Preliminary Report: Spatial Genetic Structure of Queen Conch Populations in Belize

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Main Objective: Investigate spatial genetic structure (SGS) in Belizean Queen conch (Lobatus *gigas*) populations.

Specific Objectives: 1) Determine SGS between adjacent deep and shallow water populations and 2) among nine geographic sites throughout Belize.

Rationale: The SGS work would determine the minimum number of genetically distinct clusters (*i.e*. populations) of Queen conch in the Belizean fishery. If two or more distinct populations exist, they might not be exchanging recruits at all, or would be exchanging at such a low rate as to be considered independent management units. An understanding of the relative importance of local versus regional spawners on recruitment would provide guidance on where adequate numbers of mature adults need to be maintained to sustain current levels of larval recruitment. Controlling for geography, we also have an opportunity to explore SGS in deep/shallow populations as well. Assuming geographic sites show at least moderate levels of SGS, genetic markers could be also used in future studies to test if local deep water populations are the source of shallow water recruits in geographically adjacent populations.

Sampling: Muscle tissue was non-destructively sampled by Alex Anderson from 285 Queen conch individuals between August 15 and August 18, 2017. Approximately 100 milligrams of muscle was dissected from the foot and stored in ethanol before shipment to Cornell University for genetic analysis. Nine distinct sites representing seven widespread geographic locations were sampled: 1) Turneffe_Deep (N 17.42952/43396, W 87.80244/79946, n=32), 2) Turneffe_Northeast (N 17.56423, W 87.75996, n=32), 3) Turneffe_SouthWest (N 17.36551, W 87.94231, n=33), 4) Lighthouse Reef (N 17.20846, W 87.53268, n=29), 5) San Pedro (N 17.84428, W 87.98481, n=32), 6) Glovers Reef (N 16.77439, W 87.86479, n=30), 7) Dangriga (N 16.80464, W 88.08762, n=32), 8) Laughing Caye National Park (N 16.44813, W 88.09828, n=32), 9) Sapodilla Cayes (N 16.24259, W 88.19145, n=33). The samples were collected under Belize Fisheries permit number 000048-17 and exported under CITES permit number 6661.

Genetic Methods: Genetic marker development, data collection and analyses were conducted at the Evolutionary Genetics Core Facility and the BioResource Center, both at Cornell University.

A genomic DNA library enriched for microsatellite loci was constructed and screened with DNA from a single Queen conch from Turneffe. The DNA was digested with three different 4-base restriction enzymes that generate blunt-ended fragments. The digestions were pooled, adenylated, ligated to an Illumina Truseq adapter, and enriched for simple repeats by hybridization to biotinylated synthetic oligonucleotide probes. Probe/DNA complexes were magnetically captured; the resulting products were amplified by PCR and sequenced on an Illumina Miseq platform (2 x 250 base-pair reads). Sequence data were assembled *de novo* with NGen software, and the resulting contigs were scanned for microsatellites with *msatcommander* v 1.0.8. We focused on tetrameric (4-base) loci and designed primers to 85 loci with BatchPrimer3 software. Eighty loci gave clear products upon PCR testing; these 80 were used to genotype our individual Queen conch samples from Belize.

DNA was extracted from a portion of the muscle tissue collected from each sample by a rapid Sodium Hydroxide/Tris-HCl preparation (Truett, GE et al., 2000). Each conch DNA sample was amplified at 80 microsatellite loci with a Qiagen multiplex PCR kit and uniquely barcoded with Illumina Nextera dual barcodes. The barcoded samples were pooled, size-selected with Ampure XP and sequenced on an Illumina Miseq platform (2 x 250 base-pair reads).

The sequence data for each sample was analyzed with a custom Perl script that removes low quality reads, sorts reads by the forward PCR primer sequence, merges the forward and reverse reads, and records the top two reads for each sample as the genotype at that locus (given a heterozygous allele proportion of 0.2). The haplotypes table was converted to R-format and two analyses were performed with the software package *adegenet* (Jombart, T., 2008): 1) An "unsupervised" assessment of the number of distinct genetic clusters with the SnapClust command (Beugin etal., 2018), and 2) a "supervised" discriminant analysis of principle components (DAPC). For each analysis, we analyzed all samples and all geographic sites. The likely number of clusters was inferred from the shape of the plot of the Akaike Information Criterion (AIC), for between 1 and 8 possible clusters. For DAPC, the top three discriminant functions were kept (three axes), and scatterplots were produced for all samples that represent axes 1 and 2, 1 and 3, and 2 and 3. We also exported a table of posterior assignments (the likelihood that a given sample assigns to a particular site, see Appendix) and generated a heatmap of posterior assignments for all genotyped individuals.

Results: 281 conch were successfully genotyped (less than 20% missing data) at 80 microsatellite loci. The AIC plot is shown in Figure 1. Scatterplots for all sites (three discriminate functions) and the heatmap of posterior assignments are shown in Figures 2-5:

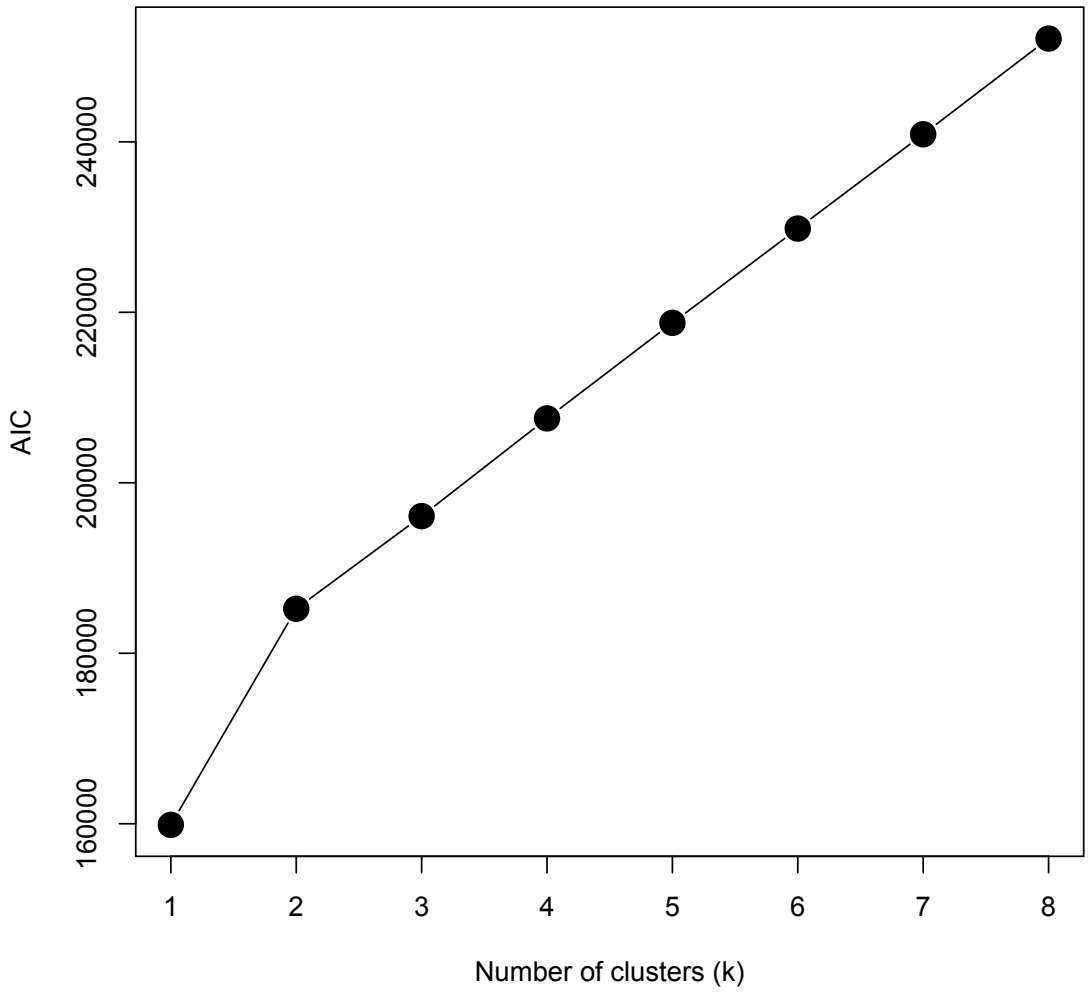

Figure 1. AIC plot from SnapClust in *adegenet*, showing optimal number of clusters (all data) =1.

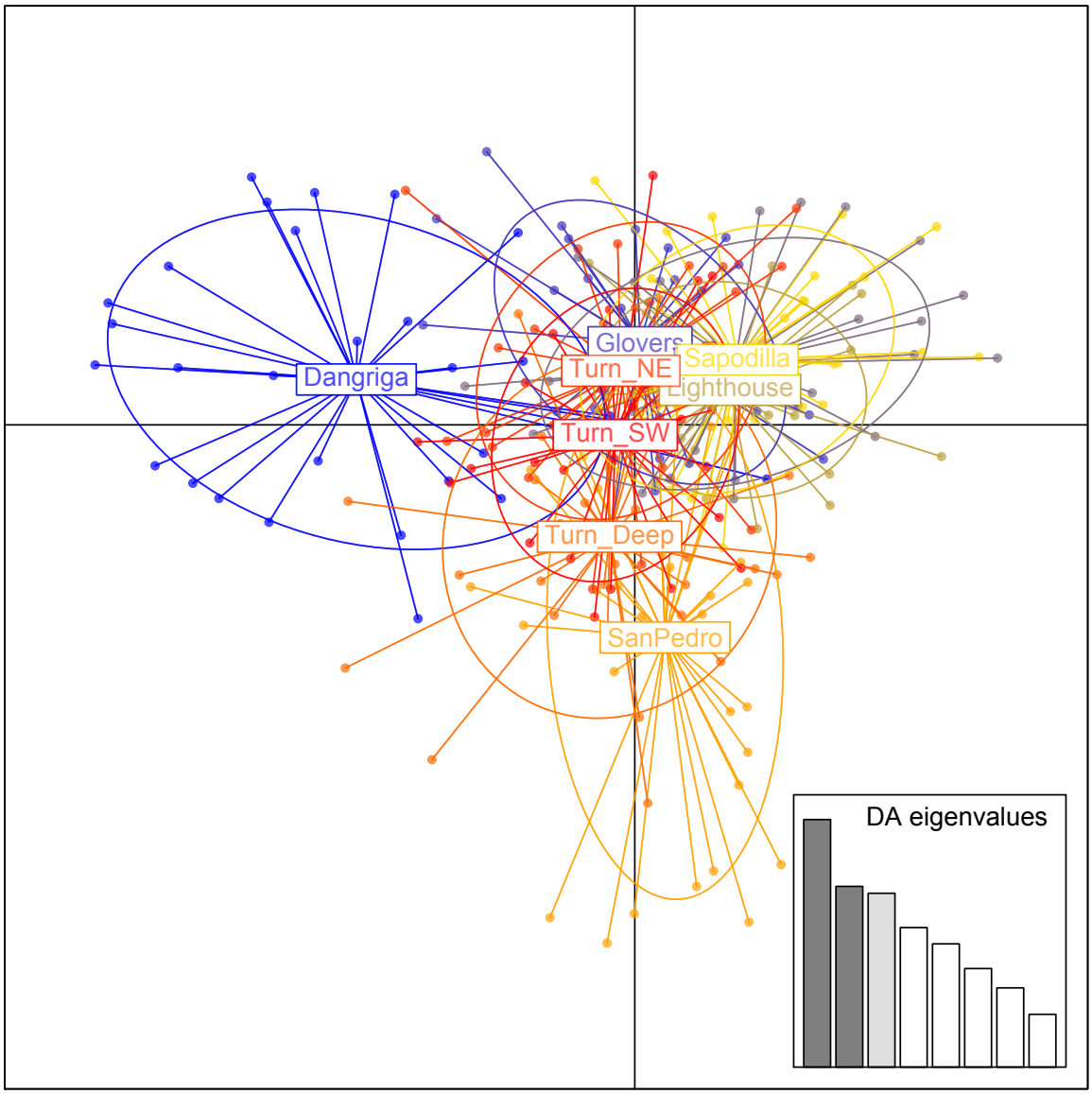

Figure 2. Scatterplot for all samples/sites. Discriminant functions (i.e. axes) 1 and 2.

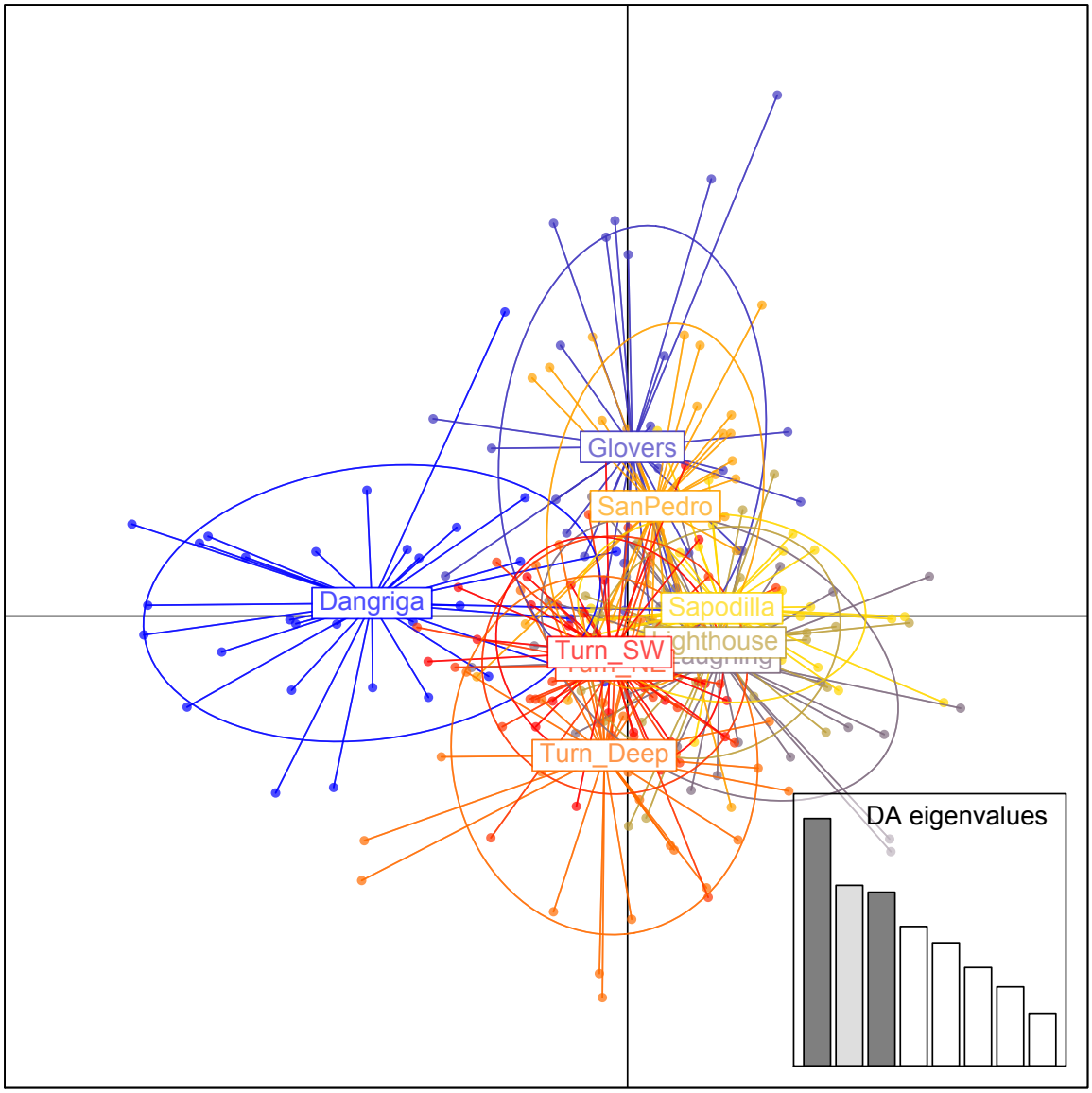

Figure 3. Scatterplot for all samples/sites. Discriminant functions (i.e. axes) 1 and 3.

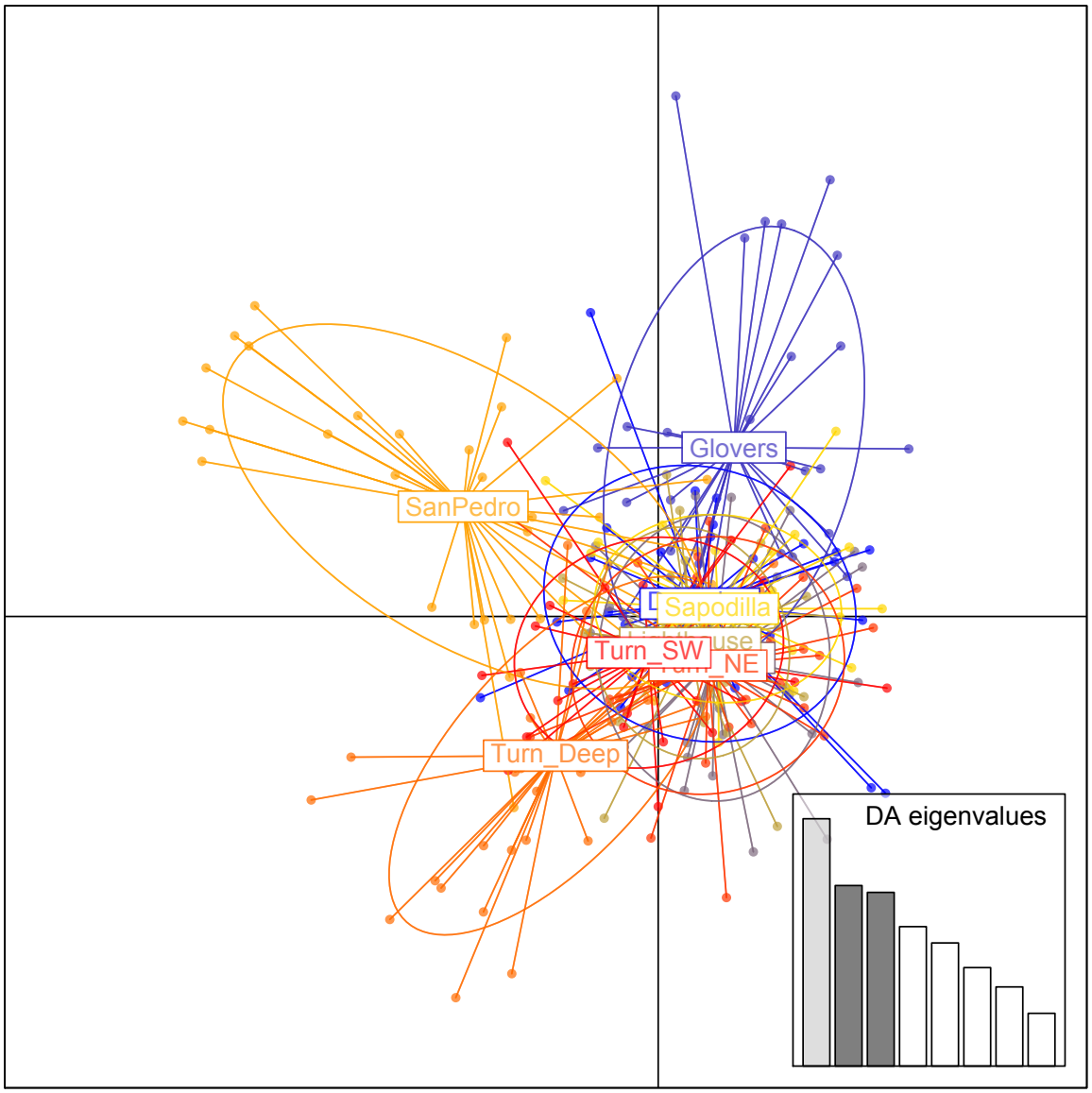

Figure 4. Scatterplot for all samples/sites. Discriminant functions (i.e. axes) 2 and 3.

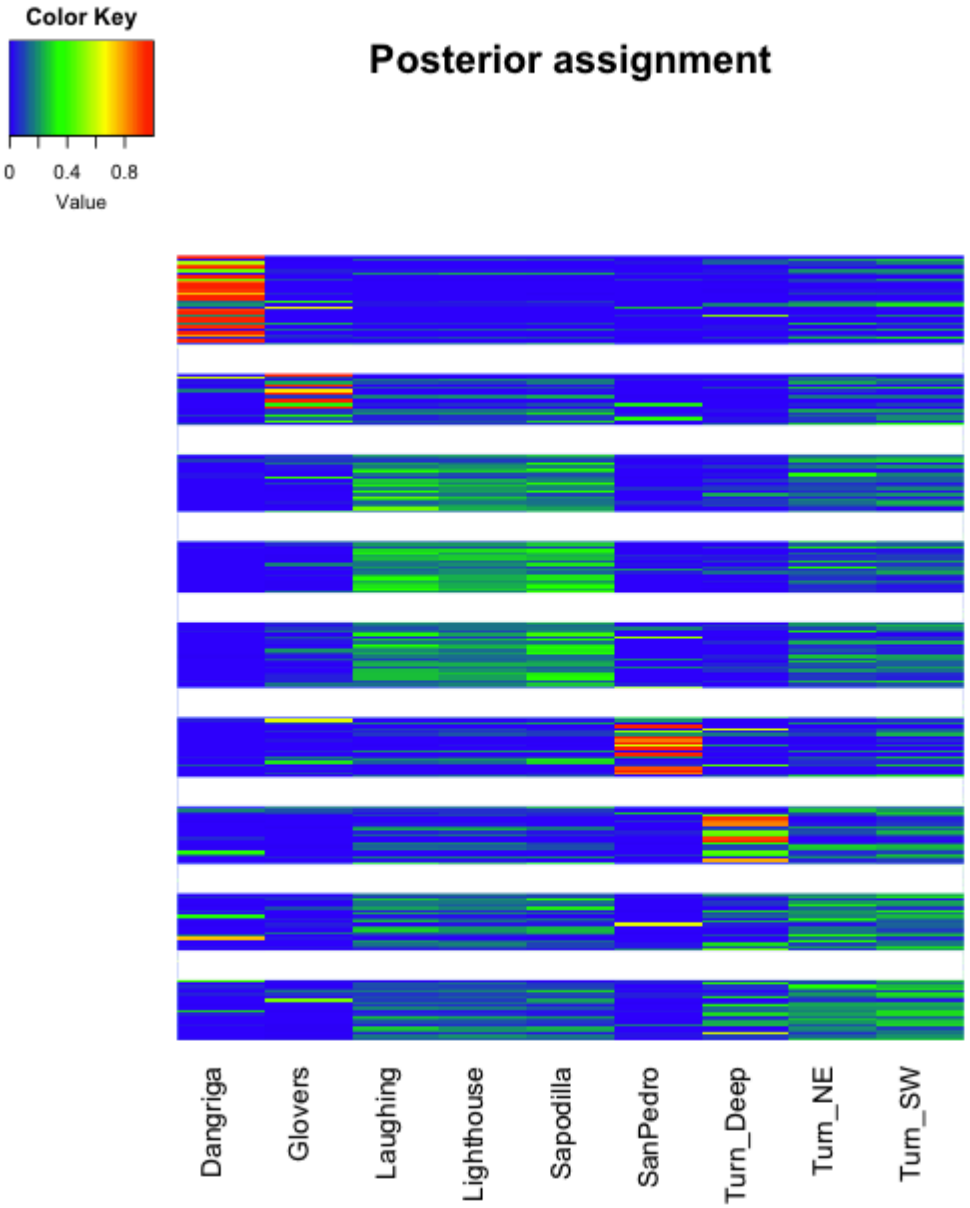

Figure 5. Heatmap of posterior assignments, all samples and geographic sites. Population labels are in the same order along the Y-axis (from top to bottom) as they are along the X-axis (left to right), meaning that blocks along the diagonal represent values for samples assigning to the site where they were collected.

Discussion: Previous studies of Queen conch genetic population structure have found some evidence of SGS, albeit over much broader areas throughout the Caribbean and Mesoamerica

(Machkour-M'Rabet et al., 2017, Truelove et al., 2016 and 2017). The data presented here are unprecedented in three respects; 1) the number of microsatellite loci analyzed, 2) the intensive sampling throughout Belize and 3) the scoring of genotypes from DNA sequence data. Traditionally, microsatellite genotypes were determined by allele *size* after fluorescent PCR and capillary electrophoresis. Alleles of equal size were assumed to be "identical by descent." Here, alleles are determined by nucleotide sequence, which includes both size (length of sequence) and nucleotide variants. Given that fragments of equal size may contain underlying polymorphism only revealed by sequencing, this approach should minimize levels of homoplasy (any mistaken assumption of identity by descent) relative to scoring allele sizes alone.

A "supervised" DAPC analysis means that sample location is taken into consideration; DAPC functions to maximize inter-group distances while minimizing within-group variances. This is in contrast to the "unsupervised" nature of the SnapClust function, which is blind to sampling location when attempting to identify the optimal number of clusters indicated by the data.

The AIC plot (Fig.1) from SnapClust in *adegenet* indicates that the most likely number of clusters is one for this dataset, consistent with a single, panmictic genetic population of Queen conch throughout the region sampled. The scatterplots and heatmap generated by the supervised DAPC analysis are generally consistent with this single cluster scenario, although there are some subtle, yet intriguing, caveats.

Four geographic sites (Dangriga, Glovers, San Pedro, and Turneffe_Deep) appear somewhat more isolated than the remaining sites. In Figures 2 and 3, the Dangriga ellipse of samples is pulling away from the central, dense cluster of points that define most of the other samples and locations. This isolation is not complete, however, since the right side of the Dangriga ellipse is embedded in the central cluster. Similar arguments can be made for SanPedro, Glovers, and Turneffe_Deep, particularly in Figure 4.

The heatmap (Figure 5) is another way of depicting the information in the scatterplots. A posterior assignment value is the likelihood that a given sample "belongs" to a particular geographic location, based on the genotype of that individual. Each row in this heatmap represents the color-coded posterior assignment values for a single individual. Every individual has nine values, one for each geographic location indicated along the bottom of the heatmap. The sum of all assignment values equals one for each sample. These values are color-coded here; blue indicates low assignment likelihoods, green and yellow represent more intermediate values, and red indicates high likelihood. The heatmap is a color-coded representation of the explicit assignment values in the Appendix.

Consider Dangriga, the uppermost block of assignments in the heatmap (the top rectangle of 31 samples by nine populations). Most individuals from Dangriga assign to Dangriga with reasonably high likelihood; hence the left uppermost block (assignment values of Dangriga genotypes to Dangriga, where they were collected) is largely a "warm" collection of reds and yellows. With some exceptions, the eight blocks to the right exhibit the "cooler" blues of lower assignment values (see the full table of posterior assignments in the Appendix, since the sample rows are difficult to fully resolve in the heatmap). Similar patterns are seen for Glovers (second block down,

second block from the left), San Pedro, and Turneffe_Deep. It's also interesting that samples from Laughing Caye, Lighthouse, and Sapodilla Cayes appear to assign to each other with roughly equal likelihood (the large, green, 3 x 3 block of values, upper left of center in the heatmap). Unfortunately, the deep water sample from Turneffe is the only deep water location sampled, so we do not know if this pattern would hold for other deep/shallow comparisons as well, or whether multiple deep water sites would show greater affinity to other deep water sites, relative to their shallow water geographic counterpart. Whether these "partially isolated" sites represent incipient population differentiation or are simply a transient pattern driven by stochastic processes is an open question that only additional sampling across multiple years would begin to resolve.

Conclusions and Recommendations: Genotypes for Queen conch (*Lobatus gigas*) at 80 microsatellite DNA loci from 281 individuals collected at nine sites across Belize indicate that the entire region represents a single panmictic population. "Quasi-panmictic" might be more appropriate, since there are some subtle hints of differentiation at four locations (Dangriga, Glovers, San Pedro, and the deep water site at Turneffe). These four sites are at best partially isolated, so we conclude that Belizean Queen conch, at the geographic sites sampled in this study, represent a single genetic population and management unit.

Lack of SGS in the region, however, does not necessarily mean that most recruitment is non-local. Theoretically, low levels of dispersal and gene flow are sufficient to genetically homogenize populations. What this means is there may be a disconnect between the (relatively low) number of recruits sufficient to homogenize allele frequencies, and the (relatively high) number of recruits needed to quickly replenish a geographic location that has been locally overharvested. If determining levels of local recruitment is a priority, we recommend intensive deep/shallow water sampling of several thousand individuals at one or two geographic sites. From the same panel of microsatellites, numbers of parent/offspring pairs could be determined, which would be a proxy for local vs non-local recruitment.

Literature Cited

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Appendix. Posterior assignments for all samples. Values >0.5 are in bold.

Sample	Dangriga	Glovers	Laughing	Lighthouse	Sapodilla	SanPedro	TA_DE	TA_NE	TA_SW
D_1	**0.99494**	0.00002	0.00001	0.00001	0.00001	0.00001	0.00196	0.00112	0.00193
D_10	0.01372	0.01346	0.12178	0.12447	0.10157	0.00747	0.11286	0.23901	0.26566
D_11	**0.56800**	0.00788	0.00517	0.00810	0.00531	0.03536	0.12162	0.07201	0.17655
D_12	**0.90722**	0.00040	0.00214	0.00132	0.00110	0.00000	0.00471	0.06165	0.02146
D_13	**0.98453**	0.00176	0.00022	0.00018	0.00025	0.00000	0.00019	0.00882	0.00406
D_14	0.47350	0.04504	0.02373	0.02608	0.02799	0.00371	0.02713	0.18452	0.18831
D_15	0.00027	0.02355	0.19283	0.23345	0.22647	0.05462	0.04529	0.08701	0.13651
D_16	**0.97673**	0.00113	0.00022	0.00026	0.00023	0.00009	0.00204	0.00908	0.01023
D_17	0.45995	0.09275	0.01187	0.01858	0.01954	0.07303	0.03299	0.09609	0.19519
D_18	**0.84103**	0.00283	0.00360	0.00346	0.00285	0.00016	0.01615	0.07069	0.05924
D_19a	**0.99964**	0.00009	0.00000	0.00000	0.00000	0.00000	0.00001	0.00011	0.00015
D_2	**0.97362**	0.00851	0.00035	0.00033	0.00055	0.00001	0.00018	0.01045	0.00599
D_20	**0.92197**	0.00228	0.00073	0.00101	0.00075	0.00148	0.01442	0.02107	0.03630
D_21	**0.74429**	0.05968	0.00786	0.00854	0.01168	0.00113	0.00471	0.08683	0.07530
D_22	**0.97424**	0.00010	0.00035	0.00021	0.00017	0.00000	0.00123	0.01785	0.00586
D_23	**0.99778**	0.00049	0.00001	0.00001	0.00001	0.00002	0.00007	0.00071	0.00090
D_24	0.14937	**0.37399**	0.03780	0.04645	0.07552	0.01645	0.00693	0.14030	0.15318
D_25	0.19292	0.01002	0.03266	0.03837	0.02696	0.01017	0.17561	0.21048	0.30281
D_26	0.04050	**0.65972**	0.00166	0.00361	0.00764	0.25900	0.00046	0.00714	0.02027
D_27	**0.90046**	0.04463	0.00107	0.00150	0.00213	0.00232	0.00147	0.01979	0.02662
D_28	**0.99834**	0.00048	0.00001	0.00001	0.00001	0.00000	0.00002	0.00065	0.00048
D_29	0.19257	0.00098	0.00393	0.00654	0.00275	0.05161	0.51209	0.05192	0.17760
D_3	**0.99939**	0.00004	0.00000	0.00000	0.00000	0.00000	0.00003	0.00027	0.00026
D_30	**0.99939**	0.00003	0.00000	0.00000	0.00000	0.00000	0.00003	0.00030	0.00025
D_31	0.18031	0.22745	0.06433	0.05503	0.09889	0.00066	0.00373	0.24355	0.12606
D_32	**0.93355**	0.00023	0.00021	0.00031	0.00015	0.00085	0.03058	0.01055	0.02356
D_5	0.01550	0.14247	0.10275	0.12754	0.15901	0.04455	0.02660	0.16312	0.21846
D_6	**0.99206**	0.00008	0.00002	0.00003	0.00002	0.00009	0.00237	0.00179	0.00355
D_7	**0.79273**	0.07883	0.00632	0.00534	0.01017	0.00007	0.00067	0.07277	0.03310
D_8	0.04327	0.04607	0.08839	0.10191	0.09852	0.01951	0.07236	0.23383	0.29613
D_9	**0.99156**	0.00061	0.00003	0.00004	0.00003	0.00054	0.00123	0.00176	0.00420
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
GL_1	0.01292	0.13670	0.12386	0.13790	0.18406	0.01712	0.01832	0.17933	0.18978
GL_10	0.47385	0.43321	0.00385	0.00511	0.01109	0.00417	0.00056	0.03337	0.03479
GL_11	0.05693	0.34520	0.08205	0.07251	0.14539	0.00109	0.00237	0.19112	0.10334
GL_12	0.00377	0.47864	0.03861	0.06109	0.10776	0.19070	0.00352	0.04048	0.07543
GL_13	0.00355	**0.54804**	0.07685	0.07292	0.18814	0.00182	0.00050	0.06815	0.04004
GL_14	0.00007	0.11796	0.15587	0.20693	0.31349	0.10219	0.00554	0.03797	0.06000
GL_15	0.00519	0.04001	0.15292	0.16767	0.17227	0.01780	0.04875	0.18134	0.21403
GL_16	0.00009	0.36483	0.09721	0.13062	0.27540	0.07135	0.00111	0.02500	0.03440
GL_17	0.00199	0.16213	0.17625	0.16001	0.28659	0.00261	0.00331	0.12535	0.08175
GL_18	0.00000	**0.97349**	0.00026	0.00055	0.00357	0.02196	0.00000	0.00006	0.00011
GL_19	0.00100	**0.88613**	0.01416	0.01685	0.05854	0.00337	0.00005	0.01108	0.00881

GL_2	0.00055	**0.98729**	0.00071	0.00111	0.00546	0.00309	0.00000	0.00084	0.00094
GL_20	0.60432	0.09761	0.01928	0.01623	0.02736	0.00019	0.00242	0.15604	0.07655
GL_21	0.00225	0.03179	0.15183	0.18253	0.17298	0.04439	0.06579	0.13994	0.20850
GL_22	0.03808	0.22519	0.10693	0.09863	0.16810	0.00218	0.00552	0.21660	0.13877
GL_23	0.01043	**0.94249**	0.00465	0.00525	0.02009	0.00074	0.00002	0.00997	0.00638
GL_24	0.02237	0.02170	0.11235	0.11726	0.10350	0.00867	0.08984	0.24904	0.27526
GL_25	0.16200	**0.69237**	0.01352	0.01161	0.03623	0.00014	0.00012	0.06018	0.02383
GL_26	0.00002	**0.99156**	0.00061	0.00084	0.00596	0.00056	0.00000	0.00025	0.00020
GL_27	0.00281	0.07332	0.17212	0.18275	0.22920	0.01359	0.01965	0.15103	0.15554
GL_2a	0.00094	**0.98139**	0.00095	0.00154	0.00671	0.00564	0.00000	0.00126	0.00156
GL_3	0.00253	**0.98936**	0.00043	0.00067	0.00328	0.00179	0.00000	0.00095	0.00099
GL_30	0.00428	0.32253	0.04083	0.06721	0.10190	0.31013	0.00749	0.04623	0.09941
GL_4	0.00052	**0.98418**	0.00137	0.00172	0.00924	0.00059	0.00000	0.00139	0.00098
GL_5	0.01152	0.06224	0.14497	0.14840	0.17279	0.00850	0.02985	0.21526	0.20648
GL_6	0.00044	0.09373	0.18230	0.20957	0.29204	0.02962	0.01026	0.08296	0.09906
GL_7	0.07219	0.32806	0.04858	0.06532	0.09535	0.05183	0.01301	0.13607	0.18958
GL_8	0.01274	0.12227	0.04212	0.06982	0.07648	0.37504	0.03673	0.07646	0.18835
GL_9	0.00059	0.42658	0.11066	0.10530	0.27051	0.00246	0.00045	0.05156	0.03190
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
LC_1	0.09042	0.02472	0.06164	0.07119	0.05973	0.01477	0.11470	0.24232	0.32052
LC_10	0.00826	0.01756	0.19692	0.14717	0.16208	0.00044	0.02445	0.28809	0.15503
LC_12	0.00001	0.00177	0.32262	0.28889	0.22668	0.00356	0.04107	0.05667	0.05874
LC_13	0.00000	0.02146	0.28553	0.22683	0.43619	0.00075	0.00049	0.01825	0.01050
LC_14	0.00210	0.03206	0.20416	0.19189	0.21728	0.00457	0.02523	0.17378	0.14893
LC_15	0.00786	0.09965	0.08380	0.12106	0.13088	0.17250	0.04574	0.11520	0.22332
LC_16	0.00000	0.00136	0.37702	0.27928	0.30452	0.00048	0.00384	0.02008	0.01341
LC_17	0.00074	0.00131	0.21280	0.17170	0.10877	0.00102	0.15953	0.18693	0.15720
LC_18	0.00566	0.35043	0.11362	0.10934	0.22556	0.00321	0.00189	0.11423	0.07606
LC_19	0.00295	0.30560	0.10811	0.13077	0.22367	0.03205	0.00508	0.08935	0.10242
LC_2	0.32671	0.01551	0.03252	0.03383	0.02851	0.00290	0.07582	0.23796	0.24624
LC_20	0.00077	0.05051	0.20400	0.21297	0.26272	0.01273	0.01730	0.11748	0.12151
LC_21	0.01161	0.09593	0.12268	0.14309	0.16939	0.02767	0.03134	0.17902	0.21927
LC_22	0.08520	0.07735	0.07989	0.08737	0.09775	0.01040	0.03937	0.25450	0.26817
LC_23	0.00036	0.14880	0.21172	0.15874	0.36071	0.00039	0.00070	0.08354	0.03504
LC_24	0.00181	0.02193	0.16845	0.19023	0.17359	0.02565	0.07036	0.14812	0.19986
LC_25	0.00002	0.03254	0.27327	0.23331	0.37787	0.00176	0.00221	0.04732	0.03170
LC_26	0.00000	0.00152	0.37535	0.27507	0.26379	0.00048	0.01084	0.04398	0.02896
LC_27	0.02869	0.01009	0.15379	0.10562	0.10507	0.00016	0.03446	0.38430	0.17781
LC_28	0.02872	0.40764	0.06902	0.07963	0.14112	0.01409	0.00478	0.12926	0.12574
LC_29	0.00024	0.00293	0.26627	0.22375	0.17263	0.00178	0.07210	0.14014	0.12015
LC_3	0.00011	0.09322	0.24594	0.17408	0.39336	0.00024	0.00055	0.06673	0.02578
LC_30	0.00005	0.00166	0.31556	0.25344	0.19299	0.00123	0.05477	0.09911	0.08121
LC_31	0.00060	0.01660	0.16580	0.20600	0.17426	0.06496	0.08758	0.10448	0.17974
LC_32	0.00002	0.00909	0.35494	0.21092	0.33054	0.00005	0.00159	0.06949	0.02335
LC_4	0.00118	0.00233	0.15573	0.15466	0.09559	0.00656	0.22629	0.15852	0.19914
LC_5	0.00000	0.00017	0.45296	0.26058	0.18488	0.00005	0.02295	0.05308	0.02533

LC_6	0.00040	0.00186	0.26501	0.20212	0.14750	0.00067	0.08379	0.17366	0.12498
LC_7	0.00363	0.03673	0.12868	0.16304	0.15203	0.06658	0.07595	0.14134	0.23202
LC_8	0.00053	0.01038	0.18563	0.21223	0.17051	0.03082	0.09814	0.11658	0.17518
LC_9	0.00000	0.00027	0.47578	0.23506	0.21143	0.00001	0.00701	0.05341	0.01703
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
LH_1	0.00882	0.22098	0.13177	0.13023	0.22128	0.00509	0.00536	0.15710	0.11939
LH_10	0.00134	0.00039	0.08824	0.08406	0.03812	0.00260	0.48234	0.13115	0.17177
LH_11	0.00439	0.04059	0.16898	0.17425	0.18883	0.01027	0.03651	0.18549	0.19067
LH_12	0.00365	0.11738	0.14935	0.16901	0.22607	0.02292	0.01624	0.13904	0.15634
LH_13	0.00015	0.23694	0.13263	0.16906	0.30679	0.05831	0.00264	0.04029	0.05320
LH_14	0.00292	0.03796	0.15805	0.18113	0.18257	0.02830	0.05146	0.15453	0.20308
LH_15	0.00014	0.02547	0.25684	0.24004	0.29867	0.00472	0.01108	0.08751	0.07553
LH_16	0.00023	0.01132	0.24656	0.24125	0.22983	0.00770	0.03817	0.10966	0.11529
LH_17	0.00365	0.06069	0.14998	0.17513	0.19352	0.03256	0.03721	0.15089	0.19636
LH_18	0.00924	0.00422	0.09227	0.09960	0.06280	0.01021	0.25967	0.19329	0.26871
LH_19	0.02170	0.05558	0.11896	0.12834	0.13735	0.01254	0.04575	0.23012	0.24966
LH_2	0.03600	0.12591	0.13047	0.11002	0.17097	0.00106	0.00780	0.26730	0.15048
LH_20	0.00100	0.05580	0.20155	0.20622	0.26130	0.01031	0.01573	0.12547	0.12260
LH_21	0.02676	0.01461	0.11991	0.11064	0.09678	0.00262	0.08120	0.29277	0.25471
LH_22	0.00000	0.00745	0.28323	0.29914	0.32852	0.01532	0.00910	0.02520	0.03204
LH_23	0.00019	0.00963	0.31535	0.21458	0.26567	0.00022	0.00781	0.12740	0.05914
LH_24	0.00004	0.01042	0.22206	0.26870	0.23707	0.05805	0.04825	0.05748	0.09793
LH_25	0.00004	0.01047	0.24400	0.27345	0.25420	0.02870	0.03673	0.06261	0.08981
LH_26	0.00443	0.00680	0.17717	0.15393	0.12461	0.00192	0.08914	0.24058	0.20141
LH_27	0.00041	0.17792	0.15405	0.18350	0.29997	0.03548	0.00498	0.06560	0.07808
LH_28	0.00409	0.00120	0.20040	0.12741	0.08813	0.00009	0.10140	0.32050	0.15678
LH_29	0.00033	0.03235	0.13471	0.19109	0.18265	0.19954	0.05086	0.06661	0.14186
LH_3	0.00107	0.00952	0.15111	0.18090	0.13488	0.04229	0.14406	0.12597	0.21020
LH_4	0.00020	0.03174	0.25219	0.23083	0.30295	0.00377	0.00914	0.09381	0.07537
LH_5	0.00002	0.00658	0.34426	0.23912	0.30556	0.00027	0.00453	0.06641	0.03324
LH_6	0.00115	0.01351	0.23485	0.20108	0.20386	0.00198	0.03216	0.17681	0.13460
LH_7	0.00028	0.01464	0.27063	0.22762	0.25709	0.00175	0.01635	0.12279	0.08883
LH_8	0.00002	0.01825	0.30427	0.23385	0.35969	0.00066	0.00232	0.05184	0.02911
LH_9	0.00002	0.00243	0.34975	0.26154	0.24570	0.00061	0.01774	0.07343	0.04877
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
SC_1	0.00008	0.12423	0.21427	0.18470	0.39163	0.00157	0.00086	0.05211	0.03055
SC_10	0.02790	0.05655	0.12051	0.12258	0.13627	0.00697	0.03764	0.25263	0.23897
SC_11	0.00000	0.00172	0.37038	0.27799	0.30617	0.00055	0.00424	0.02327	0.01568
SC_12	0.00004	0.06817	0.22738	0.22800	0.37632	0.00793	0.00281	0.04719	0.04216
SC_13	0.00009	0.01066	0.26889	0.25679	0.25889	0.00621	0.02514	0.08673	0.08660
SC_14	0.00256	0.71386	0.04510	0.04360	0.13259	0.00128	0.00016	0.03890	0.02195
SC_15	0.02897	0.02614	0.08478	0.10179	0.08549	0.02847	0.12138	0.21172	0.31126
SC_16	0.00000	0.01198	0.30265	0.25965	0.36847	0.00191	0.00263	0.03015	0.02256
SC_17	0.00728	0.01888	0.14588	0.15070	0.13391	0.00954	0.08141	0.21427	0.23814
SC_18	0.00878	0.04176	0.14709	0.15435	0.16195	0.01113	0.04515	0.20857	0.22121
SC_19	0.00003	0.01198	0.34190	0.21100	0.34045	0.00008	0.00153	0.06863	0.02441

SC_2	0.00567	0.06197	0.18622	0.16294	0.21958	0.00205	0.01281	0.20829	0.14047
SC_20	0.00590	0.02916	0.14200	0.15913	0.14895	0.02112	0.07173	0.18523	0.23679
SC_21	0.00218	0.07614	0.10896	0.15431	0.16589	0.17116	0.04129	0.09561	0.18447
SC_22	0.02965	0.12656	0.15277	0.10597	0.19404	0.00016	0.00303	0.28520	0.10261
SC_23	0.00000	0.01491	0.32252	0.21143	0.41425	0.00012	0.00041	0.02620	0.01015
SC_24	0.00056	0.10375	0.04756	0.08196	0.10111	0.53957	0.01325	0.03039	0.08186
SC_25	0.00015	0.04184	0.24357	0.23142	0.32224	0.00525	0.00706	0.08042	0.06805
SC_26	0.00841	0.02629	0.15161	0.15243	0.14807	0.00745	0.05761	0.22285	0.22529
SC_27	0.00001	0.00584	0.35258	0.23834	0.30975	0.00020	0.00377	0.06069	0.02882
SC_28	0.00004	0.02618	0.26707	0.24809	0.33735	0.00432	0.00601	0.06025	0.05068
SC_29	0.00138	0.19926	0.18197	0.14174	0.31290	0.00053	0.00101	0.11163	0.04957
SC_3	0.00011	0.09533	0.22420	0.19989	0.37419	0.00240	0.00167	0.06163	0.04057
SC_30	0.01009	0.02248	0.16027	0.14687	0.14604	0.00304	0.04806	0.25276	0.21039
SC_31	0.00382	0.10094	0.09769	0.13935	0.15639	0.16924	0.03677	0.10222	0.19358
SC_32	0.00627	0.03907	0.20090	0.15784	0.20525	0.00073	0.01314	0.24236	0.13444
SC_33	0.00035	0.01321	0.23370	0.23354	0.22260	0.00924	0.04163	0.11785	0.12790
SC_4	0.00143	0.02706	0.11307	0.15898	0.13654	0.16653	0.09392	0.09689	0.20558
SC_5	0.00092	0.00410	0.24176	0.19276	0.15719	0.00100	0.06569	0.19421	0.14238
SC_6	0.00006	0.02112	0.25735	0.25833	0.30605	0.00955	0.01259	0.06637	0.06858
SC_7	0.00018	0.04269	0.25413	0.21972	0.32857	0.00207	0.00478	0.08802	0.05984
SC_7a	0.00011	0.04952	0.25631	0.21568	0.35458	0.00153	0.00279	0.07354	0.04595
SC_8	0.02383	0.04638	0.11111	0.12285	0.12368	0.01530	0.05930	0.22976	0.26780
SC_9	0.00101	0.15591	0.14544	0.17904	0.26112	0.04969	0.00982	0.08572	0.11224
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
SP_1	0.00036	0.05485	0.06172	0.10333	0.11028	**0.51441**	0.02481	0.03469	0.09557
SP_10	0.00000	0.00018	0.00001	0.00004	0.00003	**0.99954**	0.00007	0.00001	0.00012
SP_10a	0.00000	0.00014	0.00000	0.00002	0.00002	**0.99967**	0.00005	0.00000	0.00008
SP_11	0.00001	0.00517	0.00146	0.00374	0.00370	**0.97809**	0.00192	0.00079	0.00512
SP_11a	0.00000	0.00196	0.00042	0.00120	0.00113	**0.99229**	0.00086	0.00023	0.00190
SP_12	0.03628	0.00619	0.01282	0.02301	0.01294	0.31103	0.31349	0.06053	0.22371
SP_13	0.00114	0.01564	0.19690	0.20403	0.18613	0.01206	0.06100	0.14993	0.17318
SP_14	0.00003	0.00376	0.00216	0.00530	0.00448	**0.96861**	0.00529	0.00141	0.00896
SP_15	0.00065	0.00744	0.05478	0.08978	0.05866	0.40295	0.17854	0.04890	0.15830
SP_16	0.03039	0.00866	0.08569	0.08919	0.06469	0.00676	0.17107	0.24873	0.29483
SP_17	0.00214	0.16323	0.00167	0.00409	0.00675	**0.80460**	0.00080	0.00318	0.01353
SP_18	0.00658	0.01203	0.04348	0.06950	0.04637	0.27557	0.22191	0.08325	0.24131
SP_2	0.04857	0.63273	0.00726	0.01330	0.02475	0.19279	0.00183	0.02444	0.05432
SP_20	0.00653	0.09428	0.15428	0.16026	0.20971	0.01008	0.01770	0.17808	0.16908
SP_21	0.00000	0.00082	0.00025	0.00076	0.00062	**0.99483**	0.00107	0.00015	0.00149
SP_22	0.00008	0.00018	0.08003	0.09180	0.03587	0.01445	0.61250	0.05281	0.11226
SP_23	0.00215	0.05132	0.04538	0.07743	0.07452	**0.51049**	0.04612	0.04980	0.14279
SP_24	0.00304	0.01680	0.07941	0.11651	0.08652	0.19379	0.16461	0.09966	0.23965
SP_25	0.00000	0.00003	0.00002	0.00007	0.00004	**0.99896**	0.00065	0.00001	0.00022
SP_26	0.00152	0.03698	0.00952	0.01972	0.01980	**0.82974**	0.01544	0.01341	0.05387
SP_27	0.00000	0.00023	0.00001	0.00004	0.00004	**0.99960**	0.00002	0.00000	0.00006
SP_28	0.00242	0.00373	0.01188	0.02322	0.01304	**0.61944**	0.18835	0.02457	0.11336

SP_29	0.00000	0.00003	0.00000	0.00001	0.00001	**0.99939**	0.00039	0.00001	0.00016
SP_3	0.00332	0.04123	0.16995	0.18126	0.19476	0.01444	0.03792	0.16938	0.18774
SP_30	0.00001	0.00007	0.00000	0.00001	0.00001	**0.99957**	0.00017	0.00001	0.00014
SP_32	0.02348	0.16745	0.05521	0.08389	0.09819	0.20175	0.03390	0.11195	0.22418
SP_4	0.00049	0.10391	0.18784	0.20369	0.30316	0.01665	0.00741	0.08699	0.08985
SP_5	0.00048	0.30274	0.12524	0.14686	0.28479	0.02438	0.00215	0.05497	0.05839
SP_6	0.11398	0.00292	0.01095	0.01718	0.00863	0.07091	0.44005	0.08595	0.24942
SP_7a	0.00100	0.03312	0.00491	0.01093	0.01158	**0.89228**	0.00774	0.00701	0.03143
SP_8	0.00000	0.00005	0.00001	0.00003	0.00002	**0.99908**	0.00054	0.00001	0.00026
SP_9	0.00022	0.08020	0.00812	0.01731	0.02407	**0.84027**	0.00251	0.00567	0.02162
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
TA_DE_1	0.01633	0.01882	0.11451	0.12268	0.10402	0.01146	0.10476	0.23143	0.27600
TA_DE_10	0.00268	0.00092	0.07404	0.08018	0.03928	0.00838	0.45864	0.12929	0.20660
TA_DE_11	0.00006	0.00001	0.00198	0.00382	0.00084	0.09520	**0.86791**	0.00372	0.02644
TA_DE_12	0.05596	0.00821	0.06518	0.06973	0.04899	0.00712	0.19018	0.24695	0.30767
TA_DE_13	0.00004	0.00000	0.00219	0.00298	0.00054	0.00289	**0.96853**	0.00463	0.01821
TA_DE_14	0.00002	0.00001	0.01386	0.01695	0.00411	0.00526	**0.90831**	0.01305	0.03843
TA_DE_15	0.00006	0.00009	0.08092	0.08584	0.03125	0.00648	**0.64828**	0.05076	0.09633
TA_DE_16	0.01135	0.00036	0.01568	0.02058	0.00800	0.01502	**0.69442**	0.06848	0.16612
TA_DE_17	0.10046	0.08385	0.04475	0.06387	0.06267	0.09457	0.07248	0.16738	0.30997
TA_DE_18	0.00021	0.00000	0.00576	0.00555	0.00114	0.00022	**0.94038**	0.01629	0.03044
TA_DE_19	0.02615	0.03222	0.06634	0.09125	0.07499	0.09092	0.13782	0.16387	0.31644
TA_DE_2	0.00620	0.16098	0.15692	0.14538	0.24082	0.00306	0.00556	0.16685	0.11423
TA_DE_20	0.17659	0.11358	0.06641	0.06477	0.08679	0.00270	0.01576	0.26858	0.20482
TA_DE_21	0.04686	0.08322	0.03846	0.06140	0.05881	0.25120	0.07355	0.11504	0.27147
TA_DE_22	0.00068	0.00088	0.08012	0.09652	0.04608	0.02555	0.48316	0.08752	0.17948
TA_DE_23	0.00096	0.00000	0.00396	0.00455	0.00103	0.00098	**0.92914**	0.01734	0.04203
TA_DE_24	0.01667	0.00003	0.00059	0.00122	0.00030	0.06943	**0.85245**	0.00782	0.05149
TA_DE_25	0.00017	0.00004	0.02407	0.02882	0.00880	0.00756	**0.82342**	0.03077	0.07636
TA_DE_27	0.00341	0.01609	0.17683	0.17384	0.15827	0.00664	0.06579	0.19784	0.20129
TA_DE_28	0.00010	0.00267	0.23801	0.24201	0.16709	0.01109	0.11894	0.09445	0.12565
TA_DE_29	0.00464	0.00192	0.04799	0.06369	0.03228	0.04565	0.47278	0.10043	0.23062
TA_DE_3	0.00006	0.00050	0.12485	0.14728	0.06791	0.02855	0.44137	0.06079	0.12869
TA_DE_30	0.05514	0.00000	0.00029	0.00043	0.00008	0.00121	**0.90118**	0.00897	0.03269
TA_DE_31	0.00007	0.00000	0.00209	0.00221	0.00036	0.00021	**0.97399**	0.00604	0.01503
TA_DE_32	0.00003	0.00033	0.16266	0.17653	0.08034	0.01502	0.39819	0.05852	0.10839
TA_DE_4	0.01384	0.01073	0.13711	0.12559	0.10479	0.00270	0.09247	0.27128	0.24149
TA_DE_5	0.00765	0.00809	0.15897	0.14000	0.11467	0.00203	0.09074	0.25916	0.21868
TA_DE_6	0.38949	0.00007	0.00228	0.00247	0.00083	0.00041	0.44890	0.05974	0.09582
TA_DE_7	0.15951	0.00351	0.01073	0.01660	0.00866	0.06103	0.39210	0.09255	0.25532
TA_DE_8	0.00696	0.04760	0.15605	0.16191	0.17823	0.01032	0.03621	0.19921	0.20352
TA_DE_9	0.00020	0.00005	0.03482	0.03876	0.01259	0.00509	**0.77732**	0.04229	0.08887
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
TA_NE_1	0.00016	0.02186	0.29346	0.21316	0.31046	0.00040	0.00423	0.10479	0.05148
TA_NE_10	0.02442	0.02989	0.10264	0.11521	0.10399	0.01682	0.08943	0.22929	0.28830
TA_NE_11	0.08492	0.01478	0.09132	0.07813	0.07110	0.00097	0.06335	0.34699	0.24843

TA_NE_12	0.00215	0.00123	0.05576	0.07127	0.03445	0.03479	0.50286	0.09240	0.20509
TA_NE_13	0.00673	0.04325	0.18989	0.15903	0.20109	0.00135	0.01610	0.23402	0.14855
TA_NE_14	0.00183	0.01189	0.25667	0.17787	0.20193	0.00025	0.01640	0.22588	0.10729
TA_NE_15	0.25486	0.09170	0.03136	0.04269	0.04463	0.04274	0.04833	0.17073	0.27296
TA_NE_17	0.06215	0.00032	0.02697	0.02405	0.01044	0.00050	0.48594	0.18932	0.20030
TA_NE_18	0.01144	0.28387	0.11420	0.11458	0.20570	0.00520	0.00419	0.14841	0.11242
TA_NE_19	0.05033	0.01298	0.07650	0.08173	0.06299	0.00805	0.14447	0.25599	0.30696
TA_NE_2	0.00595	0.24741	0.11744	0.13413	0.21549	0.01997	0.00734	0.12367	0.12860
TA_NE_20	0.00300	0.00674	0.13619	0.14866	0.10503	0.01560	0.17573	0.17090	0.23816
TA_NE_21	0.06359	0.05862	0.07631	0.09070	0.09043	0.02345	0.06589	0.22975	0.30127
TA_NE_22	0.00012	0.06635	0.26354	0.18357	0.37983	0.00022	0.00082	0.07599	0.02956
TA_NE_23	0.00447	0.01414	0.16607	0.16330	0.14290	0.00637	0.07819	0.20925	0.21530
TA_NE_24	0.01526	0.00919	0.15573	0.12352	0.10982	0.00069	0.06185	0.31691	0.20702
TA_NE_25	0.00106	0.10628	0.21138	0.17461	0.31087	0.00115	0.00283	0.12354	0.06828
TA_NE_26	0.06213	0.00603	0.04581	0.05388	0.03401	0.01371	0.27171	0.20045	0.31228
TA_NE_27	0.00125	0.00270	0.21830	0.17533	0.12778	0.00100	0.10355	0.20848	0.16161
TA_NE_28	0.37508	0.01000	0.02001	0.02357	0.01719	0.00670	0.12097	0.17849	0.24798
TA_NE_29	0.04012	0.04680	0.14396	0.10869	0.14290	0.00038	0.01374	0.33828	0.16512
TA_NE_3	0.00026	0.00016	0.24635	0.15343	0.07720	0.00008	0.21998	0.19209	0.11044
TA_NE_30	0.01013	0.04895	0.01765	0.03338	0.03070	0.63868	0.04586	0.03972	0.13494
TA_NE_31	0.00147	0.02897	0.22131	0.19807	0.23109	0.00293	0.02006	0.16680	0.12931
TA_NE_32	0.00055	0.01383	0.27740	0.20609	0.24423	0.00051	0.01271	0.15804	0.08664
TA_NE_4	0.00616	0.14058	0.13832	0.15413	0.21365	0.01849	0.01451	0.15245	0.16170
TA_NE_5	0.15136	0.00713	0.06013	0.05223	0.04055	0.00080	0.10184	0.33003	0.25593
TA_NE_6	0.74103	0.02957	0.01240	0.00924	0.01383	0.00004	0.00232	0.13656	0.05500
TA_NE_7	0.01073	0.00945	0.19936	0.13048	0.13641	0.00012	0.02524	0.34320	0.14502
TA_NE_8	0.00019	0.00137	0.15502	0.17432	0.09595	0.02198	0.29927	0.09158	0.16032
TA_NE_9	0.02364	0.02791	0.09476	0.11126	0.09636	0.02484	0.10711	0.21448	0.29965
Sample	**Dangriga**	**Glovers**	**Laughing**	**Lighthouse**	**Sapodilla**	**SanPedro**	**TA_DE**	**TA_NE**	**TA_SW**
TA_SW_1	0.00639	0.00266	0.02636	0.04196	0.02123	0.16933	0.45101	0.06662	0.21445
TA_SW_10	0.02218	0.13726	0.13108	0.12376	0.18317	0.00335	0.01052	0.22534	0.16334
TA_SW_11	0.00065	0.00568	0.14757	0.17835	0.12065	0.04520	0.19024	0.11162	0.20005
TA_SW_12	0.14881	0.11180	0.05938	0.06809	0.08098	0.01292	0.03031	0.23169	0.25602
TA_SW_13	0.54198	0.00800	0.01136	0.01368	0.00997	0.00498	0.08871	0.13254	0.18877
TA_SW_14	0.07192	0.02417	0.03098	0.04787	0.03517	0.15377	0.18717	0.12988	0.31908
TA_SW_15	0.00400	0.08734	0.00832	0.01734	0.02049	0.78310	0.00907	0.01500	0.05533
TA_SW_16	0.00517	0.02582	0.24165	0.14438	0.21336	0.00005	0.00522	0.27833	0.08602
TA_SW_17	0.03895	0.01639	0.06850	0.08300	0.06271	0.02619	0.17127	0.20882	0.32416
TA_SW_18	0.00654	0.01993	0.16260	0.15751	0.14860	0.00539	0.06030	0.22421	0.21492
TA_SW_19	0.03644	0.02344	0.08715	0.09836	0.08333	0.01560	0.11244	0.23701	0.30622
TA_SW_2	0.41644	0.03526	0.01545	0.02228	0.01915	0.04101	0.07629	0.12887	0.24526
TA_SW_20	0.00021	0.00115	0.11344	0.14020	0.07128	0.04365	0.39282	0.07499	0.16226
TA_SW_21	0.05543	0.01416	0.10563	0.09032	0.08170	0.00109	0.06589	0.33947	0.24631
TA_SW_22	0.03149	0.05078	0.09746	0.11257	0.11180	0.02146	0.06540	0.22456	0.28448
TA_SW_23	0.00152	0.16584	0.16457	0.17020	0.28082	0.00934	0.00523	0.10792	0.09457
TA_SW_24	0.04658	0.07784	0.07755	0.09766	0.10091	0.04242	0.05997	0.20245	0.29463

TA_SW_25	0.00160	0.00378	0.10129	0.12610	0.07551	0.04515	0.30048	0.11720	0.22889
TA_SW_26	0.00172	0.49857	0.08788	0.08866	0.21703	0.00379	0.00065	0.06017	0.04152
TA_SW_27	0.00971	0.02922	0.14060	0.14767	0.14197	0.01094	0.06348	0.21720	0.23921
TA_SW_28	0.01186	0.00086	0.07719	0.06811	0.03538	0.00112	0.35840	0.22221	0.22486
TA_SW_29	0.00166	0.00340	0.17988	0.16497	0.11452	0.00332	0.14791	0.19057	0.19377
TA_SW_3	0.27083	0.01757	0.02524	0.03248	0.02465	0.02038	0.13517	0.17931	0.29438
TA_SW_30	0.04819	0.00372	0.05071	0.05533	0.03320	0.00695	0.29616	0.21197	0.29377
TA_SW_31	0.00042	0.00730	0.25332	0.22229	0.20060	0.00266	0.04526	0.14415	0.12399
TA_SW_32	0.00536	0.00591	0.17314	0.14608	0.11626	0.00140	0.09270	0.25609	0.20308
TA_SW_33	0.00911	0.00540	0.10189	0.10884	0.07239	0.01000	0.22108	0.20200	0.26929
TA_SW_4	0.02405	0.01450	0.03980	0.06068	0.04109	0.16445	0.24006	0.11694	0.29842
TA_SW_5	0.00107	0.01692	0.26369	0.19194	0.23473	0.00041	0.01204	0.18466	0.09453
TA_SW_6	0.00116	0.01903	0.20389	0.20600	0.20028	0.00954	0.04650	0.15156	0.16203
TA_SW_7	0.00065	0.00042	0.04854	0.06144	0.02553	0.02672	0.62185	0.06357	0.15128
TA_SW_8	0.00127	0.01718	0.16920	0.19496	0.16846	0.03143	0.08426	0.13610	0.19713
TA_SW_9	0.02208	0.03987	0.12186	0.12650	0.12853	0.00870	0.05366	0.24577	0.25303