Population genetics of cobia *Rachycentron canadum*: Management implications along the Southeastern US coast

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1 Population Genetics of Cobia *Rachycentron canadum*: Management Implications along the
2 Southeastern U.S. Coast

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14 **Abstract and Key Words**

15 Cobia *Rachycentron canadum* is a pelagic, migratory species with a nearly cosmopolitan
16 distribution throughout tropical and subtropical waters. Commercial and recreational *R. canadum*
17 harvests from Gulf of Mexico and Atlantic waters have been highly variable over the past several
18 decades. Meanwhile, recreational fishing pressure on *R. canadum* has increased substantially
19 during this period, especially in areas where they exhibit annual inshore aggregations, making
20 them potentially susceptible to overfishing. Although *R. canadum* along the southeastern U.S.
21 Atlantic and Gulf of Mexico coasts is currently managed as a single fishery within U.S. waters,
22 the genetic composition of *R. canadum* in these areas is unclear. Based on a robust microsatellite
23 dataset from collections made along both coasts, the offshore groups were determined to be
24 genetically homogenous, even between the Atlantic and Gulf of Mexico. However, the two
25 sampled inshore aggregations (South Carolina and Virginia) were genetically distinct from each
26 other as well as the offshore group. The recapture of stocked fish within their release estuary 1-3
27 years post-release suggests some degree of estuarine fidelity occurs within these inshore
28 aggregations, supporting the detection of their unique genetic population structure. These results
29 complement the observed high site fidelity of *R. canadum* in South Carolina and support a recent
30 study confirming the spawning function of the inshore aggregations. Our increased
31 understanding of *R. canadum* life history will be beneficial from a management perspective, both
32 in terms of determining the appropriate scale of *R. canadum* management as well as assessing
33 potential risks from offshore aquaculture operations.

34

35 Key Words: applied fisheries research; microsatellites; Rachycentridae; spawning aggregations

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37 **Introduction**

38

39 Cobia *Rachycentron canadum* (Linneaus 1766), belonging to the monotypic Family
40 Rachycentridae (Actinopterygii: Perciformes), is a large, pelagic, migratory species with a nearly
41 cosmopolitan distribution throughout tropical and subtropical waters. The species is highly
42 prized by both recreational fisheries and aquaculture for its excellent table fare and fast growth.
43 Within the U.S., this recreationally and commercially important fish species occurs along the
44 southeastern Atlantic (SA) and Gulf of Mexico (GOM) coasts. *R. canadum* is currently managed
45 by the South Atlantic Fishery Management Council and Gulf of Mexico Fishery Management
46 Council as a single reproductive stock based on minimal tag/recapture and mitochondrial
47 fragment analysis data (Hrincevich, 1993). Most early life history information on *R. canadum*
48 comes from aquaculture research and little is known about its natural life history.

49

50 In the spring and early summer months, *R. canadum* in the western North Atlantic is thought to
51 migrate with warming waters from Florida to the Chesapeake Bay (Shaffer & Nakamura, 1989).
52 During this putative northward migration, *R. canadum* enters high salinity bays and estuaries,
53 including Port Royal Sound (PRS) and St. Helena Sound (SHS) in South Carolina (SC), Pamlico
54 Sound in North Carolina (NC; Smith, 1996), and the Chesapeake Bay (Shaffer & Nakamura,
55 1989). *R. canadum* has been reported to spawn from April through September (Lotz *et al*., 1996;
56 Smith, 1996; Burns *et al*., 1998; Brown-Peterson *et al*., 2001). Regional peaks in spawning
57 correlate with their proposed annual migration from Florida to Massachusetts, occurring in May
58 along the SC coast (Shaffer & Nakamura, 1989; Burns *et al*., 1998), June in NC (Smith, 1996),
59 and during June and July in the Chesapeake Bay region (Joseph *et al*., 1964).

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60

61 One aspect of *R. canadum* biology that has only recently been investigated is their annual inshore
62 aggregations that occur in high-salinity estuaries. The nature of these aggregations has been
63 hypothesized to be associated with either feeding or reproduction (Joseph *et al*., 1964; Richards,
64 1967; Hassler & Rainville, 1975; Lotz *et al*., 1996; Smith, 1996; Burns *et al.,* 1998) and only
65 recently has research verified the reproductive function of these aggregations through
66 documentation of the presence of eggs, newly hatched *R. canadum* larvae and reproductively
67 mature females within the PRS and SHS estuaries in SC (Lefebrve and Denson, In Review).
68 Therefore, the limited understanding of *R. canadum* life history provides conflicting expectations
69 regarding their population structure. On one hand their pelagic nature and cosmopolitan
70 distribution would indicate high potential for long distance movement and gene flow (i.e., no
71 structure expected); conversely, the presence of site-specific spawning aggregations might
72 indicate a low potential for gene flow (i.e., structure expected). As the foundation for effective
73 management of marine fishes is built upon the determination of appropriate biological population
74 segments, a better understanding of *R. canadum* biology and its population genetic structure over
75 a broad geographic area is necessary.

76

77 Commercial and recreational U.S. *R. canadum* harvests along the middle and south Atlantic have
78 been highly variable over time, but generally have been increasing since 1980 (ACCSP.org).
79 Concurrently, recreational fishing pressure on *R. canadum* has increased substantially in the last
80 decade, especially in areas where they exhibit annual inshore aggregations (SC, VA), making
81 them susceptible to overfishing during a potentially critical life stage. In these areas, fishing
82 tournaments focused solely on *R. canadum* are popular (McGlade, 2007) and 'catch and release'

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83 is the exception rather than the rule. Therefore, with continued increases in human populations
84 in coastal areas and subsequent increased fishing pressure on both offshore and inshore coastal
85 finfish populations, the South Carolina Department of Natural Resources (SCDNR) began
86 evaluating the feasibility of stocking *R. canadum* as a management option. In 2001, the SCDNR
87 began collecting *R. canadum* from the wild, developing conditioning regimes, spawning
88 broodstock in the laboratory, and producing juveniles for application to aquaculture development
89 and stock enhancement (Weirich *et al.,* 2004). In addition, efforts were made to collect life
90 history information (spawning, growth, genetics) of the wild populations during seasonal
91 migrations. Externally tagged cultured fish were also released back into the estuary from which
92 the wild broodstock had been collected as an Applied Fisheries Research Tool to monitor
93 movement, determine appropriate tag types, identify site fidelity, determine growth rates, and
94 verify annulus formation.
95
96 In 2007, shortly after Pruett *et al.* (2005) and Renshaw *et al.* (2005) published microsatellite loci
97 for *R. canadum*, we optimized three multiplexed microsatellite panels of ten loci to use as
98 genetic tags for cultured fish and population genetic analyses. Although the genetic tools were
99 not ready for use until 2007, fin clips were available from all hatchery broodstock used in the
100 program between 2004 and 2007. Here, we present population genetic data based on the 2008
101 and 2009 collections and recapture data for *R. canadum* collected from SA and GOM coastal
102 waters. Specifically, our goals are to characterize the genetic population structure of *R. canadum*
103 along the SA coast of the U.S.; determine if genetic population structure is detectable based on
104 movement patterns; document if any degree of estuarine fidelity occurs in *R. canadum*; and
105 evaluate whether genetic data support the reproductive role of their seasonal inshore

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106 aggregations. Due to the general lack of knowledge of *R. canadum* biology, our project utilizes a
107 multi-disciplinary effort over a broad geographical area to provide a holistic approach to
108 addressing current obstacles facing *R. canadum* management.
109
110 **Materials and Methods**
111
112 *Fish Spawning and Production*
113
114 Broodstock used for the production of all cultured fish were collected from the PRS estuary.
115 Spawning occurred at the Marine Resources Research Institute in Charleston, SC and Waddell
116 Mariculture Center (WMC) in Bluffton, SC, whereas all larval rearing occurred in outdoor
117 nursery ponds at WMC. Relatively small numbers of cultured fish have been produced and
118 released since 2004, with 2007 representing the largest release of ~54,000 fish (Table 1). All
119 year classes are identifiable with distinct genetic tags (see below). Genetic tags offer a non-
120 invasive, permanent approach which can be applied to all sizes of fish, including larvae, and are
121 identifiable through parentage analysis. Small juveniles were released at approximately 30 days
122 post hatch (dph), large juveniles at approximately 90 dph, and yearlings the following spring.
123 Some large juveniles and yearlings were also individually tagged with external tags prior to
124 release. Yearlings were tagged in the dorsal musculature with either 89 mm or 127 mm nylon
125 dart tags (Hallprint® Pty Ltd., Australia). Large juvenile *R. canadum* were tagged either
126 dorsally with t-bar anchor tags (Hallprint®) or in the body cavity behind the pectoral fin with an
127 anchor disc tag (Floy® Tag, Seattle WA). All *R. canadum* releases have occurred in the PRS at
128 the Trask boat landing in Bluffton, SC.

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129

130 *Sampling*

131

132 Anal fin tissue samples were collected from adult *R. canadum* at fishing tournaments, fish racks
133 donated to SCDNR's freezer/cooler program by cooperating anglers, and fish collected by
134 SCDNR personnel during the late spring and summer months of 2008 and 2009 (April-July). As
135 *R. canadum* are a federally managed species with a minimum size limit of 83.8 cm fork length,
136 fish are not expected to recruit to the fishing gear until 2-3 years of age creating a potential lag in
137 recruitment and subsequent genetic identification of up to 3 years. Collection locations were
138 provided for each specimen by participating anglers. Our 2008 collection comprised a broader
139 geographic scope than that of 2009 (Table 2). In 2008, we obtained samples from VA, North
140 Carolina (NC), SC, and the Gulf coast Florida (FL); whereas, our 2009 collection comprises
141 samples from only NC, SC, and Georgia (GA). Collected fin tissue was stored at room
142 temperature in a cell lysis/DNA stabilization solution of 8 M urea, 1 % sarkosyl, 20 mM sodium
143 phosphate, and 1 mM EDTA.

144

145 *Molecular protocols*

146

147 Total DNA was isolated from the sarkosyl-urea/tissue lysate using the SprintPrep plasmid
148 purification system (Agencourt, Beverly, MA) according to the manufacturer's instructions. Ten
149 microsatellite loci were amplified in three multiplexed polymerase chain reactions (PCR). Each
150 reaction contained 0.2 mM dNTPs, 1 x HotMaster buffer with 2.5 mM Mg^{2+}, 0.025 units
151 Hotmaster *Taq* (5 Prime, Inc., Gaithersburg, MD), and 0.5 mM $MgCl_2$, 0.20 mg/ml BSA, 0.3 μM

152 forward and reverse primers, and 1 µl of 1:10 diluted DNA isolate. All forward primers were
153 labeled with WellRed dyes; individual primer concentrations differed for each multiplexed panel
154 (Table 3). All amplifications were performed in 11 µL reaction volumes in iCyclers (Bio-Rad
155 Laboratories; Hercules, CA) using a 60˚C touchdown protocol (modified from Renshaw *et al*.,
156 2006) which consisted of three steps following initial denaturation at 94˚C for 2 min. Step 1
157 included seven cycles of 94˚C for 30 s, 60˚C for 1 min and 64˚C for 2 min. Step 2 included seven
158 cycles of 94˚C for 30 s, 57˚C for 1 min and 64˚C for 2 min. Step 3 included twenty cycles of
159 94˚C for 30 s, 54˚C for 1 min and 64˚C for 2 min, followed by a final extension at 64˚C for 60
160 min. The protocol includes substantial decreases in extension times from that of Renshaw *et al.*
161 (2006) to shorten the overall length of the protocol. All amplifications were run with two
162 negative controls. Reaction products and size standards (GenomeLab DNA Size standard Kit –
163 400; Beckman, Brea, CA) were separated using a CEQ8000 automated DNA sequencer
164 (Beckman, Brea, CA) and fragment size analysis was performed with the CEQ8000 software
165 package. All chromatograms were scored manually and genotypes were verified independently
166 by a second reader.
167
168 *Marker Statistics and Parentage Analysis*
169
170 The 2008 sample data were used to test all loci for adherence to Hardy-Weinberg Equilibrium
171 (HWE), linkage disequilibrium, and the presence of genotyping artifacts. Examinations for
172 departures from HWE and for linkage disequilibrium between loci pairs were performed in the
173 program ARLEQUIN 3.11 (Excoffier *et al*., 2005) using default parameters. The frequencies of
174 potential null alleles at each locus were estimated in CERVUS 3.0 (Kalinowski *et al*., 2007).

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175 Significance levels for all simultaneous analyses were adjusted using a sequential Bonferroni
176 correction (Rice, 1989).
177
178 To confirm the utility of the marker suite for genetic evaluation and parentage analysis (i.e.,
179 identification of genetic tags), loci were examined for genetic diversity and polymorphism, the
180 ability to distinguish between related individuals, and adherence to the principles of Mendelian
181 inheritance. Basic molecular diversity indices including number of alleles per locus (N_a), allelic
182 size range, observed heterozygosity (H_O), gene diversity (H_E; Nei, 1987), and inbreeding
183 coefficients (F_{IS}) were calculated for each locus using ARLEQUIN and GENEPOP v4.0.10
184 (Raymond & Rousset, 1995). CERVUS was used to estimate the average parent-pair and identity
185 non-exclusion probabilities for the loci suite, indices which measure the probability that a set of
186 markers will match erroneous parents to offspring and the probability that a set of markers will
187 not be able to distinguish between related individuals.
188
189 To determine whether hatchery individuals contributed to the SA *R. canadum* population(s), a
190 parentage analysis was conducted that incorporated all field samples and hatchery broodstock.
191 Simulations (n = 5) for "sexes known" parentage analysis in CERVUS consisted of 10,000
192 offspring and 8 candidate parent pairs per year (100% sampled) using allele frequencies
193 generated from all *R. canadum* samples. Critical delta values were determined using 95%
194 confidence for the relaxed criteria and 99% for the strict criteria. All parentage analyses were run
195 using the modal simulation file. The percentage of hatchery contribution is reported as $[C(W+C)^{-1}]$ 100, where C is the number of cultured individuals and W is the number of wild individuals as
197 designated by CERVUS at the strict confidence level, as no additional offspring were identified

198 with the relaxed criteria. Contribution is reported in terms of both population (all samples) and
199 yearclass (based on known-aged fish). All sampled fish identified as being of hatchery origin
200 were removed from population structure analyses.

201

202 For the Mendelian inheritance tests, 25 offspring from two parental families of the 2007 hatchery
203 production year were compared to the 2007 broodstock using PROBMAX 3.1 parentage analysis
204 software (Danzmann, 1997) to verify the contributing parental pairs. The genotypes of the
205 contributing parents were merged into a single file and imported into FAP 3.6 (Taggart, 2007), to
206 generate all the possible progeny genotypes associated with these parental crosses. A Chi-square
207 analysis (X^2) was performed to compare the observed genotypic frequencies from the progeny
208 data set with the expected genotypic frequencies from FAP.

209

210 *Population Genetic Analyses*

211

212 Due to the limited geographic distribution of our 2009 collection, our population genetic
213 analyses focused on the 2008 collection; the 2009 collection was analyzed separately to validate
214 patterns observed in 2008. For all population genetic analyses, samples were partitioned into
215 those from the inshore aggregations (defined as being captured landward of the barrier island
216 along the coast/in the estuary) and those from offshore areas (defined as captured seaward of the
217 barrier islands, mostly near wrecks or reefs) in order to detect any restriction of gene flow
218 between fish that are believed to be spawning inshore and fish captured offshore given recent
219 evidence of *R. canadum* spawning in the inshore aggregations in a SC estuary (Lefebvre and
220 Denson, In Review). An exact G-test with Markov Chain permutations, as implemented in

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221 GENEPOP, was used to test for pairwise differences in genotypic distributions among collection
222 locations. Markov chain parameters included 10,000 dememorizations, 100 batches and 5000
223 iterations per batch. Pairwise hierarchical R_{ST} statistics were calculated and an Analysis of
224 Molecular Variance (AMOVA) was conducted as implemented in ARLEQUIN with 10,000
225 iterations to determine the degree of genetic structuring occurring among states. The 2008 data
226 were tested for isolation by distance using a Mantel test of correlation between genetic (R_{ST}) and
227 geographic (coastline linear) distance matrices with 100,000 permutations implemented in
228 ARLEQUIN. Additionally, we tested the 2008 data for patterns of spatial autocorrelation using
229 both SPAGeDi (Hardy & Vekemans, 2002) and GenAlEx (Peakall & Smouse, 2006). SPAGeDi
230 analyses were performed with five spatial-categorical groups with statistics based on an ANOVA
231 approach with pair-wise R_{ST} using 10,000 permutation tests of genetic structure. GenAlEx is a
232 multivariate analysis which allows for simultaneous assessment of the spatial signal generated by
233 multiple genetic loci. Analyses were calculated at 20 km intervals up to 2040 km and statistically
234 evaluated with 99 random permutations using 2-tailed 95% CI significance tests. Finally,
235 pairwise comparisons of genotypic distributions between 2008 and 2009 collection locations
236 were conducted as described above to determine the degree of temporal genetic stability of *R.*
237 *canadum* populations along the southeastern U.S. Atlantic coast.

238

239 **Results**

240

241 For both collection years, high proportions of loci were able to be scored unambiguously,
242 resulting in low levels of missing data (2008: 97.8%; 2009: 98.2%). All loci were found to be in
243 HWE ($p > 0.07$), with no evidence of null alleles (frequency < 0.04) and no indication of linkage

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244 disequilibrium between any loci ($p > 0.17$; critical p-value after Bonferonni = 0.001). All 10 loci
245 were polymorphic with allelic richness ranging from 6 to 27, with a mean of 15.9 alleles per
246 locus (Table 4). All loci showed high levels of genetic diversity, with observed heterozygosity
247 ranging from 0.49 to 0.90, and low levels of inbreeding ($F_{IS} < 0.05$;Table 4). The X^2 test
248 comparing hatchery broodstock and offspring indicated that all loci are inherited in a Mendelian
249 fashion (Table 5).
250
251 The loci suite provides an average non-exclusion parent-pair probability of 1.3^{-7} and average
252 non-exclusion identity probability of 5.8^{-12}, signifying that the possibility of parental
253 misassignment in the parentage analysis is substantially less than 0.01% and individuals can be
254 identified confidently. Therefore, based on initial tests, this suite of microsatellite markers is
255 valuable for characterization of population genetic diversity and structure as well as parentage
256 analysis since the loci are genetically varied, adhere to the expectations of Mendelian
257 inheritance, and are able to distinguish between related individuals and correctly match offspring
258 to their parents with a high degree of confidence.
259
260 *Movement and estuarine fidelity*
261
262 Hatchery contribution to SC *R. canadum* populations: Parentage analysis identified two fish in
263 the 2008 collections that were originally stocked in 2004 in PRS. Based on otolith aging, a total
264 of 172 fish from the 2004 year class (YC) were present in the 2008 collections. Therefore, the
265 2004 stocked fish made a 1.1% contribution to the 2004YC of *R. canadum* (2004YC contribute
266 0.6% to the overall sampled SC population of *R. canadum*). From the 2009 collections, a total of

267 six stocked fish were identified, all from the 2007YC small juvenile stockings. A total of thirteen
268 2007YC fish were identified in the 2009 collections, resulting in a 46.1% contribution of stocked
269 fish to the 2007YC and a 2.3% contribution to the overall SC population of sampled *R. canadum*.
270 All of the identified stocked fish were recaptured within the PRS estuary. In addition to the
271 recapture of stocked fish in their release estuaries, three wild fish have also been recaptured
272 within the PRS estuary in multiple years. No wild recaptures have been detected among different
273 collection locations.
274
275 External tag recaptures: A total of seven tag returns have been reported from the 2004YC of
276 stocked yearling *R. canadum* (total of 95 originally stocked). Six of the fish were recaptured
277 relatively shortly after release (mean of 38 days at large); however, the last fish was reported
278 after 370 days after its release. All tag returns of 2004YC fish were recaptured within the PRS
279 estuary. From the 2005YC fish that were stocked with external tags (n = 385), a total of 69 have
280 been recaptured over a four year period. Only four of these recaptures were reported from
281 offshore areas (two from SC and two from Florida), with one collected from within Charleston
282 Harbor and the remaining 64 reported from the PRS area. Fifty-seven of the fish were recaptured
283 within one year of release (mean of 17 days at large); however, the remaining fish averaged 815
284 days at large prior to recapture. Two fish from the 2007YC release of yearling *R. canadum* (59
285 total) have also been reported from the release estuary after a mean of 25 days post-release.
286
287 *Population structure*
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289 Based on the 2008 samples, pairwise comparisons of both genotypic distributions and
290 hierarchical R_{ST}s indicated no differences among the three offshore collection locations (Table
291 6). However, the two inshore collection locations were significantly different from both each
292 other (G-test: p = 0.000; R_{ST}: 0.032, p = 0.000) and the homogenous offshore group (G-test: p =
293 0.000; R_{ST}: 0.007-0.025, $p < 0.05$); with the exception of the inshore VA and offshore NC
294 collections (Table 6). Both the AMOVA and the Mantel results were consistent with this pattern
295 with a significant portion of the molecular variance attributable to among groups (1.6%, p =
296 0.000), but no detectable isolation by distance pattern (r = -0.248, p = 0.798). No patterns of
297 spatial autocorrelation were detected using either SPAGeDi (p = 0.052-0.659) or GenAlEx ($p >$
298 0.130). Results derived from the 2009 samples were concordant with the patterns detected in
299 2008, with the SC inshore collection being significantly different from the homogenous NC and
300 SC offshore group (Table 7). Temporal within-location comparisons of the 2008 and 2009
301 collections showed no significant differences in genotypic distributions (G-test: p = 0.221-
302 0.561). Basic molecular diversity indices were similar among collection locations, with high
303 levels of genetic diversity across all loci and high levels of polymorphism (Table 8). Mean
304 number of alleles per locus ranged from 7.5-11.7 with an average allelic range of 11.5-15.4. The
305 overall average observed heterozygosity ranged from 0.691 in the SC inshore collection to 0.751
306 in the NC offshore collection.
307
308 **Discussion**
309
310 In recent years, the SCDNR has expanded its program focused on the use of genetic tools to
311 identify many types of stocked fish , specifically red drum *Sciaenops ocellatus* (Linneaus, 1766),

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312 striped bass *Morone saxatilis* (Walbaum, 1792), and spotted sea trout *Cynoscion nebulosus*
313 (Cuvier, 1830), and characterize their genetic population structure. These tools create
314 permanently identifiable tags using microsatellite markers that are useful for genetically
315 characterizing *R. canadum* populations. South Carolina's *R. canadum* research program is the
316 first to begin rigorously evaluating U.S. population(s) from a genetic perspective. Based on our
317 U.S. collections of *R. canadum* encountered along the SA and GOM coasts, tests of both
318 genotypic distributions and pairwise hierarchical R_{ST} statistics suggest the offshore groups are
319 genetically homogenous, even between the SA and GOM, which is consistent with Hrincevich's
320 (1993) findings. However, the detection of the two genetically distinct inshore aggregations (SC
321 and VA) is new information in our understanding of *R. canadum* life history. Although a
322 significant degree of genetic isolation was detected among these inshore aggregations and the
323 offshore group, the low R_{ST} statistics indicate that a low level of gene flow does occur.
324
325 We recognize population structure can be easily masked by a mixed stock effect whereby gene
326 flow is limited among population groups by differential spawning behaviors, yet intermingling
327 occurs outside of the spawning period. For example, if populations of fish spawn in unique
328 locations though intermingle and migrate with other populations during the non-spawning
329 season, the composition of non-spawning breeding stocks would appear to be homogenous in
330 terms of allele frequency distributions, whereas gene flow is restricted to individuals spawning at
331 each unique spawning site. Sampling spawning individuals at each unique spawning site would
332 reveal the true genetic structure. Although we have temporally limited our sampling to *R.*
333 *canadum's* spawning period, it is likely that the lack of detected genetic differences between the
334 VA inshore aggregation and the NC offshore samples is due to confounding effects of *R.*

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335 *canadum's* potential migration patterns. In a migrating species, the logistics of sampling
336 individuals in one location without re-sampling from the same group in another location is
337 challenging. Although temporally limiting sampling can lessen the confounding effects of such
338 migrations on population genetic evaluations, in the case of *R. canadum* their limited period of
339 accessibility coincides with both their spawning season as well as their proposed northward
340 migration. Therefore, even though our sampling was temporally limited, it is reasonable that a
341 high proportion of VA individuals were present among the *R. canadum* collected offshore of NC
342 as they were completing their migration to the VA inshore aggregation.
343
344 The genesis of *R. canadum* research in SC began with the need to collect life history information
345 to explore the potential of this species for aquaculture production and better understand the
346 impact stocking might have on a highly migratory species. The scope of our program did not
347 only encompass gathering information of basic life history and population dynamics from the
348 wild population, but also incorporated the application of tagged cultured animals to better
349 understand movement patterns and fidelity to natal estuaries. Collection of such information has
350 proven to be useful for interpreting genetic results. The detection of stocked fish from multiple
351 yearclasses of fish released within the PRS estuary was somewhat unexpected given the many
352 unknowns regarding *R. canadum* life history (i.e., juvenile habitat utilization, home ranges,
353 movement patterns, spawning migrations, etc.). Although the initial 1.1% contribution to the
354 2004YC appears low, when considering the limited number of fish originally released, we
355 interpreted these results as being positive in terms of the potential for stock enhancement to be
356 effective as a fisheries management tool for *R. canadum* and demonstrates how understanding
357 life history attributes is necessary to designing a stocking program for a highly migratory pelagic

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358 species. The much higher contribution observed in 2009 following the larger 2007YC release
359 during their first year of potential recruitment to the fishing gear provides additional support.
360 Furthermore, the recapture of these stocked fish within their release estuary 1-3 years post-
361 release suggests some degree of estuarine fidelity occurs within these inshore *R. canadum*
362 aggregations, supporting the identification of their unique genetic population structure. The
363 suggestion of estuarine fidelity is also indicated by the recapture of wild fish within the PRS
364 estuary during multiple collection years as well as the high precedence of external tag recapture
365 reports occurring within the PRS area. Therefore, these results complement both the previously
366 observed high site fidelity in SC (Hammond, 2001) and Lefebvre and Denson's (In Review)
367 documented spawning function of the inshore aggregations based on positive *R. canadum* egg
368 and larval detection within the PRS estuaries.
369
370 In the Persian Gulf and Oman Sea, Salari Aliabadi *et al.* (2008) also investigated small-scale
371 population structure in *R. canadum* using microsatellite markers. Although they report the
372 presence of three distinct genetic populations along their northern coasts, their study was likely
373 confounded by low sample size, lack of a temporal sampling design, and no corrections for
374 multiple comparisons in their analyses as they were unable to identify any potential behavioral or
375 geographic mechanisms of genetic isolation among detected groupings. In contrast, our study
376 used robust sampling and analysis approaches to provide links between the detected genetic
377 structure and several indications of mechanisms of genetic isolation (seasonal aggregations and
378 estuarine fidelity).
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380 The genetic diversity, in terms of both gene diversity and allelic richness, detected in *R. canadum*
381 along the SA coast is similar to that reported in both Iran (Salari Aliabadi *et al.,* 2008) and the
382 northern GOM (Pruett *et al.,* 2005), and all are somewhat higher than the averages reported for
383 marine fishes (DeWoody & Avise 2000). Therefore, based on the genetic characterization along
384 the southeastern Atlantic coast of the U.S., *R. canadum* appears quite genetically diverse both
385 overall and within localized areas, with temporal stability. However, the detection of discrete
386 genetic structure of *R. canadum* within this portion of their range has implications on the
387 appropriate management unit(s) for this important recreational fishery. As with many aspects of
388 *R. canadum's* life history, the answer does not appear to be straightforward. For example, a
389 recommendation based solely on information gathered from the offshore collections which
390 shows high levels of movement between the SA and GOM might include continuing the single
391 population management strategy as overfishing in one offshore area would impact other areas as
392 well. In contrast, a recommendation based solely on the inshore collections which indicate the
393 presence of distinct population segments and estuarine fidelity in *R. canadum* might favor
394 separate management of the population segments as localized fishing pressure would primarily
395 impact the local population. However, perhaps given the complicated life history of *R. canadum*,
396 a more appropriate recommendation would include a two-tiered strategy in which *R. canadum*
397 are managed as a single umbrella population for offshore activities in conjunction with inshore
398 aggregation-specific management options at the local level. Considering the genetic uniqueness
399 of the inshore aggregations, it is concerning that the majority of the fishing pressure on these
400 aggregations targets the reproductive pool on their spawning grounds.
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402 Although there is still much to learn about the intricacies of *R. canadum's* life history, the results
403 presented here are needed for informed decisions to be made regarding the future management of
404 this recreationally and commercially important species. Additionally, the accessibility of these
405 data is timely in that *R. canadum* is anticipated to be a priority species for offshore marine
406 aquaculture operations in the GOM. In the last 10 years, *R. canadum* aquaculture in the U.S. has
407 moved from research and development to the point where some companies are interested in
408 deploying offshore cages for growout production. However, only with prior knowledge of the
409 size or genetic composition of wild *R. canadum* can we begin to assess the potential risks that
410 these operations may pose to wild stocks of this species.
411

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490 Table 1. Summary of experimental releases of culture *R. canadum* by release year with data
491 presented on mean total length (TL) and number of individuals released. Small juveniles were
492 released during the summer of the production year, large juveniles were released during the fall
493 of the production year, and yearlings the following spring.

Year class	Experimental release	Mean TL (mm)	Number released
2004	Small juveniles	97	1,128
	Large juveniles	328	679
	Yearlings	496	93
2005	Small juveniles	56	3,200
	Large juveniles	230	516
	Yearlings	545	385
2007	Small juveniles	82	53,264
	Large juveniles	250	409
	Yearlings	541	59
2008	Large juveniles	249	2,000
	Yearlings	530	54
2009	Large juveniles	235	1,392

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495 Table 2. Distribution of genetic samples collected during 2008 and 2009 with known collection

496 location. Dashes indicate locations where no sampling occurred.

Location	2008	2009
Virginia inshore	35	---
North Carolina offshore	91	56
South Carolina inshore	105	114
South Carolina offshore	69	58
Florida offshore	16	---

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498 Table 3. Multiplex group, fluorescent label (dye), repeat motif, GenBank Accession #, and PCR
499 primer concentrations (μM) for 10 *R. canadum*-specific loci (modified from Renshaw *et al.*,
500 2006).

Panel	Locus	Well-Red Dye	Repeat Motif	GenBank #	[Primer]
1	Rca1-H10	D2	CA	AY850022	0.10
	Rca1-A04	D4	$(CA)_9(CACT)_4$	AY721672	0.05
	Rca1B-E02	D4	CT	AY721666	0.15
2	Rca1-A11	D4	GT	AY721673	0.05
	Rca1B-H09	D2	GATA	AY721671	0.09
	Rca1B-E08A	D3	CA	AY721667	0.11
	Rca1B-C06	D4	GATA	AY850008	0.05
3	Rca1B-D10	D3	CTAT	AY850009	0.13
	Rca1-E11	D2	CA	AY721680	0.04
	Rca1-C04	D4	GT	AY721675	0.13

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502 Table 4. Genetic diversity statistics for 10 *R. canadum*-specific microsatellite loci based on all
503 samples from the 2008 collection year. N = sample size, N_A = number of alleles, A = allelic size
504 range, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding
505 coeffiecient.

Locus	N	N_A	A	H_O	H_E	F_{IS}
Rca1-H10	487	12	119-139	0.7453	0.7495	0.006
Rca1-A04	490	13	196-206	0.6751	0.7179	0.059
Rca1B-E02	486	6	297-315	0.4958	0.5475	0.094
Rca1-A11	483	21	165-201	0.7991	0.8455	0.055
Rca1B-H09	490	18	168-224	0.8755	0.8944	0.021
Rca1B-E08A	489	14	181-229	0.6441	0.6425	-0.003
Rca1B-C06	486	22	336-404	0.8827	0.8935	0.012
Rca1B-D10	489	27	143-223	0.9079	0.9222	0.016
Rca1-E11	493	6	167-183	0.5071	0.5190	0.023
Rca1-C04	489	18	217-253	0.6666	0.6711	0.007

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507 Table 5. Mendelian inheritance statistics for two independent *R. canadum* families: X^2 values,
508 degrees of freedom (df), and p-values at each of the 10 *R. canadum*-specific microsatellite loci.
509 For two loci in Family 2, both parents were homozygous for different alleles and all offspring
510 were fixed heterozygotes, as expected (indicated by an asterisk).

	Family 1			Family 2		
Locus	X^2	df	p-value	X^2	df	p-value
Rca1-H10	2.27	1	0.131	1.33	1	0.248
Rca1-A04	0.09	1	0.764	0.50	2	0.778
Rca1B-E02	2.27	1	0.131	0.00	1	1.000
Rca1-A11	4.40	3	0.221	1.00	1	0.317
Rca1B-H09	0.00	1	1.000	5.33	2	0.069
Rca1B-E08A	1.80	2	0.406	*	---	---
Rca1B-C06	3.00	3	0.391	2.45	3	0.484
Rca1B-D10	1.00	3	0.801	0.40	1	0.527
Rca1-E11	1.22	2	0.543	*	---	---
Rca1-C04	0.00	1	1.000	3.89	3	0.273

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512 Table 6. Summary of genotypic distribution and R_{ST} pair-wise location comparison results from
513 the 2008 sample collections. VA: inshore Virginia; SC: inshore South Carolina; NC_{off}: offshore
514 North Carolina; SC_{off}: offshore South Carolina; FL_{off}: offshore Florida (Gulf of Mexico).
515 Asterisks indicate statistical significance following Bonferroni correction (critical p = 0.005).
516

Pairwise Comparison	Genotypic distribution p-value	R_{ST}
VA-SC	0.000*	0.045*
SC-NC_{off}	0.000*	0.025*
SC-SC_{off}	0.000*	0.014*
SC-FL_{off}	0.000*	0.016*
VA-NC_{off}	0.012	0.001
VA-SC_{off}	0.000*	0.011*
VA-FL_{off}	0.003*	0.025*
SC_{off}-FL_{off}	0.027	0.003
NC_{off}-SC_{off}	0.148	0.006
NC_{off}-FL_{off}	0.228	0.015

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518 Table 7. Summary of genotypic distribution and R_{ST} pair-wise location comparison results from
519 the 2009 sample collections. SC: inshore South Carolina; NC_{off}: offshore North Carolina; SC_{off}:
520 offshore South Carolina. Asterisks indicate statistical significance following Bonferroni
521 correction (critical p = 0.017).
522

Pairwise Comparison	Genotypic distribution p-value	R_{ST}
SC-NC_{off}	0.000*	0.017*
SC-SC_{off}	0.000*	0.016*
NC_{off}-SC_{off}	0.532	0.005

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524 Table 8. Summary of genetic diversity statistics for each collection location from the 2008
525 collection year. N_A = average number of alleles across loci, A = average allelic size range across
526 loci, H_O = average observed heterozygosity across loci, H_E = average expected heterozygosity
527 across loci.

Collection Location	N_A	A	H_O	H_E
VA	10.4	15.1	0.772	0.768
NC_{off}	12.9	15.4	0.751	0.759
SC	10.4	14.1	0.691	0.710
SC_{off}	11.7	14.0	0.736	0.757
FL_{off}	7.5	11.5	0.705	0.727

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