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Genetic Variation, Kinship, and Effective Population Size in a Captive Population of the Endangered Cape Fear Shiner, *Notropis mekistocholas*

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Genetic variation at 22 nuclear-encoded loci (21 microsatellites and one anonymous locus) and at mitochondrial (mt)DNA was examined among 40 Cape Fear Shiners (*Notropis mekistocholas*) sampled from the third generation of offspring raised in captivity and among 44 individuals sampled from the "wild" population from which the captive stock was originally constituted. Levels of genetic diversity in both samples were high as compared to other freshwater fishes. However, lower genetic diversity, as reflected by lower number of alleles and gene diversity (nuclear-encoded loci) and number of haplotypes and nucleon diversity (mtDNA), was found in the captive population. Kinship analyses revealed 101 significant half-sib and full-sib relationships in the sample from the captive population versus 83 in the sample from the wild population. Polygynous matings were suggested by the occurrence of 51 significant half-sib relationships that did not share mtDNA haplotypes; polyandrous matings also may have occurred but could not be inferred reliably from the analysis. Effective population size, estimated using a linkage disequilibrium approach, was 49 (95% CI: 43–56) for the captive population and was ~10 times smaller than that estimated for the wild population 528 (273–5336). Considering the low number of founders (31) originally used to constitute the captive stock, the estimate for the captive population indicates that a high proportion of the adults present at each generation actually contributed to the offspring. This suggests that relatively large effective population sizes may be achieved in captive populations of *N. mekistocholas* provided a large number of breeding adults are used.

THE Cape Fear Shiner, *Notropis mekistocholas* (Snelson, 1971), is a small, herbivorous cyprinid fish (minnow) endemic to the Cape Fear River basin in the east-central Piedmont region of North Carolina. Snelson (1971) suggested that Cape Fear Shiners may have always existed in low numbers; G. B. Pottern and M. T. Huish (Supplement to the status survey of the Cape Fear Shiner *Notropis mekistocholas*, U.S. Fish and Wildlife Service, Endangered Species Office, Asheville, NC, unpubl. 1986, 2nd supplement to the status survey of the Cape Fear Shiner *Notropis mekistocholas*, U.S. Fish and Wildlife Service, Endangered Species Office, Asheville, NC, unpubl., 1987,), however, reported declines in abundance and range for the species that were not evident for other sympatric taxa. At present, Cape Fear Shiners are reported to occur in only five localities in the Cape Fear drainage (North Carolina Wildlife Resources Commission, 1995, Cape Fear Shiner [Subsection], p. 18–20. *In:* Annual Performance Report, Nongame and Endangered Wildlife Program, vol. IV, Project E-3, Segment 12, Raleigh, NC, unpubl.). Only two Cape Fear Shiners have been found in the last decade at the two localities in the Haw River, and very recent attempts to obtain Cape Fear Shiners at a third locality, the Deep River near the bridge on SR 1456 in Moore County, have been generally unsuccessful (D. Rabon, U.S. Fish and Wildlife Service, pers. comm.). A recent, significant decline in effective population size in the two remaining populations (one in the Deep River downstream of the High Falls dam and one at the confluence of the Deep and Rocky Rivers) was inferred from variation at microsatellite markers (Saillant et al., 2004). The species is currently designated as endangered (Federal Register, 1987; Hilton-Taylor, 2000; http://www.unep-wcmc.org/species/index.htm, unpubl.); potential causes for the species decline include changes in stream morphology and flow (U.S. Fish and Wildlife Service, unpubl., 1988) and reduced habitat quality and availability (Howard, 2003).

Current conservation efforts for Cape Fear Shiners involve a program of captive propagation initiated in 1997 with the capture on 19 May of 31 adults from the Deep River, just below its confluence with the Rocky River (Confluence). The fish were held and spawned at Conservation Fisheries Inc., in Knoxville, Tennessee, and on 26 October, approximately 1100 offspring were shipped to the Edenton National Fish Hatchery in Edenton, North Carolina, for grow-out under varying conditions. These fish spawned naturally in 1998 and ~500 offspring

were sent to the North Carolina Zoological Park in Asheboro, North Carolina, in December of that year; these fish were maintained for subsequent generations at the Zoological Park independently from the conservation plan. Roughly 250 of the 500 founders survived to adulthood and were allowed to spawn naturally in holding tanks; offspring were collected at least twice by zoo personnel (J. Groves, pers. comm.). In this study, a sample of 40 offspring taken from the second spawning of the original 250 fish was assayed for the same set of 23 genetic markers used by Saillant et al. (2004). The primary objective of the study was to assess genetic diversity in the captive population in relation to the wild population from which they came. Degree of genetic relatedness and effective population size in the offspring sampled from the captive population were compared with those in the wild population. Potential use of captive stocks managed in a similar way is discussed.

Materials and Methods

Sample collection.—The 40 adult Cape Fear Shiners from the captive population (Zoo) were sampled 28 March 2002 by noninvasively removing small clips from the lower lobe of the caudal fin. Tissues were placed in 95% ethanol and returned to College Station where they were stored at −80 C. Data analysis also included a reference sample (Confluence) of 44 ''wild'' Cape Fear Shiners obtained in 1998 (15 specimens) and 2002 (29 specimens) from the same locality where the original founding stock of the captive population was taken. Details of sampling of wild individuals may be found in Gold et al. (2004) and Saillant et al. (2004). Temporal variation, that is, between sample years, in allele frequency for both 22 (21 microsatellites) nuclear-encoded loci and for mtDNA were tested via exact tests of allele distributions and found to be nonsignificant (Saillant et al., 2004). Therefore, data from the two-year samples were pooled for subsequent analyses.

Genetic assays.—All specimens were genotyped at 22 nuclear-encoded genetic markers (21 microsatellites and one anonymous locus that coamplifies with microsatellite *Nme*18A6.158). Details of PCR amplification, electrophoresis, and scoring of PCR products may be found in Burridge and Gold (2003) and Gold et al. (2004). Variation in mitochondrial (mt)DNA was assessed by directly sequencing fragments within the ND-5 (258 bp) and ND-6 (367 bp) subunits of mitochondrially encoded NADH dehydrogenase. PCR primer pairs, amplification conditions, and sequence alignment procedures may be found in Gold et al. (2004). The two mtDNA fragments were combined into unique haplotypes for data analysis.

Data analysis.—Allele frequencies, number of alleles, allelic richness (a measure of number of alleles independent of sample size), and unbiased gene diversity (Nei, 1987) were computed for each nuclear marker, using F-STAT v2.9.3 (Goudet, 1995). MtDNA haplotype frequencies, number of haplotypes, and nucleon and nucleotide diversity were computed using ARLEQUIN (vers. 2000, S. Schneider, D. Roessli, and L. Exoffier, University of Geneva, Switzerland, 2000, unpubl.). Homogeneity of allelic richness and of gene diversity between the two sampled populations was tested via Wilcoxon signed-rank tests. Conformity of genotype proportions to Hardy-Weinberg equilibrium expectations within samples was measured for each nuclear-encoded locus as Weir and Cockerham's (1984) *f,* calculated using F-STAT (Goudet, 1995). Estimates for individual nuclear-encoded microsatellites were combined to generate a weighted estimate of *f* over all microsatellites, following recommendations in Weir and Cockerham (1984). Probability of significance of departure of genotype proportions from Hardy-Weinberg expectations was assessed by a Markov-chain method (Guo and Thompson, 1992), as implemented in GENEPOP v. 1.2 and using 5000 dememorizations, 500 batches, and 5000 iterations per batch (Raymond and Rousset, 1995). Genotypic disequilibrium between pairs of microsatellites was assessed via an exact test, as implemented in GENEPOP and employing the same parameters as above for the Markov Chain.

Homogeneity of allele distributions between captive and wild populations was measured as Weir and Cockerham's (1984) θ, using F-STAT; probability of significance of observed differences in allele distributions was assessed by an exact test, as implemented in GENEPOP (same parameters as above for the Markov Chain). A 95% confidence intervals for θ, was built by bootstrap resampling other loci, as implemented in F-STAT. Homogeneity of mtDNA haplotype distributions was measured by Phi-$_{ST}$-values based on genetic distances between sequences of mtDNA haplotypes and using Kimura's (1980) two-parameter distance estimate. Significance of the distance estimates was tested via 10,000 permutations of haplotypes, as implemented in ARLEQUIN (vers. 2000, S. Schneider, D. Roessli, and L. Exoffier, University of Geneva, Switzerland, 2000, unpubl.). Sequential Bon-

ferroni correction (Rice, 1989) was applied when appropriate for all multiple tests performed simultaneously.

Kinship analyses and familial structure.—Familial structure from both captive and wild populations was inferred by testing hypotheses of pedigree relationships (i.e., full-sibs, half-sibs, unrelated), based on multilocus genotypes, between all pairs of individuals within each sample. We used the program KINSHIP v.1.3.1 (Queller and Goodnight, 1989) available at http://www.bioc.rice.edu/~kfg/GSoft.html. Given a specified pedigree relationship, KINSHIP uses the allele frequencies in the population and the genotypes of the two individuals under consideration to calculate the likelihood that this genotype combination could have been produced by the relationship specified. The likelihood calculations make the simplifying assumptions of no linkage disequilibrium, no inbreeding, and no mutation. Significance of a given relationship was assessed by comparing the likelihood of the relationship specified with the likelihood of a ''null hypothesis'' relationship and using likelihood ratio tests. Significance of the likelihood ratio was assessed by comparing observed ratios to the distributions of 1000 simulated ratios under the null hypothesis (Type I error). Type II error was assessed based on the distribution of 1000 simulated ratios under the tested hypothesis. Significant familial relationship between pairs of individuals was inferred during KINSHIP analysis when the likelihood ratio for a pair of individuals fell beyond a threshold value that corresponded to a Type I error of 0.05.

Significance of half-sib relationships was tested under the null hypothesis of pairs being unrelated. The Type I error in that case was the probability that a pair of fish matching this null hypothesis would show a higher likelihood ratio than the threshold. The Type II error (probability that a ''true'' pair of half sibs would show a lower likelihood ratio than the threshold) resulting from the threshold obtained in this analysis was 0.210 for Zoo and 0.228 for Confluence. These Type II error rates would indicate that the tests failed to detect true half-sib relationships at a probability of 0.210–0.228.

Full-sib relationships were tested under the null hypothesis of half-sib relationship. In that case, the Type I error was the probability that a pair of half-sibs would show a higher value than the threshold. Type II error rates (probability that a true pair of full sibs would show a lower likelihood ratio than the threshold) were 0.253 and 0.187 for Zoo and Confluence, respectively. These error rates would indicate that full-sib relationships could not be discriminated from half-sib relationships at a probability of 0.187–0.253. Given the high probabilities of incorrect assessment of relatedness, reliable inference of the exact familial structure of the sample is not possible because some half-sib relationships will not be detected and some full-sib relationships will be inferred to be half-sib relationships.

Effective population size.—Effective population size was inferred after Hill (1981) and Bartley et al. (1992), using an assessment of linkage disequilibrium (D). Linkage disequilibrium is the difference between the expected co-occurrence of alleles at pairs of loci and can be related to the correlation among alleles at different loci (r). In a population of limited size, D (and r) will depart from zero because of the effect of genetic drift at a magnitude that is a function of effective population size, thus allowing estimation of $N_e(D)$. The estimation assumes no immigration, selective neutrality of genetic markers (alleles), no population structure, and random sampling from the population (Bartley et al., 1992). PCR primers for two pairs of nuclear-encoded loci (*Nme*4F4.154–*Nme*4F4.222 and *Nme*2B10.166–*Nme*2B10.232) used in the study were designed from sequences in the same clone. The assumed close physical linkage at these loci could lead to bias in estimating the overall level of disequilibrium. Consequently, *Nme*4F4.154 and *Nme*2B10.166 were omitted from the analysis. Estimates of N_e and their 95% confidence intervals were computed in N_E-ESTIMATOR (vers. 1.3, D. Peel, J. R. Ovenden, and S. L. Peel, Queensland Government, Dept. of Primary Industries and Fisheries, 2004, unpubl.), following the method described in Bartley et al. (1992).

RESULTS

Genetic diversity.—Summary data of variation at each nuclear-encoded locus in both captive (Zoo) and ''wild'' (Confluence) samples are presented in Appendix 1. All nuclear-encoded loci were polymorphic. Number of alleles sampled per locus per sample ranged from two (*Nme*24B6.191, both samples) to 14 (*Nme*30F12.208, Confluence) and averaged 7.0 and 8.6 for Zoo and Confluence, respectively. Allelic richness per locus ranged from 2.0 (*Nme*24B6.191, Zoo) to 13.5 (*Nme*2B10.232, Confluence) and averaged 6.9 and 8.3 for Zoo and Confluence, respectively. Gene diversity per locus ranged from 0.049 (*Nme*24B6.191, Confluence) to 0.869 (*Nme*12D8.125, Conflu-

Table 1. Number of Pairwise Relationships Inferred from Multilocus Genotypes in Captive (Zoo) and "Wild" (Confluence) Samples of Cape Fear Shiners (*Notropis mekistocholas*).

Relationship	Zoo ($N = 40$)	Confluence ($N = 44$)
Full-sib relationships	4	0
Half-sib relationships	97	83
Half sibs not sharing mtDNA haplotype	51	70
Half sibs involved in multiple sibships	39	41

ence) and averaged 0.647 and 0.699 for Zoo and Confluence, respectively. Significant differences in both allelic richness ($P = 0.006$) and gene diversity ($P = 0.004$) were detected in pairwise comparisons between samples.

Tests of conformity to Hardy-Weinberg equilibrium expectations were nonsignificant following Bonferroni correction except for *Nme*30f12.208 in Zoo and *Nme*12D8.125 in both samples. The departure from equilibrium at *Nme*12D8.125 in Confluence is due to highly significant heterozygote deficiency (Gold et al., 2004) and may reflect occurrence of null alleles; consequently, *Nme*12D8.125 was omitted from subsequent analysis. The overall departure from Hardy-Weinberg equilibrium in Confluence was positive ($F_{IS} = 0.038$) but nonsignificant ($P = 0.206$). A significant excess of heterozygotes over all loci was found in Zoo ($F_{IS} = -0.030$, $P = 0.004$). A total of 36 of 462 pairwise tests of genotypic disequilibrium were significant ($P < 0.05$) prior to Bonferroni correction; of these, three (*Nme*2B10.166/*Nme*2B10.232, both samples; and *Nme*24B6.211/*Nme*15F2.174, Confluence) remained significant after Bonferroni correction.

A total of 15 distinct mtDNA haplotypes were detected (Appendix 2). Fourteen haplotypes were found in Confluence, whereas only seven were found in Zoo. Nucleon diversity was slightly higher in Confluence (0.796 ± 0.056) than in Zoo (0.714 ± 0.065).

Comparison of allele and haplotypes distributions between samples.—Significant heterogeneity ($P < 0.05$) of allele distributions between the two samples was detected at 14 of the nuclear-encoded loci prior to Bonferroni correction; 10 of these remained significant after Bonferroni correction. The overall degree of divergence between the two at nuclear-encoded loci was significant ($F_{ST} = 0.027$, 95% confidence interval = 0.018–0.036; $P < 0.0001$). No significant difference in haplotype distributions was detected between samples ($\text{Phi-}_{ST} = 0.010$, $P = 0.197$).

Kinship analysis.—Kinship analysis of Zoo revealed a total of 101 significant (half-sib plus full-sib) relationships, including 97 putative half-sib relationships and four full-sib relationships (Table 1). Occurrence of half-sib families was inferred conservatively by including individuals when significant relationships were found with all other individuals involved in a putative family. The size of families inferred as such was two individuals in most cases but ranged from two to four individuals. A total of 51 of the putative half-sib pairs did not share the same mtDNA haplotype. Such pairs could correspond to paternal half-sibs (i.e., individuals that shared the same sire but not the same dam). A total of 39 individuals were involved in more than one putative half-sib family, suggesting that both parents were involved in matings with multiple partners (i.e., some parents of both sexes were polygamous). However, given the high probability of undetected relationships (Type II errors), members of a given family may not show significant relationship with other members, thus resulting in fragmentation of large families and incorrect inference of family composition. Kinship analysis of Confluence revealed less frequent occurrence of related individuals, with a total of 83 significant half-sib relationships and no full-sib relationships (Table 1). The matrices of relationships for both Zoo and Confluence are available from the first author (ES).

Effective population size.—Estimates of effective population size (N_e) based on linkage disequilibrium were 49 (95% confidence interval = 43–56) for Zoo and 528 (273–5336) for Confluence.

Discussion

We assessed levels of genetic diversity in a captive stock of Cape Fear Shiners maintained at the North Carolina Zoological Park and in 44 specimens sampled from the wild population from which the captive stock was originally founded. Levels of allelic diversity as inferred

from polymorphism at 22 nuclear markers were relatively high in both samples; average number of alleles sampled were 7.0 and 8.6, whereas gene diversity averaged 0.647 and 0.699 for the captive and wild samples, respectively. These averages are nearly the same or higher than those reported by DeWoody and Avise (2000) for freshwater fishes and much higher than those encountered in other endangered vertebrates (references in Saillant et al., 2004). However, although high levels of allelic diversity were present in the captive population, both allelic richness and gene diversity were significantly lower than in the sample from the wild population. Similar results were obtained for mtDNA; only seven haplotypes were found in the sample from the captive population, whereas 14 were found in the sample from the wild population. Nucleon diversity also was lower in the sample from the captive population (0.714 ± 0.065 vs 0.699 ± 0.056).

The above results are consistent with the hypothesis that genetic diversity has been lost in the captive population because of small effective population size and concomitant genetic drift. Small effective size (and genetic drift) in the captive population also could lead to divergence in allele and haplotype frequencies. Significant differences in allele distributions between the two samples were detected at several microsatellites (14 prior to Bonferroni correction and 10 after correction) and the estimated F_{ST}-value of 0.027 between the two samples differed significantly from zero. We then asked whether genetic drift alone could account for the genetic differences observed between the two sampled populations given the very short time period involved (three generations). We used the equation (from Hedrick, 1999)

$$F = 1 - \left(1 - \frac{1}{2N_e}\right)^t$$

where F is F_{ST} among idealized populations under an infinite-island model (and assuming no migration), N_e is effective size, and t is time in generations. For N_e, we used the harmonic means of the lower ($N_e = 74$) and upper ($N_e = 112$) bounds of the confidence intervals of N_e obtained for the two samples. Assuming a starting F_{ST} of 0, an F_{ST} of 0.020 would be generated at $N_e = 74$, and an F_{ST} of 0.013 would be generated at $N_e = 112$. The estimated values of F_{ST} are close to the actual estimate of F_{ST} (0.027) obtained, indicating that genetic drift alone could have given rise to the genetic differences observed.

Small effective population sizes also can result in frequent occurrence of half and full sibs, leading to significant, pairwise genetic-relatedness coefficients (Launey et al., 2001). We examined familial structure in the two populations via KINSHIP analysis and found 101 significant half-sib/full-sib relationships in the captive population as compared to 83 in the wild population. The size of the (inferred) half-sib/full-sib families in the captive population varied from two to four individuals, suggesting that variation in family size may occur. However, the variance in family size could not be estimated because of the imprecise inference of the exact familial structure of the sample. Moreover, a high proportion of half-sib and full-sib relationships could not be detected because of the elevated Type II error rates resulting from KINSHIP analysis. It would be useful to quantify the variance in family size in the captive population, given its potential effect on reducing effective population size (Nunney and Elam, 1994).

KINSHIP analysis also provided evidence for polygynous mating in that more than half of the (putative) half-sib relationships in both samples did not share mtDNA haplotypes. Polyandrous matings also may have occurred, given that numerous individuals in both samples were involved in multiple sibships. Unfortunately, this aspect of familial structure could not be ascertained, given the incomplete assessment of familial structure resulting from KINSHIP analysis. However, many species of genus *Notropis* exhibit broadcasting spawning (Johnston and Page, 1992) where polygynous mating might occur. A polygynous versus a polygynandrous (polygyny and polyandry) mating system in *N. mekistocholas* would lead to different consequences relative to effective population size. A polygynous mating system would likely decrease effective size, whereas polygynandry would likely approach random union of gametes and thus maximize effective size (Nunney, 1993).

KINSHIP analysis assumes no inbreeding and no linkage disequilibrium. The impact of violations of these assumptions on KINSHIP analysis on populations of finite size has not been explored; however, the resulting bias is expected to be minor if panmixia occurs within the population under study (K. Goodnight, pers. comm.).

Effective population size (N_e) was estimated via the linkage-disequilibrium approach (Bartley et al. 1992) for both the captive and wild populations. The estimate for the captive population was 49 (95% confidence interval = 43–56), whereas the estimate for the wild population was 528 (95% confidence interval = 273–5336). The N_e estimate for the captive popula-

tion is five times smaller than the estimated number (~250) of breeding adults. The ratio of N_e to the number of breeding adults is ~0.20 and is well within the range of N_e:N ratios reported for vertebrates (Frankham, 1995). However, the smaller N_e just as likely may reflect the small number of founders (31) used to constitute the original stock. Indeed, the N_e estimate of 49 is close to the harmonic mean (78) of the census number of breeders used over the three successive generations of captive breeding. This may suggest that a high proportion of available breeders contributed to each generation, further suggesting that a few hundred Cape Fear Shiners used as breeders might be sufficient to generate N_e-values comparable to that of the wild population provided that captive breeding conditions are implemented as in the present study.

The N_e estimate for the wild population (528) was to close to that obtained by Saillant et al. (2004) using a coalescent approach (513, 95% confidence interval = 54–4463) for the same sample. An effective size of a few hundred is in theory needed to maintain the equilibrium between loss of adaptive genetic variation caused by genetic drift and its replacement by mutation (Franklin, 1980; Schultz and Lynch, 1997). However, Lynch and Lande (1998) argued for several reasons that the target effective size for a conservation program for an endangered species should be in the range 1000–5000. Current effective size of Cape Fear Shiners at the Confluence locality is approximately 500 and would appear to be a minimum target for conservation planning.

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APPENDIX 1. SUMMARY STATISTICS AT 22 NUCLEAR-ENCODED LOCI IN TWO SAMPLES OF CAPE FEAR SHINERS (*Notropis mekistocholas*). *N* = sample size, #A = number of alleles; AR = allelic richness, H_E = gene diversity, P_{HW} = probability of conformity to Hardy-Weinberg genotypic expectations, and F_{IS} = inbreeding coefficient.

Locality		Zoo	Confluence			Zoo	Confluence
Locus				Locus			
*Nme*4F4.154	*N*	40	44	*Nme*4F4.222	*N*	40	44
	#A	3	5		#A	8	9
	AR	2.92	4.95		AR	7.84	8.81
	H_E	0.513	0.546		H_E	0.733	0.684
	P_{HW}	0.621	0.882		P_{HW}	0.066	0.116
	F_{IS}	0.123	0.001		F_{IS}	0.080	0.103
*Nme*7B9.190	*N*	40	42	*Nme*18A6.158	*N*	39	44
	#A	7	10		#A	9	12
	AR	6.92	9.75		AR	8.95	11.16
	H_E	0.754	0.840		H_E	0.814	0.803
	P_{HW}	0.397	0.777		P_{HW}	0.020	0.205
	F_{IS}	−0.028	0.064		F_{IS}	0.023	0.095
*Nme*18C2.178	*N*	40	44	*Nme*24B6.191	*N*	40	44
	#A	9	8		#A	2	2
	AR	8.98	7.66		AR	1.99	2.00
	H_E	0.681	0.718		H_E	0.049	0.128
	P_{HW}	0.907	0.075		P_{HW}	1.000	1.000
	F_{IS}	−0.065	0.114		F_{IS}	−0.013	−0.062
*Nme*24B6.211	*N*	40	44	*Nme*25C8.208	*N*	40	43
	#A	7	10		#A	8	11
	AR	6.99	9.61		AR	8.90	10.56
	H_E	0.648	0.740		H_E	0.736	0.812
	P_{HW}	0.907	0.632		P_{HW}	0.044	0.171
	F_{IS}	−0.042	−0.044		F_{IS}	−0.088	0.112
*Nme*26G8.96	*N*	40	44	*Nme*30D2.120	*N*	40	44
	#A	5	5		#A	3	3

Appendix 1. Continued.

Locality		Zoo	Confluence			Zoo	Confluence
	AR	4.95	4.82		AR	2.92	3.00
	H_E	0.355	0.547		H_E	0.164	0.348
	P_{HW}	0.464	0.472		P_{HW}	0.249	1.000
	F_{IS}	0.132	0.169		F_{IS}	0.238	−0.112
*Nme*18A6.0	*N*	39	43	*Nme*5B10.211	*N*	40	44
	#A	3	3		#A	7	12
	AR	3.00	3.00		AR	7.00	11.67
	H_E	0.491	0.587		H_E	0.779	0.863
	P_{HW}	0.139	0.102		P_{HW}	0.325	0.996
	F_{IS}	−0.098	0.090		F_{IS}	−0.091	0.026
*Nme*27F8.230	*N*	39	44	*Nme*12D8.125	*N*	37	44
	#A	9	8		#A	7	11
	AR	8.74	7.98		AR	7.00	10.63
	H_E	0.647	0.757		H_E	0.715	0.869
	P_{HW}	0.164	0.623		P_{HW}	<0.001	<0.001
	F_{IS}	−0.070	−0.111		F_{IS}	0.547*	0.372*
*Nme*15F2.174	*N*	40	44	*Nme*30F12.208	*N*	37	42
	#A	6	6		#A	7	14
	AR	5.92	5.97		AR	7.00	13.51
	H_E	0.644	0.560		H_E	0.831	0.846
	P_{HW}	0.979	0.962		P_{HW}	<0.001*	0.017
	F_{IS}	−0.087	−0.055		F_{IS}	0.382	0.184
*Nme*33B6.125	*N*	40	44	*Nme*2B10.166	*N*	40	43
	#A	5	5		#A	7	7
	AR	5.00	5.00		AR	6.99	6.84
	H_E	0.641	0.666		H_E	0.749	0.727
	P_{HW}	0.149	0.705		P_{HW}	0.107	0.888
	F_{IS}	−0.287	−0.058		F_{IS}	−0.035	0.008
*Nme*2B10.232	*N*	40	44	*Nme*5G4.175	*N*	40	44
	#A	12	13		#A	11	13
	AR	11.84	12.93		AR	10.84	12.33
	H_E	0.866	0.843		H_E	0.797	0.812
	P_{HW}	0.958	0.050		P_{HW}	0.783	0.026
	F_{IS}	−0.040	0.084		F_{IS}	−0.160	0.132
*Nme*6A7.93	*N*	40	44	*Nme*2D5.117	*N*	40	44
	#A	11	11		#A	8	11
	AR	10.91	10.79		AR	7.92	10.72
	H_E	0.814	0.850		H_E	0.806	0.824
	P_{HW}	0.405	0.790		P_{HW}	0.033	0.747
	F_{IS}	−0.045	0.010		F_{IS}	−0.210	−0.130

* Significant after Bonferroni correction.

Appendix 2. Haplotype Frequencies and Summary Statistics for 40 Cape Fear Shiners (*Notropis mekistocholas*) Sampled from a Captive Population (Zoo) and 44 Specimens Sampled from the "Wild" Population (Confluence) from which the Captive Stock Was Founded. Haplotypes were obtained by concatenation of two sequenced fragments taken within the protein-coding ND-5 and ND-6 genes.

Gene Bank Accession (ND-5–ND-6)	Confluence	Zoo
AY396545–AY396555	19	19
AY396546–AY396555	3	1
AY396547–AY396556	4	1
AY396548–AY396556	1	0
AY396545–AY396557	1	0
AY396549–AY396558	1	0
AY396550–AY396559	4	0
AY396551–AY396560	1	0
AY396552–AY396556	4	4
AY396545–AY396561	1	0
AY396547–AY422792	2	4
AY422788–AY396555	1	6
AY396545–AY422792	1	0
AY422789–AY396559	1	0
AY396549–AY?	0	3
Number of haplotypes	14	7
Nucleon diversity	0.796 ± 0.056	0.714 ± 0.065