

GENETIC CONSIDERATIONS DURING THE EXPERIMENTAL AND EXPANDED PHASES OF SNOOK STOCK ENHANCEMENT

Michael D. Tringali[†] and Kenneth M. Leber[‡]

[†] Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute
100 Eighth Avenue S.E., St. Petersburg FL 33701

+1-727-896-8626 +1-727-823-0166 (fax) e-mail : Tringali_M@epic7.dep.state.fl.us

[‡] Mote Marine Laboratory, Center for Fisheries Enhancement, 1600 Ken Thompson Parkway, Sarasota, FL 34236

+1-941-388-4441 +1-941-388-6461 (fax) e-mail : KLeber@mote.org

ABSTRACT

A responsible approach to stock enhancement requires that negative impacts on the gene pools of wild populations be mitigated by the implementation of genetically sound breeding and release procedures. Such procedures should be readily adaptable as a stock enhancement program evolves from the experimental to the expanded phases of production and release. Common snook *Centropomus undecimalis* is a neotropical estuarine fish that constitutes a socioeconomically valuable recreational fishery in Florida (USA). Experimental releases of hatchery-reared snook are underway in southwest Florida to assess the potential for successful snook stock enhancement. Relevant genetic, demographic, and biological data are available for wild snook stocks. Herein, we apply those data and population genetic principles to develop genetic guidelines for snook stock enhancement. In Florida, snook populations are biologically and genetically divergent between the Atlantic Ocean and the Gulf of Mexico. No transfers should occur between the Atlantic and Gulf snook populations. In the Gulf, snook may be further subdivided into interconnected demes having limited genetic exchange; broodstock sources should be limited to the targeted system or an adjacent estuary. Compared to other marine and estuarine fishes, allozyme and mitochondrial DNA polymorphism is low in *C. undecimalis*. Most allozyme polymorphism is maintained in the form of rare alleles occurring at frequencies of ≤ 0.05 . During the expanded phases of stocking, it is recommended that at least 100 wild-caught adults per generation interval (*GI*), ≈ 3 years, be used to found hatchery populations and that the genetic effective sizes of those populations be ≥ 50 . This should preserve $>99\%$ of the original heterozygosity and incorporate rare alleles into hatchery populations. We modeled the potential reductive effects of stocking on the effective sizes of enhanced snook populations. Assuming 50 effective hatchery breeders are used, hatchery contributions to Atlantic or Gulf populations should not exceed 31% per *GI*. Conservatively estimating hatchling survivorship and wild spawning stock abundance, we propose stocking guidelines that satisfy this requirement.

INTRODUCTION

The common snook *Centropomus undecimalis* (Bloch) is a semicathadromous, stenothermic, euryhaline species occurring in the tropical and subtropical Western Atlantic Ocean. It is a top predator in estuarine and nearshore environments, attaining weights of up to 27 kg and lengths of up to 1.3 m (IGFA 1996). Throughout its range, the common snook is a valuable game and food fish (Tucker and Campbell 1985). In the United States, common snook occur along the southern half of the Florida peninsula and along the southeastern Texas coast. Although the species has supported commercial and recreational fisheries in Texas coastal lagoons in the past, it is only rarely landed

there at this time because of overharvest and adverse environmental factors (Matlock and Osburn 1987). In Florida, common snook continues to represent an important component of the sport fishery, ranking among the top three species specifically targeted by recreational anglers (Muller and Murphy 1998). Declines during the late 1970s and early 1980s resulted in its designation as a species of "special concern" by state fishery managers; harvest is currently regulated by permit requirements, prohibition of sale, strict bag and size limits, gear restrictions, and seasonal closures. Nonetheless, approximately 1.6 million common snook were caught by Florida anglers during 1997, of which at least 200,000 were harvested. Despite the increasingly stringent regulations, the annual rate of

harvest has increased fourfold during the last decade (Muller and Murphy 1998). Additional harvest controls have recently been proposed by the Florida Marine Fisheries Commission and await approval by the Florida Cabinet. During the early 1980s, the interest in stock enhancement as a potential management tool for the Florida common snook fishery intensified. Mariculture programs were initiated at the University of Miami Rosenstiel School of Marine and Atmospheric Sciences, Harbor Branch Oceanographic Institute (HBOI), Mote Marine Laboratory (MML), and Florida Marine Research Institute (FMRI). Propagation of common snook in captivity proved to be a difficult, stepwise process in which significant problems associated with broodstock handling, egg production, bacterial infection, and larval and juvenile feeding had to be overcome (Anonymous 1993). Recently, through collaborative effort, MML, HBOI, and FMRI were able to refine breeding techniques for common snook (Kennedy *et al.* 1998) and to rear sufficient numbers of hatchlings for controlled release into juvenile nursery habitats (S. Serfling, MML, pers. commun.). This achievement raised the possibility that cultured snook may be used to enhance overexploited stocks in Florida or to offset losses caused by degradation of critical habitat and natural, acyclic perturbations (e.g., cold kills, red tide).

It has been recommended that incipient stocking programs adopt an experimental approach (Leber 1999), predicated upon the involvement of many scientific subdisciplines, adherence to the scientific method, and the use of "active-adaptive" management. Currently, pilot studies involving small-scale releases of common snook are being conducted in southwest Florida to determine optimal release strategies, e.g., size at release, timing of release, stocking densities, critical habitat assessment (Leber *et al.* 1997). During this "experimental" stocking phase, survival, growth, and recruitment of cultured fish to local (or non-local) populations will be assessed (Leber and Arce 1996, Leber *et al.* 1998). If, after completing the experimental phase for common snook, stock enhancement appears to be a useful tool for the overall management of the Florida common snook fishery, the stocking program could progress, rapidly or gradually, into an "expanded" phase of production and release.

A responsible approach to marine stock enhancement requires that potential negative impacts upon the gene pools of wild populations be mitigated through the use of genetically sound breeding and release protocols (Blankenship and Leber 1995). Consequently, researchers and managers at MML and FMRI seek to include genetic considerations into the overall management plan for their developing snook program. Herein, we integrate population genetic principles and baseline information on genetic diversity, population structure, and demographics of wild snook stocks to address genetic hazards and to develop a

preliminary genetic risk management strategy for the snook enhancement program. We begin by reviewing the general types of genetic concerns that are most relevant to marine stock enhancement programs.

GENETIC HAZARDS

Some level of genetic exchange must be anticipated between native and hatchery stocks for marine stock enhancement programs. There are numerous ways in which cultured organisms can have a direct genetic impact on recipient stocks (reviewed by Utter 1998). The majority of genetic hazards may be grouped into three categories. We define genetic "Type I" hazards as those that occur by way of hatchery-mediated translocation of exogenous genes into native populations. Hatchery progeny derived from breeders belonging to a genetically divergent stock may, upon release, interbreed with conspecific or even congeneric members of the recipient stock (Leary *et al.* 1995, Sheridan 1995). The admixing of genetically discrete stocks (Altukhov and Salmenkova 1987) can break down local adaptations through introgression of maladapted genes or by disruption of coadapted genomes, thereby affecting the fitness of the native stock (outbreeding depression; c. f., Templeton 1986, Waples 1995). For example, interracial crosses between even- and odd-year returning pink salmon have resulted in decreased survivorship and increased bilateral asymmetry in F_2 hybrids (Gharrett and Smoker 1991).

If genetic stock structure in a candidate species has been characterized, genetic hazards associated with intraspecies introgression may be minimized through judicious broodstock source selection (Hindar *et al.* 1991, Philipp *et al.* 1993). This approach reduces Type I hazards but does not mitigate all genetic risks.

Genetic hazards in the second category (Type II hazards) may be broadly defined as those stemming from genetic changes in a hatchery population, irrespective of the source of broodstock, that directly result from the processes of broodstock sampling, breeding, and rearing. Typically, the number of breeders selected to found the hatchery stock represents a small percentage of the available breeders in the source population. When insufficient numbers of breeders are used, sampling error can cause large stochastic differences in allelic and genotypic frequencies (Taniguchi and Sugama 1990) or reduced levels of genetic variation in hatchery broods compared to the wild stock (Bartley and Kent 1990). Hatchery populations can also be genetically compromised if the initial broodstock sampling fails to capture a sufficient range of phenotypic variability available in the source population (Leary *et al.* 1986). Other types of genetic changes to hatchery populations include artificial selection and domestication (Kohanne and Parsons 1988) and inbreed

ing depression (Tave 1993). Artificial selection, domestication, stochastic allele frequency changes, and reduced levels of variation can occur in the F_1 generation. However, hatchery populations must usually be propagated over multiple generations without sufficient input of additional wild genotypes before experiencing the deleterious effects of inbreeding.

The third category of genetic hazard (Type III) is represented by a singular mechanism --- the possible genetic swamping of natural populations through successful enhancement efforts. This mechanism can lead to post-stocking alterations in the native gene pool even when hatchery populations lack Type I and Type II genetic risk factors. Because of the disproportionate contribution of hatchery-derived progeny to the gene pool of a supplemented stock, an inevitable reduction occurs in the genetically "effective" population size of the admixed (enhanced) stock in the following generation (Ryman and Laikre 1991). The effective population size (N_e) represents the hypothetical abundance (number of individuals) in an ideal population (i.e., randomly mating, demographically constant, devoid of selection, migration, and mutation) that would undergo genetic change at the same rate as an actual population of abundance N . The magnitude of N_e in an admixed population composed of hatchery and wild stocks is a function of the original effective population size of the wild stock ($N_{e,w}$), the effective number of breeders in the hatchery stock ($N_{e,h}$), and the relative contribution of reproductively mature hatchery offspring (x) to the admixed population. According to the Ryman/Laikre model

$$N_e = \left[\frac{x^2}{N_{e,h}} + \frac{(1-x)^2}{N_{e,w}} \right]^{-1} \quad (\text{Eq. 1})$$

Reductions in N_e , if severe, can result in substantial allelic and genotypic frequency changes over time and, depending upon future population abundance (Waples and Do 1994), excessive loss of genetic diversity. Tringali and Bert (1998) evaluated the sensitivity of the model parameters $N_{e,h}$, $N_{e,w}$, and x over a range of values that may be typical for marine stock enhancement programs. The parameter x , a function of the number of cultured fish stocked, was shown to exert the greatest influence on the effective population sizes of supplemented marine populations. By using the model to quantitatively assess the Type III risk level for two marine species having highly disparate population dynamics and genetic structures (i.e., red drum and Atlantic sturgeon), Tringali and Bert (1998) underscored the relationship between species life history and the potential genetic impact of stock enhancement.

BIOLOGICAL AND GENETIC RESOURCES IN FLORIDA COMMON SNOOK

Population Dynamics and Biology

Largely because of its popularity as a game fish and food fish, common snook has been extensively studied in Florida. Consequently, many biological, demographic, and life history traits for the species have been well characterized. Many of these traits differ between common snook from the Atlantic and Gulf of Mexico waters of Florida. From tagging studies (reviewed by Tringali and Bert 1996), extensive movement by adult common snook has been documented along Florida Atlantic nearshore waters --40% of 1,947 individuals recaptured had dispersed 50-350 km from their site of release. In contrast to the extremely vagile members of the Florida Atlantic common snook population, members of the Florida Gulf population exhibit a strong philopatric behavior within natal estuaries -- 99.5% of 2,053 common snook tagged in Gulf estuaries were recaptured <10 km from their release site, regardless of the time interval between tagging and recapture. Important biological differences also occur between Atlantic and Gulf common snook, including growth rate, natural mortality, female longevity, age at maturity (Taylor *et al.* 1998a), and annual reproductive cycle (Taylor *et al.* 1998b). Because these biological traits typically have significant components of additive genetic variation and high heritabilities in fishes (Hard 1995), the inherent differences distinguishing these groups of common snook should be viewed as a genetic resource.

Atlantic and Gulf populations also differ in total abundance and in abundance trends (Muller and Murphy 1998). From 1988-1998, the average total abundance for the exploitable portion of the Atlantic population (ages 3+) was estimated to be 410,000. Annual abundance estimates have declined since 1993 ($N = 506,000$) to a 10-year minimum of 250,000 in 1998. In the Gulf, the average total abundance (age 3+) between 1988 and 1998 was estimated to be 607,000; annual abundances have fluctuated considerably around that mean. Currently, the exploitable Gulf population is thought to be composed of 850,000 snook and the breeding population, which contains a portion of 2-year-old snook (Taylor *et al.* 1998a), may be in excess of 1 million.

Genetic Structure and Diversity

Population structure and genetic diversity in common snook were examined by Tringali and Bert (1996) using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis and allozyme electrophoresis. Application of neighbor-joining cluster analysis to between-sample mtDNA sequence divergence values revealed that common snook populations are genetically

subdivided in Florida between Atlantic and Gulf waters (Figure 1). Florida Gulf samples showed a high degree of mtDNA similarity to Caribbean samples; these samples formed a group that was divergent from the Florida Atlantic group. Using the nucleotide divergence values for pairwise comparisons of mtDNA haplotypes from Tringali and Bert (1996), we performed a hierarchical analysis of molecular variance to calculate Φ statistics (analogous to F statistics) and to estimate components of genetic variance (Table 1). The majority of mtDNA variance is apportioned within samples. However, a significant amount of the total variance (~10%) is partitioned between the Atlantic group and the Gulf/Caribbean group, providing statistical support for the hypothesis that these groups represent genetically divergent populations. Components of variance among samples within the Atlantic, Gulf, and Caribbean groups, respectively, were not different from zero (negative variances and Φ statistics are allowed by the AMOVA procedure), indicating that

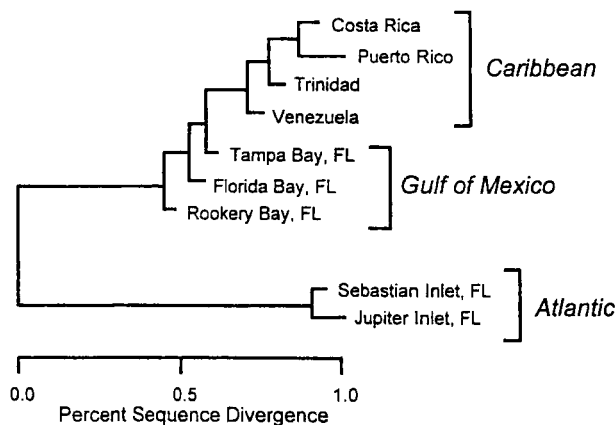


Figure 1. Geographic relationships among common snook *Centropomus undecimalis* based on a neighbor-joining analysis (RESTSITE computer program, version 1.2; Nei and Miller 1990) of between-sample mtDNA sequence divergences [tree redrawn from Tringali and Bert (1996)]. See Figure 3 for the collection locations of the Florida samples.

the mitochondrial genomes of common snook are relatively homogeneous on a regional basis. A hierarchical analysis of geographic structure based on allozymes using F statistics (Weir and Cockerham 1984) was generally concordant with the mtDNA hypothesis that gene flow between Florida Atlantic snook and those from other regions is restricted, although the sample from Florida Bay could not be assigned with statistical certainty to either the Atlantic or the Gulf population (Tringali and Bert 1996).

Tringali and Bert (1996) observed that allozyme and mtDNA polymorphism is generally low in common snook. In their allozyme survey of 187 Florida common snook (49 Atlantic, 138 Gulf), the average number of alleles per locus for the 31 presumptive genetic loci examined was approximately 1.4 for the Atlantic population and 1.6 for the Gulf population. The average heterozygosity value, H_o , for all loci was 0.027 (± 0.010) for the Atlantic population and 0.033 (± 0.013) for the Gulf population. Measures of allozyme diversity for each sample are given in Table 2. For each locus, the majority of alleles other than the most common allele occurred at very low frequency (<0.05 ; Figure 2A). Because the probability of sampling alleles diminishes as allele frequency decreases (Figure 2B), it is likely that many alleles occurring at frequencies ≤ 0.01 were not detected. Thus, the actual distribution is most likely U-shaped -- highly skewed toward very rare alleles and very common alleles at the expense of intermediate-frequency alleles (Chakraborty *et al.* 1980).

All measures of mtDNA variability are very low in common snook (Table 2; see also Wilson *et al.* 1997). Nucleotide diversity is an order of magnitude below values for the majority of marine and estuarine perciform fishes, e.g., red drum, sheepshead (FMRI, unpublished data), black drum, spotted seatrout, red snapper, and greater amberjacks (Gold and Richardson 1998), but similar to diversity values for other lower percoid fishes that, like snook, are sequential hermaphrodites (Gold and Richardson 1998). Both nucleon (h) and nucleotide sequence diversities (p) were higher in Atlantic samples than in Gulf samples. The disparate p values between these two populations suggests that the effective (female)

Table 1. Hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) among mitochondrial DNA composite haplotypes of common snook from Florida. Analysis based on data presented in Tringali and Bert (1996). nc = not calculated.

Variance Component	Φ statistic and value	Variance	% of total	P^a
Between Atlantic and Gulf/Caribbean	$\Phi_{CT} = 0.099$	0.006	9.98	0.014
Among samples within Atlantic	$\Phi_{SC} = -0.251$	-0.024	-29.13	0.487
Among samples within Gulf/Caribbean	$\Phi_{SC} = -0.068$	-0.007	-18.87	0.998
Within samples	nc	0.083	138.79	nc

^a Probability of obtaining a more extreme random value, based on 5,000 permutations.

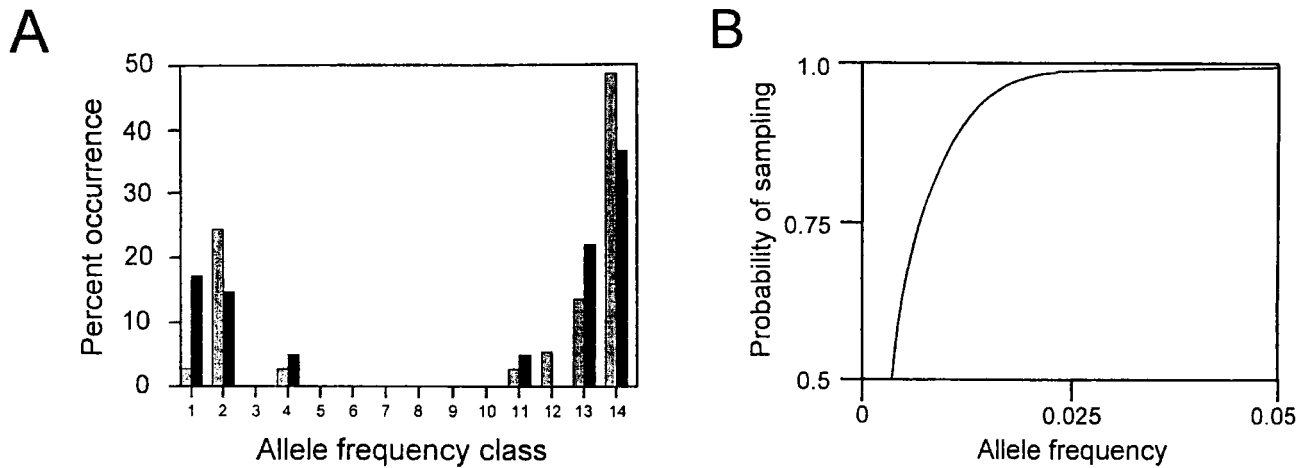


Figure 2. (A) Observed distribution of allozyme allele frequencies in common snook *Centropomus undecimalis* from the Florida Atlantic (light bars; $n=49$ individuals) and Florida Gulf (dark bars; $n=138$ individuals). Alleles were allocated by frequency of occurrence into 14 classes: $0.0 < 0.01$, $0.01 < 0.05$, $0.05 < 0.1$, $0.1 < 0.2$, $0.2 < 0.3$, $0.3 < 0.4$, $0.4 < 0.5$, $0.5 < 0.6$, $0.6 < 0.7$, $0.7 < 0.8$, $0.8 < 0.9$, $0.9 < 0.95$, $0.95 < 0.99$, $0.99 < 1.0$ (see Chakraborty et al. 1980). Protein electrophoretic procedures and a list of the 31 presumptive loci surveyed may be found in Tringali and Bert (1996). (B) Single-locus probability of sampling an alternate allele (y -axis) when it occurs in a population at a given frequency (x -axis) and when 138 individuals are sampled.

Table 2. Summary of allozyme and mitochondrial DNA variation in *Centropomus undecimalis* from Florida. Except for the average number of allozyme alleles per locus (\bar{n}_a), results appear in Tringali and Bert (1996). \bar{H}_o = average proportion of heterozygotes per locus; P_{95} = percentage of loci in which the frequency of the most common allele did not exceed 95%; h = mtDNA nucleon (haplotype) diversity; p = percent mtDNA nucleotide sequence divergence. Locations of samples are depicted in Figure 3.

Location	Allozyme			mtDNA	
	\bar{n}_a	P_{95}	\bar{H}_o	h	p
<i>Atlantic coast</i>					
Sebastian Inlet	1.23	13	0.022	0.83	0.28
Jupiter Inlet	1.29	3	0.024	0.82	0.16
<i>Gulf of Mexico</i>					
Florida Bay	1.24	7	0.023	0.50	0.06
Rookery Bay	1.42	10	0.032	0.63	0.08
Tampa Bay	1.32	7	0.025	0.33	0.08

population size has remained higher in the Atlantic population (~32,000 females) than in the Gulf population (~11,000 females) for an ecologically meaningful period of time.

To summarize the biological and genetic data, common snook in Florida are regionally divided into two populations occurring in Atlantic and Gulf of Mexico waters. Each population contains unique biological and genetic resources that should be preserved. Because common snook in Florida Gulf waters are highly philopatric, the Gulf population may be further subdivided into loosely-

connected demes that occasionally exchange migrants among adjacent estuaries. Gene flow among common snook within the respective Atlantic and Gulf populations appears to be sufficiently high to homogenize neutral genetic variation over time. However, the gene pools of localized demes along the Florida Gulf coast may still be temporally affected by a large-scale stock enhancement program. Accordingly, the genetic-management goals for snook stock enhancement in Florida should be focused on the conservation of within-population diversity and between-population divergence.

RECOMMENDATIONS FOR GENETIC MANAGEMENT OF SNOOK STOCK ENHANCEMENT

The common snook stock enhancement program being conducted by MML and FMRI in southwest Florida is currently in the experimental release phase. Between April 1997 and April 1998, approximately 25,000 cultured snook were tagged and released into various juvenile nursery habitats in Sarasota Bay (K. Leber, S. Serfling, and B. Halstead, unpublished data). Assessments of the various release treatments are ongoing. Monitoring studies by MML have shown that cultured snook can contribute significantly to the abundance of juvenile snook (up to 30%) in net samples from stocked nursery habitats 1 year after release (N. Brennan, K. Leber, and S. Serfling, unpublished data). Cultured juvenile snook so far have exhibited strong release-site fidelity, as would be expected for wild Gulf snook. The husbandry and stocking technologies for snook are rapidly progressing to a point at which the large-scale stocking of hatchery-reared snook could be considered an optional management tool for the Florida snook fishery. Because the nature and potential severity of genetic impacts upon enhanced populations change as stocking programs evolve from experimental to expanded phases of production and release, we evaluate the genetic concerns of these phases separately.

Experimental Stocking Phase

During the experimental stocking phase, managers of the common snook enhancement program should avoid transferring genetic material between subdivided stocks (Type I risks). Tringali and Bert's (1996) genetic stock identification for wild snook populations provides the baseline information needed for broodstock source selection. However, there remain two caveats concerning the genetic characterization of common snook (see Grant *et al.* 1999). First, geographic patterns in adaptive traits (e.g., disease resistance, thermal tolerance, timing of spawning) might be masked in assays of presumably neutral markers (Utter *et al.* 1993, Conover 1998) such as those employed in the genetic study of common snook. Second, fine scale stock structure (e.g., among samples within regions) may not always be detected in mtDNA RFLP and allozyme analyses, especially when the sampled genetic diversity is low (Brunner *et al.* 1998). Accordingly, we advocate a conservative approach regarding broodstock source selection.

Therefore, mindful of the genetic, biological, and behavioral differences among common snook, we recommend that the species be divided into multiple conservation units in Florida. Hatchery-mediated genetic exchange between Florida Atlantic and Gulf populations should be strictly avoided. For stock enhancement programs involving Gulf common snook, we recommend that hatchery

broodstock be obtained from the recipient spawning stock or collected from systems adjacent to the operational estuary (Figure 3). An exception to this guideline is needed for the southernmost system, i.e., Florida Bay/Florida Keys. Because common snook eggs, larvae, and juveniles are absent from this system (Peters 1993), Tringali and Bert (1996) posited that local adult stocks may be a mixed stock composed of individuals from both the Atlantic and the Gulf. A detailed study of stock composition in this area is ongoing and, until more is known, snook from this area should not be used to stock any other system. Finally, because of the high vagility of Florida Atlantic common snook, it appears that geographic constraints pertaining to broodstock source could be relaxed in that region.

Although it appears that cultured snook released during MML's pilot studies may contribute significantly to localized juvenile abundance in certain nursery habitats, we estimate that contributions of reproductively mature cultured snook to any local breeding subpopulation will be minimal ($\ll 5\%$) during the course of the experimental stocking phase. Managers of the stocking program currently use wild adults captured from local common snook populations for broodstock. Because only indigenous genotypes are propagated at MML, Type I hazards have been eliminated during the experimental phase. Because newly collected wild snook will be used to produce each generation of hatchery progeny, the Type II hazards relating to inbreeding depression and hatchery adaptation incurred by hatchery fish over multiple generations of captive propagation will also be eliminated (Utter 1998). Type II genetic changes that can occur in the F_1 generation (including artificial selection/domestication, allele frequency shifts, and diversity reductions) remain a possibility for hatchery broods of common snook. However, because of the limited hatchery input to wild stocks (Ryman and Laikre 1995), these changes are unlikely to significantly impact locally adapted gene pools in wild snook unless the experimental phase continues over multiple generation intervals.

Though unlikely to impact the recipient population, fitness reductions in hatchery offspring could affect the outcomes of tests of the various release strategies. Captive propagation imposes very different selection pressures than does natural reproduction (Doyle 1983) and some level of domestication in hatchery broods is almost inevitable (Waples 1999). In studies in which fitness differentials in performance traits have been documented between hatchery-derived and wild fish, hatchery-derived fish typically exhibit poorer performance in the natural environment, suggesting that natural selection has already optimized most genotypic states in those wild populations (Hindar *et al.* 1991). Therefore, culture protocols should be implemented that increase the likelihood that hatchery snook have fitness potentials that are similar to those of