Population Genetic Analyses of Scamp

SEDAR68-SID-04

26 July 2019
Updated: 22 August 2019

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Please cite this document as:

Darden, T. and M. Walker. 2019. Population Genetic Analyses of Scamp. SEDAR68-SID-04. SEDAR, North Charleston, SC. 14 pp.

Population Genetic Analyses of Scamp

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July 26, 2019, updated August 20, 2019

Sample Collections:

The SCDNR Genetic Tissue Collection currently houses 1,601 archived genetic samples of Scamp. Tissue types include heart, muscle, gonad, gill, fin, and otoliths. All soft tissues are stored in a sarcosyl-urea preservation solution (8M urea, 1% sarcosyl, 20 mM sodium phosphate, 1 mM EDTA) until processing. Whole otoliths are individually stored dry in small paper envelopes. For the current project, a total of 823 samples were selected for genetic analyses, including those collected along the Gulf of Mexico and southeastern U.S. Atlantic coasts with available samples ranging from North Carolina south along the Atlantic coast around the Florida peninsula into the Gulf of Mexico westward to Panama City, FL and a sample set from Gulf of Mexico waters in Mexico (Table 1; Figure 1). Collections occurred from 1996 through 2018 with similar temporal representation throughout U.S. waters. Evaluations of length and year class compositions among collections verified that multiple cohorts were present (10-13 year classes in those with age data available) and length ranges were similar across collection years and locations (Tables 2 & 3).

Table 1. Regional distribution and collection years of Scamp samples available for genetic analyses.

Location	Collection Years	Initial Sample Sizes
NC	2000-2018	51
SC	1996-2018	272
GA	1996-2018	90
FL (Atlantic)	1999-2018	45
FL (Keys)	1998-2002	76
FL (Gulf)	2000-2017	130
FL (Panhandle)	1999-2017	135
GOM (Mexico)	1999	24
Grand Total		823

Table 2. Summary of sample sizes by year class for reach regional collection of Scamp genetic samples. Unknown indicates age data was not available.

Year Class	Sample Size
FL (Atlantic)	
unknown	26
1992	2
1993	1
1994	1
1995	1
1996	1
1997	1
1998	1
2000	1
2001	2
2003	3
2006	3
2008	2
FL (Gulf)	
unknown	130
FL (Panhandle)	
unknown	135
FL Keys	
unknown	76
GA	
unknown	25
1994	7
1997	2
1999	1
2000	2
2001	15
2002	5
2003	9
2005	2
2006	3
2008	13
2010	1
2012	5
GOM	
unknown	24
NC	
unknown	17
1994	1
1996	1
1997	1
2001	5
2002	4
2003	9
2006	2
2008	3
2010	2
2012	6
SC	
unknown	7
1993	1
1994	31
1997	5
2000	2
2001	89
2002	12
2003	66
2006	23
2008	15
2009	2
2010	3
2012	16
Grand Total	823

Table 3. Summary of sample sizes and TL ranges (mm) by collection year for each regional collection of Scamp genetic samples.

Collection Year	Sample Size	Minimum TL (mm)	Maximum TL (mm)
FL (Atlantic)	45	411	900
1999	1	411	411
2000	23	476	782
2009	1	638	638
2010	1	763	763
2011	4	479	619
2012	2	622	767
2013	2	527	610
2014	1	664	664
2017	4	482	900
2018	6	472	900
FL (Gulf)	130	433	715
2000	65	442	715
2001	46	---	---
2002	16	433	455
2017	3	460	538
FL (Panhandle)	135	196	750
1999	34	289	631
2000	32	196	750
2001	32	242	580
2002	31	235	643
2003	4	433	510
2017	2	414	620
FL Keys	76	220	710
1998	4	461	640
1999	12	435	710
2000	1	410	410
2001	58	220	655
2002	1	---	---
GA	90	338	872
1996	1	338	338
2000	2	561	620
2006	17	453	715
2007	2	553	710
2009	4	458	624
2010	9	465	725
2012	5	477	695
2013	10	505	660
2014	1	675	675
2015	5	557	712
2016	4	514	604
2017	13	387	843
2018	17	514	872
GOM	24	527	810
1999	24	527	810
NC	51	344	892
2000	1	479	479
2007	2	463	489
2009	1	457	457
2013	1	344	344
2014	5	423	721
2015	4	453	523
2016	2	703	823
2017	17	409	880
2018	18	470	892
SC	272	335	900
1996	2	335	494
1997	15	367	521
1998	5	382	508
2000	4	561	668
2005	2	440	505
2006	74	379	855
2007	57	369	900
2009	2	454	717
2010	2	590	650
2011	2	679	712
2012	3	530	676
2013	9	538	736
2014	21	341	662
2015	10	432	703
2016	5	440	685
2018	59	422	857
Grand Total	823	196	900

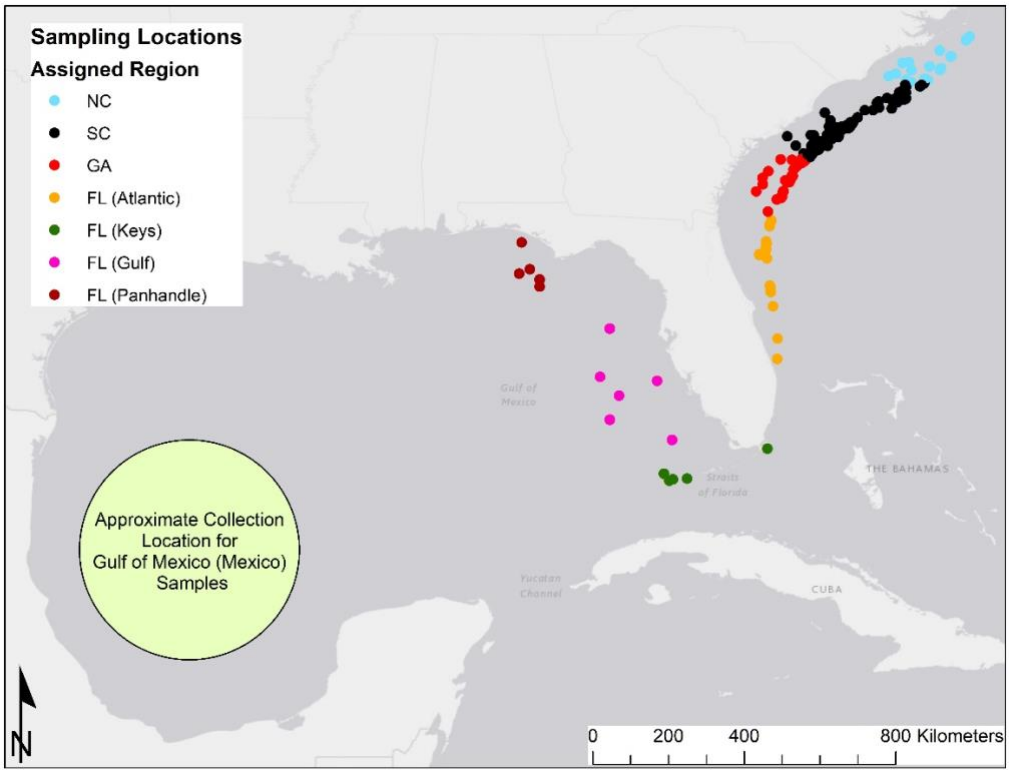

Figure 1. Geographic distribution of Scamp sample collections.

Genetic Protocols and Analyses:

The sarcosyl-urea preservative simultaneously stabilizes sample DNA and serves as a preliminary cell lysis solution. Dry otolith samples were subjected to a proteinase K cell lysis overnight prior to DNA isolation. All DNA isolation, microsatellite amplification, and genotyping methods followed standard laboratory procedures. Briefly**,** DNA was isolated from all soft tissue samples using a carboxylate-modified magnetic bead isolation procedure and from all otoliths using the Promega Wizard SV Genomic DNA Isolation Kit. Fifteen polymorphic microsatellite loci were then amplified via polymerase chain reaction (PCR) in three multiplexed groupings. These polymorphic loci were selected from peer-reviewed literature and were originally developed for species other than Scamp (Table 4). All PCRs were conducted in 11 µL reactions with 1x HotMaster buffer with 2.5 mM Mg^{2+}, 0.2 mM dNTPs, 0.3 units 5 Prime HotMaster *Taq* polymerase (Qiagen), 2.0 mM $MgCl_2$, 0.3 µM forward and reverse primers, and 1 µL of 1:10 diluted DNA template. Forward primers for all loci were labelled with WellRED fluorescent dyes (Beckman Coulter, Inc.). Thermal cycling for PCR used a standard protocol consisting of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturing at 94°C for 30s, annealing at 61°C for 30s, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 60 min. Both size standards (Genome Lab DNA Size standard kit 400) and reaction products were separated with a Beckman CEQ 8000/GenomeLab GeXP (Beckman Coulter, Inc.), with fragment size analysis performed with CEQ Fragment Analysis Software. All chromatograms were scored manually by two

independent readers. Discrepancies between readers were resolved in conference, or samples were rerun to obtain an unambiguous genotype for all individuals.

Table 4. Microsatellite loci amplified in Scamp for the current study with the peer-reviewed paper of origin, species for which each locus was designed, number of alleles (N_A) and the number of individuals assayed (N) in the final data set.

Locus Name	Source	Target Species	N_A	N
Mros9	Jackson et al. 2014	Leopard grouper	26	765
Mros10	Jackson et al. 2014	Leopard grouper	32	805
Mti17	Renshaw et al. 2012	Tiger grouper	7	782
Mros12	Jackson et al. 2014	Leopard grouper	22	790
Mti7	Renshaw et al. 2012	Tiger grouper	8	801
Mbo48	Zatcoff et al. 2004	Black grouper	8	805
Mros13	Jackson et al. 2014	Leopard grouper	54	784
Mros7	Jackson et al. 2014	Leopard grouper	33	792
Est49B	Bernard et al. 2012	Nassau grouper	6	796
Mros03	Jackson et al. 2014	Leopard grouper	24	760
Mbo88	Zatcoff et al. 2004	Black grouper	25	776
Mti18	Renshaw et al. 2012	Tiger grouper	7	665
Est338	Bernard et al. 2012	Nassau grouper	21	639
Est376	Bernard et al. 2012	Nassau grouper	24	769
Est290	Bernard et al. 2012	Nassau grouper	15	807
		Averages	20.8	769.1

All individuals successfully genotyped at 10 or more loci were included in the data set for initial analyses. The data set was evaluated for linkage disequilibrium among loci in both combined and regional collection scales in Arlequin 3.5.1.2 (Excoffier and Lischer 2010) and GENEPOP 4.7.2 (Rousset 2008); no loci were identified to be consistently linked across locations or data set groupings. Screening for duplicate genotypes was conducted using GenAlEx 6.5 (Peakall & Smouse 2006, 2012); no duplicate genotypes occurred within the data set. Sibship analyses as implemented in the software Colony 2.0.6.4 (Jones & Wang 2010) were conducted to identify any potential large family groups within the data set that could confound further genetic structure analyses. Two simulation models were run using settings of polygamous breeding, updating allele frequencies, 0.0001 genotyping error, and FPLS likelihood method for medium run lengths with 3 replicates per model. The simulations models differed in their assumption of priors; one model was run with no knowledge of priors or putative family sizes and the other was conducted with weak priors with small putative family sizes. Results were consistent both among model replicates as well as between models with only 35 full sibs identified in the entire data set with only 6 of those having >90% probability of sibship and an additional 3 ranging from 80-89% probability. All family groups identified included only 2 individuals; therefore, no confounding effects from family structure were anticipated in further analyses.

Standard population genetic statistical analyses were applied to the resulting sample data set. Population genetic structure throughout the collection range was assessed via evaluations of Hardy Weinberg

equilibrium (HWE) in GENEPOP and Arelquin, AMOVA analyses in Arlequin, pairwise F_{ST}-style statistics calculated in GenAlEx 6.5, and with the clustering algorithms implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). Iterative AMOVA (F_{ST}-based) analyses were conducted to evaluate areas of genetic discontinuity in the data sets with potential location groupings under two-population scenarios. Pairwise comparisons of sample locations and HWE were conducted initially at the smallest geographic scale and locations were combined sequentially to represent the smallest number of homogenous groupings. The clustering model assignment employed in the program STRUCTURE using a hierarchical approach with the assistance of the web-based software Structure Harvester 0.6.94 (Earl et al. 2012) was used to identify the most appropriate number of distinct populations (K) of each run. Simulations were run with both the locprior (collection location) and no locprior parameter settings for all analyses, with five replicates for each K, the length of the burn-in period set at 20,000, and number of Markov chain Monte-Carlo reps after burn-in set at 20,000. All analyses were conducted from K=1 to K= # collection locations included +1. Samples that showed homogenous ancestry patterns were removed from the data set and STRUCTURE was run iteratively until K=1 was the most appropriate assignment for each cluster (based on combined evaluation of the Evanno method and log likelihood plots). Effective number of migrants per generation and year (based on 16.5 year generation time) were calculated for each resulting homogenous cluster.

Results and Conclusions:

All 823 selected samples met the genotyping inclusion criteria, resulting in an average of 14.2 loci/sample genotyped across collection regions (Table 5). All loci in all collection regions were in HWE with the exception of 2 loci in the FL(Panhandle) region; therefore, all loci were retained in the analyses.

Table 5. Sample sizes and average number of loci genotyped per sample for each collection region in the initial data set. Final sample sizes are shown for each collection region following removal of samples of questionable genetic ancestry (see below).

Location	Initial sample sizes	Average number of loci genotyped	Final sample sizes
NC	51	14.0	49
SC	272	14.3	269
GA	90	14.4	90
FL (Atlantic)	45	14.3	45
FL (Keys)	76	14.8	73
FL (Gulf)	130	13.5	130
FL (Panhandle)	135	14.7	132
GOM (Mexico)	24	13.4	21
Grand Total	823	14.2	809

As initial pairwise F_{ST}-style comparisons of collection regions showed similar trends and magnitudes across estimates of F_{ST}, G_{ST}, and D_{EST}, only F_{ST} results are presented here. Although no pairwise comparisons were statistically significant following Bonferonni correction, there is an obvious pattern with the GOM(Mexico) region showing greater differentiation will all other regional comparisons (Table 6). Also noted is that the FL(Panhandle) region showed an inconsistent pattern relative to the matrix in its degree and probability of differentiation from the FL(Gulf) region.

Table 6. Pairwise collection region matrix of F_{ST} values (below the diagonal) and associated p-values (above the diagonal). All comparisons with GOM (Mexico) region are shown in purple and the FL(Panhandle) and FL(Gulf) comparison are shown in red.

	GOM (Mexico)	**FL (Panhandle)**	**FL (Gulf)**	**FL Keys**	**FL (Atlantic)**	**GA**	**SC**	**NC**
GOM (Mexico)	---	***0.011***	***0.014***	***0.006***	***0.023***	***0.011***	***0.005***	***0.011***
FL (Panhandle)	***0.009***	---	***0.017***	0.224	0.145	0.138	0.225	0.109
FL (Gulf)	***0.011***	**0.003**	---	0.834	0.596	0.395	0.141	0.484
FL Keys	***0.011***	0.003	0.003	---	0.900	0.332	0.708	0.819
FL (Atlantic)	***0.012***	0.004	0.004	0.004	---	0.656	0.259	0.343
GA	***0.010***	0.003	0.003	0.003	0.004	---	0.482	0.198
SC	***0.010***	0.002	0.002	0.002	0.004	0.002	---	0.425
NC	***0.013***	0.004	0.004	0.004	0.006	0.005	0.003	---

Based on the initial pairwise comparisons, the data set was collapsed to three groups including GOM(Mexico), FL(Panhandle), and Atlantic (which includes all regions from NC south through FL(Gulf)) for further evaluation. STRUCTURE analyses with and without the locprior parameter produced consistent results; therefore, only the results from the locprior-based analyses are presented here as the patterns are more clearly visualized in the STRUCTURE plots. Structure Harvester indicated 2 genetic ancestries present in the resulting collapsed data set (Figure 2). However, evaluation of the ancestry plot by individual indicated the lack of a geographic signature; instead indicating the presence of a strong genetic signal in scattered individuals (Figure 3). Although we cannot distinguish between the signature representing a unique Scamp ancestry that is not otherwise sampled in our data set versus a unique species ancestry (see Supplement below), given prior discussions regarding potential morphological identification concerns the samples with the unique signatures were removed from the data set. Nine samples showed more than 50% unique ancestry (3 GOM, 3 FL(Panhandle), 3 FL(Keys)) and 5 samples displayed 5-12% unique ancestry (3 SC, 2 NC). The resulting data set was re-evaluated and represents the 'final' data set in Table 5.

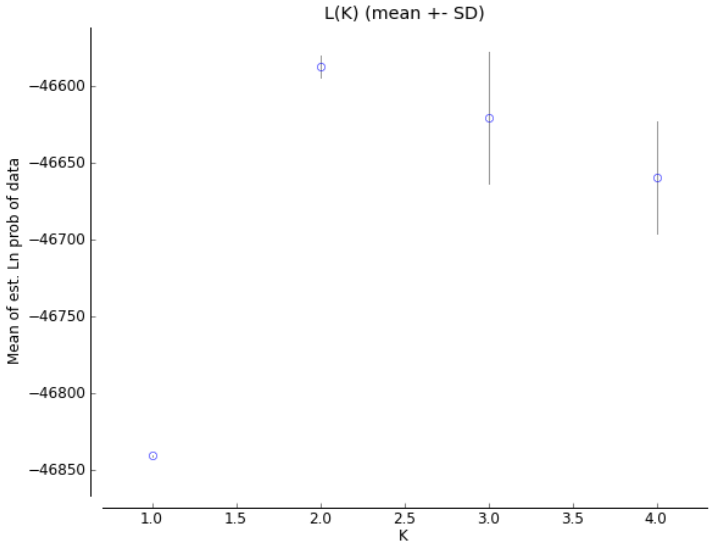

Figure 2. Resulting Structure Harvester Mean Ln Probability plot from the complete initial Scamp data set indicating a most likely K=2 genetic ancestries.

Figure 3. Population ancestry plot for the complete data set based on STRUCTURE results of K=2. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Collections are geographically oriented from Mexico on left to North Carolina on the right.

Although the trend remains in the pairwise F_{ST} indicating a change in differentiation between the GOM(Mexico) and FL(Panhandle) regions (Table 7), both pairwise F_{ST} and STRUCTURE analyses (Figures 4 and 5) statistically indicate the samples in our final data set represent a single genetic population of Scamp throughout the U.S. Atlantic and Gulf of Mexico waters. The iterative AMOVA analyses also did not detect any significant partitioning of genetic variation among any of the regions within our sample set. The strongest (but non-significant) break was identified between the FL(Panhandle) and FL(Gulf) regions, explaining 0.45% (p=0.354) of the variation in the data set. The levels of genetic differentiation detected between the GOM (Mexico) and Atlantic group translated into effective number of migrants (N_em) of 1.7 individuals per year between these populations (27.5 individuals/generation). Therefore, the Scamp samples included in our project data set represent a single genetic stock.

Table 7. Pairwise matrix of F_{ST} values (below the diagonal) and associated p-values (above the diagonal). Comparisons of GOM (Mexico) with Atlantic region are shown in purple.

	GOM (Mexico)	FL (Panhandle)	Atlantic
GOM (Mexico)	---	0.070	***0.034***
FL (Panhandle)	0.009	---	0.099
Atlantic	***0.009***	0.001	---

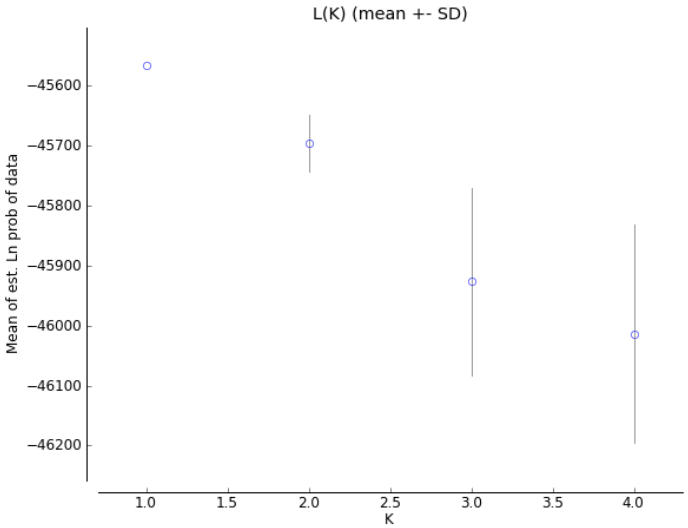

Figure 4. Resulting Structure Harvester Mean Ln Probability plot from the final Scamp data set indicating a most likely K=1 genetic ancestry.

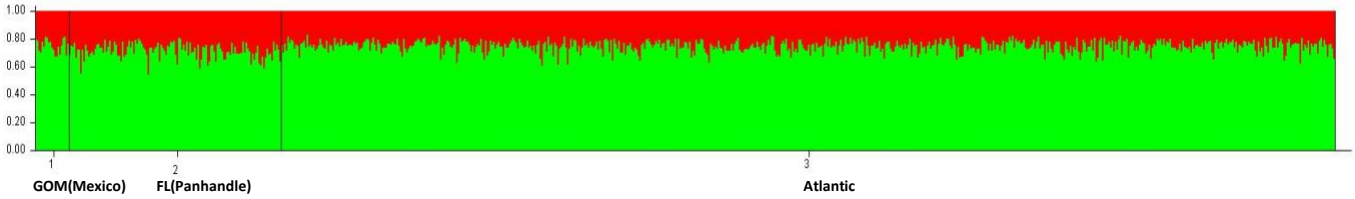

Figure 5. Population ancestry plot for the final data set based on STRUCTURE results of K=2 for visualization purposes of the true K=1 ancestry. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Collections are geographically oriented from Mexico on left to North Carolina on the right.

Supplements:

Sensitivity Analyses:
Two types of sensitivity analyses were also conducted with the final data set to evaluate (1) any potential masking influence from non-spawning season movements and (2) STRUCTURE model run parameters. The final data set was partition with spawning season defined as February to August and March to May, with any samples collected outside of these windows removed. Total sample sizes were reduced to 543 and 218 samples, respectively (Table 8). Pairwise comparisons and STRUCTURE analyses were run as described above using the three collapsed regions.

Table 8. Scamp samples sizes following partitioning by sample season windows.

	Spawning Window	
Collection	Feb-Aug	Mar-May
GOM (Mexico)	21	21
FL (Panhandle)	87	32
Atlantic	435	165
Total	543	218

Single genetic groups were recovered from analyses of both partitioned data sets using both pairwise comparisons (Tables 9 & 10) and STRUCTURE assessments (Figure 6). Even with the substantially reduced samples sizes with the March to May window, both results are consistent with the gene flow patterns and magnitudes detected in the full data set. Therefore, results suggest a lack of influence of time of collection on characterization of stock structure in Scamp and are in agreement with observations of high site fidelity in this species.

Table 9. Pairwise matrix of F_{ST} values (below the diagonal) and associated p-values (above the diagonal) for the data set restricted by a February to August spawning window. Comparisons of GOM (Mexico) with Atlantic region are shown in purple.

	GOM (Mexico)	FL (Panhandle)	Atlantic
GOM (Mexico)	---	0.096	***0.021***
FL (Panhandle)	0.009	---	0.121
Atlantic	***0.009***	0.002	---

Table 10. Pairwise matrix of F_{ST} values (below the diagonal) and associated p-values (above the diagonal) for the data set restricted by a March to May spawning window. Comparisons of GOM (Mexico) with Atlantic region are shown in purple.

	GOM (Mexico)	FL (Panhandle)	Atlantic
GOM (Mexico)	---	0.204	***0.050***
FL (Panhandle)	0.012	---	0.385
Atlantic	***0.009***	0.005	---

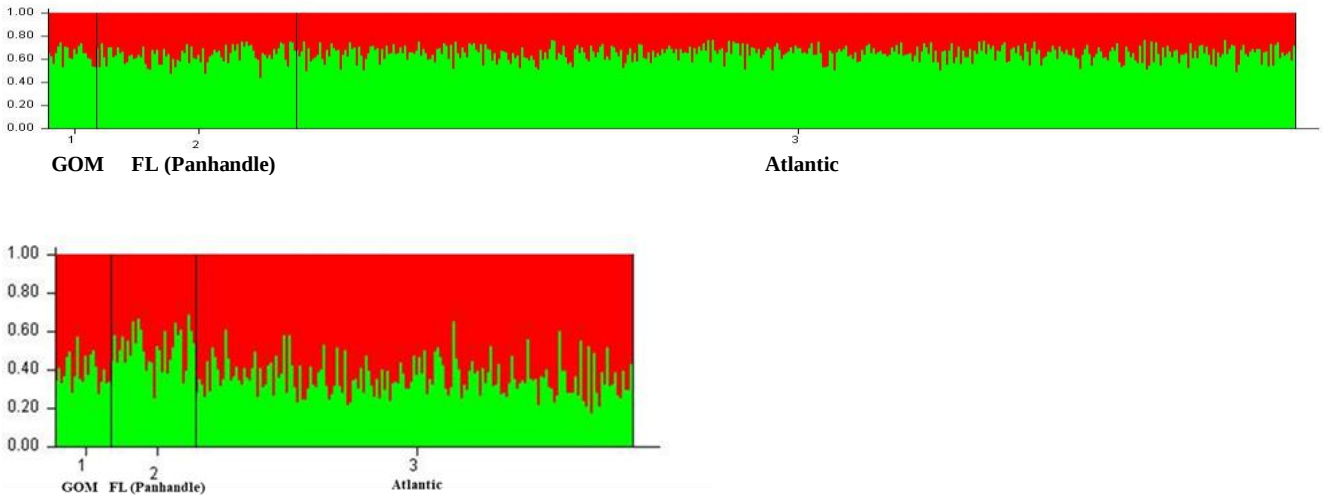

Figure 6. Population ancestry plots for the final data set partitioned by spawning windows based on STRUCTURE results of K=2 for visualization purposes of the true K=1 ancestry. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Collections are geographically oriented from Mexico on left to North Carolina on the right. Top bar plot represents the February to August spawning window; bottom plot represents the March to May spawning window.

Sensitivity analyses were conducted to evaluate potential influences of model run lengths and admixture parameter. In comparison to the above Admixture models with 20,000 burnin period and 20,000 Monte Carlo repetitions, a longer Admixture simulation with 100,000 burnin period and 100,000 Monte Carlo repetitions was conducted. Additionally, a simulation with no Admixture was run with 20,000 burnin and 20,000 Monte Carlo repetitions. All other parameters remain the same, including the Location Prior setting. All runs resulted in K=1 ancestry with similar model convergence for the Admixture models (Figure 7). Therefore, we found no impacts of evaluated model parameters on results for Scamp genetic gene flow patterns.

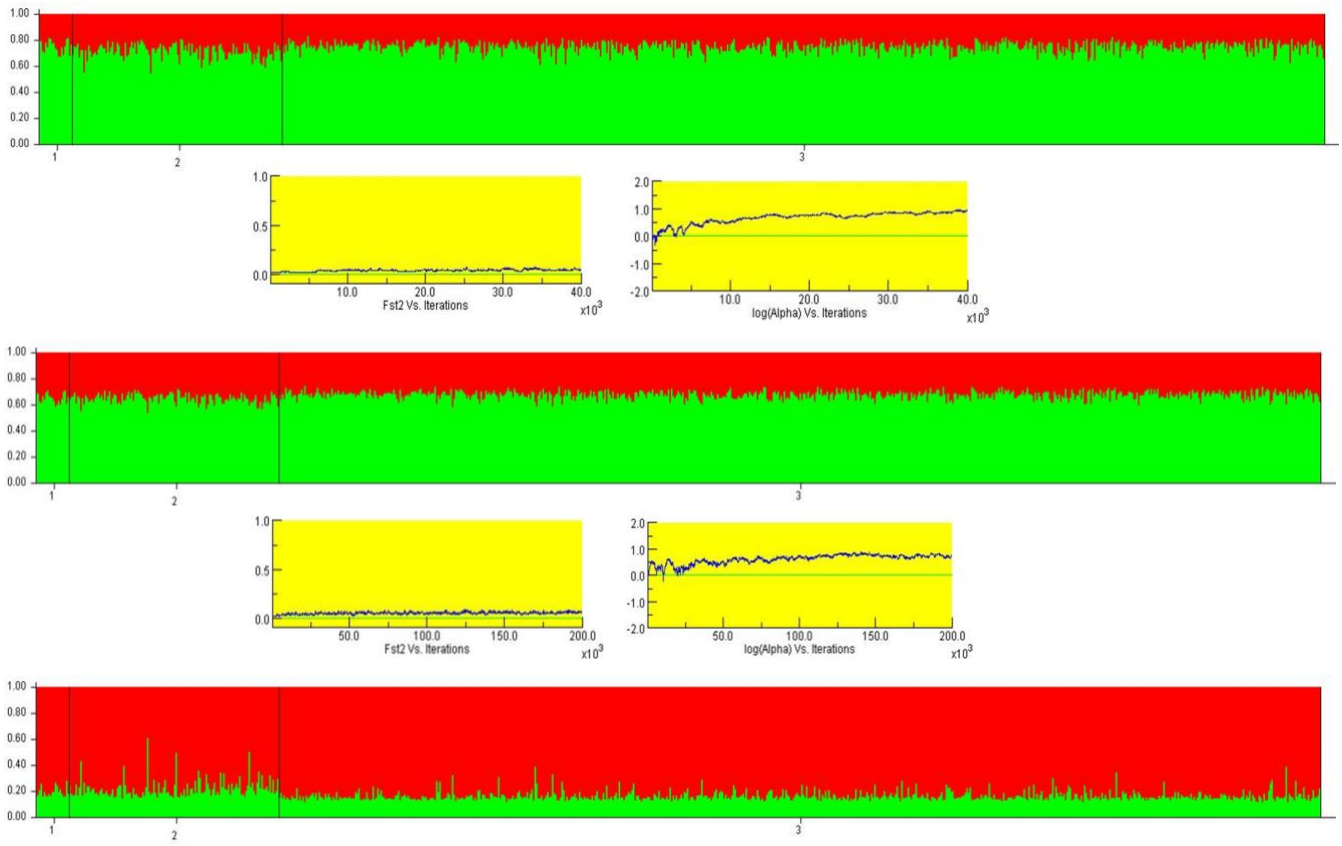

Figure 7. Population ancestry and model convergence plots for the final data set based on STRUCTURE results of K=2 for visualization purposes of the true K=1 ancestry. For bar plots, each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Collections are geographically oriented from Mexico on left to North Carolina on the right. For model convergence plots, variation in Fst2 and log(Alpha) are tracked in relation to run iteration. Top plots represent the original model configuration (Admixture 20,000/20,000); middle plots represent the long run (Admixture 100,000/100,000); bottom plot represents the no Admixture model (20,000/20,000).

Putative Yellowmouth Group Misidentifications:

In attempt to elucidate the origin of the unique ancestry samples from our initial evaluations, we genotyped 18 archived Yellowmouth Grouper samples (9 from SCDNR, 9 from FFWC) using our Scamp microsatellite panel. Resulting genotypes were combined with the original grouped Scamp data set (new samples were modeled as a separate collection location) and STRUCTURE analyses were conducted as described above (admixture, location priors, 20,000/20,000, K from 1-5, 5 replicates). Results indicated K=2 genetic ancestries in the data set, similar to the prior analysis without the Yellowmouth Grouper samples. Only 50% of the 'known' Yellowmouth Grouper samples were assigned to the second ancestry (Figure 8). However, the second ancestry assignments in the Yellowmouth Grouper samples aligned with the unique ancestry signature in the Scamp samples from the original evaluation, suggesting those samples represent a Yellowmouth Grouper signature instead of an unsampled Scamp population. The three SC samples in the original data set (with lowest % unique ancestry of those removed) showed a much lower % Yellowmouth Grouper ancestry in the new analysis, but strengths of remaining sample

signatures were similar. Additionally, the presence of mixed ancestry in several samples from the Scamp data set suggest the occurrence of both misidentification (95-100% ancestry) as well hybridization (10-50% ancestry) between the two species. No relationship of misidentification with fish length is apparent from this data set (Table 10), with lengths ranging from 330 to 758 mm TL.

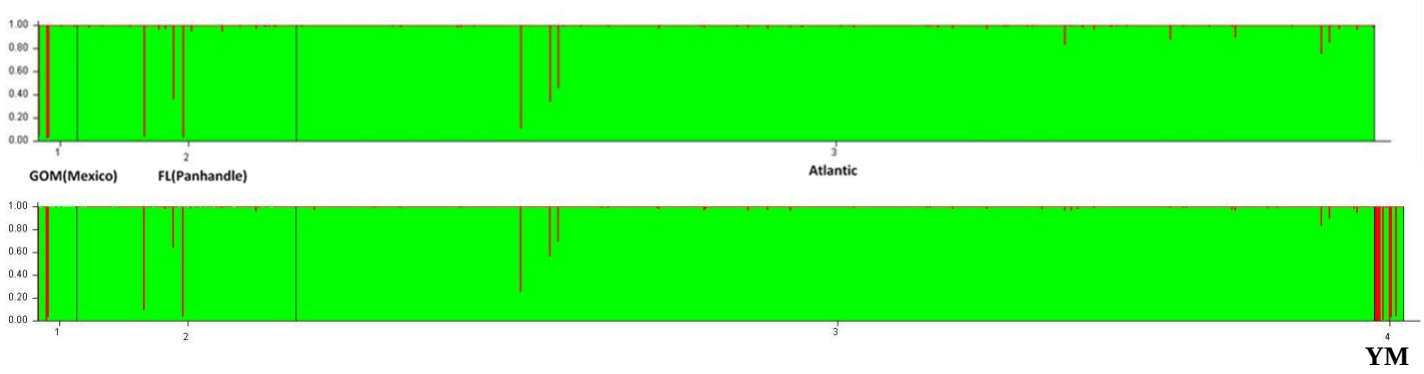

Figure 8. Population ancestry plots based on STRUCTURE results of K=2 for visualization purposes of the true K=1 ancestry in both data sets. The top plot represents results from the original grouped data set; the bottom plot represents results from the original data set plus the new Yellowmouth Grouper (YM) samples as a separate location. For bar plots, each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Scamp collections are geographically oriented from Mexico on left to North Carolina on the right; Yellomouth Grouper samples are not in any geographical order.

Table 10. Length of Scamp samples assigned to unique ancestry in the original grouped data set, shown by collection location.

Collection	TL (mm) of Samples
GOM (Mexico)	650, 655, 758
FL (Panhandle)	468, 509, 750
FL Keys	330, 361, 635
SC	351, 369, 571
NC	564, 605

Literature Cited:

Bernard, A. M., K. A. Feldheim, V. P. Richards, R. S. Nemeth, and M. S. Shivji. 2012. Development and characterization of fifteen novel microsatellite loci for the Nassau grouper (*Epinephelus striatus*) and their utility for cross-amplification on a suite of closely related species. Conservation Genetics Resources 4(4):983-986.

Earl, D. A. and B. M. von Holdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2):359-361.

Excoffier, L., and H.E.L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Jackson, A., A. Manguia-Vega, A.Lain, S. Stokes, A. Williams, and G. Bernardi. 2014. Isolation and characterization of fifteen microsatellite loci in Leopard grouper (*Mycteroperca rosacea*) via 454 pyrosequencing. Conservation Genetics Resources 6: 185-187.

Jones, O. and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Kalinowski S.T., M. L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1106.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

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

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

Renshaw, M., R. Nemeth, J. Gold. 2012. Isolation of microsatellite markers from tiger grouper (*Mycteroperca tigris*) and characterization in yellowfin grouper (*Mycteroperca venenosa*), coney (*Cephalopholis fulva*), and red hind (*Epinephelus guttatus*). Conservation Genetic Resources 4(4): 1049-1054.

Rousset, F. 2008. GENEPOP'007: a complete reimplementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Res.* 8:103–106.

Zatcoff, M. S., A. O. Ball, and G. R. Sedberry. 2004. Population genetic analysis of red grouper,Epinephelus morio, and scamp, *Mycteroperca phenax*, from the southeastern U.S. Atlantic and Gulf of Mexico. Marine Biology 144(4):769-777.