiation were conducted among the three South Carolina estuaries (Winyah Bay, Charleston Harbor, and Port Royal Sound), North Carolina (Pamlico Sound), Georgia, and Florida (Indian River Lagoon). The analysis for genetic structure was performed as described above for the temporal examinations (using G-tests and F_{ST}). An Analysis of Molecular Variance (AMOVA; F_{ST}-like) was also conducted in Arlequin with 10,000 permutations to partition the genetic variation among collection locals. Finally, a Bayesian Clustering analysis was performed in the program STRUCTURE 2.3 (Pritchard et al. 2000) to identify the appropriate number of populations (K). Three iterations were run with K set from 1 to 7. A burn-in of 10,000 replicates, followed by

10,000 replicates of the Markov Monte Carlo (MCMC) simulation, was run under the Admixture model with Correlated allele frequencies. Sampling locations were used as priors; all other parameters were set at default values. The program STRUCTURE HARVESTER (Earl and vonHoldt 2012) was utilized to compile and sort the data. The most appropriate value of K was identified using ΔK (i.e. the second-order rate change between successive K values; Evanno et al. 2005).

No significant spatial differentiation (p≥0.003; Bonferroni correction for 15 comparisons) was detected between sites (NC, WB, CHS, PRS, GA, FL) during the non-spawning period (Nov. 1 – June 30; Table 4). During the spawning period (July 1- Oct. 31), no significant spatial differentiation (p≥0.003; Bonferroni correction for 15 comparisons) was detected among the southern sites (WB, CH, PRS, GA, FL); however, low but significant genetic structure was detected between North Carolina and the majority of the southern sites (WB, CH, PRS, GA; Table 5). The among-site component of variation produced by AMOVA during the non-spawning season was very low (0%) and not significant (F_{ST} = -0.0002, df = 5, P = 1.000). The among-site component of variation produced by AMOVA during the spawning season was low (0.26%) but significant (F_{ST} = 0.0013, df = 5, P = 0.024). The program STRUCTURE partitioned the samples into one group for the non-spawning season (Figure 3a) and two groups for the spawning season (Figure 3b), with NC being separate from the southern sites (WB, CH, PRS, GA, FL). Taken together, these results indicate that a genetic break exists between NC and SC during the spawning season. The genetic distinction between NC and FL is not strongly significant, but the weak structure may possibly be due to smaller sample sizes from the FL collections. However, the low F_{ST} (<0.004) values show that the genetic structure is weak and that gene flow is still occurring between regions is still occurring. As significant genetic structure occurs between regions (NC vs. SC/GA/FL) during the spawning season, but not the non-spawning season, all analyses below this point are performed within each region for the spawning groups (NC and SC/GA/FL) and with all regions combined for the non-spawning group (NC, SC, GA, FL).

Table 4. Chi-square values from G-tests (below the diagonal) and pair-wise F_{ST} values (above the diagonal) comparing adult red drum from six sites along the southeastern coast of the U.S. during the non-spawning season (Nov. 1 – June 30; Table 4). Probability values are shown in parentheses.

Site	FL	GA	PRS	CHS	WB	NC
FL	**X**	-0.0034 (0.551)	-0.0093 (0.897)	-0.0030 (0.304)	-0.0058 (0.542)	-0.0027 (0.328)
GA	10.11 (0.860)	**X**	-0.0062 (0.975)	0.0004 (0.240)	-0.0003 (0.389)	-0.0007 (0.193)
PRS	17.42 (0.359)	12.48 (0.710)	**X**	-0.0024 (0.865)	-0.0015 (0.815)	-0.0014 (0.899)
CHS	17.64 (0.345)	9.98 (0.868)	18.88 (0.275)	**X**	0.0015 (0.243)	-0.0000 (0.563)
WB	15.2 (0.510)	8.60 (0.929)	23.84 (0.093)	24.85 (0.072)	**X**	0.0009 (0.242)
NC	12.38 (0.717)	6.75 (0.978)	16.14 (0.443)	14.23 (0.582)	11.67 (0.766)	**X**

Table 5. Chi-square values from G-tests (below the diagonal) and pair-wise F_{ST} values (above the diagonal) comparing adult red drum from six sites along the southeastern coast of the U.S. during the spawning season (July 1- October 3). Probability values are shown in parentheses. Comparisons that are significant before correction are shown in bold (critical $P \leq 0.05$); comparisons that are significant after correction are in red (critical $P \leq 0.003$ following Bonferroni correction).

Site	FL	GA	PRS	CHS	WB	NC
FL	**X**	0.0009 (0.328)	0.0001 (0.791)	0.0008 (0.439)	0.0019 (0.654)	0.0037 (0.061)
GA	14.61 (0.553)	**X**	0.0009 (0.122)	0.0005 (0.343)	0.0008 (0.262)	**0.0039 (0.001)**
PRS	9.19 (0.905)	19.45 (0.246)	**X**	0.0001 (0.102)	-0.0001 (0.103)	**0.0020 (<0.001)**
CHS	14.38 (0.570)	15.93 (0.458)	22.47 (0.129)	**X**	0.0002 (0.126)	**0.0025 (<0.001)**
WB	10.77 (0.823)	17.09 (0.380)	19.80 (0.230)	21.34 (0.166)	**X**	**0.0013 (0.001)**
NC	24.42 (0.081)	**34.93 (0.004)**	**--- (<0.001)**	**--- (<0.001)**	**79.31 (<0.001)**	**X**

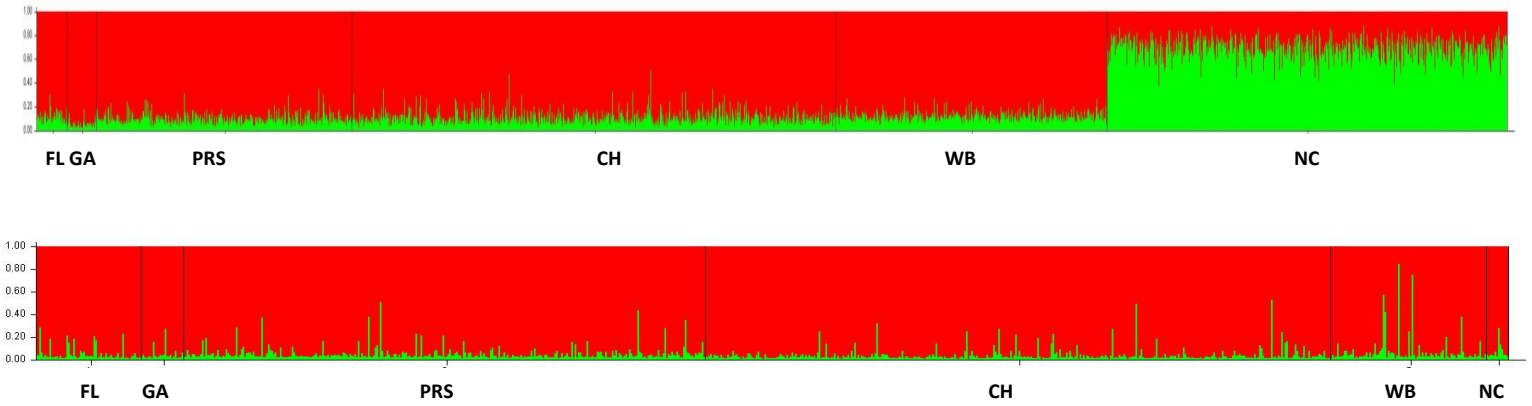

Figure 3. STRUCTURE Bar plots depicting K=2 for the spawning season (top) and non-spawning season (bottom). Individuals are shown in thin vertical lines (colors represent the proportion of estimated ancestry from each of the clusters for each individual). The numbered segments represent sample sites; sites labeled as follows: FL=Florida, GA=Georgia, PRS=Port Royal Sound, CH=Charleston Harbor, WB=Winyah Bay, NC=North Carolina.

Isolation by Distance

To identify if the significant structure between regions (NC vs. SC/GA/FL) during the spawning season was due to a stepping stone mutation, a mantel test using F` and geographic distance was performed in the program Arlequin 3.11. The regression of F` or (Fst/1-Fst) estimates for pairs of subpopulations on geographic distance has been shown to be the appropriate method to test for stepping stone structure (Rousett 1997). Geographic distance was calculated using the average sample location for each of the six sampling locations. The distance was then measured between each of the six average sampling sites using the measure function in Google Earth. The results show no significant correlation (p=0.934) suggesting an isolation by distance genetic pattern does not occur in adult red drum along the southeastern US coast.

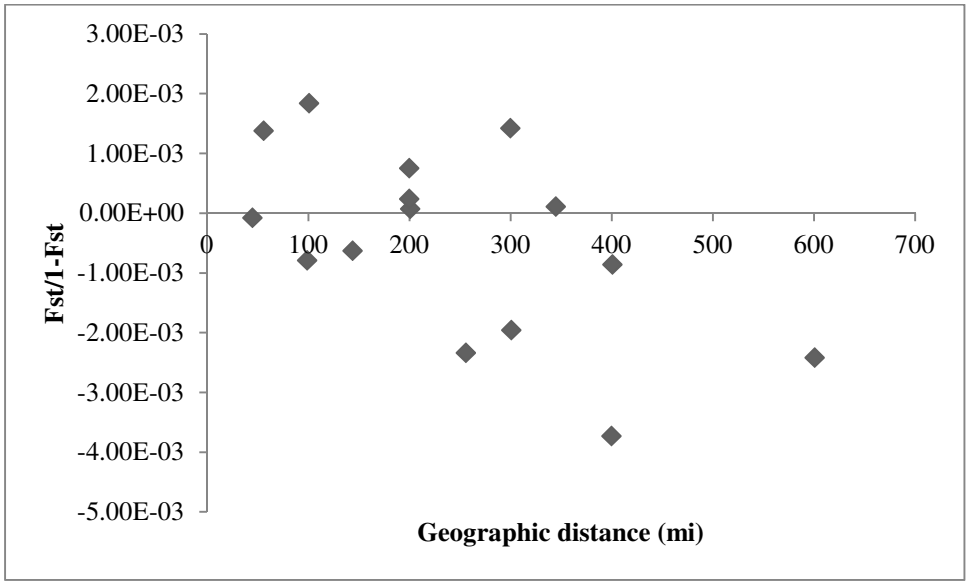

Figure 4. Relationship of genetic and geographic distances in adult red drum along the southeastern US Atlantic coast.

Genetic Diversity

Using Arlequin 3.11, GenePop 4.1, and FSTAT 2.9.3.2 (Goudet 1995; 2001), basic molecular diversity indices including number of alleles per locus (N_a), allelic size range, allelic richness (R), observed heterozygosity (H_O), expected heterogygosity (H_E), and F_{IS} values were calculated for all loci, within each region for the spawning group and with all regions combined for the non-spawning group.

For all loci, heterozygosity (observed and expected) was moderate (>0.50) to high (>0.80), both within regions (spawning groups) and with all regions combined (non-spawning group; Table 6). Average observed heterozygosity (taken across loci) was moderately high within regions for the spawning groups (Northern group = 0.74, Southern group = 0.74) and with all regions combined for the non-spawning group (Non-spawning group =0.74). Average expected heterozygosity (taken across loci) was also moderately high within regions for the spawning groups (Northern

group = 0.74, Southern group = 0.75) and with all regions combined for the non-spawning group (Non-spawning group =0.75). The number of alleles ranged from 5 - 24 and allelic richness ranged from 5.0 - 24.0 (Table 6). Some loci had a lower numbers of alleles and allelic richness (<10), while other loci had moderate (>10) to high (>20) numbers of alleles and allelic richness. Levels of inbreeding (F_{IS}) were low (<0.10) for all loci, both within regions (spawning groups) and with all regions combined (non-spawning group; Table 6). Average F_{IS} (taken across loci) was very low within regions for the spawning groups (Northern group = 0, Southern group = 0.006) and with all regions combined for the non-spawning group (Non-spawning group =0). No large differences between regions were seen, with respect to heterozygosity, number of alleles, allelic richness, or inbreeding.

Table 6. Summary of per-locus genetic diversity statistics for each region during the spawning and non-spawning periods. N = sample size; N_A = number of alleles, A = allelic size range, R = allelic richness; H_O = observed heterozygosity, H_E = expected heterozygosity; F_{IS} =inbreeding coefficients (Weir and Cockerham, 1984); P = probability of divergence from Hardy-Weinberg equilibrium (HWE).

Locus		Northern Spawning Group (NC)	Southern Spawning Group (SC, GA, FL)	Non-spawning Group (NC, SC, GA, FL)
Soc014	N	549	1466	830
	Na	16	18	17
	A	110-146	110-146	108-142
	R	15.989	17.92	17.00
	H_O	0.852	0.82469	0.79373
	H_E	0.819	0.81489	0.8116
	F_{IS}	-0.041	-0.012	0.022
	P	0.8706	0.8029	0.3266
Soc017	N	544	1461	847
	Na	14	16	16
	A	110-140	106-134	106-136
	R	14.00	15.965	15.980
	H_O	0.8511	0.83025	0.82881
	H_E	0.84711	0.84744	0.84407
	F_{IS}	-0.005	0.020	0.018
	P	0.6073	0.037	0.223
Soc029	N	550	1469	865
	Na	5	5	5
	A	127-135	127-135	127-135
	R	5.00	5.00	5.00
	H_O	0.61273	0.62968	0.61387

Locus		Northern Spawning Group (NC)	Southern Spawning Group (SC, GA, FL)	Non-spawning Group (NC, SC, GA, FL)
	H_E	0.61199	0.61825	0.62124
	F_{IS}	-0.001	-0.018	0.012
	P	7334	0.7816	0.6494
Soc060	N	550	1440	848
	Na	6	5	5
	A	154-169	154-166	154-166
	R	5.987	5.00	5.00
	H_O	0.62727	0.61806	0.5967
	H_E	0.60365	0.61262	0.6082
	F_{IS}	-0.039	-0.009	0.019
	P	0.8878	0.353	0.4693
Soc129	N	548	1410	866
	Na	22	24	22
	A	111-191	103-199	111-219
	R	21.973	24.00	21.957
	H_O	0.89599	0.90638	0.90531
	H_E	0.91014	0.91344	0.91475
	F_{IS}	0.016	0.008	0.010
	P	0.0829	0.7188	0.4975
Soc243	N	551	1451	856
	Na	7	8	9
	A	88-109	85-109	85-109
	R	7.00	7.972	8.969
	H_O	0.65154	0.70159	0.69509
	H_E	0.6951	0.71366	0.70411
	F_{IS}	0.063	0.017	0.013
	P	0.0029	0.4506	0.0309
Soc083	N	547	1454	853
	Na	17	19	21
	A	118-156	118-156	118-160
	R	16.993	18.939	20.918
	H_O	0.86837	0.87414	0.87104
	H_E	0.86922	0.87624	0.8738
	F_{IS}	0.001	0.002	0.003

Locus		Northern Spawning Group (NC)	Southern Spawning Group (SC, GA, FL)	Non-spawning Group (NC, SC, GA, FL)
	P	0.5095	0.7325	0.4598
Cne612	N	543	1454	836
	Na	9	12	14
	A	119-141	117-155	118-157
	R	9.00	11.939	13.964
	H_O	0.55493	0.55915	0.60048
	H_E	0.52554	0.58157	0.58866
	F_{IS}	-0.051	0.039	-0.20
	P	0.7298	0.0008	0.1451

Effective Population Size

Contemporary (parental generation) estimates of the genetic effective population size (N_e) were estimated for the northern (NC) and southern regions (FL, GA, SC) during spawning season and for all regions combined during the non-spawning season using the single-sample program LDNe 1.2 (Waples 2006). Genetic drift generates non-random associations among unlinked loci; LDNe analyzes this linkage disequilibrium between a set of loci to determine contemporary N_e for a single time point. Minimal allele frequencies for inclusion were set at default values (0.01, 0.02, and 0.05), but only the <0.02 frequency is reported, as the exclusion of alleles with frequencies <0.02 is recommended for sample sets larger than 100 individuals (Waples and Do 2010). A random mating model was assumed and confidence intervals were calculated using parametric procedures. It must be stated that there are a few caveats to the estimates of N_e in this study with regards to the assumptions inherent to the linkage disequilibrium method (i.e. no mutation, selection, overlapping generations, or migration). Mutation rate is not expected to be a concern for short-term estimates (Waples and Do 2010) and microsatellite markers are generally deemed to be selectively neutral, so these two assumptions have been met. However, red drum do exhibit overlapping generations and migration is likely occurring between localities (particularly during the non-spawning season), so the N_e values calculated here should only be taken as a rough estimate of true population N_e.

Results from the LDNe single time point estimates of N_e show that North Carolina (N_e = 2,478) is orders of magnitude smaller than the southern region (FL, GA, SC). In fact, the southern region was too large for LDNe to produce estimates of N_e, resulting in a negative number (Table 7). Negative estimates are given by LDNe when there is no evidence of disequilibrium caused by genetic drift due to a finite number of parents; in this case, the data cannot prove that the population is not "very large" (Waples and Do 2010). The upper confidence intervals (CI) on all values were unbounded (i.e. ∞), also indicating that the effective number of breeders is likely large ($N_b \geq 1{,}000$). Large populations do not typically have well-defined upper bounds on N_e (or N_b) estimates in LDNE even with robust sample sizes ($n \geq 200$; Waples and Do 2010). These results are agreement with the stock abundance estimates from the 2009 SEDAR stock

assessment, which found that the southern region was much larger in terms of abundance than the northern region. The northern region was estimated to contain over 3 million fish in 2007 and the southern region was estimated to contain about 6-7 million (ASFMC 2009). While LDNe provides an estimated range for the effective size, future analysis should include methods that account for overlapping generations and migration; these methods require age data and a sample sets representative of the full generation time for adult red drum, which were not available for this study.

Table 7. Estimates of effective population size (N_e) for red drum from each region during the spawning and non-spawning periods. Estimates of infinity are often obtained when N_e is large ($N_e \geq 1{,}000$).

Region	N_e estimate (95% confidence interval)	Sample size
Northern spawning group (NC)	2,478 (1,080 - ∞)	551
Southern spawning group (SC, GA, FL)	∞ (109,356 - ∞)	1,473
Non-spawning group (NC, SC, GA, FL)	∞ (7,370 - ∞)	869

Movement

To serve as a power analysis for our loci suite, Cervus 3.0 (Kalinowski et al. 2007) was used to estimate the identity non-exclusion probability, which measures the probability that a set of markers will not be able to distinguish between related individuals. The loci suite (all 8 loci together) provides an average non-exclusion identify probability of 9.70^{-12}, signifying that the possibility of incorrectly matching individuals to one another is substantially less than 0.01%. As such, genotypes from all samples were compared to one another using Microsatellite Toolkit, an Add-in for Excel. Samples which matched at all 8 loci were considered to be recaptures, although pit tag information was compared as well when available.

A total of 109 recaptures (involving 51 individuals) were found. Thirty-nine individuals were both captured and recaptured during the spawning season (Table 8). There were 19 individuals taken during the non-spawning season, meaning that they were either captured, recaptured, or both outside of the designated spawning window (Table 9). For both the spawning and non-spawning seasons, there was no indication of large-scale (between-state) movement, and most individuals were recaptured at or near the site of their first capture (Table 8; 9). However, the small sample sizes of recaptures, most found within Charleston Harbor, suggest that a more extensive analysis should be performed when larger numbers of recaptures have been obtained.

Table 8. Capture and recapture locations for adult red drum on the southeastern coast of the U.S. during the spawning season.

Original Capture Location	Recapture Location	# Fish
Charleston Jetties	Charleston Jetties	33
	St. Helena Sound	1
	Winyah Bay	1
Offshore Charleston	Charleston Jetties	1
Port Royal Sound	Port Royal Sound	1
Winyah Bay	Winyah Bay	1
Ocracoke, NC	Ocracoke, NC	1

Table 9. Capture and recapture locations for adult red drum on the southeastern coast of the U.S. during the non-spawning season.

Original Capture Location	Recapture Location	# Fish
Charleston Jetties	Charleston Jetties	15
Offshore Charleston	Charleston Jetties	1
Port Royal Sound	Port Royal Sound	1
Winyah Bay	Winyah Bay	1
Indian River Lagoon, FL	Indian River Lagoon, FL	1

Conclusions

The evaluation of genetic diversity and effective population size in this study provides important information on the genetic characteristics of adult red drum along the southeastern coast of the U.S. Decreases in population size (i.e. census size) have recently been linked to a reduction in genetic diversity for a number of marine fishes (Hauser and Carvalho 2008). Diminished genetic diversity, along with inbreeding, can increase the risk of extinction by negatively impacting a species' fitness and capacity to respond to environmental stochasticity (Saccheri et al. 1998; Frankham et al. 2002; Keller and Waller 2002; Reed and Frankham 2003; Frankham 2005), making measurements of genetic diversity valuable indicators of overall genetic 'health'. We found that genetic diversity, as measured by the degree of polymorphism (N_a, R) and the heterozygosity (H_O) of our loci suite, was high (N_a: 5-24 alleles per locus; R: 5.0-24.0; H_o: 0.74) and that inbreeding was very low (<0.007). Effective population size is one of the most important measures in conservation biology (Frankham 2005; Waples 2002), as low N_e has been shown to lead to reduced fitness and an increased likelihood of extinction. We found that estimates of effective size were on the order of several thousand individuals. Estimates are above the minimum number of 50 recommended to avoid significant inbreeding and maintain short-term fitness (Franklin 1980) of a population and are within the minimum values recommended to maintain the evolutionary potential (i.e., quantitative trait heritability; Frankham 1995) and long-term viability of a population (N_e = 500-1,000; Franklin and Frankham 1998 or N_e = 1,000-5,000; Lynch and Lande 1998).

Understanding genetic population structure can aid in effective management by serving as a tool to identify proper management units. Results show that *S. ocellatus* found along the southeastern Atlantic coast are genetically homogenous during the months of November to June, outside of the spawning window; but are genetically distinct during the spawning months (July to October). This indicates that a genetic break exists between NC and SC, which is in agreement with life history data (red drum from NC are typically larger and live longer than SC red drum). From North Carolina to the Chesapeake, adult red drum were seen to grow approximately 200mm (TL) larger than in the southern Atlantic region (South Carolina to Southern Florida) with a maximum age of ~60 compared to ~ 40 in the south. Our results are also supported by the observed limited movement seen in our genetic recapture data. It is possible that the limited suitable estuarine habitat for red drum at the boarder of NC and SC may potentially have caused the slight divergence in genetic structure. Additionally, Cape Hatteras or the changes in the direction of the gulf stream in this area may also provide a large geographic barrier that limits interaction between the two stocks. There was no significant isolation-by-distance, so the genetic difference between NC and the southern states was not correlated with geographical distance.

Our results support the current assessment strategy of separating NC and SC into different units (northern vs. southern regions; ASFMC 2013). However, it should be kept in mind that the low F_{ST} values show that the genetic structure is weak (i.e. gene flow between regions is still occurring), which may possibly be due to the rare straying of adults or the occasional dispersal of spawns.

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