

SUPPLEMENTARY FILE S2—Molecular Protocols and Procedures

Reproductive Success Dynamics Could Limit Precision in Close-Kin Mark–Recapture Abundance Estimation for Atlantic Goliath Grouper (*Epinephelus itajara*)

Michael D. Tringali

Fish and Wildlife Research Institute, FL Fish and Wildlife Conservation Commission,
100 Eighth Avenue S.E., Saint Petersburg, FL 33701, USA; mike.tringali@myfwc.com

Molecular markers for this study

Multilocus genotypes were built with microsatellite DNA allelic data over 36 loci. Twenty-nine of the loci were isolated and characterized in-house from a goliath grouper library as described in Seyoum et al. (2013); assay procedures and the analytical suitability of these markers, including conformance to Hardy-Weinberg and population genotypic equilibrium expectations, were reported therein. The remaining seven loci were developed in-house as well, but from a Red Grouper (*Epinephelus morio*) library. Procedures are described in below, with cross-species amplification testing and QAQC information for goliath grouper provided.

Isolation and characterization of red grouper (*Epinephelus morio*) microsatellite markers used in the Atlantic Goliath Grouper study.

Genomic screening of the red grouper library was conducted using the PCR-based isolation of microsatellite arrays (PIMA) method of Lunt et al. (1999), with modifications as reported in Seyoum et al. (2005). Briefly, 50-100 ng of the total DNA was used as the template for multiple randomly amplified polymorphic DNA (RAPD) polymerase chain reactions, PCRs, each with three different RAPD primers following procedures described in Seyoum et al. (2005). The PCR products were purified (Agilent Technologies, Santa Clara, CA) and cloned into plasmid T-vectors (Bluescript PBC KS-, Agilent Technologies). Recombinant colonies were screened by performing PCRs (12.5- μ L total reaction volume) containing T3 and T7 vector primers and three repeat-specific primers [5'-(AC)₈-3', 5'-(AG)₈-3', 5'-(AGC)₅-3', 5'-(ACT)₁₂-3', 5'-(ACC)₆-3', as described in Seyoum et al. (2005)]. Colonies having two or more bands when the PCR product was electrophoresed through 1.5% low-EEG agarose gels were flagged as microsatellite candidates and were transferred to new agar plates for overnight growth at 37°C. Another round of PCR amplification was performed with the selected colonies using only vector primers; the resulting amplification products were purified and cycle-sequenced from both directions using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc. Foster City CA). Sequencing products were visualized on an Applied Biosystems Prism™ 3130XL Genetic Analyzer.

Primers were designed for 33 candidate loci by using the OligoPerfect™ Designer (Invitrogen) with annealing temperature between 58°C and 60°C. Loci were designed such that cloned fragment sizes of the seminal specimen generally occurred within one of three ranges (i.e., 90-115 bp, 125-170 bp, or 190-300

bp). Forward primers were 5'-end-labeled with a fluorescent dye that corresponded to one of the size ranges. PCR was conducted with 100 ng of DNA and three primers, one from each of the three fragment-size category, in 12.5- μ l reaction volume following the step-down protocol described in Seyoum et al. (2016). Fragments were visualized on an ABI 3130XL genetic analyzer alongside ROX-labeled size standard GeneScan-500 (Applied Biosystems, Inc.) and genotyped using GENEMAPPER software version 4.0 (Applied Biosystems, Inc.).

Cross-amplifications were performed for 388 specimens of *E. itejara* from the Florida Atlantic and Florida Gulf of Mexico. Seven loci (**Table S1**) were judged to be reliable and sufficiently informative (variable) for project goals.

Table S1. Characteristics of 7 polymorphic microsatellite loci in 50 specimens of Red Grouper (*Epinephelus morio*) from Tampa Bay, Florida.

Locus	Primer Sequence (5'→3') Forward/Reverse	Repeat motif	Allele sizes red grouper	Allele sizes goliath grouper	GenBank Accession
Em14	CTCCCAAAAGGATTATACAGAGGA AAATGAGTAAGAGGCTTGATGCT	(AC) ₆ /(CA) ₂₂	125-169	119-141	KX833917
Em15	GTGCATGCATGTATCTGTGTAAGA ACAGGGTCAGTGGAGTGAGATT	(AC) ₉	189-202	192-210	KX833918
Em22	GCATGCTTTACATGGTTGT AGTGGAGATGTGTGTGTG	(AC) ₈	104-126	106-124	KX833924
Em23	GATGTGGCTTCCTCTAAAAACA TTTATCTGTAGGCCTATCGTTGC	(TG) ₁₁	139-147	158-190	KX833925
Em24	TTTAAGGGACTTCTCTTCAGC ACACTTTATTGATCCCACGATT	(CA) ₈	208-234	224-242	KX833926
Em28	TCCTGCGTCTTATTCAAACATTA GAGTGTGAGTGGTAAAAGATTG	(TG) ₁₄	109-129	109-123	KX833930
Em33	ATTGTCGTTGGGTAGTGTACAGA GGACATGATCAGTGATATTGGTG	(GT) ₁₁	250-308	266-286	KX833935

General assay procedures for the current study

Total genomic DNA was isolated using the PUREGENE DNA Purification Kit (Gentra Systems, Minneapolis, Minnesota), according to manufacturer's instructions. PCR was performed using the primer pairs in **Table S1** and from **Table 1** of Seyoum et al. (2013). Template-free negative controls were employed in all PCR reactions. Following PCR, a mixture containing 1.0 μ L of PCR product, 12.5 μ L Hi-Di formamide, and 0.15 μ L GSROX500 was denatured (95°C, 4 m) and snap-cooled on ice. Fragments were visualized on a 3130XL Genetic Analyzer (Applied Biosystems) and processed using GENEMAPPER software v4.0.

Numbers of alleles, allelic richness (AR), observed and expected heterozygosities (H_O and H_E), gene diversities, and inbreeding coefficients (F_{IS}) were determined using GENEPOL v4.7 and FSTAT v2.9.3.2; exact tests for Hardy-Weinberg equilibrium within loci and linkage disequilibrium between loci were conducted using GENEPOL v4.7 (Goudet 1995; Raymond and Rousset 1995; Rousset 2008). Single locus

F_{IS} estimates were computed as in Weir and Cockerham (1984) and averaged over all loci. No departures from HWE were detected and, based on probabilities for pairwise estimates of linkage disequilibrium, following sequential Bonferroni adjustment to exact test results, there was no table-wide support for a hypothesis of nonindependence between any locus-pair. The observed value of F_{IS} was 0.021 and significantly positive ($p = 0.00139$). Allelic richness (AR) is displayed in **Figure S1**; single-locus gene diversities in **Figure S2**; green color = goliath grouper loci; red color = red grouper loci. Over all loci, the average number of alleles was 6.75; gene diversities for polymorphic loci ranged from 0.207 to 0.863 (mean = 0.575).

Figure S1. Allelic richness.

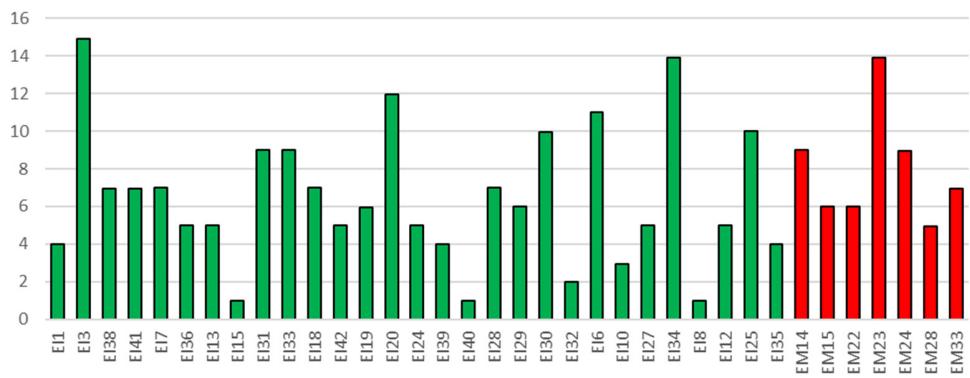
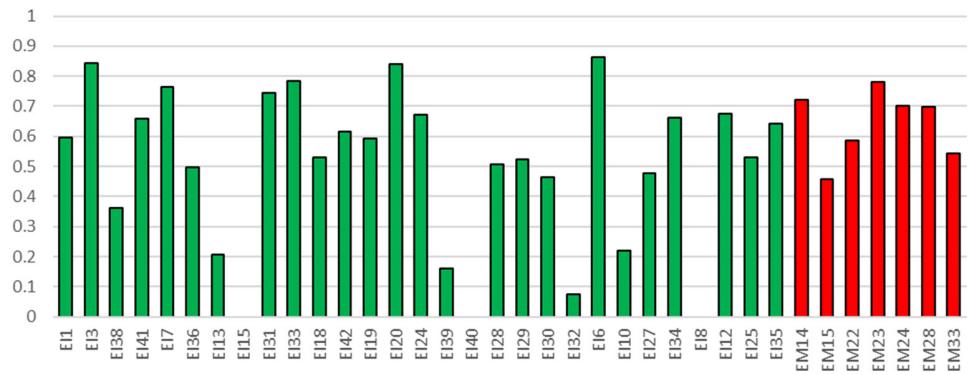


Figure S2. Single-locus gene diversities.



In silico adjustment of possible sources of estimation bias

For the goliath grouper sample, the 2011, 2012, and 2103 sample groups contained fish collected from the same locality that ranged in age from 4 – 19 yr. Given a generation length of ~20 yr, it is appropriate to treat the pooled combination of all three groups as comprising single mixed-age, adult population sample. In silico adjustments were made for mixed-age sampling and physical linkage among loci as follows:

Mixed-age samples: The raw point estimate of N_e could in theory be downwardly biased to a degree because in all studied populations it was based on ‘mixed-age’ adult samples. That is,

additional (artefactual) linkage disequilibrium could have been created by sampling across multiple year classes if those year classes originated with slightly differing allele frequencies. Waples et al. (2014) showed that a downward bias of up to 20-30% might be generally expected given $\tau/T \approx 3$ for the studied subpopulation. Therefore, a bias adjustment was applied to raw estimates of N_e for goliath grouper.

Physical linkage: Removal of genotypes from recaptured goliath grouper eliminated the opportunity for downward bias in the N_e estimate due to redundancy. However, LD in physically linked loci can be elevated by limited recombination and if it is not accounted for, it can cause estimates of N_e to be biased downward. As noted above, there was no table-wide support for a hypothesis of linkage disequilibrium between any locus-pair. Further, it was shown in Waples et al. (2016) that the magnitude of bias in N_e is strongly and negatively correlated with the number of chromosomes and their length, providing two possible methods for bias adjustment. Goliath grouper is known to have 24 chromosomes. Using the relationship based on the number of chromosomes, a correction factor of $1/0.794 = 1.259$ was obtained.

Table S2. Primary data for genetically surveyed specimens.

Fish #	Date	Site Name	Latitude	Longitude	TL cm	Age
123	6/20/2011	Bahia Honda Bridge	24°39.491	81°17.326	144	7
124	6/20/2011	Bahia Honda Bridge	24°39.491	81°17.326	110	5
125	6/20/2011	Bahia Honda Bridge	24°39.491	81°17.326	157	6
126	6/20/2011	Bahia Honda Bridge	24°39.491	81°17.326	127	4
127	6/20/2011	Bahia Honda Bridge	24°39.491	81°17.326	131	5
128	6/27/2011	Sun Tug	26°58.478	80°00.094	143	8
129	6/27/2011	Sun Tug	26°58.478	80°00.094	140	7
130	6/27/2011	Sun Tug	26°58.478	80°00.094	201	13
131	6/27/2011	Sun Tug	26°58.478	80°00.094	191	
132	6/27/2011	Sun Tug	26°58.478	80°00.094	180	12
133	6/27/2011	Sun Tug	26°58.478	80°00.094	179	9
134	6/27/2011	Sun Tug	26°58.478	80°00.094	148	8
135	6/27/2011	Sun Tug	26°58.478	80°00.094	180	13
136	6/27/2011	Sun Tug	26°58.478	80°00.094	149	9
137	6/27/2011	MG111	26°58.640	80°01.496	104	4
138	6/27/2011	MG111	26°58.640	80°01.496	191	10
139	6/27/2011	MG111	26°58.640	80°01.496	129	7
140	6/27/2011	MG111	26°58.640	80°01.496	187	
141	6/27/2011	MG111	26°58.640	80°01.496	176	13
142	6/27/2011	MG111	26°58.640	80°01.496	170	9
143	6/27/2011	MG111	26°58.640	80°01.496	173	11
144	6/27/2011	MG111	26°58.640	80°01.496	141	8

145	6/28/2011	3-Hole	27°00.215	80°03.991	145	8
146	6/28/2011	3-Hole	27°00.215	80°03.991	168	
147	6/28/2011	3-Hole	27°00.215	80°03.991	134	
148	6/28/2011	3-Hole	27°00.215	80°03.991	183	
149	6/28/2011	3-Hole	27°00.215	80°03.991	179	11
150	6/28/2011	3-Hole	27°00.215	80°03.991	117	6
151	6/28/2011	3-Hole	27°00.215	80°03.991	127	
152	6/28/2011	Tunnels	26°56.78	80°00.88	142	7
153	6/28/2011	Tunnels	26°56.78	80°00.88	158	9
154	6/28/2011	Tunnels	26°56.78	80°00.88	122	7
155	6/28/2011	Tunnels	26°56.78	80°00.88	127	7
156	6/28/2011	Tunnels	26°56.78	80°00.88	155	
157	6/28/2011	Tunnels	26°56.78	80°00.88	161	8
158	6/28/2011	Tunnels	26°56.78	80°00.88	137	8
159	6/28/2011	Tunnels	26°56.78	80°00.88	152	7
160	6/29/2011	Zion Train	26°57.81	80°00.40	154	
161	6/29/2011	Zion Train	26°57.81	80°00.40	168	8
162	6/29/2011	Zion Train	26°57.81	80°00.40	166	9
163	6/29/2011	Zion Train	26°57.81	80°00.40	152	7
164	6/29/2011	Zion Train	26°57.81	80°00.40	173	10
165	6/29/2011	Zion Train	26°57.81	80°00.40	165	11
166	6/29/2011	Zion Train	26°57.81	80°00.40	150	10
167	6/29/2011	Zion Train	26°57.81	80°00.40	155	
168	6/29/2011	Zion Train	26°57.81	80°00.40	186	9
169	6/29/2011	Zion Train	26°57.81	80°00.40	215	15
171	6/29/2011	Zion Train	26°57.81	80°00.40	187	
172	6/29/2011	Zion Train	26°57.81	80°00.40	177	
173	6/29/2011	Zion Train	26°57.81	80°00.40	180	
174	6/29/2011	Zion Train	26°57.81	80°00.40	138	8
175	8/12/2011	3-Hole	27°00.215	80°03.991	133	7
177	8/12/2011	Tunnels	26°56.81	80°02.047	145	
178	8/12/2011	Tunnels	26°56.81	80°02.047	136	7
179	8/12/2011	Tunnels	26°56.81	80°02.047	132	9
180	8/12/2011	Tunnels	26°56.81	80°02.047	165	7
181	8/13/2011	3-Hole	27°00.223	80°03.993	220	16
182	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	170	9
183	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	189	13
184	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	215	11
185	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	201	12

186	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	192	11
187	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	151	5
188	8/14/2011	South FAD and assoc. rock piles	26°54.979	80°02.827	126	4
189	8/14/2011	North FAD	26°57.864	80°03.693	192	9
190	8/14/2011	North FAD	26°57.864	80°03.693	185	8
191	8/15/2011	Gulfland			123	4
192	8/15/2011	3-Hole	27°00.223	80°03.993	207	10
193	8/15/2011	3-Hole	27°00.223	80°03.993	171	9
194	8/15/2011	3-Hole	27°00.223	80°03.993	136	7
195	8/15/2011	3-Hole	27°00.223	80°03.993	154	10
196	8/15/2011	3-Hole	27°00.223	80°03.993	155	6
197	8/15/2011	3-Hole	27°00.223	80°03.993	167	7
203	9/10/2011	3-Hole	27°00.223	80°03.993	182	11
204	9/10/2011	3-Hole	27°00.223	80°03.993	153	8
205	9/11/2011				186	10
206	9/11/2011				177	11
207	9/11/2011				186	10
208	9/11/2011				178	11
209	9/11/2011				176	8
210	9/11/2011				183	9
211	9/12/2011	MG111	26°58.640	80°01.496	180	7
212	9/12/2011	MG111	26°58.640	80°01.496	200	14
213	9/12/2011	MG111	26°58.640	80°01.496	195	17
214	9/12/2011	MG111	26°58.640	80°01.496	170	10
215	9/12/2011	MG111	26°58.640	80°01.496	210	
216	9/12/2011	MG111	26°58.640	80°01.496	128	6
217	9/12/2011	MG111	26°58.640	80°01.496	169	7
218	9/12/2011	MG111	26°58.640	80°01.496	162	8
219	9/12/2011	MG111	26°58.640	80°01.496	166	9
220	9/12/2011	MG111	26°58.640	80°01.496	162	6
221	9/12/2011	MG111	26°58.640	80°01.496	161	11
224	9/30/2011	Zion Train	26°57.769	80°00.444	162	9
225	9/30/2011	Zion Train	26°57.769	80°00.444	163	7
226	9/30/2011	Zion Train	26°57.769	80°00.444	181	12
227	9/30/2011	Zion Train	26°57.769	80°00.444	160	9
228	10/2/2011	Zion Train	26°57.769	80°00.444	196	
229	10/2/2011	Zion Train	26°57.769	80°00.444	188	9
230	10/2/2011	Zion Train	26°57.769	80°00.444	150	9
231	10/2/2011	Zion Train	26°57.769	80°00.444	162	9

232	10/2/2011	Zion Train	26°57.769	80°00.444	165	11
233	10/2/2011	Zion Train	26°57.769	80°00.444	156	9
234	10/2/2011	Zion Train	26°57.769	80°00.444	164	7
235	10/2/2011	Zion Train	26°57.769	80°00.444	177	11
246	3/31/2012	3-Hole	27°00.223	80°03.993	156	12
247	3/31/2012	3-Hole	27°00.223	80°03.993	178	10
248	3/31/2012	3-Hole	27°00.223	80°03.993	145	8
256	7/15/2012	Following Long liner boat	27°02.77	80°02.04	180	12
258	7/15/2012	Following Long liner boat	27°02.77	80°02.04	145	8
259	7/15/2012	Following Long liner boat	27°02.77	80°02.04	156	
260	7/15/2012	Following Long liner boat	27°06.3	80°02.6	142	9
262	7/15/2012	Following Long liner boat	27°06.3	80°02.6	186	9
266	7/16/2012	3-Hole	27°00.223	80°03.993	134	6
267	7/16/2012	3-Hole	27°00.223	80°03.993	127	5
268	7/16/2012	3-Hole	27°00.223	80°03.993	188	12
269	7/16/2012	3-Hole	27°00.223	80°03.993	162	
270	7/16/2012	3-Hole	27°00.223	80°03.993	188	17
272	7/16/2012	3-Hole	27°00.223	80°03.993	174	11
274	7/16/2012	Zion Train	26°57.769	80°00.444	188	12
275	7/16/2012	Zion Train	26°57.769	80°00.444	180	
276	7/16/2012	Zion Train	26°57.769	80°00.444	169	11
279	7/16/2012	Zion Train	26°57.769	80°00.444	171	10
280	7/16/2012	Zion Train	26°57.769	80°00.444	156	8
281	7/16/2012	Zion Train	26°57.769	80°00.444	192	12
283	7/16/2012	Zion Train	26°57.769	80°00.444	182	
284	7/16/2012	Zion Train	26°57.769	80°00.444	162	11
285	7/16/2012				189	10
287	7/17/2012				160	11
290	7/17/2012	Zion Train	26°57.769	80°00.444	187	
291	7/17/2012	Zion Train	26°57.769	80°00.444	153	5
292	7/17/2012	Zion Train	26°57.769	80°00.444	176	14
293	7/17/2012	Zion Train	26°57.769	80°00.444	213	19
300	8/6/2012	3-Hole	27°00.223	80°03.993	183	10
301	8/6/2012	3-Hole	27°00.223	80°03.993	172	
302	8/6/2012	3-Hole	27°00.223	80°03.993	168	
303	8/6/2012	3-Hole	27°00.223	80°03.993	194	10
304	8/6/2012	3-Hole	27°00.223	80°03.993	146	6
306	8/18/2012	Sun Tug	26°58.478	80°00.094	132	5
307						

308	8/18/2012	Sun Tug	26°58.478	80°00.094	148	8
309	8/18/2012	Sun Tug	26°58.478	80°00.094	168	12
310	8/19/2012	MG111	26°58.640	80°01.496	181	10
311	8/19/2012	MG111	26°58.640	80°01.496	175	9
312	8/19/2012	MG111	26°58.640	80°01.496	189	8
314	8/19/2012	MG111	26°58.640	80°01.496	143	8
315	8/19/2012	MG111	26°58.640	80°01.496	173	10
316	8/19/2012	MG111	26°58.640	80°01.496	139	5
317	8/19/2012	MG111	26°58.640	80°01.496	176	11
318	8/19/2012	MG111	26°58.640	80°01.496	152	9
319	8/19/2012	MG111	26°58.640	80°01.496	185	8
320	8/19/2012	MG111	26°58.640	80°01.496	167	9
321	8/19/2012	MG111	26°58.640	80°01.496	163	8
323	8/31/2012	Zion Train	26°57.769	80°00.444	145	7
326	8/31/2012	Zion Train	26°57.769	80°00.444	172	7
327	8/31/2012	Zion Train	26°57.769	80°00.444	163	9
328	8/31/2012	Zion Train	26°57.769	80°00.444	143	10
329	8/31/2012	Zion Train	26°57.769	80°00.444	178	12
330	8/31/2012	Zion Train	26°57.769	80°00.444	166	10
332	8/31/2012	Zion Train	26°57.769	80°00.444	166	9
333	8/31/2012	Zion Train	26°57.769	80°00.444	160	11
334	8/31/2012	Zion Train	26°57.769	80°00.444	175	10
335	8/31/2012	Zion Train	26°57.769	80°00.444	200	14
336	8/31/2012	Zion Train	26°57.769	80°00.444	115	5
337	8/31/2012	Zion Train	26°57.769	80°00.444	140	9
338	8/31/2012	Zion Train	26°57.769	80°00.444	149	10
340	8/31/2012	Zion Train	26°57.769	80°00.444	166	10
341	8/31/2012	Zion Train	26°57.769	80°00.444	170	10
342	8/31/2012	Zion Train	26°57.769	80°00.444	146	8
343	8/31/2012	Zion Train	26°57.769	80°00.444	180	11
344	8/31/2012	Zion Train	26°57.769	80°00.444	167	11
345	8/31/2012	Zion Train	26°57.769	80°00.444	147	9
347	9/1/2012	Zion Train	26°57.769	80°00.444	162	7
348	9/1/2012	Zion Train	26°57.769	80°00.444	162	9
349	9/1/2012	Zion Train	26°57.769	80°00.444	168	9
350	9/1/2012	Zion Train	26°57.769	80°00.444	143	12
351	9/1/2012	Zion Train	26°57.769	80°00.444	211	19
352	9/1/2012	Zion Train	26°57.769	80°00.444	181	10
353	9/1/2012	Zion Train	26°57.769	80°00.444	178	9

354	9/1/2012	Zion Train	26°57.769	80°00.444	163	11
355	9/1/2012	Zion Train	26°57.769	80°00.444	167	9
356	9/1/2012	Zion Train	26°57.769	80°00.444	163	10
357	9/1/2012	Zion Train	26°57.769	80°00.444	158	7
359	9/1/2012	Zion Train	26°57.769	80°00.444	195	14
360	9/1/2012	Zion Train	26°57.769	80°00.444	189	13
361	9/1/2012	Zion Train	26°57.769	80°00.444	172	8
362	9/1/2012	Sun Tug	26°58.478	80°00.094	174	15
363	9/1/2012	Sun Tug	26°58.478	80°00.094	163	8
365	9/1/2012	Sun Tug	26°58.478	80°00.094	162	10
366	9/1/2012	Sun Tug	26°58.478	80°00.094	177	10
367	9/1/2012	Sun Tug	26°58.478	80°00.094	167	10
372	9/2/2012	Tunnels	26°56.81	80°02.047	148	9
373	9/2/2012	MG111	26°58.640	80°01.496	129	9
374	9/2/2012	MG111	26°58.640	80°01.496	186	12
375	9/2/2012	MG111	26°58.640	80°01.496	177	10
376	9/2/2012	MG111	26°58.640	80°01.496	122	7
377	9/2/2012	MG111	26°58.640	80°01.496	168	10
378	9/2/2012	MG111	26°58.640	80°01.496	158	9
380	9/2/2012	MG111	26°58.640	80°01.496	158	10
382	9/2/2012	MG111	26°58.640	80°01.496	189	14
383	9/2/2012	MG111	26°58.640	80°01.496	200	13
384	9/2/2012	MG111	26°58.640	80°01.496	185	11
385	9/2/2012	MG111	26°58.640	80°01.496	167	8
389	9/2/2012	MG111	26°58.640	80°01.496	154	11
390	9/2/2012	MG111	26°58.640	80°01.496	174	8
391	9/2/2012	MG111	26°58.640	80°01.496	157	9
393	9/2/2012	MG111	26°58.640	80°01.496	225	16
394	9/2/2012	MG111	26°58.640	80°01.496	155	10
395	9/2/2012	MG111	26°58.640	80°01.496	197	17
396	9/2/2012	MG111	26°58.640	80°01.496	161	8
397	9/2/2012	MG111	26°58.640	80°01.496	178	11
398	9/2/2012	MG111	26°58.640	80°01.496	152	10
400	9/2/2012	MG111	26°58.640	80°01.496	157	8
401	9/2/2012	Sea Inspector			149	9
402	9/2/2012	Sun Tug	26°58.478	80°00.094	122	6
403	9/2/2012	Sun Tug	26°58.478	80°00.094	171	10
404	9/2/2012	Sun Tug	26°58.478	80°00.094	182	11
405	9/15/2012	Zion Train	26°57.769	80°00.444	148	8

406	9/15/2012	Zion Train	26°57.769	80°00.444	164	9
407	9/15/2012	Zion Train	26°57.769	80°00.444	156	9
409	9/15/2012	Zion Train	26°57.769	80°00.444	173	8
410	9/15/2012	Zion Train	26°57.769	80°00.444	152	9
411	9/15/2012	Zion Train	26°57.769	80°00.444	182	11
412	9/15/2012	Zion Train	26°57.769	80°00.444	187	15
413	9/15/2012	Zion Train	26°57.769	80°00.444	208	12
414	9/15/2012	Zion Train	26°57.769	80°00.444	125	11
416	9/15/2012	Sun Tug	26°58.478	80°00.094	158	11
417	9/15/2012	Sun Tug	26°58.478	80°00.094	194	12
418	9/15/2012	Sun Tug	26°58.478	80°00.094	160	9
419	9/16/2012	Zion Train	26°57.769	80°00.444	182	10
420	9/16/2012	Zion Train	26°57.769	80°00.444	167	8
422	9/16/2012	Zion Train	26°57.769	80°00.444	192	16
424	9/16/2012	Zion Train	26°57.769	80°00.444	158	10
425	9/16/2012	Zion Train	26°57.769	80°00.444	155	9
426	9/16/2012	Zion Train	26°57.769	80°00.444	167	9
427	9/16/2012	Zion Train	26°57.769	80°00.444	145	11
428	9/16/2012	Zion Train	26°57.769	80°00.444	180	9
429	9/16/2012	3-Hole	27°00.223	80°03.993	133	7
430	9/16/2012	3-Hole	27°00.223	80°03.993	142	8
431	9/16/2012	3-Hole	27°00.223	80°03.993	105	4
432	9/16/2012	Sun Tug	26°58.478	80°00.094	155	10
433	9/16/2012	Sun Tug	26°58.478	80°00.094	154	10
437	9/16/2012	Zion Train	26°57.769	80°00.444	125	6
438	9/16/2012	Zion Train	26°57.769	80°00.444	168	9
439	9/16/2012	Zion Train	26°57.769	80°00.444	177	12
440	9/16/2012	Zion Train	26°57.769	80°00.444	162	11
443	9/16/2012	Zion Train	26°57.769	80°00.444	161	13
444	9/16/2012	Zion Train	26°57.769	80°00.444	171	11
445	9/16/2012	Zion Train	26°57.769	80°00.444	163	9
446	9/16/2012	Zion Train	26°57.769	80°00.444	159	7
447	9/28/2012	Sun Tug	26°58.478	80°00.094	160	10
448	9/28/2012	Sun Tug	26°58.478	80°00.094	189	13
449	9/28/2012	Sun Tug	26°58.478	80°00.094	170	12
451	9/28/2012	Sun Tug	26°58.478	80°00.094	170	11
453	9/28/2012	Sun Tug	26°58.478	80°00.094	185	12
454	9/28/2012	Zion Train	26°57.769	80°00.444	166	9
455	9/28/2012	Zion Train	26°57.769	80°00.444	183	11

456	9/28/2012	Zion Train	26°57.769	80°00.444	159	10
457	9/28/2012	Zion Train	26°57.769	80°00.444	157	9
458	9/28/2012	Zion Train	26°57.769	80°00.444	160	8
459	9/28/2012	Zion Train	26°57.769	80°00.444	136	10
461	9/28/2012	Zion Train	26°57.769	80°00.444	176	12
464	9/29/2012	Zion Train	26°57.769	80°00.444	135	6
465	9/29/2012	Zion Train	26°57.769	80°00.444	155	8
466	9/28/2012	Sun Tug	26°58.478	80°00.094	166	10
467	9/30/2012	Zion Train	26°57.769	80°00.444	158	8
469	9/30/2012	MG111	26°58.640	80°01.496	162	10
470	9/30/2012	MG111	26°58.640	80°01.496	160	9
477	8/18/2012	Sun Tug	26°58.478	80°00.094	168	
478	8/18/2012	Sun Tug	26°58.478	80°00.094	152	9
479	8/18/2012	Sun Tug	26°58.478	80°00.094	205	
480	8/18/2012	Sun Tug	26°58.478	80°00.094	185	
481	8/18/2012	Sun Tug	26°58.478	80°00.094	198	11
482	8/18/2012	Sun Tug	26°58.478	80°00.094	126	5
483						
484	8/8/2013	Zion Train	26°57.769	80°00.444	167	9
485	8/8/2013	Zion Train	26°57.769	80°00.444	167	11
486	8/8/2013	Zion Train	26°57.769	80°00.444	181	
488	8/8/2013	Zion Train	26°57.769	80°00.444	164	
489						
493	8/8/2013	Zion Train	26°57.769	80°00.444	188	11
494	8/8/2013	3-Hole	27°00.223	80°03.993	152	
495	8/8/2013	3-Hole	27°00.223	80°03.993	129	107
496	8/8/2013	3-Hole	27°00.223	80°03.993	138	9
497	8/8/2013	3-Hole	27°00.223	80°03.993	167	
498	8/8/2013	3-Hole	27°00.223	80°03.993	168	7
499	8/8/2013	3-Hole	27°00.223	80°03.993	102	4
500	8/8/2013	3-Hole	27°00.223	80°03.993	123	6
501	8/8/2013	Gulf Land	27°04.822	80°05.133	160	9
502	8/8/2013	Gulf Land	27°04.822	80°05.133	126	7
503	8/8/2013	Tunnels	26°56.81	80°02.047	118	6
504	8/8/2013	Tunnels	26°56.81	80°02.047	123	6
505	8/8/2013	Tunnels	26°56.81	80°02.047	150	10
506	8/9/2013	Sun Tug	26°58.478	80°00.094	153	10
507	8/9/2013	Sun Tug	26°58.478	80°00.094	178	14
508	8/9/2013	MG111	26°58.640	80°01.496	190	12

509	8/9/2013	MG111	26°58.640	80°01.496	191	14
510		FL Keys	24.63828	-82.94952		
511		FL Keys	24.659045	-81.280365		
512		FL Keys	24.659045	-81.280365		
513		FL Keys	24.659045	-81.280365		
514		FL Keys	24.659045	-81.280365		
515		FL Keys	24.659045	-81.280365		

References for Supplementary File S2

- Goudet, J. (1995). Fstat (version 1.2): A computer program to calculate f- statistics. *Journal of Heredity*, 86(6), 485–486.
- Lunt, D.; Hutchinson, W.; Carvalho, G. An efficient method or PCR-based isolation of microsatellite arrays (PIMA). *Mol. Ecol.* **1999**, 8, 891–894.
- Raymond, M., & Rousset, F. (1995). GENEPOL (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248-249.
- Rousset, F. (2008). Genepop'007: a complete reimplementation of the GENEPOL software for Windows and Linux. *Molecular Ecology Resources*, 8, 103-106.
- Seyoum, S.; Tringali, M.D.; Sullivan, J.G. Isolation and characterization of 27 polymorphic microsatellite loci for the common snook, *Centropomus undecimalis*. *Mol. Ecol. Notes*. **2005**, 5, 922–927.
- Seyoum, S.; Tringali, M.D.; Barthel, B.L.; Puchulutegui, C.; Davis, M.C.; Collins, A.B.; Craig, M.T. Isolation and characterization of 29 polymorphic microsatellite markers for the endangered Atlantic goliath grouper (*Epinephelus itajara*), and the Pacific goliath grouper (*E. quinquefasciatus*). *Cons. Gen. Res.* **2013**, 5, 729–732.
- Seyoum, S.; Puchulutegui, C.; McBride, R.S. Isolation and characterization of 24 polymorphic microsatellite loci for the study of genetic population structure of the sheepshead *Archosargus probatocephalus* (Actinopterygii, Perciformes, Sparidae). *BMC Res. Notes* **2016**, 9, 251. DOI 10.1186/s13104-016-2058-7
- Waples, R. S.; Antao, T.; Luikart, G. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics*, **2014**, 197, 769–780.
- Waples, R. K.; Larson, W. A.; Waples, R. S. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, **2016a**, 117, 233–240.