Genetic Characterization of Red Hind, *Epinephelus guttatus*, Collected from Three Spawning Aggregations in Western Puerto Rican Waters - Variation in Allozymes

ROCKY WARD[1], MIGUEL FIGUEROLA[2], BRIAN E. LUCKHURST[3], IVONNE R. BLANDON[1], and WILLIAM J. KAREL[1]

[1]*Perry R. Bass Marine Fisheries Research Station*
Texas Parks and Wildlife Department
HC 02 Box 385
Palacios, Texas 77465 USA
[2]*Fisheries Research Laboratory*
Department of Natural Resources
P. O. Box 3665
Mayaquez, Puerto Rico
[3]*Division of Fisheries*
Department of Agriculture, Fisheries and parks
P. O. Box CR 52, Crawl CR BX
Bermuda

ABSTRACT

Red hind (*Epinephelus guttatus*) is a heavily exploited reef fish which form short-term spawning aggregations beginning in January in western Puerto Rican waters. Reproductively active individuals were collected from three aggregations and examined for variation in genetic characteristics using horizontal starch-gel electrophoretic techniques. Eighteen putative loci were found to be informative. A fixation value (F_{ST}) of 0.022 was estimated, indicative of minimal population subdivision. Estimates of Nei's unbiased genetic distance (Nei 1978)suggested red hind are nearly identical genetically within this limited grouping of aggregations. These results suggest red hind spawning aggregations, at least within the context of the sampled groupings, may not represent biologically significant genetic subdivisions.

KEY WORDS: Red hind, genetic variation, population genetics

INTRODUCTION

Growth and recruitment overfishing have been implicated in recent declines of grouper stocks in tropical Atlantic waters (Sadovy 1994). Declines have often been precipitous (Olsen and LaPlace 1978) and recoveries of fisheries have been minimal (Butler et al. 1993). As fisheries for preferred species such as Nassau grouper (*Epinephelus striatus*) and red grouper (*E. morio*) become unproductive, local efforts often are shifted towards other species such as red hind (Heemstra and Randall 1993). Increased pressure on these stocks increase the probability that they too will decline. The potential for overexploitation is increased in some grouper species, including red hind, by the formation of spawning aggregations which are

heavily targeted by local fishermen (Aguilar-Perera and Aguilar-Davila 1996, Coleman et al. 1996). Overfishing of spawning aggregations have been suggested to result in reduced age at sexual maturity and changes in sex-ratio among some protogynous hermaphroditic groupers (Coleman et al. 1996) resulting in increased ratios of females to males within an aggregation. One immediate consequence of changes away from a 1:1 sex ratio is to lower effective population size, with concomitant effects on important genetic factors such as inbreeding, genotypic diversity, and population structuring.

Little information is available on levels of genetic variability or population structure in protogynous hermaphroditic spawners such as red hind. This information is important to the understanding of effects of overfishing on red hind populations and has far-reaching implications for the evolutionary potential of an exploited population (Soulé 1980, Smith and Chesser 1981). Equally important, the effects of aggregation formation during reproduction on genetic structure may be critical to understanding stock structure in species employing this strategy and becomes crucial as proposals for artificial propagation and enhancement are made. Small-scale stocking of Nassau grouper already has occurred without determination of existing stock structure (Roberts et al. 1995), putting at risk the genetic integrity of this species. As natural stocks continue to decline and culture techniques are improved, such attempts will become attractive to management agencies (Brulé et al. 1996, Fukuhara 1989, Tucker 1994, Tucker and Woodward 1994). It is critical that such programs, if pursued, be guided by adequate knowledge of genetic variation within populations and genetic differentiation among populations.

As a first step in providing this information, the present study has three basic goals. First, to assess the genetic variability inherent in red hind spawning aggregations. Second, to examine differentiation among aggregations by comparing polymorphic genetic markers. Third, to generate a baseline with which future genetic data may be compared.

METHODS

Electrophoretically detectable allelic variants were surveyed in samples collected from three spawning aggregations located off the west coast of Puerto Rico. Collection sites are described in Sadovy, et al. (1994). Sampled aggregations were close enough geographically that within group differences could be attributed to normal variability and not to broad-based differences across the distribution of the species. This was important in assessing spatial stability of the genetic parameters chosen to characterize the aggregations.

During June - October of 1996, red hind were collected using fish traps from each breeding aggregation. All fish were immediately placed on ice until transported to the Laboratorio de Investigaciones Pesqueras in Mayaqüez, Puerto Rico. Whole fish were maintained at -20°C until shipment to the Perry R. Bass Marine Fisheries Research Station (PRBMFRS) where individuals were measured and samples of skeletal muscle, liver, heart, kidney, fin, gill, and eye were excised. Tissues were

homogenized in an equivalent ammount of buffer (0.01 M tris-HCl, 0.001 M EDTA, pH 7.5; modified from Selander et al. 1971) and centrifuged for 10 minutes at 10,000 rpm. Supernatants were stored at -80°C until needed for electrophoretic examination.

Variation in electrophoretic characteristics of proteins was assayed using horizontal starch gels (Selander et al. 1971, Morizot and Siciliano 1984, Murphy et al. 1996) prepared as 12% suspensions of hydrolyzed starch (StarchArt Company, Smithville, Texas). Gel and electrode buffers used were tris-citrate, pH 8 (TC-8; Selander et al. 1971); tris-borate-EDTA, pH 8 (TVB; Selander et al. 1971); tris-citrate/borate (POU; Selander et al. 1971; tris-citrate, pH 7 (TC-7; Ayala et al. 1972); amine-citrate, pH 6.1 (AC-6; Clayton and Tretiak 1972); and lithium hydroxide, pH 8.2 (RW; Ridgway et al. 1970). Histochemical staining followed, with minor modifications, the methods of Manchenko (1994). Protein nomenclature and abbreviation followed the recommendations of Manchenko (1994) and electromorph designation followed Shaklee et al. (1990). Loci were abbreviated like the proteins they govern, but in italic form with a terminal asterisk. Allele codes were italicized and proceded by an asterisk. The allele of each locus which migrated most anodally was designated **A* and all other alleles were sequentially labeled in accordance with their mobility.

A gene locus was considered polymorphic for statistical purposes if the most common allele frequency was 0.99 or less in any of the three aggregations. Gene frequencies and summary statistics were determined using the Fortran program BIOSYS-1 (Swofford and Selander 1981). Heterogeneity chi-square tests of genotype distributions were used to test for gene frequency differences among spawning aggregations. In all chi-square tests comparing gene frequencies among samples or testing conformation to Hardy-Weinberg expectations were calculated using the computer program GenePop (Raymond and Rousset 1995) and appropriate corrections for significance values of multiple simultaneous tests were employed (Miller 1966).

Partitioning of genetic variation into within population (F_{IS}) and between population (F_{ST}) components followed Weir and Cockerham (1984). Chi-square was used to test significance of *F*-statistics. Calculation of chi-square followed Waples (1987). The average number of migrants exchanged between spawning aggregations was calculated as $N_em = ((1/ F_{ST})-1)/4$. The variance of N_em was estimated by jackknifing loci (Reynolds et al. 1983, Weir and Cockerham 1984) to obtain 95% confidence limits for F_{ST} and then employing these values to estimate N_em. Nei's genetic distance (Nei 1978) was determined and examined as an index of population differentiation (Rogers 1972)

Correlation analysis (SAS Institute 1985) were used to examine trends in heterozygosity and allele frequency across aggregations. Significance of relationships between degrees west longitude and degrees north latitude with genetic variation were determined by the probability associated with Spearman's correlation coefficient. Probabilities less than 0.05 were considered statistically significant (Snedecor and Cochran 1980).

RESULTS

Consistently scorable electromorphs were resolved for 75 enzymes and structural proteins allowing genotypes to be determined for 106 putative gene loci. Eighteen loci were scored as polymorphic at one or more aggregations (Table 1) and were used in statistical analyses. The same common allele predominated for all loci at all aggregations.

Table 1. Gene frequencies at 18 polymorphic loci examined in three spawning aggregations of red hind in Puerto Rican waters. Means of measures of genetic variability (with standard errors in parentheses) are provided.

Locus/allele	Abrir la Sierra	Bajo el Cico	Tourmaline
*AAT-1**			
N	35	47	62
**A*	0.057	0.064	0.048
**B*	0.943	0.936	0.952
*CAT-1**			
n	35	47	60
**A*	0.314	0.372	0.350
**B*	0.686	0.628	0.650
*EST-1**			
n	34	48	63
**A*	1.000	1.000	0.992
**B*	0.000	0.000	0.008
*EST-2**			
n	34	48	63
**A*	0.132	0.188	0.095
**B*	0.868	0.813	0.905
*GD-1**			
n	34	48	63
**A*	0.000	0.021	0.032
**B*	1.000	0.979	0.968
*GP-2**			
n	33	48	66
**A*	0.727	0.833	0.523
**B*	0.273	0.167	0.477
*G6PD-3**			
n	36	50	64
**A*	0.111	0.120	0.242
**B*	0.889	0.880	0.750
**C*	0.000	0.000	0.008
*GRI-2**	34	51	74
**A*	0.088	0.216	0.277
**B*	0.912	0.775	0.723
**C*	0.000	0.010	0.000
*IDDH-1**			
n	34	50	61
**A*	0.044	0.080	0.098
**B*	0.956	0.920	0.902
*LDH-1**			
n	35	48	63
**A*	0.043	0.021	0.016
**B*	0.014	0.010	0.016
**C*	0.943	0.969	0.968

Table 1 (continued)

Locus/allele	Abrir la Sierra	Bajo el Cico	Tourmaline
*PEPA**			
n	33	50	63
**A*	0.000	0.010	0.000
**B*	1.000	0.090	1.000
*PEPB**			
n	33	50	63
**A*	0.000	0.000	0.008
**B*	0.000	0.000	0.992
*PEPD**			
n	33	45	60
**A*	0.348	0.378	0.317
**B*	0.606	0.578	0.658
**C*	0.045	0.044	0.025
*PEPF**			
n	28	35	39
**A*	0.196	0.057	0.205
**B*	0.714	0.757	0.654
**C*	0.089	0.186	0.141
*PEPT-1**			
n	33	50	63
**A*	0.015	0.000	0.008
**B*	0.985	1.000	0.984
**C*	0.000	0.000	0.008
*PEPT-2**			
n	33	50	63
**A*	0.000	0.010	0.000
**B*	1.000	0.990	1.000
*PFK**			
n	34	48	63
**A*	0.971	1.000	0.992
**B*	0.029	0.000	0.008
*PGLM-1**			
n	33	50	63
**A*	0.076	0.050	0.095
**B*	0.924	0.950	0.905
Mean alleles / locus	1.86 (0.16)	2.00 (0.16)	2.17 (0.15)
Polymorphic loci %	72.22	77.78	72.22
Heterozygosity			
Direct Count	0.125 (0.032)	0.139 (0.032)	0.170 (0.037)
Expected	0.163 (0.041)	0.170 (0.042)	0.198 (0.042)

The percentage of polymorphic loci ($P_{0.99}$) ranged from 72.22% at Abrir la Sierra and Tourmaline to 77.78% at Bajo el Cico. Significant deviations from Hardy-Weinberg (Table 2) were detected in 7 of 54 (13.0 %) possible comparisons. No locus was found to deviate from Hardy-Weinberg expectations in all aggregations (Table 2), though *EST-2** was not in Hardy-Weinberg at Abrir la Sierra ($\chi^2 = 14.59$, df = 1, $P < 0.01$) and Bajo el Cico ($\chi^2 = 5.27$, df = 1, $P = 0.04$). In nearly every instance, loci failing to meet Hardy-Weinberg expectations had

observed frequencies of heterozygous genotypes which were less than expected. Heterozygote deficiencies may be attributed to one or more of four factors: negative heterosis, the Wahlund effect, the presence of null alleles, or misscoring of heterozygous individuals as homozygous.

Table 2. Breeding aggregation/locus combinations failing to meet Hardy-Weinberg expectations.

Aggregation	Locus	*P*	allele	Observed	Expected
Abrir la Sierra	*EST-2**	0.008	AA	3	0.600
			AB	4	8.800
			BB	31	28.600
	*IDDH**	0.040	AA	1	0.040
			AB	1	2.920
			BB	36	35.040
	*PEPD**	0.001	AA	6	1.813
			AB	5	12.693
			AC	0	0.680
			BB	24	20.433
			BC	3	2.240
			CC	0	0.040
Bajo el Cico	*EST-2**	0.038	AA	4	1.577
			AB	10	14.845
			BB	35	32.577
	*PEPF-1**	<0.001	AA	2	0.464
			AB	3	7.732
			AC	3	1.340
			BB	34	28.608
			BC	4	10.052
			CC	3	0.804
Tourmaline	*CAT**	0.022	AA	11	6.674
			AB	20	28.651
			BB	34	29.674
	*GP-2**	0.006	AA	24	18.186
			AB	21	32.628
			BB	20	14.186

Three loci, *CAT**, *LDH-1**, and *PFK**, exhibited changes in allele frequency and average heterozygosity from north to south across the three aggregations which were not statistically significant. This is partially attributable to the limited array of units of analysis; however the three loci that did change in allele frequency did not vary by more than 10% between peripheral aggregations.

Heterogeneity chi-square examinations indicated allele frequencies at *GP-2**, *G6PD-3**, and *GPI-2** were not distributed homogeneously among the three aggregations (Table 3) as were allele frequencies of combined loci. Distributions of allele frequencies for each of the 15 other loci were not found to differ significantly

from homogeneity. The three loci exhibiting statistically significant differences in allele frequencies among aggregations were not the same loci which had apparent (though statistically insignificant) clines in allele frequency.

Table 3. F-statistics (Weir and Cockerham 1984) estimating within-group structuring (F_{IS}) and between-group structuring (F_{ST}), gene-flow estimates (NeM, effective number of migrants per generation), and heterogeneity chi-square tests (degrees of freedom in parentheses) in three spawning aggregations of red hind in Puerto Rican waters. *P < 0.05; **P < 0.01;***P < 0.001; n.s., not statistically significant.

Locus	F_{IS}	F_{ST}	Chi-square
AAT-1*	-0.061 n.s.	0.001 n.s.	0.247 (2) n.s.
CAT-1*	0.202 *	0.003 n.s.	0.597 (2) n.s.
EST-1*	-0.008 n.s.	0.005 n.s.	1.306 (2) n.s.
EST-2*	0.356 **	0.012 n.s.	3.988 (2) n.s.
GD-1*	-0.028 n.s.	0.010 n.s.	2.197 (2) n.s.
GP-2*	0.267 **	0.078 **	25.514 (2) ***
G6PD-3*	0.098 n.s.	0.028 *	9.798 (4) *
GPI-2*	-0.066 n.s.	0.040 **	11.907 (4) *
IDDH-1*	0.056 n.s.	0.007 n.s.	1.760 (2) n.s.
LDH-1*	-0.036 n.s.	0.004 n.s.	1.318 (4) n.s.
PEPA*	-0.010 n.s.	0.007 n.s.	1.927 (2) n.s.
PEPB*	-0.008 n.s.	0.005 n.s.	1.322 (2) n.s.
PEPD*	0.333 **	0.004 n.s.	1.867 (4) n.s.
PEPF*	0.243 **	0.017 n.s.	8.923 (4) n.s.
PEPT-1*	-0.014 n.s.	0.005 n.s.	1.540 (4) n.s.
PEPT-2*	-0.010 n.s.	0.007 n.s.	1.927 (2) n.s.
PFK*	-0.025 n.s.	0.013 n.s.	3.490 (2) n.s.
PGLM-1*	-0.085 n.s.	0.005 n.s.	1.638 (2) n.s.
Mean	0.173 *	0.022 n.s.	81.266 (48) **
Unbiased F_{ST}		0.0244 ± 0.0048	
N_eM		48.87 (6.00 -249.75)	

The standardized variance of allele frequencies, F_{ST}, suggested little genetic differentiation among the three spawning aggregations of red hind from waters off western Puerto Rico. F_{ST} estimates for three loci, *GP-2**, *G6PD-3**, and *GPI-2**, were found to be significant but the mean F_{ST} for all loci was 0.022 and insignificant (Table 3). Several loci had statistically significant F_{IS} estimates and mean F_{IS} was significant, suggesting some degree of within aggregation genetic structuring. High levels of gene flow were inferred among spawning aggregations in Puerto Rican waters. Quantitative estimation of the effective number of migrants per generation (N_em), calculated using the fixation index of Wright (1943), ranged from 6 to 250 individuals per generation (Table 3).

DISCUSSION

Red hind collected from three spawning aggregations off the western coast of Puerto Rico did not demonstrate patterns or levels of allozymic variability suggestive of genetic differentiation between aggregations. Tentatively, red hind spawning

aggregations are viewed as spatially distinct components of a single local population. This does not suggest the species is panmictic. The present study is limited to the issue of genetic differentiation of a small group of spatially separate but proximate aggregations of reproductive individuals.

Failure to find locally-important genetic variation in a reef fish such as red hind is not surprising. Nonanadromous marine fishes often have been shown to exhibit less genetic divergence between geographic locales than freshwater and anadromous species (Gyllensten 1985, Smith et al. 1990, Neigel 1994). Extended planktonic life-stages and highly mobile adults are life-history characteristics that predict limited or absent population subdivision among marine fish species (Shulman and Bermingham, 1995). When biologically significant population differentiation is found in marine fishes (e.g., Bell et al. 1982, King and Pate 1992, Gold et al. 1999) physical barriers exist or existed historically, behavioral and life history characteristics operate which result in natal area fidelity, or reproductive strategies effectively enhance stochastic processes (Hedgecock 1994, Chapman et al. 1999).

Much of what is know about grouper life history argues against formation of significant population structuring. Red hind eggs and early larvae are planktonic and subject to appreciable dispersion from spawning sites (Smith 1961, Beaumariage and Bullock 1976, Colin 1982, Jory and Iverson 1989). Unless natural selection is operating across the distribution of the species or if fidelity is shown to aggregate sites it is not surprising that local structuring is minimal or absent. The present data suggest levels of gene flow among aggregations is of sufficient magnitude to mask any effects of selective and stochastic processes operating within the aggregates examined.

Based on these interpretations, management decisions treating the three aggregations as a single management unit may be justified. It may also be reasonable to generalize from this study to suggest that other spatially proximate red hind spawning aggregations will tend to be genetically similar and representative of the same population, and that if enhancement efforts are deemed necessary nearby aggregates are the best sources of donor individuals. These results do not justify transfer of red hind from one portion of the species' distribution into another prior to a thorough investigation of the genetic resources of potentially impacted populations. Surveys of mitochondrial DNA variation in this species are currently underway and may provide greater resolution of among-aggregates genetic differentiation (Avise 1987) and information concerning stock structure in red hind over a wider portion of its range and across time.

An additional issue should be addressed. Exploited species, by their very nature, may be characterized by genetic variation that is biased and which may fail to adequately describe the species prior to overfishing. Overfishing may alter life-history and population parameters making conclusions about within and/or among population variation tentative. When possible, genetic surveys should be conducted prior to biologically significant anthropogenic impacts. When this is not possible results must be interpreted with considerable caution.

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