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Genetic changes in natural populations caused by the release of cultured fishes

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Genetic Changes in Natural Populations Caused by the Release of Cultured Fishes

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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Dedication

To my family and friends, for their unwavering kindness, patience, and support…
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Genetic Changes in Natural Populations Caused by the Release of Cultured Fishes

Michael Dominic Tringali

ABSTRACT

Genetic changes likely occur in wild fish populations as a consequence of interactions with cultured fish, but to what extent do those changes threaten the maintenance of natural genetic diversity and population viability? Following a review and categorization of numerous processes suspected of being agents of post-release genetic change in recipient wild populations (Chapter 1), I focus on risks relating to the magnitude and duration of releases – but with a twist. That is, I assume that the mean fitness of released, cultured individuals does not differ from that of the recipient natural population. Throughout, attention is devoted to potential post-release changes in inbreeding \( N_{el} \) and variance \( N_{ev} \) effective population sizes – indicators of expected rates of population-level change in inbreeding and drift variance, respectively. The reductive effect that large-scale releases exert on \( N_{el} \) in recipient populations can be significant. The effect is shown to be a threshold process (Chapter 2) and thus suggestive of an approach for determining risk-adverse stocking (or release) rates. This approach is utilized in Chapter 3, which describes genetic recommendations for an incipient marine stocking program. Several discordant contemporary \( N_{el} \) models are examined mathematically and by computer simulation (Chapter 4). I show that certain published results pertaining to the effect of multiple paternity on \( N_{el} \) are erroneous; a general model
is described which accounts for inbreeding and relatedness in and among parents. That model is utilized in an empirical study of gene correlation in a hatchery cohort (Chapter 5). Propagation-related causes of reductions in $N_{ef}$ are also investigated in this cohort. Finally, extending mutational meltdown theory to accommodate fluctuating population sizes and recessive selective effects, I show that when large reductions in $N_{ev}$ occur (such as those that accompany admixtures of cultured and wild fish), the expected time to population inviability is significantly reduced (Chapter 6). Although a more comprehensive theoretical approach is needed, a precautionary inference may be drawn – aquaculture-induced reductions in $N_e$, even though they may be transient, can lead to adverse genetic impacts. Avoidance of $N_e$-reductions cannot be accomplished, in a practical sense, without considering the stocking or release rates of cultured fish.
Chapter One
Identifying and Managing Genetic Risks from Aquaculture

Introduction

Genes gain a variety of new forms and functions through mutation and other internal molecular processes. Random forces, natural selection, mating systems, migration patterns, and dispersive processes all combine to package genetic variation within individuals and to partition it among groups of individuals. The manner in which genetic material is packaged and partitioned influences individual morphology, physiology, and life history, and affects population dynamics. As a result, numerous, diverse, interconnected populations and species of wildlife exist that are largely suited to their current environment and perhaps capable of adapting to changing environments. Therefore, natural genetic diversity within and between wildlife populations is a valuable resource.

My research focuses on genetic changes that might occur in natural fish populations as a consequence of interactions with cultured fishes. This is a broad subject with a long history of scientific attention and concern. It is a subject of considerable complexity, characterized by processes whose outcomes are, by nature, uncertain. Yet, it is a subject that must ultimately be distilled into simple, effective management policies and practices. As documented below, conservation biologists have relied on population
genetic theory and empirical information (albeit limited) to propose that the introduction of exogenous or artificially altered genomes of cultured fish into natural populations can compromise genetic integrity and population fitness (the mean fitness of individuals in the population). Indeed, even when the relationship between cultured and wild fishes is non-introgressant, it has been suggested that the presence of cultured fish in natural populations can indirectly lead to detrimental genetic impacts.

In this introductory chapter, I describe various activities that result in the admixture of cultured and wild fishes. I offer an opinion regarding the question “at what hierarchical level should we guard against negative genetic impacts?” I categorize and explain the numerous processes posited to cause detrimental genetic changes in admixed (cultured + wild) populations. Illustrative examples of interactions between cultured fish and wild populations of marine, freshwater, and anadromous fishes are provided. In subsequent chapters, I explore in detail certain genetic processes, generally unaccounted for in management practices, which may impact recipient populations.

Activities Involving the Release of Cultured Fishes

Cultured fish may integrate with wild conspecifics or impact other species through a variety of aquaculture-based activities. For natural populations, it is of little consequence whether the release of cultured organisms was sanctioned or unsanctioned, intentional or accidental, a potential for genetic impact ensues. Nonetheless, the potential for and nature of genetic impact usually varies with respect to the type of aquacultural activity involved. These activities may be broadly categorized as follows:
**Restoration or Enhancement of a Natural Stock.** Cultured fishes may be released for the purpose of rehabilitating or supplementing depleted, over-harvested, or under-producing populations. By definition, these activities are conducted within the population’s historical range. Stock restoration represents the attempt to maintain demographic stability and minimize genetic damage in a non-viable population until such time that natural production is self-sustaining. Unless the underlying causes for the population’s threatened or endangered status (e.g., critical habitat loss, pollution, excessive harvest) are also addressed, this activity is not likely to succeed. Stock enhancement represents an attempt to capitalize on underutilized carrying capacity within a population. Because of the nature of stock-enhancement and -restoration operations, infusion of genetic material from cultured fish is to be expected. Indeed, the levels (or rates) of genetic influx from cultured fish into recipient populations are directly related to the success criteria of these programs.

**Reintroduction.** In areas within a species historical range where wild fish are extirpate, attempts to may be made to reestablish self-sustaining breeding populations with fish bred in captivity. Through subsequent emigration from areas of release, the released, cultured fish or their descendants may come into contact with extant populations.

**Establishment/ Maintenance of ‘Put-and-Take’ Fisheries.** As implied in the name, put-and-take aquaculture is the propagation and release of harvestable-sized fishes with the objective of subsequent recreational or commercial harvest. This activity has
been extended to include the release of under-sized fish that, in turn, grow to harvestable sizes (put-grow-and-take aquaculture). Ocean- or sea-ranching activities, when they are open-water or open-system, belong to this category. By definition, put-and-take and put-grow-and-take fisheries are maintained by continued propagation and release rather than by subsequent production from released organisms. Some released organisms, however, may escape harvest and form self-sustaining feral populations or integrate reproductively with natural populations (via immigration or translocation, if the activity occurs outside of the normal range of the natural population). In contrast to stock enhancement and restoration, the amount of genetic influx from cultured fish into natural populations is inversely related to the program’s goal, which is to provide fish for harvest.

**Purposeful Introductions.** Purposeful introductions may involve the release of cultured organisms into habitats and locations outside the normal range of the species from which those organisms were derived. Sometimes these releases are used to create self-sustaining, fishable populations in man-made water bodies or severely altered habitats within the organisms normal or historic range. In other instances, introduced fish are used prescriptively as ‘natural’ control measures for undesirable plants or insects. Although cultured fish may be initially segregated from natural populations, the transference of genetic material into natural populations may occur through immigration or translocation by some extrinsic process (e.g., flooding).

**Commerce Aquaculture.** Commerce aquaculture refers to the production of fish for the purpose of sale as food or aquarium commodities or for *ex situ*
research/educational purposes. Commerce aquaculture excludes commercial operations involved in restoration/enhancement, purposeful introductions, or put-and-take aquaculture. Commercial operations involving net-pen rearing or other closed-system aquaculture are included in this category. Whereas any releases resulting from commerce aquaculture will be, by definition, unintended, an operation’s proclivity for escapement must be considered. Such escapement may be chronic (low level, systemic) or acute (massive, infrequent, non-systemic).

Natural Gene Pools as Genetic Units

The first step in effective resource management is to identify the management goal. The goal addressed implicitly in this dissertation – the preservation and maintenance of the genetic diversity and fitness of natural populations of marine, anadromous, and freshwater fishes – relates to all activities involving the release of cultured fish, whether such activities are purposeful or accidental. The next step is to identify and delineate operational units. For example, having a goal of sustaining fisheries at maximum harvest levels, fisheries managers identify ‘unit stocks’ as groups of fish for which demographic dynamics (i.e., recruitment rates, fishing and natural mortalities, etc.) are largely independent of other such groups (Cushing 1981). Similarly, for genetic management, we should seek distinct operational units for which the genetic dynamics are largely independent, at least for relevant time periods. The delineation of such units fosters creation of tangible, actionable management practices, e.g., identifying
source locations when assembling a captive brood stock and establishing stocking boundaries.

Throughout this dissertation, a natural population (or, equivalently, stock) will be taken to mean a genetically distinct group of fish that naturally interbreeds. This definition is intended to hold even though individuals within the group may be further subdivided into transient geographic or temporal units (demes). A gene pool is taken to mean the total genetic information possessed by members of a natural population. Importantly, my definition of natural population/stock extends beyond that which may be generally inferred from studies of ‘genetic stock structure’ (see reviews by Waples 1987, Shaklee and Bentzen 1998, DeWoody and Avise 2000). Although useful for some purposes (e.g., estimating migration rates and divergence times), such studies rely on selectively neutral markers (or an assumption thereof) to compare estimated levels of gene flow or divergence in light of putatively ‘homogenizing’ levels (sensu Wright 1978). Genotypic distributions at neutral loci, however, represent only a portion of the genetic information contained in gene pools (e.g., Pfrender et al. 2000, Merilä and Crnokrak 2001). For a group of fish to evolve into a genetically distinct unit, gene flow within the group must be sufficient not only to homogenize neutral allele frequencies but also to overcome localized selective forces (Slatkin 1975, Felsenstein 1977). Critical, adaptive genetic differences can exist among individuals that are homogeneous with respect to surveyed neutral alleles, and such differences should not be ignored (Ihssen et al. 1981, Utter et al. 1993, Conover 1998, Lynch 1996, Grant et al. 1999) when partitioning management units. At the same time, we should recognize that populations can be naturally transient (Hanski and Gilpin 1997) and that clinal variation in adaptive
traits (May 1975, Nagalaki 1975, Huey et al. 2001), if it occurs, may present a particular problem.

What evidence, then, apart from genetic surveys of neutral alleles, should be used to partition groups of fish into distinct units for which the genetic dynamics are largely independent? It may be tempting for biological, morphological, ecological, or behavioral differences to be taken as proxies for genetic distinctiveness (Utter et al. 1993, e.g., Haddon and Willis 1995). Indeed, traits such as growth rate, survivorship, disease resistance, longevity, age-at-maturity, and annual reproductive cycle putatively have significant components of additive genetic variation and high heritabilities in certain fishes (Hard 1995). Nonetheless, it should be remembered that phenotypic properties are the product of both the genotype and the environment. Genotypes may vary in their penetrance and/or expressivity (e.g., Tave 1993). Penetrance refers to the proportion of individuals in a group that show an expected phenotype under a specific set of environmental conditions. Expressivity refers to the range of phenotypes expressed by a genotype under a given set of environmental conditions or over a range of environmental conditions. Accordingly, assemblages of fish may be genetically homogenous but phenotypically different (i.e., “plastic”), sometimes considerably so (e.g., Angus 1983, Shultz and Warner 1991, Weeks 1993, Mittelbach et al. 1999), because they inhabit disparate environments. Such differences should not be confused with adaptive traits (Lynch 1996). However, phenotypic plasticity itself may be adaptive. For example, fitness differentials in brook trout (Salvelinus fontinalis) have been linked to size-dependent differences in life history traits through environmentally-induced variation in juvenile growth rate (Hutchings 1996).
On occasion, adaptive genetic variation may be directly apparent in biomolecular surveys (e.g., Powers et al. 1991, Jordon et al. 1992, Kim et al. 1999), as when selection acts directly on surveyed gene products or on the products of closely linked genes. In other cases, relationships between phenotypic and genetic variation have been derived by inference, such as when intraspecific biological differences are geographically consistent with differences at multiple, neutral loci. In the widely studied salmonid family, genetic divergences in neutral markers among fish from different locations or habitats are often accompanied by differences in performance related traits (e.g., Riddell et al. 1981, Hansen and Jonsson 1991, Rintamakikinnunen and Valtonen 1996, Donaghy and Verspoor 1997). In the common snook, *Centropomus undecimalis*, individuals from the Florida Atlantic coast differ from those of the Florida Gulf coast in growth rate, longevity, and maturity schedule; these differences were concomitant with among-coast divergences in allozyme and mitochondrial DNA (mtDNA) markers (Tringali and Bert 1996). When direct or indirect evidence is lacking, the genetic basis for observed phenotypic variation must be uncovered directly by common-garden experimentation (e.g., Tave 1993, Conover et al. 1997) or by the mapping of quantitative trait loci (e.g., Danzmann et al. 1999, Sakamoto et al. 1999). In the absence of direct empirical testing, it seems that observed phenotypic variation should be assumed to be adaptive, as a precaution, when the consequence of being wrong could be great. Potential consequences of transgressing natural stock boundaries by released, cultured fish are described in the next sections.
There are numerous ways in which released, cultured fish can have direct or indirect genetic impacts on natural stocks. Direct impacts are defined as those genetic changes that follow from the transference of genetic material from cultured organisms into wild gene pools. Indirect impacts are defined as those genetic changes in natural stocks that are caused by released organisms or related to their presence, but that occur in the absence of genetic mixing (Waples 1991, Utter 1998). I neglect indirect genetic impacts hereafter, partly because I wonder whether the typically associated demographic concerns are not of a more immediate importance. Topically, the subject of direct genetic concerns for cultured fish/wild fish interactions encompasses a broad range of possibilities. Nonetheless, categorization of these concerns helps in the consideration and management of them. For this purpose, I group them into two categories:

- **Risks stemming from the translocation of non-indigenous genes**
- **Risks stemming from propagation-related genetic changes in cultured fish**

I do not intend to suggest that concerns in these categories are mutually exclusive – any given crop of cultured fish may be subject to concerns from both. However, to manage for genetic impacts, root causes must be understood and it is in the root cause that concerns within these two categories differ. I spend the remainder of this section describing the genetic concerns within each category, presenting empirical evidence and supportive information.
Translocation of Non-indigenous Genes into Distinct Populations. Impacts of this type may be expected when broodfish from non-native (genetically divergent) populations are used to found captive stocks or produce fish that are released into native populations. Unless cultured fish are made sterile prior to their release, the potential for genetic exchange between them or their descendants and wild conspecifics must be considered.

Growing evidence shows that many fishes are highly adapted to local environmental conditions and that natural selection operates at various life history stages (e.g., Stearns and Sage 1980, Smith et al. 1989, Taylor 1991, Powers et al. 1991, Carvalho 1993, Philipp and Claussen 1995, Magurran 1998, Conover 1998, Haugen and Vøllestad 2000, Secor et al. 2000). It is commonly thought that the admixing of genetically divergent stocks can break down local adaptations through two mechanisms – the introgression of maladapted alleles and the disruption of coadapted genomes. Maladapted alleles are those at a particular locus conveying a lower probability, relative to mean fitness, that an individual will survive and reproduce in a given environment. Genomic coadaptation is a natural process whereby selection operates to maintain harmoniously interacting genes in a single genome (c.f., Templeton 1986). When members from genetically divergent stocks interbreed, the ability of their progeny to survive and reproduce may be adversely impacted such that mean fitness may decline. The loss of fitness from maladated alleles or disruption of genomic coadaptation is referred to as ‘outbreeding depression’ (see Lynch 1991).

Gordan and Gordan (1957) first demonstrated the disruption of a coadapted gene complex in the freshwater platy Xiphophorus maculatus. When individuals from isolated
river basins were interbred and those progeny were mated back to individuals from ‘pure’
stocks, the production and distribution of macromelanophores in the second generation
was disrupted to the point of lethal malignancy. In fishes and other animals, the
disruption of coadaptation may be manifested in the form of developmental instability in
‘hybrid’ crosses (Graham and Felley 1983, Clarke 1993). The degree of instability and,
thus, potential for disruption of coadaptation, generally increases with increased genetic
divergence (Leary et al. 1985). Because of possible heterosis and because gene
complexes generally remain intact in the hybrid (F1) generation, the effects of disrupted
coadaptation are not expected to be greatest until later (backcrossed) generations (Lynch
1991), where, unfortunately, relevant data are often not available.

Examples of aquaculture-mediated inter-stock genetic transfers having a potential
for outbreeding effects are well documented. For example, inter-stock crosses between
even- and odd-year returning pink salmon (Oncorhynchus gorbuscha) have resulted in
decreased survivorship and increased morphological defects in the F2 generation
(Gharrett et al. 1999). Captive broodstock from genetically divergent spring-run chinook
salmon (O. tshawytcha) have been inadvertently used in a stocking program for the
perilously endangered winter-run chinook (Hedrick et al. 2000), although the impact is
yet to be determined. Alleles from straying fall-run hatchery chinook have introgressed
into the threatened spring-run population in the Sacramento River (Banks et al. 2000),
potentially disrupting natural run timing. Based on empirical evidence of fitness
differences in performance traits among geographically and genetically discrete
subspecies, Philipp and Claussen (1995) cautioned that widespread inter-stock transfers
among largemouth bass, Micropterus salmoides, could negatively impact locally adapted
populations throughout the eastern and central United States. Farm-raised Atlantic salmon (*Salmo salar*), derived from Norwegian stocks, exhibited poor survival and a reduced incidence of parr maturity compared to native fish in rivers in western Ireland (McGuinnity et al. 1997); progeny of wild (native) × farmed (non-native) *S. salar* generally had intermediate performance in those fitness-related traits. Skaala et al. (1996) reported that *in situ* survival rates for juvenile offspring of crosses between released, non-indigenous, farm-reared brown trout (*Salmo trutta*) and wild *S. trutta* were one-third lower than those of wild juveniles.

*Propagation-related Genetic Changes in Cultured Fish.* Concerns in this category are associated with genetic changes in artificially propagated fish that may result from inappropriate breeding and/or rearing protocols. In this category, (founding) broodfish need not have originated from genetically divergent stocks to pose a genetic risk. Cultured individuals, cohorts, or stocks may contain 1) insufficient levels of genotypic and/or phenotypic variation compared to the wild stock, 2) inbred genomes caused by the continued propagation of related individuals, 3) maladapted genomes caused by artificial or domestication selection, or 4) altered ratios of sex-determining genes.

Typically, the numbers of breeders selected to found a captive brood stock represent a small percentage of the available breeders in source populations. When small numbers of founding breeders are used, large stochastic differences in allelic and genotypic frequencies (e.g., Taniguchi and Sugama 1990, Hansen et al. 1997, Koskenin et al. 2002) and reduced levels of genetic variation may be expected in cultured lots,
cohorts, or stocks (e.g., Taniguchi et al. 1983, Ferguson et al. 1993, Bartley et al. 1995, Clifford et al. 1998) compared to the source natural population. Cultured stocks can also be genetically underrepresented if the initial sampling for broodfish fails to capture a sufficient range of heritable phenotypic variability available within the source population (Leary et al. 1996).

Cultured fish may be individually compromised when breeding schemes foster mating between related broodfish. Like most sexual organisms, fish populations contain significant amounts of ‘hidden’ genetic variation in the form of rare recessive alleles (Nevo 1978; e.g., Launey and Hedgecock 2001). Harmful (even lethal) recessive alleles persist in a gene pool despite natural selection because of the protection conferred to them when in the heterozygous state. Inbreeding in a hatchery setting can bring deleterious partially recessive alleles together in homozygous genotypes, thereby exposing them to greater selective forces. This may lead to a form of reduced fitness known as ‘inbreeding depression’ (see Lynch 1991, Crnokrak and Roff 1999). Among cultured fishes, inbreeding depression has been manifested in empirical studies as lethal or harmful morphological deformities such as missing opeculi (e.g., Brachydanio rerio, Mrakovic and Haley, 1979; Cichlosoma nigrofasciatum, Winemiller and Taylor 1982) or caudal deformities (e.g., Oreochromis niloticus, Mair 1992). Other typical problems experienced by inbred cultured fish include poor physiological adaptation, slow growth, high mortality, low reproductive output or success, abnormal mechanical function, and developmental instability (e.g., Kincaid 1983, Kinghorn 1983, Leary et al. 1985, Chilcote et al. 1986, Ferguson and Drahushchak 1990, Leider et al. 1990). Among vertebrate animals, inbreeding depression may generally have a greater impact when manifested in
life history traits than in morphological traits (DeRose and Roff 1999). A variety of structured breeding programs have been recommended based on pedigree- (e.g., Fernández and Caballero 2001) or marker-assisted (e.g., Toro et al. 1999, Wang and Hill 2000) minimization of inbreeding.

Potentially detrimental genetic changes in cultured fish or within cultured stocks may arise through artificial selection or domestication (Kohanne and Parsons 1988, Doyle et al. 1995). The selective response (change in a trait under selection) is a function of selection differential (the difference in the mean phenotype of the selected group and that of the whole population) and the heritability of the trait (the fraction of trait variance attributable to additive genetic variance). The goal of artificial selection is the achievement of a targeted selective response for a particular trait, such as faster growth or earlier maturity (see Tave 1993). Domestication, in the context of aquaculture, is usually unintended and is characterized by selective changes in traits that befit captive environments – e.g., changes in reproductive biology (e.g., Chilcote et al. 1986, Crandell and Gall 1993, Danzmann et al. 1994, Chebanov and Ridell 1998), growth and survival (e.g., Crandell and Gall 1993, Reisenbichler and McIntyre 1977), morphology, or behavior (e.g., Ruzzante and Doyle 1991, Berejikian 1995, Berejikian et al. 1996). In many cases, multiple, correlated responses to domestication or intentional selection may be exhibited by farmed fishes. For example, farmed Atlantic salmon in Norway, studied by common-garden experimentation, differed from wild salmon in growth, morphology, behavior, parr maturity and smolting rates. Fleming et al. (2000) demonstrated that the lifetime success of escaped, farmed Atlantic salmon was on average 81% lower than that of native Norwegian salmon. As with translocation of non-indigenous genes,
propagation-related divergence in fitness-related traits between farmed and wild fish can result in outbreeding depression (Reisenbichler and McIntyre 1997, Currens et al. 1997, Reisenbichler and Rubin 1999). Often, crosses between farm-raised and wild fish lead to intermediate levels of performance in F1 progeny (e.g., Einum and Fleming 1997), although heterosis is occasionally observed (see Thornhill 1993).

Finally, in those cultured fishes where sex is determined or influenced by environmental factors (e.g., channel catfish [Patiño et al. 1996], Japanese flounder [Yamamoto 1999], sockeye salmon [Craig 1996], tilapia [Lester et al. 1989]), a risk ensues when sex-reversal rates vary in the hatchery and in the wild. For example, Kanaiwa and Harada (2002) investigated the case in which a species has male heterogametic sex determination (XX female – XY male) but in which some juvenile XX fish develop as functional males (‘reverse sex’) depending upon pre-maturity environmental conditions. In their study, they identified various hatchery-breeding practices, many which are common in stocking programs involving sexually labile species, which could lead to the extinction of the Y gene in natural populations.

*How Much is too Much?*

Intuitively, there may be some level of genetic mixing between cultured and wild fish that may be negligible in terms of the potential for genetic impact, regardless of the individual fitness of the cultured fish. In this section, I address issues pertaining to the magnitude and duration of release activities – i.e., when is the proportion of cultured fish in a recipient natural population too high and when have releases of cultured fish
continue for too long? These questions will be explored in the context of *genetic swamping*, which refers *sensu lato* to a process whereby the genetic influx (in this case, from cultured fish) exceeds the level at which a population can maintain its natural genetic state. This occurs as alleles from cultured fish replace, sometimes not so gradually, those from wild fish in the recipient populations.

Short-term genetic risks from swamping must be considered in light of the potential impact of cultured fish on the balance between migration, drift, and selection: assessment of long-term risks also necessitates consideration of spontaneous mutation. Cultured fish may be viewed as a novel class of immigrants (entering via stocking, straying, or escapement) that, at a practical minimum, will likely have higher levels of interrelatedness compared to that of random individuals in the recipient stocks and, at a maximum, will have inbred and/or genetically divergent, maladapted genomes. Although it may not be strictly true, gene flow typically has been assumed to be unidirectional – cultured fish → wild fish – by those who have considered this process (e.g., Felsenstein 1997, Tufto 2001, Withler et al. 2003; *but see* Lynch and O’Hely 2001).

Immigration rates, $m_c$, are typically considered in terms of those cultured fish that actually contribute reproductively to the admixture; differentials in lifetime reproductive success among cultured and wild fish are accounted or sometimes ignored in analyses. Immigration rates of cultured fish can be used to predict the rate of replacement of ‘neutral’ wild alleles. Specifically, the time $t_{p,w}$ in generations for which the proportion, $p_w$, of wild alleles remains in an admixed population is $t_{p,w} = (-\ln p_w)/m_c$ (Chakraborty and Leimar 1987). For example, given a 5% immigration rate, approximately 10% of wild alleles will be replaced by alleles from immigrants in only two generations. In
approximately 13 generations, more than half of the wild alleles will have been replaced.
Given \( m_c = 30\% \), 95% of wild alleles will have been extirpated in approximately 10
generations.

Empirical evidence confirms that allelic replacement can occur quickly. In
Trinidad, genotypes from cultured *Poecilia reticulata* introduced in an unoccupied
upstream location had nearly completely replaced those of downstream native
populations in less than 35 years (Shaw et al. 1992). Ten years after the commencement
of stocking (2-3 generations), \( \sim 20\% \) of the alleles in the admixed population of the
endangered Lake Saimaa (Finland) grayling (*Thymallus thymallus*) were of hatchery
origin (Koskinen et al. 2002). On the other hand, a low occurrence of cultured fish in
natural spawning populations does not necessarily translate to an immediate or significant
impact on genotype frequencies in the following generation. A small number of
hatchery-derived adult Columbia River fall-run chinook stray into Snake River spawning
populations. Marshall et al. (2000) determined through genetic monitoring that the
reproductive contribution of Columbia River hatchery strays to out-migrating juvenile
Snake River chinook was not appreciable. It is not known whether this is because
Columbia River strays do not spawn in appreciable amounts or because Columbia River
\( \times \) Snake River progeny suffer early mortality.

Population genetic theory indicates that wild alleles can be replaced with those
conferring a lower fitness. Felsenstein (1997; *see also* Felsenstein 1977) has shown that
stocking over concurrent generations will overwhelm the process of natural selection
when the immigration rate (relative contribution of cultured vs. wild individuals) exceeds
the average selective difference between genotypes \( (m_c > s) \). Lynch and O'Hely (2001)
further observed that stochastic events associated with small populations are expected to be more powerful than natural selection in the segregation dynamics of maladapted genes which, in theory, could result in extinction of the local population. Tufto (2001) developed a demographic/evolutionary model to explore the demographic impact of immigration of cultured fish into natural populations, applying it to Atlantic salmon stocks in Norway. In his model, selection differentials at quantitative trait loci known to exist between cultured and wild salmon were employed. His results indicated that continued immigration of cultured salmon at a rate of 15% or more would be sufficient to drive wild salmon populations to extinction.

The process of allelic replacement would, in theory, also operate on wild beneficial alleles, possibly leading to reduced levels of quantitative genetic variance and the consequent loss of adaptive potential in changing environments (see Lynch 1996). Many adaptive traits are polygenic (under the control of multiple gene loci) and the forces of selection are distributed over these loci. Accordingly, it is thought that most adaptive alleles have very small individual selective effects \( s < 0.05 \), Keightley and Hill 1988, Houle 1989). If so, immigration rates exceeding 5% would be sufficient, in theory, to overwhelm the selective retention of these alleles and subject population gene frequencies completely to the process of random genetic drift (and further immigration).

Lastly, levels of inbreeding in the recipient population may be inflated when family sizes of the cultured portion of the admixture are significantly larger that those of the wild portion. As noted earlier, inbreeding depression may result from the exposure of deleterious recessive traits when the coancestry of a mating pair is recent. Ryman and Laikre (1991) provided a method of predicting post-immigration changes in levels of
inbreeding via a simple admixture model for inbreeding effective population numbers (Wright 1931). The ‘Ryman-Laikre’ model has been employed for particular species within the context of stocking (Waples and Do 1994, Ryman et al. 1995a, Hedrick et al 2001, Thorpe et al. 1995). This mechanism is explored in greater detail in the next chapter.

Although immigration rates of cultured fish have been the topic of research (see above citations) and discussion (e.g., Ryman et al. 1995b, Withler et al. 2003), they are typically not addressed in governmental policies or other regulatory practices. However, policies typically abound with guidelines pertaining to broodstock origin and breeding/rearing practices. To me it seems, from a management perspective, that immigration rates – i.e., the scale of releases – should merit the same degree of attention as broodfish origin and progeny fitness. Perhaps there remain difficulties in reaching consensus opinions on what the limitations on rates should be. Perhaps in other cases there exists a belief that if the mean fitness of individuals in the cultured crop is not appreciably lower than that of fish in the recipient stock (assuming that condition can be established) then releases, in any amount and for any duration, can proceed with impunity. But can they?
Chapter 2

Risk to Genetic Effective Population Size should be an Important Consideration in Fish Stock-Enhancement Programs

Introduction

Habitat destruction, pollution, and exploitation have taken a toll on many wild fish populations in North America. Indeed, half of all exploited marine fish populations in the U.S.A. for which abundance trends are known are in a state of decline (NOAA, 1992). To combat the declines, resource managers are increasingly using hatchery-based rehabilitative measures (stocking programs) for fish species having ecological or economic value. Salmonid fishes have been the subject of intensive stocking efforts for decades (Hynes et al., 1981). More recently, wild populations of white and striped bass (Morone spp.), mullet (Mugil cephalus), American shad (Alosa sapidissima), red drum (Sciaenops ocellatus), weakfish (Cynoscion spp.), snook (Centropomus spp.), and sturgeon (Acipenser spp.) have been targeted for stock enhancement or restoration by various organizations in North America (Edwards and Henderson, 1987; Landau, 1992; FDEP, 1993).

Since the early 1980s, an increasing number of anomalous genotypic and phenotypic traits have been observed in hatchery populations (reviewed by Allendorf and
Ryman, 1987). Scientists became fearful that these traits, the majority of which were deemed undesirable (Hindar et al., 1991), would be transferred to the wild populations undergoing supplementation (e.g., Ryman, 1981a; Cross and King, 1983; Leary et al., 1985; Simon et al., 1986, Taniguchi et al., 1983). Although general awareness of these troubles has been somewhat enhanced by national and international conferences on the subject (e.g., FAO and UNEP, 1981; STOCS [Hynes et al., 1981]; Fish Gene Pools [Ryman, 1981b]), genetically unsound breeding and stocking practices still continue in many stock-enhancement programs.

Two issues receive the majority of attention in the genetic management plans of stock-enhancement programs. First, genetic identities and viabilities of wild fish populations may be eroded by the transplantation of nonnative fish (or their hatchery-derived offspring). Although geneticists have encouraged those engaged in stock enhancement to obtain broodstock directly from the population into which their progeny will be released (Krueger et al., 1981; Hindar et al., 1991, Ryman et al., 1995), many still utilize nonnative fish in their supplementation programs (F. Utter, pers. comm.). Second, hatchery fish may be deficient in overall genetic variability and this deficiency may ultimately reduce genetic variability in the population into which they are released. To maintain sufficient genetic variability in hatchery populations, a total of 50-200 genetically effective breeders is typically recommended to found hatchery populations (Ryman and Ståhl, 1980; Frankel and Soulé, 1981; Hynes et al., 1981; Kincaid, 1983). Populations so founded will retain, in theory, at least 99% of the heterozygosity present in the wild population.
In addition to artificial introgression and hatchery inbreeding, a third issue is of importance for protecting supplemented wild populations from hatchery-induced genetic erosion. In a simple model, Ryman and Laikre (1991) described the reductive effect of stock enhancement on the genetically effective sizes of the supplemented animal populations. They observed that, because of this effect, genetic variation in supplemented populations might be at risk even when presumably adequate numbers of breeders are used. This risk is especially great for animals having both high and varying reproductive rates, such as fishes. Nevertheless, this consideration is generally ignored in conventional breeding and stocking guidelines for fish (but see Waples and Do, 1994; Campton, 1995; Thorpe et al., 1995).

In this paper, we relate the model of Ryman and Laikre (1991) to the practice of fishery stock enhancement and we discuss the genetic risks associated with the reduction of effective population sizes. We then present contrasting case studies of red drum and Gulf sturgeon, fishes with greatly differing life histories, population dynamics, and population genetic characteristics. Both species are currently the subjects of stock enhancement activities, and protocols developed for the captive breeding of individuals of each species have generally followed conventional genetic guidelines. We demonstrate that, in some cases, although the released hatchery fish collectively may satisfy existing genetic recommendations and retain >99% of the heterozygosity initially present in the wild population, the genetic future of the population into which they are released could still be in considerable jeopardy.
Ryman and Laikre’s Model

The genetically effective population size \( N_e \) has been defined as the size of a hypothetical ideal population (randomly mating; devoid of selection, immigration, and mutation) that would undergo the same amount of genetic change as the actual (nonideal) population (Wright, 1969). The definition is applicable to any population, wild or captive. Operationally, \( N_e \) has been equated to the inverse of the probability \( P \) that two homologous genes in two randomly mating individuals were derived from a single parent in the preceding generation (Crow and Kimura, 1970). Any variance in family size within a population (e.g., that which is likely to be induced during the operation of a stock-enhancement program) will cause \( P \) to increase and, therefore, \( N_e \) to decrease. The rate of decrease in \( N_e \) depends upon 1) the original effective size of the wild population \( (N_{e,o}) \), 2) the effective size of the introduced hatchery population \( (N_{e,h}) \), and 3) the relative hatchery contribution \( (x) \) to the mixed population. Ryman and Laikre (1991) offered the following equation to predict an admixed population's total effective size \( (N_e') \) following a single generation of stock enhancement:

\[
\frac{1}{N_e'} = \sum_{i=1}^{n} \frac{x_i^2}{N_{e,i}}
\]

where \( x_i \) is the relative percentage of offspring contributed by \( N_{e,i} \) effective breeders in each contributing group \( (i = 1, 2, \ldots n) \); in our case, \( n = 2 \), the hatchery and wild breeding
groups. It follows from the model that \( N_e \) is always less than \( \sum N_{e,i} \) when the offspring contributions of the various groups are disproportional to their effective sizes.

The model input parameters \( x \), \( N_{e,h} \), and \( N_{e,o} \) can be often estimated using commonly available fishery and hatchery data. De facto estimates of \( x \) are commonly made in supplementation programs using either genetic methods (e.g., Utter and Seeb, 1990) or tag/recapture methods (e.g., Gausen and Moen, 1991). Estimates of effective hatchery population sizes are also possible (e.g., Taniguchi et al., 1983; Bartley et al., 1992; Kincaid, 1995); these should consider variances in brood and family sizes (Pudovkin et al., 1996). Estimates of \( N_{e,o} \) may be generated prior to stocking using the most applicable method for estimating \( N_e \) (reviewed by Waples, 1991; Jorde and Ryman, 1995). However, these estimates are only reasonably accurate when applied to small effective population sizes (\( N_e < 1,000 \)) and are only precise when both sample sizes and the number of loci examined are large. When a genetic estimate of \( N_{e,o} \) is lacking, Ryman and Laikre (1991) point out that it can be at least roughly approximated using fishery estimates of spawning-stock abundance. Fortuitously, depending on the anticipated value of \( x \), only a crude estimate (order of magnitude) of \( N_{e,o} \) may be required because, for a given \( N_{e,h} \), values of \( N_e \) converge rapidly as the hatchery contribution increases for \( N_{e,o} \) values that are within orders of magnitude of each other. For example, if hatchery fish reared from a total of 50 effective breeders comprise 10% or more of the admixed population, it ultimately makes little difference in \( N_e \) whether the original effective size of the wild population was 10,000 or 100,000 (Fig. 2-1).
Potential Effect of Reduced $N_e$

The potential effects of lowered effective population sizes to both single-locus polymorphism and quantitative (polygenic) variation are important. Selectively-neutral (single) gene loci generally contain many alleles per locus, including some that may become adaptive in a changing environment (Lande and Barrowclough, 1987). Low-frequency alleles at neutral loci are subject to extinction in small supplemented
populations, especially those populations that fail to maintain their poststocking abundances over time (the "supplementation and crash scenario" of Waples and Do [1994]). Poststocking population crashes are likely when the underlying causes of the initial decline are not corrected (Meffe, 1992). A supplemented population that does not sustain its abundance is affected genetically by the initial swamping of wild-population alleles by those from hatchery fish and also by future drift-associated changes caused by the combined effects of the crash and the hatchery-induced reduction in $N_e$. The severity of the effects varies depending on the degree of genetic structure present in the population. For marine fish populations, two extreme types of genetic units can be described (Fig. 2-2A). Type I units may be characterized as discrete populations; reproductive isolation is sufficient to allow genetic divergence from neighboring populations. Type II units may be characterized as subunits (demes) within a Type I population; gene flow may be sufficient to homogenize genetic variability among neighboring demes. Over time, the effect of stocking upon discrete populations (Type I) may be the complete replacement of alleles derived from the wild population with the much smaller subset of alleles derived from the hatchery breeders (Waples and Do, 1994; Fig. 2-2B).
Figure 2-2. Genetic population structures of marine fish and effects of Waples and Do (1994) "supplementation and crash" scenario on genetic variability in populations having these structures. A) The two basic types of "genetic units" in which supplemental stocking programs may operate. B) Potential genetic effects of supplemental stocking upon Type I and II genetic units under the supplementation and crash scenario.

Supplemental stocking leads to an increased population abundance but a reduced effective population size ($N_e$). Contributions of hatchery fish (1) may cause swamping of the genomes of small units with alleles derived from hatchery breeders (hatched areas) (2). When the breeding program ends, abundance declines to pre-stocking levels (3). As a consequence of the lowered abundance and small $N_e$, the altered allele frequencies (in
which wild-stock alleles [unhatched areas] now exist at low frequency) are subject to
high levels of random drift. In Type II units, allelic loss due to random drift may be
countered by immigration from neighboring demes; in Type I units, allelic extinction
may be high (4) (Chakraborty and Leimar, 1987).

Because most major phenotypic changes in species result from the accumulation
of polygenic mutations (Lande, 1981), maintenance of sufficient quantitative variability
will, in theory, preserve the potential for adaptive evolution (Soulé, 1986). A multitude
of life history traits (e.g., growth, fecundity, age-at-maturity) and threshold traits (e.g.,
disease resistance, incidence of morphological deformities) are polygenic (Hard, 1995).
Loss of quantitative variability has been associated with adverse effects to some of these
traits (Riggs, 1990). Once quantitative variability is lost, a population must regain and
sustain a high abundance for many successive generations ($10^3$) until that variability is
replaced by new mutations (Lande and Barrowclough, 1987). A sustained effective
population size of $\geq 500$ (Franklin, 1980) has been generally suggested as necessary to
conservive sufficient levels of genetic variance in quantitative characters on an
evolutionary time scale. Populations of relatively small effective size ($<100$) could, in
theory, retain sufficient levels of quantitative variability on a short time scale ($\geq 200$
generations) if they are subdivided and/or subject to continued immigration (Lande,
1995). On the other hand, Lande (1994) demonstrated that populations with effective
sizes on the order of $10^3$ might still incur a substantial risk of extinction due to chance
fixation of new, mildly deleterious mutations.
Because a threshold effective population size of 500 has been recommended in most management plans for threatened and endangered species (Anonymous, 1984; Seal and Foose, 1984; Foose et al., 1995), we adopt that value. Figure 2-3 depicts for given numbers of effective hatchery breeders the relative hatchery contributions ($x\%$) that would reduce the effective size of a supplemented population to 500. For all fish populations with an original effective size greater than 500, a relative hatchery contribution of less than 17% should not drive the total effective population size to or below 500, provided sufficient numbers of effective hatchery breeders ($N_{e,h} \geq 50$) are employed. If $N_{e,o}$ is approximately 2,000, the relative contribution could be increased to $\sim 30\%$ using 50 effective breeders, or to $\sim 42\%$ using 100 effective breeders before the threshold $N_e$ was reached. Thus, even using the often-recommended 100 effective hatchery breeders, and essentially regardless of the original effective size of the wild population, hatchery contributions larger than $\sim 45\%$ (a common occurrence in many supplemented fish populations [Hindar et al., 1991]) result in $N_e$ below 500.

**Case One: Red Drum**

In the United States, red drum (*Sciaenops ocellatus*) support one of the most valuable fisheries in the estuaries and nearshore waters of the Gulf of Mexico and Atlantic Ocean (Mercer, 1984; Swingle et al., 1984). Because of recent declines in red
Figure 2-3. Relative hatchery contribution ($x\%$) required to reduce the overall effective size ($N_e$) of a population to below 500 individuals as a function of the original effective size ($N_{e,o}$) of the native population. The numbers superimposed on the curves indicate the value of the effective hatchery population size ($N_{e,h}$) component of the mixed population.

drum abundance and an apparent high rate of annual mortality in young age-classes (Goodyear, 1989; Murphy and Taylor, 1990), state and regional management agencies concluded that red drum were overharvested (SAFMC, 1990). Consequently, state agencies in Alabama, Florida, South Carolina, and Texas began to study the feasibility of supplemental stocking as a component of management for this fishery. Agencies in Florida and Texas are currently engaged in large-scale, red drum stock-enhancement
programs (FDEP, 1993; McEachron et al., 1995); the numbers of hatchery-reared red drum stocked in the waters off Alabama and South Carolina are relatively small.

Red drum is a marine species that ranges from central Mexico throughout the northern and eastern Gulf of Mexico and in the Atlantic Ocean to Cape Cod, Massachusetts. Adult red drum populations are pelagic and highly dispersing (Overstreet, 1983). In September and October, adults congregate at the mouths of inlets and passes to spawn (Matlock, 1987). Eggs and larvae are transported by tidal currents to estuarine nurseries. Juvenile red drum inhabit the estuary until age 3 or 4, at which time they join offshore adult populations (Murphy and Taylor, 1990). Red drum are long-lived (>30 yrs [Murphy and Taylor, 1990]) and iteroparous. At the peak of fishery exploitation (1986), the census size of the adult spawning stock in the Gulf of Mexico was estimated to be 7 million individuals (Nichols, 1988); based on nearly identical values of female effective population sizes between red drum inhabiting the Gulf and Atlantic, Gold et al. (1993) estimated an equivalent abundance for red drum in Atlantic waters.

Wild red drum have been the subject of numerous genetic studies (e.g., Ramsey and Wakeman, 1987; Bohlmeyer and Gold, 1991; Gold and Richardson, 1991; Gold et al., 1993), each with a general goal of identifying geographic ranges of populations. Levels of allozyme and mitochondrial DNA (mtDNA) variability are relatively high in red drum, compared to other fishes. It appears that red drum may be weakly subdivided into at least two populations, one inhabiting the Gulf of Mexico and the other the Atlantic Ocean. Genetic differentiation between Gulf and Atlantic red drum is minimal; subdivision is suggested by frequency differences in nuclear and mtDNA genotypes.
rather than by distinct phylogeographic differences (cf. Avise et al., 1987). However, genotype-frequency differences may be typical indicators of stock subdivision in marine fish species (Tringali and Bert, 1996). Here, as a conservative approach, we consider Gulf and Atlantic red drum populations each to be self-contained and noninterbreeding; we examine the stocking activity occurring only in Atlantic waters.

Fishery managers in Florida seek to supplement or restore red drum in selected embayments along Florida's Atlantic coast where juvenile and subadult abundances are low (FDEP, 1993). The genetic units of red drum that hatcheries deal with are essentially subgroups (demes) of the larger Atlantic population (Type II, Fig. 2-2A). Whereas gene flow should be sufficiently high throughout the species range (Bohlmeyer and Gold, 1991; Gold et al., 1993) to homogenize genetic variation in the Atlantic over time, natural recruitment to particular areas (e.g., Biscayne Bay, Florida) may not sustain local fisheries because of high fishing pressure or environmental challenge (FDEP, 1993). Therefore, red drum hatchery managers in Florida are assessing the potential of stock enhancement to mitigate localized declines in wild red drum abundance. They must do so without affecting the genetic composition of the larger red drum Atlantic population (Willis et al., 1995).

Because of the high abundances of red drum populations, techniques for estimating contemporary $N_{e,o}$ via disequilibrium or temporal methods (Waples, 1990; Waples and Teel, 1990) are untenable. Nevertheless, because of the relative insensitivity of Ryman and Laikre's model to $N_{e,o}$ in this case (see Fig. 2-1), it is sufficient to know that contemporary $N_{e,o}$ values for red drum probably fall within the range of $10^5$-$10^6$. We use the long-term value of 372,000 (Gold et al., 1993) as a conservative estimate of $N_{e,o}$.
for red drum. Because the long-term $N_{e,o}$ approximates the harmonic mean of the individual generational values over time (Crow and Kimura, 1970), the contemporary $N_{e,o}$ of red drum will probably be >372,000 and the potential effect on $N_e$ in this case study will not be underestimated.

To estimate the relative contribution of hatchery-reared red drum to the wild adult breeding populations, we considered the numbers of hatchlings released and their probability of survival to reproductive age. Much of the total annual mortality ($M$; comprising both natural and fishing mortality) in subadult red drum (age 1-3) is related to fishing pressure in estuaries (Murphy and Taylor, 1990). When red drum migrate offshore (out of the fishery), the annual survival ($1 - M$) presumably increases to that of other long-lived fishes (80-90% [Hoenig, 1983]). To date, the state hatchery in Florida has released approximately 1-1.5 million fingerling red drum into each of two Florida estuaries (B. Halstead, pers. comm.) per generation (3 yr [Gold et al., 1993]) for two succeeding generations. Currently, the facility in South Carolina does not intend to release appreciable numbers of red drum (W. Jenkins, pers. comm.). In Florida, the vast majority of red drum are released at approximately 6 to 12 wks of age, when they are 25-60 mm long (standard length). Based on age-specific mortality estimates of wild red drum (Anonymous, 1993; Vaughan, 1995), we assumed survival rates for released fingerlings of 30-40% for age 6 wks to 1 yr, 60% for age 1-2, and 80% for ages 2-3 and 3-4. Recruitment of red drum into spawning populations is 98% complete by age 4 (Vaughan, 1995). Thus, approximately 100,000-175,000 hatchlings may join the migrating schools of neritic adults ($N \approx 7$ million) each generation, comprising a relative contribution of 1.5-2.5% per generation.
The procedures employed by managers of Florida's red drum hatchery are generally typical of marine hatcheries in the United States throughout the Gulf of Mexico and Atlantic coastal regions. To reduce the potential for inbreeding, the Florida hatchery attempts to use 50-100 breeders per generation in their assisted-spawning protocols (FDEP, 1993). Nevertheless, because their spawning protocols and release strategies for red drum probably lead to high variance in family sizes and relatively low effective hatchery population sizes, we assessed a broad range of values for $N_{e,h}$ (5-50). Our analyses of the spawning and stocking procedures in Florida's red drum hatchery indicate that 5-10 actual breeders may be required for every desired effective breeder (M. Tringali and T. Bert, unpubl. data). Note: it has subsequently been determined that 2-3 actual red drum broodfish are required for every desired effective breeder, as reported in Chapter 5. Over a given generation interval, the Florida hatchery likely employed 50 or more effective breeders in their Atlantic stocking program. MDT

Using the above values for $N_{e,o}$ (372,000), $N_{e,h}$ (5-50), and $x$ (1.5-2.5%), we predicted the potential effect of the Florida stocking program upon the effective size of the Atlantic red drum population (Fig. 2-4A). When values of $N_{e,h}$ are sufficiently high and hatchery contributions are modest, $N_e$ is projected to be in the range of 10,000-200,000. Therefore, there is no apparent risk of a genetic bottleneck or a reduction in quantitative variation. In general, both the large population sizes and the relative lack of genetic substructure in wild red drum populations work concertedly to offset risks to both single-locus and quantitative genetic variation. The potential for genetic swamping (cf. Campton, 1995) appears unlikely for the Atlantic red drum population. At relative contributions of 2% per generation, stocking would have to continue for many successive
generations (~50 [Chakraborty and Leimar, 1987]) for severe changes to occur in the genetic composition of wild red drum in the Atlantic. By the criteria set forth earlier, it appears that red drum stock-enhancement programs are genetically feasible. However, it is important that adequate numbers of effective breeders be used and that per-generation contributions be modest. It would also be prudent to monitor single-locus genetic variation for changes within the supplemented population over time, especially if the supplementation effort is to continue over multiple generations.

Case Two: Atlantic Sturgeon

Nearly all chondrostean species (sturgeon and paddlefishes) are endangered or threatened (Birstein, 1993). Atlantic sturgeon (Acipenser oxyrhynchus) are included in this group of primitive bony fishes. Vladykov (1955) divided Atlantic sturgeon into two subspecies. The Atlantic subspecies (A. o. oxyrhynchus) ranges from Labrador, Canada, to the eastern coast of central Florida (Backus, 1951). The Gulf of Mexico subspecies (A. o. desotoi), commonly called the Gulf sturgeon, has been recorded from the Mississippi River to the southwestern coast of Florida (Huff, 1975; Wooley and Crateau, 1985). Lack of suitable riverine habitat and warm water temperatures along southern coastal Florida and in the Florida Straits reportedly maintain the geographic integrity of the putative subspecies (Rivas, 1954), although secondary genetic introgression (from A. o. desotoi into A. o. oxyrhynchus) may have occurred in the past (Bowen and Avise, 1990).

Acipenser oxyrhynchus is a long-lived anadromous species. Adult sturgeon are oceanic shelf-dwellers except when they annually migrate to the upper portions of rivers
during spawning season (April-October). Juveniles remain in fresh water for at least a year and make the transition to a neritic existence as they age. Male sturgeon mature at ages 7-9 and females at ages 9-12 (St. Pierre et al., 1994). Reported maximum ages for sturgeon differ, but individuals >40 yrs of age have been documented (Huff, 1975).

*Acipenser oxyrhynchus* has been harvested by man for several millennia (Ritchie, 1969) and has been intensively exploited commercially for more than a century. Fishing pressure peaked in the 1890s, when total annual landings reached 3.3 million kg (approximately 70,000 fish). However, by 1927, annual landings had dropped to below 50,000 kg (approximately 1,000 fish), and they have since remained at that level (Smith et al., 1980). Currently, sturgeon are considered rare, and many populations have completely disappeared from their historical breeding grounds (Murawski and Pacheco, 1977). Deteriorating water quality and prolific damming (preventing access of sturgeon to spawning sites) are also blamed for the precipitous decline in abundance (U.S. Army Corps of Engineers, 1978). In the Atlantic and its associated rivers, commercial harvest of *A. o. oxyrhynchus* continues, but it is highly regulated (St. Pierre et al., 1994); in the Gulf of Mexico and its associated rivers, *A. o. desotoi* has been assigned federal threatened-species status (Bentzien, 1991). Because of the Gulf sturgeon's dire situation, we focus on them in this analysis.

Fishery records indicate that Gulf sturgeon have undergone approximately 7-10 successive generations of greatly reduced abundance. The Suwannee River, Florida, supports the vast majority of the remaining Gulf sturgeon (Huff, 1975). Based on mark-recapture studies, the current census of adult Gulf sturgeon in the Suwannee River has been estimated to be 2,250-4,000 (Hollowell, 1980; USFWS and GSMFC, 1995). Since
1979, census estimates within the next most abundant drainages, the Apalachicola and Choctawhatchee rivers, Florida, have remained at approximately 100-300 individuals, but not all recaptured sturgeon had reached maturity (Crew, 1979; Wooley and Crateau, 1985; USFWS, 1992). Citings or landings of adult Gulf sturgeon in other Gulf of Mexico drainages are rare (USFWS and GSMFC, 1995). Compared to other marine and diadromous fishes, the total abundance of the subspecies is remarkably low.

There is insufficient data regarding the effective population size of the Gulf subspecies; an estimate of $N_{e,o}$ is greatly needed. Tagging and other data suggest that juvenile and adult sturgeon are capable of traveling considerable distances (Huff, 1975); however, adults may return repeatedly to the same drainage to spawn (Wooley and Crateau, 1985). Molecular genetic studies indicate that at least the female component of *A. o. oxyrhynchus* is composed of subdivided populations. Stabile et al. (1996) observed significant differences in mtDNA sequences between Gulf sturgeon from rivers east of the Choctawachee (the Suwannee, Ochlockonee, and Apalachicola composed a single population) and those from the more westerly drainages (see their Fig. 2-1). Moreover, the Gulf sturgeon occupying some of these more westerly drainages appeared to be further subdivided. The significant genetic differences among Gulf sturgeon occupying various regions suggests that they are composed of several Type I units (Fig. 2-2A), with little or no gene flow between populations (Stabile et al., 1996). If gene flow among males is similarly structured, effective population sizes of Gulf sturgeon may be much less than the total abundance of breeders ($N_{e,o} \ll N$). Here, we will assume the effective size for the eastern Gulf of Mexico (Suwannee, Ochlockonee, and Apalachicola) sturgeon population to be 2,500 (approximately half of the total adult census size [Nunney and
Elam, 1994]), but we caution that the value could be lower (Mace and Lande, 1991; Hedgecock, 1994).

Low effective population sizes of Gulf sturgeon may already be the cause of reduced genetic diversity in that subspecies. Bowen and Avise (1990) observed very little overall mtDNA sequence diversity ($p < 0.0001$) in the Gulf sturgeon compared to that of the Atlantic subspecies ($p = 0.0017$). In general, mtDNA diversity is higher ($p \approx 0.05-0.005$) in other marine and anadromous fish species (Tringali and Bert, 1996). In addition, Miracle and Campton (1995) observed no nucleotide substitutions in a 268-base-pair portion of the mtDNA control region of 16 Suwannee River individuals, and Stabile et al. (1996) identified only two polymorphic sites among 203 base pairs in the mtDNA control regions of 33 Suwannee River sturgeon (however, sequence variation was slightly higher in Gulf sturgeon from some of the more westerly drainages). Finally, Chapman et al. (1993) reported no verified RAPD (random amplified polymorphic DNA) polymorphisms in 20 Suwannee River individuals assayed with eight RAPD primers. It seems evident that little genetic diversity exists in Gulf sturgeon in general, particularly in the eastern Gulf population. The genetic diversity that remains should be conserved.

The U.S. Fish and Wildlife Service and Gulf States Marine Fisheries Commission have developed a recovery and management plan for Gulf sturgeon containing the tiered objectives of 1) preventing further reduction of existing populations and 2), when populations become self-sustaining, implementing directed fisheries (USFWS and GSMFC, 1995). In addition to reducing the incidental by-catch of sturgeon, improving water quality, and restoring habitat, managers are studying the possibility of employing large-scale stocking as a means of attaining these objectives. Breeding-and-release
guidelines to be used for Gulf sturgeon have been formulated by the Atlantic Sturgeon Aquaculture and Stocking Committee (ASASC [St. Pierre et al., 1994]). Large-scale propagation and stocking of sturgeon, including Gulf sturgeon, is technically feasible (Landau, 1992; Chapman et al., 1993); indeed, Russian fish culturists have released more than a billion juvenile common sturgeon (*Acipenser sturio*) into the Azov, Black, and Caspian seas since 1975 (less than two generations) (Barannikova, 1987). Perhaps the greatest challenge remaining for culturists of Gulf sturgeon is that of collecting sexually mature broodstock. Chapman et al. (1993) were able to collect an average of only 7-8 potential breeding pairs from the Suwannee River in a single season – much less than the 50-100 breeding pairs required to satisfy conventional considerations relating to inbreeding. However, citing a protracted generation interval for sturgeon (~10 yr), authors of the ASASC breeding protocol allow for annual paired matings of 10 different parents (5 of each sex) for a period of 10 years to achieve a "generation effective hatchery population size" of 100 individuals. Nonetheless, because some variance must occur in stocking abundances, survival rates, and reproductive rates between cohorts, the actual $N_{e,h}$ will be some fraction of the 100 actual parents used (Waples, 1990). Therefore, we considered a range of 50-100 effective breeders in our simulation.

The ASASC plan recommends that stocking be limited to 50,000 offspring per breeding pair, or 250,000 sturgeon per river (probably the Suwannee), per year and that it continue for a minimum of 10 years. Thus, the Suwannee River would receive 2.5 million hatchery-spawned sturgeon per generation. Most natural mortality of sturgeon occurs during the first 120 days (St. Pierre et al., 1994). Age at stocking was not specified; however, >120 days is typical. For Gulf sturgeon, fishing mortality has nearly
been eliminated. Therefore, assuming a survival rate of 50% for the remainder of the first year and 80-85% annually thereafter (Hoenig, 1983), approximately 250,000 sexually mature hatchery-spawned sturgeon may integrate with the wild population during the required 10-year stocking period. Thus, within a single generation, sturgeon cultivated in the hatchery could constitute 98% of the total breeding population.

In stark contrast to that of red drum, the potential effect of stocking upon the effective size of the eastern Gulf sturgeon population is dramatic. All reasonable parameter estimates \( N_{e,h} \leq 75, N_{e,o} = 2500, \) and \( x > 75\% \) yield \( N_e \) values below 150 (Fig. 2-4B). The parameter most responsible for the troubling result is \( x \). Because Gulf sturgeon breeding populations are very small, the potential contribution of hatcheries would be quite large. With the levels of stocking recommended in the ASASC protocol, \( N_e \) will be reduced to nearly \( N_{e,h} \) (~50 individuals) within a single generation. A bottleneck of this magnitude would likely result in a substantial loss of polygenic variation and a reduced adaptive potential. Limiting the number of fish stocked would not significantly alleviate this effect. Even if culturists released only 1,000 juvenile offspring per breeding pair (totaling 5,000 juveniles) per yr for 10 yr into the Suwannee River, \( x \) would still exceed 50% (after correcting for mortality), producing an unacceptable reduction in \( N_e \) (to ~290 individuals).

In addition to lost quantitative variation, the practice of "overstocking" small natural populations like the Gulf sturgeon poses risks to single-locus variation. Two outcomes of the same mechanism (genetic swamping) may be realized. First, rare alleles will not be well represented in a hatchery breeding population of \( \leq 100 \) individuals.
Figure 2-4. Potential reduction in the effective size of (A) the Atlantic population of red drum (*Sciaenops ocellatus*) and (B) the Gulf sturgeon (*Acipenser oxyrhynchus desotoi*) predicted using Ryman and Laikre's (1991) model. Justification for the ranges of input parameter values (original effective population size \(N_{e,o}\), hatchery contribution \(x\%), and effective hatchery population size \(N_{e,h}\)) are given in the text. The more darkly shaded areas depict the most likely range of outcomes for the ongoing stocking of red drum in the Atlantic or the proposed stocking of Gulf sturgeon.

(Sjögren and Wyöni, 1994). Thus, when hatchery-reared progeny outnumber native fish in the wild, the frequencies of those alleles initially missing in hatchery breeders will be much smaller in the admixed population than they were in the original wild population.
In a sense, rare alleles will become even rarer after stocking. Because of the increased rate of genetic drift associated with a diminutive effective population size, a multitude of these alleles could be lost in one or two generations. Second, alleles of moderate frequency may be stochastically overrepresented in hatchery offspring (e.g., C. Crawford and T. Bert, unpubl. data), and their frequencies would be artificially inflated in the wild (Hindar et al., 1991). Indeed, this phenomenon forms the operational basis for many genetic tagging programs (Gharrett and Seeb, 1990). With very low effective population sizes, it is theoretically possible for random drift to drive these alleles to fixation in less than $3N_e$ generations (Kimura, 1955). The result would be similar for alleles that are mildly deleterious (Lacy, 1987). Neither of these two outcomes of genetic swamping is consistent with the goals of conserving diversity in gene pools and maintaining the genetic identities of natural populations of Gulf sturgeon.

The genetic risks to Gulf sturgeon would be greatly compounded should the supplemented population not sustain its larger abundance when stocking was discontinued. Stock-enhancement programs have unquestionably led to contemporaneous gains in production, but their ability to yield lasting and self-sustaining increases in population abundance has been largely unproven (Matthews and Waples, 1991; Nehlson et al., 1991). Such increases are usually tied to permanent improvements in survivorship and to the carrying capacity of the environment. Thus, the stated goal (USFWS and GSMFC, 1995) of reimplementing a Gulf sturgeon fishery following population recovery (to fishable levels) might be counterproductive if the recovery was due principally to supplemental stocking.
Concluding Remarks

Despite warnings of the risk to effective sizes of natural populations posed by artificial supplementation (Ryman and Laikre, 1991; Ryman et al., 1995), this consideration has yet to be widely incorporated into fish stocking policies. As demonstrated in our case studies, the parameter values needed to assess the risk to $N_e$ are generally available. Resource managers have known for more than a decade that the genetic effective sizes of managed populations should be maintained at or above a threshold value. Our application of Ryman and Laikre's (1991) model clearly shows that even in the instances when the effective number of hatchery breeders equals 100, almost no natural fish population can absorb a hatchery contribution of more than 45% without a drastic and potentially genetically harmful reduction in their effective size. Yet large-scale releases of artificially cultured fish into natural systems continue despite evidence that such fish, generation after generation, may compose the majority of individuals in many populations. The numbers of fish stocked (intentionally or accidentally) in some populations of the species (e.g., more than 10 million Atlantic salmon, 130 million American shad, 1 billion common sturgeon, 4 billion Pacific salmon) are typical of hatchery release programs world wide (Hynes et al., 1981). In many instances, the ability to artificially propagate and release fish has far exceeded the capacity of natural populations to genetically withstand such input.

When Ryman and Laikre's model is used to evaluate the genetic effects of stocking plans, it is of paramount importance to consider the population biology and genetic stock structure of the target fish species. For example, generation times in fishes
greatly differ, spawning may be semelparous or iteroparous, and homing tendencies may range from strong to nonexistent. These factors, and many more, affect the degree of genetic risk posed to a population targeted for rehabilitation. We purposely selected our case studies to illustrate the contrast in genetic consequences of stock enhancement for two species with pronounced differences in life history and population demography. Our simulations suggest that if hatchery contributions are expected to be large, managers should be cognizant of the potential genetic risks when supplementing species that are subdivided into discrete populations of even moderate effective size. On the other hand, modest hatchery contributions made to local demes within geographically widespread populations should not result in reduced genetic diversity in the long term. We should also note that, in some instances, it might be possible to mitigate the genetic effects described above. For example, selective harvest of hatchery-released fish from an admixed population would still allow a harvest benefit but, because the hatchery contribution would be lower, the depression in $N_e$ may be reduced.

Finally, a general conclusion pertaining to the management of threatened or endangered fish populations may be drawn from this analysis. Almost by definition, abundance in such populations will be so low that even a limited input of cultured fish may constitute an overwhelming portion of the admixture. In these situations, the original effective population size could be reduced to nearly $N_{eh}$ in a single generation. The concomitant genetic effects of this phenomenon are greatly amplified should the supplemented populations fail to sustain the gains in abundance realized from stocking. Accordingly, it may be more prudent to exhaust other means (e.g., restoring habitat and water quality, circumventing man-made obstacles [e.g., Hendricks, 1995], and reducing
incidental fishing mortality) to naturally rehabilitate these populations before, rather than in conjunction with, attempting to supplement them by artificial propagation.
Chapter 3

Genetic Considerations During the Experimental and Expanded Phases
of Snook Stock Enhancement

Introduction

The common snook, *Centropomus undecimalis* (Bloch), is a semicatadromous, 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 et al. 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 overharvestation 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 Rosenstiel School of Marine and Atmospheric Sciences, Harbor Branch Oceanographic Institute (HBOI), Mote Marine Laboratory (MML), and Florida Department of Environmental Protection (FDEP). 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 FDEP were able to refine breeding techniques for common snook (e.g., Kennedy et al. 1998) and to rear sufficient numbers of hatchlings for controlled release into juvenile nursery habitats (S. Serfling, MML, pers. comm.). 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, the survival, growth, and recruitment of cultured fish to local (or non-local) populations will be assessed (e.g., 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 (Blankenship and Leber 1995) requires that potential negative impacts upon the gene pools of wild populations be mitigated through the use of genetically sound breeding and release protocols. Consequently, managers at MML and FDEP 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 the 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 the disruption of coadapted genomes, thereby affecting the fitness of the native stock (outbreeding depression; c.f. Templeton 1986, Waples 1995). For example, inter-race crosses between even- and odd-year returning pink salmon have resulted in decreased survivorship and increased bilateral asymmetry in F2 hybrids (Gharrett and Smoker 1991).

If genetic stock structure in a candidate species has been characterized, genetic hazards associated with intra-species 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 the 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 (e.g., Taniguchi and Sugama 1990) or reduced levels of genetic variation in hatchery broods compared to the wild stock (e.g., 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 inbreeding depression (Tave 1993). Artificial selection, domestication, stochastic allele frequency changes, and reduced levels of variation can occur in the F1 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

$$N_e = \frac{x^2}{N_{e,h}} + \frac{(1-x)^2}{N_{e,w}}$$

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), the 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 gamefish and foodfish, 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 ten-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 (Fig. 3-1). 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 \( \Phi \) statistics (analogous to \( F \) statistics) and to estimate components of genetic variance (Table 3-1). 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 \( \Phi \) statistics are allowed by the AMOVA procedure), indicating that 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
Figure 3-1. 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 3-3 for the collection locations of the Florida samples.

Florida Bay could not be assigned with statistical certainty to either the Atlantic or the Gulf population (Tringali and Bert 1996).

<table>
<thead>
<tr>
<th>Variance Component</th>
<th>Φ statistic and value</th>
<th>Variance</th>
<th>% of total</th>
<th>P (^a)</th>
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<td>Between Atlantic and Gulf/Caribbean</td>
<td>Φ(_{CT}) = 0.099</td>
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<td>9.98</td>
<td>0.014</td>
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<td>Among samples within Atlantic</td>
<td>Φ(_{SC}) = -0.251</td>
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<tr>
<td>Among samples within Gulf/Caribbean</td>
<td>Φ(_{SC}) = -0.068</td>
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<tr>
<td>Within samples</td>
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<td>138.79</td>
<td>nc</td>
</tr>
</tbody>
</table>

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

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, \(H_\text{o}\), 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 3-2. For each locus, the majority of alleles other than the most common allele occurred at very low frequency (<0.05; Fig. 3-2A). Because the probability of sampling alleles diminishes as allele frequency
Table 3-2. Summary of allozyme and mitochondrial DNA variation in *C. undecimalis* from Florida. Except for the average number of allozyme alleles per locus (\(n_a\)), results appear in Tringali and Bert (1996). \(H_o\) = average percentage of loci heterozygous in an individual; \(P_{95}\) = 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 3-3.

<table>
<thead>
<tr>
<th>Location</th>
<th>Allozyme</th>
<th>mtDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n_a)</td>
<td>(P_{95})</td>
</tr>
<tr>
<td><strong>Atlantic coast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sebastian Inlet</td>
<td>1.23</td>
<td>13</td>
</tr>
<tr>
<td>Jupiter Inlet</td>
<td>1.29</td>
<td>3</td>
</tr>
<tr>
<td><strong>Gulf of Mexico</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida Bay</td>
<td>1.24</td>
<td>7</td>
</tr>
<tr>
<td>Rookery Bay</td>
<td>1.42</td>
<td>10</td>
</tr>
<tr>
<td>Tampa Bay</td>
<td>1.32</td>
<td>7</td>
</tr>
</tbody>
</table>

decreases (Fig. 3-2B), it is likely that many alleles occurring at frequencies \(\leq 0.01\) 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).
Figure 3-2. (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 (\( P_s \)) an alternate allele (\( y \)-axis) when it occurs in a population at a given frequency (\( x \)-axis) and when 138 individuals are sampled.

All measures of mtDNA variability are very low in common snook (Table 3-2; 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 [FDEP, 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) 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.
The common snook stock-enhancement program being conducted by MML and FDEP 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 (e.g., Brunner et al. 1998). Therefore, 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 (Fig 3-3). 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 (≤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. Moreover, 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 F1 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 in 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 wild snook, and these protocols should be continuously evaluated and adjusted, if necessary, during the experimental phase.

Accordingly, we recommend that managers of the common snook stocking program continue using wild-caught adults as broodstock. To capture within-population phenotypic diversity, broodstock should be systematically sampled from the recipient population over the course of the protracted breeding season (April-September [Taylor et al. 1998b]) and from various spatially and environmentally separated spawning aggregates (e.g., those from barrier island inlets, passes to secondary [within-estuary] embayments, and mouths of coastal rivers [Peters et al. 1998]). Egg production for
common snook is usually accomplished by fertilizing the eggs of strip-spawned females, which may have undergone hormone treatment to induce egg maturation, with the sperm of one or more males (Wallace et al. 1993). To minimize the risk of F1 domestication, family sizes should be equalized (Allendorf 1993) so that hatchery selection will operate only through fitness differentials among different genotypes within families of full- or half-sibs (depending on the mating scheme employed). Sources of potential artificial selection during rearing should be identified and avoided. For example, a particular concern for snook reared at high density may be cannibalism of slower growing individuals by faster growing individuals. Mitigation of this problem may require segregation of progeny by size during rearing or by reducing rearing densities.

*Expanded Stocking Phase.* Should the common snook stock-enhancement program expand to the production phase in Florida, additional captive-propagation and stocking guidelines will be necessary. During breeding and rearing (Type II processes), the objective should be to produce hatchery broods that are similar to wild stocks with respect to both adaptive and selectively neutral variation. Intraspecific genetic variability in common snook is low; therefore, to propagate a sufficient amount of within-population genetic diversity, a minimum of 100 hatchery breeders ($N_h$) should be used per generation interval (3 yrs). This strategy should maintain natural allele frequencies and preserve 99.5% of the original heterozygosity and the majority of allelic diversity present in the source population (Allendorf and Ryman 1987).

Due to the potential for genetic swamping in common snook, risks associated with Type III (Ryman/Laikre) hazards should be minimized. To do so, we recommend that a minimum of 50 effective hatchery breeders be used per generation interval. To achieve a ratio of at least 0.5 effective breeders to actual hatchery breeders, attention to parental sex ratio and to family size variance will be required (Crow and Denniston 1988;
Kincaid 1995).  \( N_{e,h}/N_h \) ratios ranging 50-75% have been achieved in other hatchery programs through the use of genetically efficient protocols (e.g., Hedrick and Hedgecock 1994).

Stocking guidelines were formulated as follows.  Adopting a minimum-allowable \( N_e \) value of 500 for the enhanced stock (FAO/UNEP 1981; Tringali and Bert 1998), we first used the Ryman/Laikre model (equation 3-1) to estimate maximum relative contributions \( (x_{\text{max}}) \) to recipient wild stocks (subpopulations) for values of \( N_{e,h} \).  We then estimated the maximum-allowable number of juvenile hatchlings \( (H_{\text{max}}) \) that should be stocked in a subpopulation of known abundance by using the expression

\[
x_{\text{max}} = \frac{H_{\text{max}} \cdot S_r}{(H_{\text{max}} \cdot S_r) + N_w},
\]

where \( S_r \) is the pre-recruitment survival rate (i.e., the anticipated survival rate of released juvenile cultured snook to reproductive age) and \( N_w \) is the spawning stock abundance of the recipient subpopulation.  As a conservative measure, the genetic structure model used for broodstock-source selection was used to define the range of subpopulation abundances.

For \( N_{e,h} \) values of 50 and 75, maximum relative contributions of hatchery-released snook should be limited to 31.5% and 38.7%, respectively, during a generation interval.  For these two values of \( x_{\text{max}} \), Figure 3-4 depicts the maximum-allowable stocking limits for values of \( S_r \) between 5-15% and values for \( N_w \) between 100,000-500,000 (based on annual estimates [Muller and Murphy 1998]).  We anticipate that the parameter ranges modeled in Figure 3-4 would be applicable to the majority of stocking activities during an expanded phase of snook production.  Notably, the relatively small increase in \( N_{e,h} \) from 50 to 75 allows a significant increase in the maximum number of
Figure 3-4. Maximum-allowable number of cultured snook ($H_{max}$) per generation interval for survival rates ($S_r$) prior to recruitment to the breeding subpopulation between 5-15% and subpopulation abundances of wild common snook ($N_w$) between 100,000-500,000. Estimates generated for $N_{e,h}$ values of (A) 50 and (B) 75 breeders.
hatchlings that could be released. For example, assuming a pre-recruitment survival rate of 15% for hatchery-reared snook, up to 840,000 hatchlings could be propagated from 75 effective breeders and stocked into a wild spawning stock of 200,000 individuals (per GI) compared to only 614,000 hatchling snook propagated from 50 effective breeders. This results in a 27% increase in the stocking limit for snook, potentially increasing the rate at which a declining stock could be rebuilt.

Finally, we recommend that a genetic monitoring program for supplemented snook populations be incorporated into the overall management plan during an expanded stocking phase, should it occur, or if small-scale (experimental) releases in particular waterways occur repeatedly over multiple generation intervals. Components of the monitoring program should include the characterization of genetic diversity and composition in hatchery broods and periodic genetic sampling of the recipient stock to evaluate any fluctuations in gene frequencies and reductions in pre-stocking levels of genetic diversity that may be associated with hatchery releases. Available allozyme and mtDNA genotype frequency data for wild snook stocks (Tringali and Bert 1996; Wilson et al. 1997) should be useful in this process. However, because of the low level of genetic variation found in those data, additional genetic markers may be required for certain analyses.

Concluding Remarks

A growing body of evidence demonstrates that cultured marine organisms can make substantial contributions to fisheries landings in some coastal marine species (Leber et al. 1998, Masuda and Tsukamoto 1998, Rimmer and Russell 1998). However, the effectiveness of marine stock enhancement as a resource management tool
remains a hotly debated subject in the United States (Radonski and Loftus 1995; Travis et al. 1998). The potential for negative genetic consequences typically and justifiably ranks high among the list of concerns. We have adopted a conservative approach throughout our assessment of genetic risk and in the formulation of risk-adverse guidelines for snook stock enhancement. We anticipate that genetic risk management will be an ongoing process within the program, subject to refinement and amendment as more information becomes available. Our general conclusion from this preliminary assessment is that cultured snook, propagated and released according to the preceding guidelines, are not likely to have significant short- or long-term impacts on the genetic composition or diversity of wild snook populations in Florida.
Chapter 4
Measuring Gene Correlations among F1 Progeny in Complex Systems of Nonrandom Mating

Introduction

Inbreeding is the term used to describe mating among related (consanguineous) individuals. Within populations, the consequence of inbreeding may be measurable excesses of homozygous genotypes in relation to Hardy-Weinberg expectations. Broader forms of non-random mating, e.g., demic or geographic cohesion, can also lead to similar departures from Hardy-Weinberg genotypic proportions (Wright 1951). The inbreeding coefficient \( f \) represents the probability that two alleles in an individual are autozygous (derived from the same gene in a common ancestor) whereas the average inbreeding coefficient \( F \) represents the fraction by which average heterozygosity has been reduced in a single, unstructured population.

As described and referenced in the introductory chapter (see page 13), it is often proposed and borne out by empirical data that severe reductions in heterozygosity may lead to reductions in individual fitness, thereby lowering mean population fitness. Briefly, the gene pools of outcrossing populations contain significant amounts of “hidden” genetic variation in the form of rare recessive alleles (Nevo 1978; e.g., Launey and Hedgecock 2001). Theory suggests that deleterious recessive alleles will persist in a
gene pool despite natural selection because of the protection conferred to them when in the heterozygous state. Inbreeding exposes a greater portion of deleterious recessive alleles to selection, potentially leading to a form of reduced fitness known as inbreeding depression (e.g., Ferguson and Drahushchak 1990, Coltman et al. 1999). In finite populations, gamete sampling error causes allele frequencies to drift toward allele fixation or loss. Thus, random genetic drift may further increase levels of homozygosity for some fraction of the deleterious recessive alleles segregating in the population (see Chapter 6). The parallel and possibly synergistic actions of inbreeding and drift may reduce population viability and increase the risk of extinction in very small populations (Lande 1995). The rate at which heterozygosity decreases in a population over time is inversely proportional the inbreeding effective number \((N_e)\) of the population.

As described in Chapter 2, a potential form of genetic impact related to inbreeding has come to be known as the “Ryman-Laikre effect” (Waples and Do 1995, Hedrick et al. 2000, Hedgecock and Koykendal in press) and relates specifically to changes in gene correlations within and among individuals in a population following supplementation. As noted by Ryman and Laikre (1991), the inbreeding effective number for an admixed population (composed of captive-bred and wild individuals) may be predicted from a mixture model via three variables; the inbreeding effective numbers of the original wild and hatchery-derived component populations, respectively, and the relative abundance of hatchery-derived individuals in the mixture. The focus of the present chapter is on one of the model’s variables – the inbreeding effective number of the hatchery-derived population. Obtaining a reliable estimate of \(N_{e1}\) for a discrete group of captive-bred (cultured) organisms may be challenging. If breeding procedures are structured, i.e., if
individual gamete contributions are systematically controlled via paired or otherwise prescribed matings, then *prima facie* estimates of $N_{ef}$ are often computed through simple pedigrees. However, the various *unstructured* tank- or pond-spawning protocols employed in many stock enhancement programs represent complex and largely unpredictable systems of nonrandom mating.

In this chapter, I address theoretical considerations that apply to the estimation of $N_{ef}$ for breeding systems used at many fish stock-enhancement facilities. Some of the analytical results, however, may be generally relevant to any captive-bred or wild population for which there is subdivision and internal breeding structure. General definitions and terminology used throughout appear in Appendix A. Organisms are assumed to be diploid and to reproduce sexually without self-fertilization. In a captive setting, P1 broodfish may be subdivided into several discrete spawning groups (cages, tanks or ponds). During a production season, each spawning group may produce multiple lots; each female within a spawning group may produce multiple clutches. Group spawning may be induced (e.g., through photo-thermal manipulation or hormone treatment in fish hatcheries) but reproductive contributions of individuals within groups are not generally controlled. Accordingly, some level of genetic polygyny and multiple paternity may occur within groups and these may vary among groups. Potential immediate intragroup relationships among progeny are full sibling, maternal and paternal half-sibling, and non-sibling. By definition, full and half-sibling relationships do not extend beyond progeny groups. The broodfish may themselves by inbred or related to some degree. Sex ratios of parents within spawning groups may be unequal and numbers of progeny from each brood and from each progeny group may be disproportionate.
Ultimately, offspring from all progeny groups are admixed (e.g., via their release into a single nursery) and thus constitute a hatchery cohort, which is a constituent of the admixed hatchery/wild population. Marine organisms can be highly fecund; therefore, if stocking is successful, there will be many more offspring than captive-bred parents – the admixed population will likely grow.

Genetically, the above set of circumstances represents a complex scenario of non-random mating in which gene correlations can only be understood by considering probabilities of kinship at the appropriate hierarchical level. One of my objectives (see Chapter 5) was to accurately estimate the inbreeding effective number of a captive-bred cohort under these circumstances. Unfortunately, nearly 70 years after the theoretical concept was introduced by Wright (1931), application of $N_{ef}$ to real biological situations remains confounded by a lack of concordance among various mathematical models.

**Background**

To determine effective numbers in subdivided populations in either natural or captive settings, gene correlations within individuals ($F$), among individuals within groups (intragroup, $\theta$ [also known as the coefficient of coancestry]), and among individuals from different groups (intergroup, $\alpha$) must be considered (e.g., Chesser 1991a,b). Initially I assume that the brood stock was obtained from a relatively large outcrossing population; thus, gene correlations relating to $F$ and $\alpha$ will be negligible in the P1 generation. In such cases, the effective number of breeders for a progeny group can be viewed in terms of the gene correlations accrued among F1 offspring within the
group. Thus, an initial (single generation) $N_{el}$ can be approximated by the coancestral (intragroup) effective number, $N_{e\theta}$ (Chesser et al. 1993; Sugg and Chesser 1994). Often, models for $N_{e\theta}$, including those examined herein, incorporate the simplifying assumption that sexes of progeny are determined randomly with a probability of 0.5. Thus, to examine discordance in these models, there is no need to distinguish between intragroup gene correlations of like-sexed and different-sexed progeny (i.e., $\theta_{ff} = \theta_{mm} = \theta_{mf}$). I make this assumption also out of necessity, because stocking (and censusing) of hatchlings often occurs prior to the onset of sexual differentiation.

With parentage data available, the coancestral effective number of a progeny group (generation F1) can be computed directly by considering the expected coancestry ($\theta_{F1}$) of progeny born within the group. The coancestry of two individuals ($\theta_{x,y}$) is defined as the probability that homologous genes drawn at random, one from each of the $x$th and $y$th individuals, are identical by descent (i.e., derived from the same gene in a common ancestor)(Malécot 1948). This coefficient equates to the inbreeding coefficient for any progeny (generation F2) that may be produced by these two individuals. The coancestries of progeny pairs in an F1 progeny group can be represented by the following matrix, where $N$ is the total number of progeny. That is,

\[
\begin{matrix}
\theta_{1,2} & \theta_{1,3} & \ldots & \theta_{1,N} \\
\theta_{2,3} & \ldots & \theta_{2,N} \\
\vdots & \vdots & \ddots & \vdots \\
\theta_{N,N-1}
\end{matrix}
\]
The expected coancestry of the progeny group is the mean of the values in this matrix. The coancestral effective number of a progeny group (Chesser et al. 1993) equates to $1/2 \theta_{FJ}$.

Chesser et al. (1993) and Sugg and Chesser (1994) developed recurrence equations to obtain general, instantaneous, and asymptotic expressions for the effective number in subdivided populations for various systems of breeding. Their expressions have been applied in the contexts of molecular evolution (Chesser 1998a), molecular ecology (Chesser and Baker 1996, Chesser 1998b), and conservation biology (Pope 1996, Dobson et al. 1997). In Chesser et al. (1993), a model for inbreeding effective number was presented ostensibly for application to cases of genetic polygyny, variable family sizes, unequal numbers of male and female breeders, and population structure. Sugg and Chesser (1994) extended the general model of Chesser et al. (1993) to incorporate a term for multiple paternity within broods. Mathematical formulae presented in that study indicated that multiple paternity can increase the inbreeding effective number of the spawning group up to twofold compared to systems that involve only one male mate per female.

Building on the work of Kimura and Crow (1963), Crow and Denniston (1988) derived general expressions for inbreeding effective number which putatively apply to cases of unequal numbers of male and female breeders and variable family size when $\theta_{ff} = \theta_{nm} = \theta_{nf}$. Their expressions apply only to single, unstructured populations. Others (e.g., Hill 1979; Caballero 1995; Nagylaki 1995; Wang 1997) have extended the models of Kimura and Crow to include circumstances such as overlapping generations, covariance in the numbers of male and female progeny per parent, and migration among
subpopulations. Notably, Wang (1996) reported that the model of Crow and Denniston (1988) is not appropriate for cases in which abundance in the F1 generation exceeds that in the P1 generation – often the principle goal in stock restoration/enhancement.

The general expressions developed by Chesser and coworkers, Crow and Denniston (1988), and Wang (1996) differ considerably and lead to disparate conclusions with respect to the effects of population growth and internal mating structure on $N_e \theta$. In the following section, I briefly describe each model and examine concordance or convergence between models under relevant circumstances. All investigators derived their models under the assumption that breeders in the P1 (initial) generation were not inbred or related. In a later section, I extend expressions for $N_e \theta$ to the case where P1 parents might be inbred and related.

*Descriptions and Comparative Analyses of the Models*

In our case, we are considering multiple, subdivided spawning groups whose progeny are admixed prior to their attaining reproductive maturity to form a single cohort. Because they are discrete (isolated), each spawning group may be considered a single population and their progeny group may be described by a representative inbreeding effective number prior to pooling. Upon pooling, within-cohort gene correlations will exist as a result of subdivision in the parental generation. These will be accounted for generically in a later section when the inbreeding effective number of the entire cohort is considered. In the models of Chesser and his coworkers, breeding parameters were averaged over multiple (all) spawning groups. However, below I treat
each spawning group as a single population and I make separate estimates of relevant probabilities and of $N_e \theta$ for each spawning group. This approach allows a direct comparison with the models of Crow and Denniston (1988) and Wang (1996). To simplify the process of introducing parameters, I begin with the model of Chesser et al. (1993).

Chesser et al. (1993). To account for intragroup breeding dynamics, Chesser et al. (1993) derived the parameters $\phi_f$ and $\phi_m$, which reportedly describe average probabilities of single maternity and single paternity within spawning groups, respectively. For $s$ spawning groups, breeding parameters involved in the estimation of $\phi_{f,j}$ and $\phi_{m,j}$ for the $j$th group are

- $n_j$: number of potential female breeders in the $j$th spawning group.
- $m_j$: number of potential male breeders within the $j$th spawning group.
- $k_j$: average number of progeny produced by females in the $j$th spawning group.
- $\sigma^2_{k,j}$: variance in number of progeny produced by females in the $j$th spawning group.
- $b_{i,j}$: average number of females mated by the $i$th male in the $j$th spawning group that result in surviving progeny.
- $b_j$: average number of females mated by each male in the $j$th spawning group that result in surviving progeny.
\( \sigma^2_{b,j} \) variance in number of females mated by each male in the \( j \)th spawning group that result in surviving progeny.

Herein, \( k_i \) is defined as the number of offspring produced by the \( i \)th female that survive to reproduce. \( N_{S,j} \) is defined as the total number (abundance) of offspring that survive to reproduce from the \( j \)th spawning group, where for each group \( N_{S,j} = \sum k_i \) for \( i = 1 \) to \( n \) = \( k_j n_j \). Thus, the total abundance of the hatchery cohort (\( N_C \)) at the time of recruitment to the breeding population is defined as \( N_C = \sum N_{S,j} \) from \( j = 1 \) to \( s \).

The parameter \( \phi_{f,j} \) represents the probability that two randomly chosen progeny produced from within the \( j \)th spawning group were the offspring of a given female. This probability was given by Chesser et al. (1993) to be \( (\sigma^2_{k,j} + k_j (k_j - 1))/(k_j (k_j n_j - 1)) \). The parameter \( \phi_{m,j} \) represents the probability that two randomly chosen progeny produced from within the \( j \)th spawning group were sired by the same male. Chesser et al. (1993) defined this probability as \( m(\sigma^2_{b,j} + b_j (b_j - 1))/(n_j (n_j - 1)) \). For \( \phi_{f,j} \) and \( \phi_{m,j} \), values of zero indicate each offspring produced from within a given spawning group was the product of a different female or male, respectively (impossible for \( N_{S,j} > n_j \) or \( m_j \), respectively), whereas values of one indicate that all progeny produced from within a given spawning group were the product of a single female or male, respectively. When we consider a single, isolated population, Chesser et al.’s (1993) expression for coancestral effective number reduces to

\[
N_{e\theta,j} = \frac{4}{2\phi_{f,j} + (1 - \phi_{f,j})\phi_{m,j}},
\]

4-2
Expressed in terms of expected coancestry, equation 4-2 becomes

\[
\theta_{r1} = \frac{1}{4}\phi_{f,j} + \frac{1}{8}(1-\phi_{f,j})\phi_{m,j},
\]  

4-3

*Crow and Denniston (1988).* When there are separate sexes, unequal numbers of males and females, and variance in the number of progeny per parent, Kimura and Crow (1963) noted that the inbreeding effective number of a single isolated population is

\[
\frac{1}{N_{el}} = \frac{1}{4N_{el,f}} + \frac{1}{4N_{el,m}},
\]  

4-4

where \(N_{el,m}\) and \(N_{el,f}\) are the inbreeding effective numbers of male and female parents, respectively. By considering progeny from a particular spawning group, we can consider expressions stemming from equation 4-4 to be analogous to coancestral effective numbers. For individual sexes, the coancestral effective number can be obtained from the number of parents \(N_r\) of sex \(r\) \((r = m\ or f)\) and the means and variances of progeny production. Specifically,

\[
N_{e\theta,r} = \frac{N_r\mu_r - 1}{\mu_r - 1 + \frac{\sigma^2_r}{\mu_r}},
\]  

4-5
where $\mu_r$ and $\sigma^2_r$ are the mean and variance of progeny production for sex $r$. Crow and Denniston’s general expression (equation 4-4) can be rederived for individual spawning groups in terms of breeding parameters that are analogous to those of Chesser et al. (1993). Using equation 4-5, the female coancestral effective number of the $j$th spawning group can be estimated by

$$N_{e\theta,j} = \frac{n_j k_j - 1}{k_j - 1 + \frac{\sigma^2_{k,j}}{k_j}} = \frac{k_j (n_j k_j - 1)}{k_j (k_j - 1) + \sigma^2_{k,j}} = \frac{1}{\phi_{f,j}}$$

To obtain an analogous expression for $N_{e\theta,m}$, I employ these additional demographic parameters:

$h_j$ average number of progeny produced by males in the $j$th spawning group.

$\sigma^2_{h,j}$ variance in number of progeny produced by males in the $j$th spawning group.

As noted, Chesser et al. (1993) express $\phi_m$ (the probability that randomly chosen progeny are the product of a given male parent) in terms of the mean and variance of the numbers of females mated by each male. This probability can be more generally obtained, however, in terms of the mean and variance in male progeny production. That is, $\phi_{m,j} = \phi'_{m,j} = \frac{(\sigma^2_{h,j} + h_j (h_j - 1))/(h_j (h_j m_j - 1))}{\phi'_{m,j}}$ where the prime (′) in $\phi'_{m,j}$ is used to distinguish it from the probability given in Chesser et al. (1993). Note that this probability is independent of
the number of females in a given spawning group. As in equation 4-6, it can be shown that \( N_{e\theta m} = 1/\phi'_m \) and the general expression (equation 4-4) of Crow and Denniston (1988) for the \( j \)th spawning group becomes

\[
N_{e\theta,j} = \frac{4}{\phi_{f,j} + \phi'_{m,j}},
\]

A similar expression, conditioned on \( n, m \gg 1 \), was also obtained by Nagylaki (1995, his equation 15). Equation 31 of Wang (1997) also simplifies to equation 4-7 above for the general case considered here. Expressed in terms of expected coancestry, equation 4-7 becomes

\[
\theta_{fj} = \frac{1}{8} \phi_f + \frac{1}{8} \phi'_m.
\]

In its full form, equation 4-7 is

\[
N_{e\theta,j} = 4 + \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} + \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right].
\]

Chesser et al. (1993) versus Crow and Denniston (1988). If they are to be considered generally applicable, models for the estimation of \( N_{e\theta j} \) should exhibit concordance or convergence with classical models under simplifying assumptions.

Consider a spawning group consisting of \( n_j \) females and \( m_j \) males. When all individuals
of the same sex have an equivalent expectation of leaving progeny, Wright (1969) has shown that the effect of unequal sex ratios on the coancestral effective number is given by

\[ N_{e\theta,j} = \frac{4n_j m_j}{n_j + m_j}, \quad 4-10 \]

It has been asserted (e.g., Chesser et al. 1993, Wang 1996, Sugg and Chesser 1994, etc.) that the general expression of Chesser et al. (1993) is convergent with that of Crow and Denniston (1998) when applied to a single, randomly mating population. We can examine the behavior of \( \phi_{f,j} \) and \( \phi_{m,j} \) under assumptions of an equal expectation of offspring for like-sexed individuals. In cases of random mating, when the distributions of \( k_j \) and \( h_j \) are binomial, then \( \sigma^2_{k,j} \) and \( \sigma^2_{h,j} \) equal \( k_j (1 - (1/ n_j)) \) and \( h_j (1 - (1/ m_j)) \), respectively. When the distributions are Poisson, then \( \sigma^2_{k,j} \) and \( \sigma^2_{h,j} \) equal \( k_j \) and \( h_j \), respectively. Thus, the probabilities \( \phi_{f,j} \) and \( \phi_{m,j} \) may be approximated by \( 1/n_j \) and \( 1/m_j \) when conditioned for random mating – i.e., when associated variances are binomial (Crow and Denniston 1988; Nagylaki 1995) or Poisson. Substituting these approximations into equation 4-7 gives

\[ N_{e\theta,j} \approx 4 \div \left[ \frac{1}{n_j} + \frac{1}{m_j} \right], \quad 4-11 \]
which, upon simplification, gives a result that is identical to that obtained equation 4-10. Therefore, the expression of Crow and Denniston is convergent with Wright’s (1969) general expression under conditions for which the approximations $1/n_j$ and $1/m_j$ may be applied. We can also condition $\phi_{f,j}$ and $\phi_{m,j}'$ for the case in which sex ratios and family sizes are equal and all possible matings occur. In this case, maternal variance in progeny production within spawning groups is zero and $\phi_{f,j} = k_j(k_j - 1)/(k_j(n_j - 1)) \approx 1/n_j$ when $k_j$ is sufficiently large. Similarly, variance in paternal production within spawning groups is zero and $\phi_{m,j}' \approx 1/m_j$ when values of $h_j$ are sufficiently large. For this case, $N_{eB,j} = N_{B,j}$, as expected from equation 4-10.

In contrast, inspection reveals that the expression of Chesser et al. (1993) is not strictly concordant with those of Wright (1969) or Crow and Denniston (1988) and converges with those expressions only under certain conditions. Again, I assume that individuals of the same sex have an equal expectation of leaving progeny. I first note that the probability $\phi_m$ of Chesser et al. (1993) is non-general and can yield values $> 1$. For example, consider a spawning group in which 2 females are mated with 4 males to produce 160 progeny as follows:

<table>
<thead>
<tr>
<th>Dam1 x Sire1 → 20 progeny</th>
<th>Dam2 x Sire1 → 20 progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam1 x Sire2 → 20 progeny</td>
<td>Dam2 x Sire2 → 20 progeny</td>
</tr>
<tr>
<td>Dam1 x Sire3 → 20 progeny</td>
<td>Dam2 x Sire3 → 20 progeny</td>
</tr>
<tr>
<td>Dam1 x Sire4 → 20 progeny</td>
<td>Dam2 x Sire4 → 20 progeny</td>
</tr>
</tbody>
</table>
According to the formula of Chesser et al. (1993), the probability that random progeny within this progeny group were the product of a particular sire would be 4, which is incorrect. This probability was expanded from an earlier version given in Chesser et al. (1991) to include mean and variance in the number of females mated by each male. Their original probability, defined as \((\sum b_i^2 - b_i)/(n^2 - n)\) for \(i = 1\) to \(m\), is also incorrect. Substituting the conditional approximations for \(\phi_{f,j}\) and \(\phi'_m\) (\(1/n\) and \(1/m\), respectively) into Chesser et al.’s (1993) general expression (equation 4-2 above) yields, after simplification,

\[ N_{e\theta,j} = \frac{4n_j m_j}{2m_j + n_j - 1}, \quad 4-12 \]

which is concordant/convergent neither with Wright (1969) nor with Crow and Denniston (1988), even when sex ratios are equal and mating is random, except in the trivial case where there is a single breeding pair.

In the special case of harem polygyny, in which a single male contributes reproductively to the \(j\)th spawning group, \(\phi'_m = 1\). In this case, all offspring will be full sibs or paternal half-sibs and both equation 4-7 (Crow and Denniston [1988]) and equation 4-2 (Chesser et al. [1993]) reduce to

\[ N_{e\theta,j} = \frac{4}{1 + \phi_{f,j}}, \quad 4-13 \]
Thus, when all females have an equal expectation of producing offspring, equation 4-13 becomes

\[ N_{e0,j} = \frac{4}{1 + \frac{1}{n_j}} \geq \frac{4n_j}{n_j + 1}, \]

as expected. In the special case of polyandry, in which a single female contributes reproductively to the \( j \)th spawning group, \( \phi_{f,j} = 1 \). In this case, all offspring will be full sibs or maternal half-sibs and the expression of Crow and Denniston (1998) (equation 4-7 above) reduces to

\[ N_{e0,j} = \frac{4}{1 + \phi_{m,j}^j}, \]

When all males have an equal expectation of producing offspring, equation 4-15 becomes

\[ N_{e0,j} = \frac{4}{1 + \frac{1}{m_j}} \geq \frac{4m_j}{m_j + 1}, \]

as expected. However, the expression of Chesser et al. (1993) (equation 4-2 above) reduces to \( N_{e0j} = 2 \), regardless of the number of contributing parents, which is incorrect except, again, when there is a single breeding pair.
For the special case of monogamy, we must recall definitions for spawning groups/progeny groups. The criterion for spawning-group membership is that individuals must potentially interbreed. As a consequence, full and half-sib relationships among progeny are confined to within progeny groups. Therefore, although it may seem trite, we consider each monogamous breeding pair to be a distinct spawning group, $s$ equals the number of breeding pairs, and $k_i = N_{S,j}$. Collectively, progeny from a group of monogamous breeding pairs represent a cohort, where $N_C = \sum N_{S,j} = \sum k_i$ in this case. Here, I define $m_C$ and $n_C$ as the total number of male and female P1 breeders in the cohort. I assume that members of the progeny group, upon maturing, choose mates randomly from within the cohort. In other words, the effective number for the cohort derived by monogamous mating can be viewed as an intergroup effective number ($N_{e\alpha,C}$) in which male dispersal ($d_m$) and female dispersal ($d_f$) is complete ($d_m = d_f = 1$). The probability $P$ that two randomly chosen homologous genes from two randomly chosen progeny within a monogamous brood were derived from the same P1 parent is $1/N_{e\theta} = 0.5$; for each breeding pair, $N_{e\theta} = N_{B,j} = 2$. The probability that two progeny were randomly chosen from the same brood ($\phi_{f,C}$) may be obtained from $\sigma^2_{k,C} + k_C(k_C - 1))/(k_C(k_Cs - 1)$, where $k_C$ and $\sigma^2_{k,C}$ are the mean and variance in individual dam production (in this case, brood size) for a cohort. Taking the inverse of the product of these probabilities, the intergroup effective number for a cohort of progeny derived from $s$ breeding pairs is

$$N_{e\alpha,C} = \frac{N_{e\theta}}{\phi_{f,C}} = \frac{2}{\phi_{f,C}} = 2 \times \left[ \frac{\sigma^2_{k,C} + k_C(k_C - 1)}{k_C(k_Cs - 1)} \right].$$
Noting that \( s = n_C (= m_C) \), convergence between equation 4-17 and the expression of Crow and Denniston (1988; herein, equation 4-7) becomes apparent. It is also apparent that male reproductive data would be unnecessary if the appropriate female reproductive data were available and vice-versa. That is, we can define \( \phi_{m,C} \) as the probability that two randomly chosen progeny in a cohort shared a father; thus, \( \phi_{m,C} = \sigma^2_{h,C} + h_C(h_C - 1)/(h_C s - 1) \), where \( h_C \) and \( \sigma^2_{h,C} \) are the mean and variance in individual sire production for a cohort based on paternity data. For the special case of monogamy, \( \phi_{f,C} = \phi_{m,C} \) and, thus, \( N_{e\alpha,C} \) is also equivalent to \( 2/\phi_{m,C} \).

*Sugg and Chesser (1994).* Sugg and Chesser (1994) stated that the general expression of Chesser et al. (1993, equation 4-2 above) “assumes single paternity”. However, in Figure 2 of Chesser et al. (1993), the authors use the expression to depict model space that would seemingly require multiple paternity, i.e., concurrently high \( \phi_f \) and low \( \phi_m \). As indicated above, equation 4-2 contains a non-general probability and converges by happenstance with other models in cases where \( \phi_m \) is precisely unity – harem polygyny. Considering expected gene correlations of offspring within spawning groups (full-sibling, half-sibling, and non-sibling) caused by variable family size, polygyny, and multiple paternity, Sugg and Chesser (1994) reported that the coancestral effective number may be approximated by

\[
N_{e\theta,j} = \frac{4}{\phi_{f,j}(1 + \phi_{w,j}) + \phi_{m,j}(1 - \phi_{w,j})},
\]

4-18
where $\phi_{w,j}$ is the probability that random born progeny within a brood are the product of a single male. For a single, isolated population (here, a single spawning group), Sugg and Chesser (1994) defined this probability as $l_j (\sigma^2_p + p(p-1)/(k_j (k_j - 1))$, where $l_j =$ average number of males mated by each female in the $j$th spawning group, $p =$ average number of progeny sired by a single male in a brood, and $\sigma^2_p =$ variance in number of progeny sired by a single male in a brood. Note that when $\phi_{w,j} = 1$, equation 4-18 is identical to equation 4-2. Sugg and Chesser (1994) attempted to generalize the probability $\phi_{m,j}$ of Chesser et al. (1993), redefining it as $m_j (\sigma^2_{b,j} + b_j(b_j - 1)/(l_j n_j (n_j - 1)))$. Herein, this probability will be denoted $\phi''_{m,j}$ to distinguish it from $\phi_{m,j}$ and $\phi'_{m,j}$. Expressed in terms of coancestry, equation 4-18 becomes

$$\theta_{f,j} = \frac{1}{8}[\phi_{f,j} (1 + \phi_{w,j})] + \frac{1}{8}[\phi'_{m,j} (1 - \phi_{f,j})].$$

4-19

This term, which appears in the middle row of Sugg and Chesser’s (1994) transition matrix $T$ and the middle element of their constant vector $C$, differs considerably from the term specified in equation 4-8 but converges with equation 4-3 when $\phi_{w,j} = 1$.

If we apply Sugg and Chesser’s amended probability $\phi''_{m,j}$ to the pedigree example above, we obtain 1, which is incorrect. Note also that the term $\phi_{w,j}$ (and the average term provided in Sugg and Chesser 1994) does not account for variance in $p$ among broods, which could seemingly lead to significant inaccuracies in Sugg and Chesser’s expression for $N_e \theta$, especially in the unstructured breeding systems considered here. Although ultimately unnecessary (see below), a more accurate expression for $\phi_{w,j}$
can be obtained as follows. I define the parameter $\phi_{w,i}$ as the probability that randomly chosen progeny from the brood of the $i$th female were the offspring of a single male. This probability may be determined by \((\sigma^2_{p,i} + p_i (p_i - 1))/(p_i (k_i - 1))\) where $p_i$ and $\sigma^2_{p,i}$ represent the mean and variance of the number of progeny sired by a single male in the $i$th brood. Values of zero indicate that each offspring in a given brood was the product of a different male (impossible for $k_i > m_j$) and values of one indicate that all progeny of given a brood were sired by a single male. Further, I define $\varphi_i$ as the probability that randomly chosen progeny were the offspring of the $i$th female. This probability may be determined by \((k_i / N_S)\). Summing the products of these two probabilities over all broods in the $j$th spawning group gives the overall probability of single paternity within broods for the spawning group, i.e., $\phi'_{w,j} = \sum \varphi_i \phi_{w,i}$ from $i = 1$ to $n$ (the new probability is again distinguished by a prime).

Confusion regarding Sugg and Chesser’s (1994) breeding parameters may be evident in empirical studies. For example, Dobson et al. (1997) employed Sugg and Chesser’s (1994) model to examine inbreeding in black-tailed prairie dogs. Using $\phi_w = \frac{1}{(\sigma^2_p + p(p - 1))/(k(k - 1))}$, Dobson et al. computed a value $\phi_w = 1.00$. Mindful that a value of unity for $\phi_w$ should indicate single paternity within broods (over a given female’s reproductive lifetime), their $\phi_w$ value conflicts with the other reproductive values given, e.g., 1.25 successful male mates per female.

For the special case of monogamy, we recall that a single breeding pair represents a discrete spawning group. In this case, $\phi_{w,j} = \phi'_{w,j} = \phi_{m,j} = \phi'_{m,j} = \phi''_{m,j} = 1$ and equation 4-18 becomes $N_{e\theta_j} = 2/\phi_{r,j} = 2$, which, although not very useful, is correct when $P1$
parents are not inbred/related. As with the expression of Crow and Denniston, equation 4-18 can be licentiously applied as an intergroup effective number to achieve concordance with equation 4-17. However, that concordance occurs only when/because male reproductive parameters/probabilities are not relied upon. In contrast, when male reproductive parameters are substituted for female reproductive parameters, the general expression of Crow and Denniston still obtains.

Wang (1996) versus Crow and Denniston (1988). Based on probabilities of random F1 progeny being full sibs or nonsibs, Wang (1996) developed a recurrence equation for $F$, assuming each P1 male mates at random with an equal number of P1 females. The solution to that equation yielded an expression for $N_{e\theta}$ (his equation 21) which putatively may be applied to a spawning group having unequal numbers of males and females. However, discordance between the model of Wang (1996) and that of Crow and Denniston (1988) can be readily observed in cases where $\theta_{ff} = \theta_{mm} = \theta_{mf}$. Here, the equation of Wang (1996) simplifies to

$$N_{e\theta,j} = \frac{16n_j m_j}{N_{b,j} (2 + \sigma_{k,h}^2)}, \quad 4-20$$

where $\sigma_{k,h}^2$ is the total variance in progeny production as given in Wang (1996). When $n = m$, and $\sigma_{k,h}^2 = 0$, equation 20 above further simplifies to
\[ N_{e\theta,j} = \frac{8n_jm_j}{m_j + n_j} = 2N_{B,j}, \tag{4-21} \]

regardless of the number of parents in the spawning group or of differences between spawning-group and progeny-group abundance. The general expression of Crow and Denniston (equation 4-7) predicts that, for a range of \( N_{B,j} \) and \( N_{S,j} \), \( N_{e\theta} \) should equal twice the number of males and females in the spawning group when population abundance is constant (\( N_{B,j} = N_{S,j} \)) and the numbers of male and female parents are sufficiently large (Figure 4-1). This occurs because \( \phi_{f,j} \) and \( \phi_{m,j} \) converges to approximately \( 2/n_j \) and \( 2/m_j \), respectively, in this case. Otherwise, \( N_{e\theta,j} \) converges asymptotically to a value that is approximately equal to the number of male and female parents. Thus, Crow and Denniston’s values of \( N_{e\theta,j} \) become increasingly discordant with those of Wang (1996) as the ratio \( N_{S,j}/N_{B,j} \) increases.

The expression of Wang (1996) for \( N_{e\theta,j} \) simplifies to \( 8n_j/(1 + n_j) \) and \( 8m_j/(m_j + 1) \) in cases of harem polygyny and polyandry, respectively, when \( \sigma^2_{k,h} = 0 \), regardless of differences between \( N_{B,j} \) and \( N_{S,j} \). As before, the expressions of Crow and Denniston (equations 4-13 and 4-15 above) converge with that of Wang (1996) only when \( N_{B,j} = N_{S,j} \). Wang (1996) concluded that the expression of Crow and Denniston (1988) was correct only when population size was constant and \( n = m \). An alternate hypothesis is that the expression of Crow and Denniston (1988) is more general than that given by Wang (1996). These competing hypotheses will be examined in a later section.
Figure 4-1. Hypothesized relationship between the coancestral effective population number ($N_{e\theta}$), numbers of breeders ($N_B$), and population growth rate ($N_S / N_B$), where $N_S$ is the abundance of the progeny group at maturity. Plotted lines are based on the model of Crow and Denniston (1988). Short dash, $N_B = 2$; medium dash, $N_B = 4$; long dash, $N_B = 10$; unbroken line, $N_B = 20$; dotted line, $N_B = 40$; dash/dotted line, $N_B = 100$.

*Inbred and/or Related Breeders*

Here, I extend expressions for $N_{e\theta}$ to allow breeders in the P1 generation to be inbred and related. The resulting expressions allow estimation of instantaneous inbreeding effective numbers ($N_{e\theta}$), which apply to offspring of the F1 progeny group.
The coefficient of coancestry between two F1 progeny may be determined using Wright’s (1921) method of path analysis. $F_{P1}$ is defined as the expected inbreeding coefficient for P1 parents; $\theta_{P1}$ is defined as the expected coancestry for pairs of P1 parents. Sires and dams in the P1 generation may themselves be products of a nonrandomly mating population; thus $F_{P1}$ and $\theta_{P1} \geq 0$. Gene correlations in generation F1 accrue through different pathways in cases of monogamous and multiple paternity mating and we must examine expected coancestries for progeny groups ($\theta_{F1}$) for both cases. To do so, we consider the four types of coefficients of coancestries – i.e., those for pairs of progeny that are full sibs, maternal and paternal half-sibs, and that share neither parent. The path diagram and gene pathways for two individuals that are full sibs are

For two F1 progeny that are maternal half-sibs, the path diagram and gene pathways may be depicted as

For two F1 progeny that are paternal half-sibs, the path diagram and gene pathways may be depicted as
Pathways for gene correlations for paternal half-sibs can be determined in a similar manner. For two F1 progeny that do not share a parent, the four pathways that contribute to gene correlations are

Replacing individual terms for P1 inbreeding and coancestry with expected terms, conditional expressions for each type of coancestry among F1 progeny are summarized in Table 4-1.

Next, I consider four additional probabilities: $\psi_{FS}$, the probability that two random individuals in the progeny group are full sibs; $\psi_{MH}$, the probability that two random individuals in the progeny group are maternal half-sibs; $\psi_{PH}$, the probability that two random individuals in the progeny group are paternal half-sibs; and $\psi_{UR}$, the probability that two random individuals in the progeny group share neither parent. The expected coancestry in the progeny group is

$$\theta_{F1} = \theta_{MH}\psi_{MH} + \theta_{FS}\psi_{FS} + \theta_{PH}\psi_{PH} + \theta_{UR}\psi_{UR}.$$

4-22
Table 4-1. Conditional equations for expected coancestry among F1 progeny – randomly chosen full sibs \((\theta_{FS})\), maternal half-sibs \((\theta_{MH})\), paternal half-sibs \((\theta_{PH})\), and progeny who share no parent \((\theta_{UR})\). For Case 1, P1 parents are neither inbred nor related; for Case 2, P1 parents may be inbred but not related; for Case 3, coefficients of inbreeding and coancestry may be >0 for P1 parents. \(F_{P1}\) represents the average inbreeding coefficient for P1 parents. \(\theta_{P1}\) represents the average coefficient of coancestry between P1 parents.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\theta_{FS})</td>
<td>(1/4)</td>
<td>(1/4(1 + F_{P1}))</td>
<td>(1/4(1 + F_{P1} + 2\theta_{P1}))</td>
</tr>
<tr>
<td>(\theta_{MH}, \theta_{PH})</td>
<td>(1/8)</td>
<td>(1/8(1 + F_{P1}))</td>
<td>(1/8(1 + F_{P1}) + 3/4\theta_{P1})</td>
</tr>
<tr>
<td>(\theta_{UR})</td>
<td>0</td>
<td>0</td>
<td>(\theta_{P1})</td>
</tr>
</tbody>
</table>

Note that 1) \(\psi_{FS}\) equates to the product of \(\phi_{f,j}\) and \(\phi_{m,j}'\) within a single progeny group, 2) \(\psi_{MH}\) and \(\psi_{PH}\) equate to \((\phi_{f,j} - \psi_{FS})\) and \((\phi_{m,j}' - \psi_{FS})\), respectively, and 3) \(\psi_{UR}\) equates to \([1 - (\psi_{MH} + \psi_{PH} + \psi_{FS})]\). Collecting terms, equation 4-22 can be rewritten as

\[
\theta_{F1} = \theta_{MH} \left[ \phi_{f,j} - (\phi_{f,j} \cdot \phi_{m,j}') \right] + \theta_{FS} \left( \phi_{f,j} \cdot \phi_{m,j}' \right) + \theta_{PH} \left[ \phi_{f,j} - (\phi_{f,j} \cdot \phi_{m,j}') \right] \\
+ \theta_{UR} \left[ 1 - \phi_{f,j} + \phi_{m,j}' - (\phi_{f,j} \cdot \phi_{m,j}') \right] .
\]

In conjunction with our breeding parameters (probabilities that two individuals share dams and sires, respectively), the conditional expectations for \(\theta_{FS}, \theta_{MH}, \theta_{PH},\) and \(\theta_{UR}\) in
Table 4-1 can be employed in equation 4-23 to obtain the full expression for the expected coancestry of a group, i.e.,

$$\theta_{F1} = \left[ \frac{1}{8} (1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} - \left[ \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right] \right] + $$

$$\left[ \frac{1}{4} (1 + F_{p1} + 2 \theta_{p1}) \right] \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} \cdot \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right] + $$

$$\left[ \frac{1}{8} (1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \left[ \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} - \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} \cdot \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right] \right] + $$

$$\left[ \frac{1}{8} (1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \left[ 1 - \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} + \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right] \right] \right] + $$

$$\left[ \frac{1}{8} (1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \left[ 1 - \left[ \frac{\sigma^2_{k,j} + k_j (k_j - 1)}{k_j (k_j n_j - 1)} + \frac{\sigma^2_{h,j} + h_j (h_j - 1)}{h_j (h_j m_j - 1)} \right] \right] \right] + $$

The expression for the inbreeding effective number for the $j$th group can be written as

$$N_{el,j} = \frac{4}{\phi_{f,j} \cdot [1 + F_{p1} - 2 \theta_{p1}] + \phi_{m,j} \cdot [1 + F_{p1} - 2 \theta_{p1}] + 8 \theta_{p1}}$$

The above expression converges to that of Crow and Denniston (equation 4-7 above) when $P1$ parents are not inbred/related and is appropriate for random mating structures. Mindful of progeny-group constraints, it is applicable to all forms of polygyny, polyandry, and polygynandry. However, recalling that each monogamous breeding pair should be considered a discrete spawning group, equation 4-25 is not overly useful for...
cases of monogamously mating populations. Therefore, we must use path analysis to develop an expression for the “intergroup” effective number for this case by using the above approach. Noting that, in this case, $\psi_{FS} = \phi_{f,C}$ and $\psi_{UR} = (1 - \psi_{FS}) = (1 - \phi_{f,C})$, substitution into equation 4-22 yields

$$\theta_{F_1} = \left[ \frac{1}{4}(1 + F_{p_1} + 2\theta_{p_1}) \right] \left[ \frac{\sigma^2_{k,C} + k_C(k_C - 1)}{k_C(k_Cn_C - 1)} \right] + \left[ \theta_{p_1} \left[ 1 - \left[ \frac{\sigma^2_{k,C} + k_C(k_C - 1)}{k_C(k_Cn_C - 1)} \right] \right] \right]$$

$$= \frac{1}{4}(1 + F_{p_1} + 2\theta_{p_1})\phi_{f,C} + \theta_{p_1}(1 - \phi_{f,C}) \quad \text{4-26}$$

It follows that the inbreeding effective number for a population whose members mate monogamously, expressed in terms of maternal reproductive data, is

$$N_{eI} = 2 + \left[ \frac{\sigma^2_{k,C} + k_C(k_C - 1)}{k_C(k_Cn_C - 1)} \right] \left[ 1 + F_{p_1} - 2\theta_{p_1} \right] + 4\theta_{p_1}$$

$$= \frac{2}{\phi_{f,C}(1 + F_{p_1} - 2\theta_{p_1}) + 4\theta_{p_1}} \quad \text{4-27}$$

which equates to equation 4-17 in the absence of inbreeding/relatedness among P1 parents. We can now use the expressions given in equations 4-25 and 4-27 to directly compare $N_{eI}$ for multiple paternity and monogamous mating structures. For multiple paternity, when $n_j = m_j$, all possible matings occur, and $\phi_{fj} = \phi_{m,j}$, equation 4-25 becomes
\[ \theta_{F1} = \left[ \frac{1}{8}(1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \cdot \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} - \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} \right]^2 \right] \\
+ \left[ \frac{1}{4}(1 + F_{p1} + 2\theta_{p1}) \right] \cdot \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} \right]^2 + \right.
\]
\[ \left. \left[ \frac{1}{8}(1 + F_{p1}) + \frac{3}{4} \theta_{p1} \right] \cdot \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} - \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} \right]^2 \right] \cdot \theta_{p1} \left[ 1 - \left[ \frac{2\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} - \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} \right]^2 \right] \right] \right] \]

which simplifies to

\[ N_{el,j} = 2 + \left[ \frac{\sigma^2_{k,j} + k_j(k_j - 1)}{k_j(k_j n_j - 1)} \right] \cdot [1 + F_{p1} - 2\theta_{p1}] + 4\theta_{p1} \]
\[ = \frac{2}{\phi_{f,j}(1 + F_{p1} - 2\theta_{p1}) + 4\theta_{p1}} \]

Comparing equation 4-29 to equation 4-27 under conditions in which \( n_j, k_j, \sigma^2_{k,j}, m_j, h_j \), and \( \sigma^2_{h,j} \) for a multiple paternity case are identical to \( n_C, k_C, \sigma^2_{k,C}, m_C, h_C \), and \( \sigma^2_{h,C} \) for a monogamy case, it is obvious that there is no difference in the respective inbreeding effective numbers. This equivalence is expected to hold regardless of the number of male mates per female (and of the number of female mates per male) in the multiple paternity case.
Although it was useful conceptually in the derivation of instantaneous expressions, inspection reveals that $\psi_{FS}$ is a nuisance parameter – it is eliminated from expressions by simplification. This fact will become important in the next section.

When levels of inbreeding and relatedness among P1 parents are known, the instantaneous inbreeding effective number can be computed. For example, we can expect that a spawning group having a coancestral effective number $N_e \theta = 20$ will have an inbreeding effective number $N_{eI} = 10.1$ when $\theta_{P1}$ and $F_{P1}$ are 0.025. Figure 4-2 depicts the relative effects of $F_{P1}$ and $\theta_{P1}$ on $N_{eI}$. Overall, the potential impact of $\theta_{P1}$ on $N_{eI}$ is much greater than that of $F_{P1}$. Levels of P1 inbreeding and relatedness below $10^{-3}$ and $10^{-4}$, respectively, have a negligible impact on $N_{eI}$ but higher levels can lead to significant reductions. Thus, inbreeding and relatedness in the P1 generation can be safely neglected in single-generation expressions for $N_{eI}$ when recent inbreeding effective numbers are greater than $\approx 5000$ and are expected to exert only a minimal effect when recent $N_{eI}$ is greater than $\approx 500$.

_Pooling Progeny from Isolated Spawning Groups_

I now return to the objective of estimating $N_{eI,C}$ under general circumstances (beyond monogamy) in which multiple parents were isolated into discrete spawning groups but progeny were admixed prior to reproductive maturation. I assume that members of progeny groups, upon maturing, choose mates randomly from within the cohort. Thus, the inbreeding effective number can be viewed as an intergroup effective number for which $d_m = d_r = 1$ (see Wang 1997 for a partial-pooling treatment in which
Figure 4-2. Three-dimensional surface plot depicting the influences of inbreeding ($F$) and relatedness ($\theta$) in generation P1 on the instantaneous inbreeding effective number ($N_{eI}$). The graph was produced by using $\phi_f = \phi'_m = 0.1$ in Equation 25. The $x$ and $y$ axes were scaled using the common logarithm.

dispersal rates are $<1$ and dispersal occurs according to island-model criteria). A cohort derived by monogamous mating merely represents a special case of the above circumstances.

Defining the relative abundance of progeny produced by the $j$th spawning group as $N_{Sj}/N_C$, the probability that two randomly chosen F1 individuals were both derived from the $j$th spawning group may be defined as $\omega_j = N_{Sj}(N_{Sj}-1)/N_C s(N_C s -1)$. As noted, the probability that two randomly chosen genes from these same two individuals were derived from the same P1 individual within the $j$th group is $1/ N_{eI,j}$. Summing the
products of these two probabilities over all groups and taking the inverse, the inbreeding effective number for the cohort is

\[ N_{et,c} = \left[ \sum_{j=1}^{s} \frac{\omega_j}{N_{et,j}} \right]^{-1}. \quad 4-30 \]

Substituting equation 4-25 into equation 4-30 yields the general expression for the intergroup inbreeding effective number for a cohort upon admixture

\[ N_{et,c} = \left[ \sum_{j=1}^{s} \frac{\omega_j \cdot \left[ \phi_{j,j} \cdot \left[ 1 + F_{p1} - 2\theta_{p1} \right] + \phi_{m,j} \cdot \left[ 1 + F_{p1} - 2\theta_{p1} \right] + 8\theta_{p1} \right]}{4} \right]^{-1}. \quad 4-31 \]

Equation 4-31 can be proved using equation 4-22 from the path analysis. For multiple groups, the expression for the cohort-wide probability \( \psi_{FS} \) is lengthy and not given here. However, recalling that the expression for inbreeding effective number does not rely on the parameter \( \psi_{FS} \), equation 4-25 can be rewritten on the basis of individual male and female reproductive contributions, without information pertaining to spawning-group structure. Using cohort-wide reproductive parameters, equation 4-25 becomes
\[ N_{el,C} = \frac{4}{\left[ \frac{\sigma_{k,C}^2 + k_C (k_C - 1)}{k_C (k_C n_C - 1)} \right] \cdot \left[ 1 + F_{p_1} - 2\theta_{p_1} \right] + \left[ \frac{\sigma_{h,C}^2 + h_C (h_C - 1)}{h_C (h_C m_C - 1)} \right] \cdot \left[ 1 + F_{p_1} - 2\theta_{p_1} \right] + 8\theta_{p_1}} \]

\[ = \frac{4}{\phi_{f,C} \cdot \left[ 1 + F_{p_1} - 2\theta_{p_1} \right] + \phi_{m,C}' \cdot \left[ 1 + F_{p_1} - 2\theta_{p_1} \right] + 8\theta_{p_1}} \quad .4-32 \]

Invoking the additive law of probabilities, it can be seen that equation 4-31 and 4-32 are convergent. When generations do not overlap, a discretized form of equation 4-32 can be written as

\[ N_{el,t} = 4 \pm \left[ \frac{\sigma_{k,t-2}^2 + k_{t-2} (k_{t-2} - 1)}{k_{t-2} (N_{t-1} - 1)} \cdot \left[ 1 + F_{t-2} - 2\theta_{t-2} \right] + \right] \cdot \left[ \frac{\sigma_{h,t-2}^2 + h_{t-2} (h_{t-2} - 1)}{h_{t-2} (N_{t-1} - 1)} \cdot \left[ 1 + F_{t-2} - 2\theta_{t-2} \right] + 8\theta_{t-2} \right] \]  

4-33

where \( t \) = generation and \( N_{t-1} \) is the census number of breeders in generation \( t-1 \).

The above general expression (equation 4-32) reduces to equation 4-27 for cases of monogamy, because in this case \( \phi_{f,C} = \phi_{m,C}' \). It reduces to equation 4-17 when P1 parents are not inbred/related. Notably, predicted \( N_{el} \) values from equation 4-32 are equivalent to those from equation 4-27 for all conditions in which \( n_C, k_C, \sigma_{k,C}^2, m_C, h_C, \) and \( \sigma_{h,C}^2 \) for a multiple paternity case are identical to \( n_C, k_C, \sigma_{k,C}^2, m_C, h_C, \) and \( \sigma_{h,C}^2 \) for a monogamy case regardless of the number of male mates/female and number of female mates/male in the multiple paternity case.
General Summary of Model Analyses

**Mating Structures.** Inspection has confirmed that Crow and Denniston’s expressions for inbreeding effective size are convergent for cases of monogamy, random mating, and multiple paternity with equal family sizes and sex ratios when $\theta_{ff} = \theta_{nm} = \theta_{mf}$. Nagylaki (1995) also considered the special case of monogamy and concluded that for $\theta_{ff} = \theta_{nm} = \theta_{mf}$ and $n = m$, inbreeding effective numbers do not differ from that for the case in which all possible matings occur and variance in offspring production is binomial.

Using path analysis, I have further shown that increasing the number of male breeders per female beyond unity has no apparent independent effect on the inbreeding effective number of a progeny group when P1 parents are inbred and/or related. All expressions from the Kimura/Crow family of models reduce to the classical model of Wright (1969) under the appropriate simplifying assumptions. In contrast, the general expressions of Chesser and coworkers lead to a very different conclusion regarding the effect of multiple paternity on the initial $N_{ef}$. Wang (1997) identified a consistent error in the recurrence equations in the series of papers by Chesser and coworkers. That is, for a subdivided population where $s$ is the number of subpopulations and $k$, $n$, and $m$ are population (breeding group) averages, Chesser and coworkers gave the probability that two males were taken at random from the same subpopulation to be $(kn-1)/(kns-1)$. Wang noted that the correct probability is $(km-1)/(kms-1)$. Because I am considering a single, isolated population ($s = 1$), this error should not affect the comparative analysis nor conclusions pertaining to single versus multiple paternity – both the correct and incorrect probabilities reduce to 1. However, I have shown that their recurrence equations contain other non-
general or incorrectly specified probabilities ($\phi_{m,j}$ and $\phi'_{m,j}$) and one probability ($\phi_{w,j}$) that may be insufficiently accurate in general cases. Equation 4-18 provides a dubious estimator for $N_{ef}$ for cases of harem polygyny. Neither expression – equation 4-2 nor equation 4-18 – represents a tenable estimator for $N_{ef}$ for ideal cases of polyandry and neither is likely to yield accurate estimates for cases of polygyandry. Given discordance between models and potentially important problems in the recurrence equations of Chesser and coworkers, a quantitative examination of the effects of multiple paternity, polygyny, and polyandry on the inbreeding effective number is warranted.

*Growing versus Stable Populations.* The model of Crow and Denniston (1988) exhibited concordance or convergence with the classical model of Wright (1969) for all cases considered. The more general (instantaneous) expressions for $N_{ef}$ (equations 4-25 and 4-32) derived in this chapter using path analysis reduce to the model of Crow and Denniston for the special case of non-inbred/non-related parents but are discordant with the expression of Wang (1996). Because progeny-group abundance is typically much larger than spawning-group abundance in restoration/enhancement programs, discrepancies between the models of Wang (1996) and Crow and Denniston (1988) are be relevant and should be examined.

To evaluate the accuracy and general applicability of the contemporary models for captive breeding scenarios that involve multiple paternity and increasing abundance, I conducted several stochastic simulations in which inbreeding effective numbers were determined for hypothetical progeny groups whose breeding dynamics conformed to relevant cases. In some cases, I examined groups characterized by the small numbers of
breeders and extremely high variances in family sizes expected in some unstructured breeding programs. The simulation algorithm was designed to directly provide the probability that randomly chosen homologous alleles from random chosen progeny from representative progeny groups are derived from the same parent. The probabilities $\phi$, $\phi'$, and $\psi_{FS}$ were simultaneously computed for each group.

**Stochastic Simulations**

The approach and procedures for the simulations are described in Appendix B. For simplicity, I assume P1 parents are not inbred/related such that $N_e\theta$ and $N_e\alpha$ may be taken as instantaneous inbreeding effective numbers.

**Single Paternity versus Multiple Paternity.** For an ideal case of single paternity (Simulation 1), I considered a spawning group in which 10 dams (D1 – D10) each mated with a different sire (S1 – S10) to produce 100 progeny (p) apiece:

\[
\begin{align*}
D1 & \times S1 \rightarrow 100 \; p \\
D2 & \times S2 \rightarrow 100 \; p \\
& \ldots \\
D10 & \times S10 \rightarrow 100 \; p
\end{align*}
\]

Thus, the mating scheme is one of monogamy. For a corresponding ideal case of multiple paternity (Simulation 2), I considered a spawning group in which 10 dams mated with each of 10 sires in which each mating produced 10 progeny:
For Simulations 1 and 2, it can be seen that progeny distributions were formed such that any differences in $N_{el}$ were solely attributable to differences in $b$ and $l$ among the two groups. For the progeny distribution in Simulation 2, all possible matings occurred; values for $\phi'_{w,j}$ (and $\phi_{w,j}$) and $\phi'_{m,j}$ (all computed from formulae given in the text) were intentionally low – 0.091 and 0.100, respectively. Results from stochastic simulations are given in Table 4-2. As can be seen from the simulations, there was no difference in the inbreeding effective numbers for the two cases. Both values were nearly identical and were not significantly different from the value $N_{el} = 20.182$, which was estimated using equations 4-7 and 4-17 (rewritten from Crow and Denniston 1988).

For a non-ideal case of single paternity (Simulation 3), I considered a spawning group in which 5 dams each mated with a different sire to produce varying broods:

D1 x S1 → 250 p, D2 x S2 → 210 p, D3 x S3 → 180 p, D4 x S4 → 160 p, D5 x S5 → 150 p
For a corresponding non-ideal case of multiple paternity (Simulation 4), I considered a spawning group in which 5 dams mated with each of 5 sires in which each mating produced varying numbers of progeny:

\[
\begin{align*}
D1 \times S1 & \rightarrow 50 \text{ p}, \\
D2 \times S1 & \rightarrow 50 \text{ p}, \\
D3 \times S1 & \rightarrow 50 \text{ p}, \\
D4 \times S1 & \rightarrow 50 \text{ p}, \\
D5 \times S1 & \rightarrow 50 \text{ p} \\
D1 \times S2 & \rightarrow 50 \text{ p}, \\
D2 \times S2 & \rightarrow 40 \text{ p}, \\
D3 \times S2 & \rightarrow 40 \text{ p}, \\
D4 \times S2 & \rightarrow 40 \text{ p}, \\
D5 \times S2 & \rightarrow 40 \text{ p} \\
D1 \times S3 & \rightarrow 50 \text{ p}, \\
D2 \times S3 & \rightarrow 40 \text{ p}, \\
D3 \times S3 & \rightarrow 40 \text{ p}, \\
D4 \times S3 & \rightarrow 40 \text{ p}, \\
D5 \times S3 & \rightarrow 40 \text{ p} \\
D1 \times S4 & \rightarrow 50 \text{ p}, \\
D2 \times S4 & \rightarrow 40 \text{ p}, \\
D3 \times S4 & \rightarrow 40 \text{ p}, \\
D4 \times S4 & \rightarrow 40 \text{ p}, \\
D5 \times S4 & \rightarrow 20 \text{ p} \\
D1 \times S5 & \rightarrow 50 \text{ p}, \\
D2 \times S5 & \rightarrow 40 \text{ p}, \\
D3 \times S5 & \rightarrow 30 \text{ p}, \\
D4 \times S5 & \rightarrow 20 \text{ p}, \\
D5 \times S5 & \rightarrow 10 \text{ p}
\end{align*}
\]

As in the ideal case above, progeny distributions were constructed such that any differences in \( N_{el,j} \) were solely attributable to differences in \( b \) and \( l \) among the two groups. That is, the numbers of offspring per parental pair were chosen so that individual variance in offspring production were identical for each case. For the progeny distribution in Simulation 4, computed values for \( \phi_{w,j} \) and \( \phi_{m,j} \) were 0.163 and 0.207, respectively. Results from stochastic simulations are given in Table 4-2. Again there was no difference in the inbreeding effective numbers for the two cases; both values were nearly identical to each other and were not significantly different from the predicted value \( N_{el} = 9.686 \) (equations 4-7 and 4-17).

**Harem Polygyny and Polyandry.** I examined the case of harem polygyny by way of four 1-sire/3-dams simulations (Simulations 5-8) having increasing levels of maternal
variance in progeny production. Progeny distributions from the following pedigrees were used in simulations:

Pedigree 5: S1 x D1 → 320 p, S1 x D2 → 330 p, S1 x D3 → 350 p
Pedigree 6: S1 x D1 → 290 p, S1 x D2 → 300 p, S1 x D3 → 410 p
Pedigree 7: S1 x D1 → 150 p, S1 x D2 → 250 p, S1 x D3 → 600 p
Pedigree 8: S1 x D1 → 50 p, S1 x D2 → 150 p, S1 x D3 → 800 p

In these simulations, $\sigma^2_{k,j}$ ranged from slightly less than binomial variance (Simulation 5) to a level of variance on the order of $k_j^2$ (Simulation 8). Results from stochastic simulations are given in Table 4-2. Simulated values of $N_{ef}$ are also plotted in Figure 4-3 and are in good agreement with predicted values from equation 4-13 above, which was rewritten for the special case from expressions of Crow and Denniston (1988) and of Chesser et al. (1993). Notably, values of $N_{ef}$ predicted using the expression of Wang (1996) are in general disagreement with simulated values. Indeed, with high variances in family size, the expression of Wang predicts inbreeding effective numbers < 2 for the case modeled here, which is not possible.

Cases of polyandry can be considered easily by “reversing” the sexes of parents used in the pedigrees for Simulations 5-8. For example, the pedigree from Simulation 5 would, upon reversal, contain a single dam and 3 sires and the pedigree becomes:

Pedigree 5B: D1 x S1 → 320 p, D1 x S2 → 330 p, D1 x S3 → 350 p
Importantly, reversing sexes of parents in pedigrees 5-8 does not change values of $N_{ef}$ in simulations under the general conditions assumed herein. Accordingly, the expression of Crow and Denniston (1988) holds for cases of polyandry and that of Wang (1996) remains in disagreement. For polyandry, the expressions of Chesser and coworkers (equations 4-2 and 4-18 above) also provide incorrect results because of the incorrectly specified probabilities for paternal gene correlations.

**Growing versus Stable Population Abundances.** Here I used simulations to examine the effect of population growth in the F1 generation on inbreeding effective size. Because $N_{ef}$ is unaffected by $b$ and $l$, cases of single paternity with equal family sizes were modeled for simplicity. I considered a spawning group in which 10 dams each mated with a different sire to produce “$p$” progeny:

\[
\begin{align*}
\text{D1 x S1} & \rightarrow p \\
\text{D2 x S2} & \rightarrow p \\
\text{...} & \\
\text{D10 x S10} & \rightarrow p
\end{align*}
\]

where $p = 2, 4, 6, 8, 10, 13, \text{ and } 16$, respectively, for Simulations 9 – 15. For the cases examined in Simulations 9 – 15, Wang’s (1996) model predicts that all values of $N_{ef}$ should equate to 40. In contrast, Crow and Denniston’s (1988) model (equation 4-7) predicts that values should equate to 40 when spawning-group and progeny-group abundances are equal (Simulation 9) and converge to approximately 20 as the ratio of spawning-group to progeny-group abundance increases. Results are given in Table 4-2. As shown in Figure 4-4, all simulated values of $N_{ef}$ were in good agreement with those predicted by the expression of Crow and Denniston (1988) and diverged from those
predicted by the expression of Wang (1996) when populations underwent positive growth.

**Pooling Progeny from Isolated Spawning Groups.** Here, equations 4-31 and 4-32 are supported through stochastic simulation (Simulation 16). I considered a cohort formed by admixing progeny from two isolated spawning groups. The first spawning group consisted of 10 sires and two dams. For this group, all possible matings occurred and each mating resulted in 10 progeny; thus, \( N_{S,j} = 200, \phi_{j} = 0.49749, \) and \( \phi'_{m,j} = 0.09548. \) The second spawning group consisted of 2 sires and 2 dams. For this group, all possible matings occurred where

\[
D1 \times S1 \rightarrow 400 \text{ p, } D1 \times S2 \rightarrow 100 \text{ p, } D2 \times S1 \rightarrow 100 \text{ p, } D2 \times S2 \rightarrow 200 \text{ p;}
\]

thus, \( N_{S,j} = 800, \phi_{j} = \phi'_{m,j} = 0.53066. \) Progeny from both progeny groups were admixed completely to form an F1 cohort, where \( N_C = 1000, \phi_{C} = 0.35936, \) and \( \phi'_{m,C} = 0.34334. \) Using equation 4-31, we predict \( N_{el,C} = 5.69016; \) using equation 4-32, we predict \( N_{el,C} = 5.69231. \) Results for Simulation 16 are given in Table 4-2. The simulated value for \( N_{el,C} (5.70939) \) is not significantly different from the predicted values.
Table 4-2. Stochastic simulations for the probabilities $\phi_f$, $\phi^\prime_m$, $\psi_{FS}$, and $P$. The approach and procedures for the simulations are described in Appendix B. Pedigrees for each case and definitions for parameters are given in the text. For each case, 20 replicate simulations were conducted; for each simulation, 1000 trials were conducted. For all probabilities, means and standard errors from replicated simulations are reported.

Simulation 1 – Monogamy with equal family sizes.

Female breeding parameters: $n_C = 10$, $k_C = 100$, $\sigma^2_{k,C} = 0$, $l_j = 1$

Male breeding parameters: $m_j = 10$, $h_C = 100$, $\sigma^2_{h,C} = 0$, $b_j = 1$

Simulated probabilities: $\phi_{f,C} = \phi^\prime_{m,C} = \psi_{FS} = 0.09585 \pm 0.00227$

$N_{eI,j}$ from simulated $\psi_{FS}$: 20.866

$P$ from simulation: 0.04775 ± 0.00159

$N_{eI,j}$ from simulated $P$: 20.942

Simulation 2 – Multiple paternity with equal family sizes.

Female breeding parameters: $n_j = 10$, $k_j = 100$, $\sigma^2_{k,j} = 0$, $l_j = 10$

Male breeding parameters: $m_j = 10$, $h_j = 100$, $\sigma^2_{h,j} = 0$, $b_j = 10$

Simulated probabilities: $\phi_f = 0.09970 \pm 0.00126$, $\phi^\prime_m = 0.09575 \pm 0.00185$, $\psi_{FS} = 0.00905 \pm 0.00063$

$N_{eI,j}$ from simulated $\phi_f$ and $\phi^\prime_m$: 20.466

$P$ from simulation: 0.04800 ± 0.00152

$N_{eI,j}$ from simulated $P$: 20.833
Table 4-2 (Continued)

**Simulation 3** – Monogamy with unequal family sizes.

Female breeding parameters: \( n_C = 5, \quad k_C = 190, \quad \sigma^2_{k_C} = 1320, \quad l_j = 1 \)

Male breeding parameters: \( m_C = 5, \quad h_C = 190, \quad \sigma^2_{h_C} = 1320, \quad b_j = 1 \)

Simulated probabilities: \( \phi_{k,C} = \phi_m' = \psi_{FS} = 0.20530 \pm 0.00259 \)

\( N_{e_l,j} \) from simulated \( \psi_{FS} \): 9.742

\( P \) from simulation: 0.1039 ± 0.00219

\( N_{e_l,j} \) from direct simulation: 9.625

**Simulation 4** – Multiple paternity with unequal family sizes.

Female breeding parameters: \( n_j = 5, \quad k_j = 190, \quad \sigma^2_{k,j} = 1320, \quad l_j = 5 \)

Male breeding parameters: \( m_j = 5, \quad h_j = 190, \quad \sigma^2_{h,j} = 1320, \quad b_j = 5 \)

Simulated probabilities: \( \phi = 0.20720 \pm 0.00259, \quad \phi_m' = 0.20875 \pm 0.00287, \quad \psi_{FS} = 0.04205 \pm 0.00139 \)

\( N_{e_l,j} \) from simulated \( \phi \) and \( \phi_m' \): 9.617

\( P \) from simulation: 0.10270 ± 0.00213

\( N_{e_l,j} \) from direct simulation: 9.737

**Simulation 5** – Harem polygyny with \( \sigma^2_{k,j} < \text{binomial} \).

Female breeding parameters: \( n_j = 3, \quad k_j = 333.3333, \quad \sigma^2_{k,j} = 155.5555 \)

Male breeding parameters: \( m_j = 1, \quad h_j = 1000, \quad \sigma^2_{h,j} = 0 \)

Simulated probabilities: \( \phi_m' = 1, \quad \phi = \psi_{FS} = 0.33520 \pm 0.00374 \)

\( N_{e_l,j} \) from simulated \( \phi \) and \( \psi_{FS} \): 2.996
Table 4-2 (Continued)

$P$ from simulation: $0.33620 \pm 0.00465$

$N_{ct,j}$ from direct simulation: 2.974

Simulation 6 – Harem polygyny with $\sigma^2_{k,j} > \text{binomial}$.

Female breeding parameters: $n_j = 3$, $k_j = 333.3333$, $\sigma^2_{k,j} = 2955.5555$

Male breeding parameters: $m_j = 1$, $h_j = 1000$, $\sigma^2_{h,j} = 0$

Simulated probabilities: \( \phi'_m = 1 \), \( \phi = \psi_{FS} = 0.34265 \pm 0.00351 \)

$N_{ct,j}$ from simulated $\phi$ and $\psi_{FS}$: 2.979

$P$ from simulation: $0.33580 \pm 0.00299$

$N_{ct,j}$ from direct simulation: 2.978

Simulation 7 – Harem polygyny with $\sigma^2_{k,j} \gg \text{binomial}$.

Female breeding parameters: $n_j = 3$, $k_j = 333.3333$, $\sigma^2_{k,j} = 68888.8888$

Male breeding parameters: $m_j = 1$, $h_j = 1000$, $\sigma^2_{h,j} = 0$

Simulated probabilities: \( \phi'_m = 1 \), \( \phi = \psi_{FS} = 0.54110 \pm 0.00378 \)

$N_{ct,j}$ from simulated $\phi$ and $\psi_{FS}$: 2.596

$P$ from simulation: $0.38740 \pm 0.00289$

$N_{ct,j}$ from direct simulation: 2.581

Simulation 8 – Harem polygyny with $\sigma^2_{k,j} \gg \text{binomial}$.

Female breeding parameters: $n_j = 3$, $k_j = 333.3333$, $\sigma^2_{k,j} = 110555.5555$

Male breeding parameters: $m_j = 1$, $h_j = 1000$, $\sigma^2_{h,j} = 0$
Table 4-2 (Continued)

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Single paternity with equal sex ratio and family sizes and $N_{Sj}/N_{Bj} = 1$.</th>
<th>Single paternity with equal sex ratio and family sizes and $N_{Sj}/N_{Bj} = 2$.</th>
<th>Single paternity with equal sex ratio and family sizes and $N_{Sj}/N_{Bj} = 3$.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female breeding parameters:</td>
<td>$n_C = 10$, $k_C = 4$, $\sigma^2_{k, C} = 0$</td>
<td>$n_C = 10$, $k_C = 4$, $\sigma^2_{k, C} = 0$</td>
<td>$n_C = 10$, $k_C = 6$, $\sigma^2_{k, C} = 0$</td>
</tr>
<tr>
<td>Male breeding parameters:</td>
<td>$m_C = 10$, $h_C = 4$, $\sigma^2_{h, C} = 0$</td>
<td>$m_C = 10$, $h_C = 4$, $\sigma^2_{h, C} = 0$</td>
<td></td>
</tr>
<tr>
<td>Simulated probabilities:</td>
<td>$\phi_{f, C} = \phi_{m, C} = \psi_{FS} = 0.04940 \pm 0.00152$</td>
<td>$\phi_{f, C} = \phi_{m, C} = \psi_{FS} = 0.07505 \pm 0.00142$</td>
<td></td>
</tr>
<tr>
<td>$N_{elj}$ from simulated $\psi_{FS}^a$:</td>
<td>40.486</td>
<td>26.649</td>
<td></td>
</tr>
<tr>
<td>$P$ from simulation:</td>
<td>0.02440 $\pm$ 0.00092</td>
<td>0.03600 $\pm$ 0.00126</td>
<td></td>
</tr>
<tr>
<td>$N_{elj}$ from direct simulation:</td>
<td>40.984</td>
<td>27.778</td>
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</tr>
</tbody>
</table>
Table 4-2 (Continued)

Male breeding parameters: \( m_C = 10, \quad h_C = 6, \quad \sigma^2_{h,C} = 0 \)

Simulated probabilities: \( \phi_{f,C} = \phi_{m,C}' = \psi_{FS} = 0.08775 \pm 0.00209 \)
\( N_{eI,j} \) from simulated \( \psi_{FS} \)\(^a\): 22.792

\( P \) from simulation: 0.04430 ± 0.00141
\( N_{eI,j} \) from direct simulation: 22.573

Simulation 12 – Single paternity with equal sex ratio and family sizes and \( N_{S,j} / N_{B,j} = 4 \).

Female breeding parameters: \( n_C = 10, \quad k_C = 8, \quad \sigma^2_{k,C} = 0 \)
Male breeding parameters: \( m_C = 10, \quad h_C = 8, \quad \sigma^2_{h,C} = 0 \)
Simulated probabilities: \( \phi_{f,C} = \phi_{m,C}' = \psi_{FS} = 0.08675 \pm 0.00120 \)
\( N_{eI,j} \) from simulated \( \psi_{FS} \)\(^a\): 23.055

\( P \) from simulation: 0.04330 ± 0.00118
\( N_{eI,j} \) from direct simulation: 23.095

Simulation 13 – Single paternity with equal sex ratio and family sizes and \( N_{S,j} / N_{B,j} = 5 \).

Female breeding parameters: \( n_C = 10, \quad k_C = 10, \quad \sigma^2_{k,C} = 0 \)
Male breeding parameters: \( m_C = 10, \quad h_C = 10, \quad \sigma^2_{h,C} = 0 \)
Simulated probabilities: \( \phi_{f,C} = \phi_{m,C}' = \psi_{FS} = 0.09305 \pm 0.00183 \)
\( N_{eI,j} \) from simulated \( \psi_{FS} \)\(^a\): 21.494

\( P \) from simulation: 0.04575 ± 0.00148
\( N_{eI,j} \) from simulated \( P \): 21.858
Table 4-2 (Continued)

**Simulation 14** – Single paternity with equal sex ratio and family sizes and $N_{S,j} / N_{B,j} = 6.5$.

Female breeding parameters: \( n_C = 10, \quad k_C = 13, \quad \sigma^2_{k,C} = 0 \)

Male breeding parameters: \( m_C = 10, \quad h_C = 13, \quad \sigma^2_{h,C} = 0 \)

Simulated probabilities: \( \phi_C = \phi'_m = \psi_{FS} = 0.09270 \pm 0.00234 \)

\( N_{e,C} \) from simulated $\psi_{FS}$: \( 21.575 \)

$P$ from simulation: \( 0.04730 \pm 0.00208 \)

\( N_{e,C} \) from direct simulation: \( 21.142 \)

**Simulation 15** – Single paternity with equal sex ratio and family sizes and $N_{S,j} / N_{B,j} = 8$.

Female breeding parameters: \( n_C = 10, \quad k_C = 16, \quad \sigma^2_{k,C} = 0 \)

Male breeding parameters: \( m_C = 10, \quad h_C = 16, \quad \sigma^2_{h,C} = 0 \)

Simulated probabilities: \( \phi_C = \phi'_m = \psi_{FS} = 0.09405 \pm 0.00176 \)

\( N_{e,C} \) from simulated $\psi_{FS}$: \( 21.265 \)

$P$ from simulation: \( 0.04765 \pm 0.00131 \)

\( N_{e,C} \) from direct simulation: \( 20.986 \)

**Simulation 16** – Pooled progeny groups.

Female breeding parameters: \( n_C = 4, \quad k_C = 250, \quad \sigma^2_{k,C} = 27500 \)

Male breeding parameters: \( m_C = 12, \quad h_C = 83.333, \quad \sigma^2_{h,C} = 21722.222 \)

Simulated probabilities: \( \phi = 0.35860 \pm 0.00300, \quad \phi'_m = 0.33945 \pm 0.00372, \quad \psi_{FS} = 0.22005 \pm 0.00295 \)

\( N_{e,C} \) from simulated $\phi$ and $\phi'_m$: \( 5.730 \)
Table 4-2 (Continued)

<table>
<thead>
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<th></th>
<th>Value</th>
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</thead>
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<tr>
<td>$P$ from simulation:</td>
<td>$0.17515 \pm 0.00280$</td>
</tr>
<tr>
<td>$N_{el,C}$ from direct simulation:</td>
<td>5.709</td>
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</tbody>
</table>

*aComputed using approach described in Appendix B.

Figure 4-3. Inbreeding effective number ($N_{el}$) for a case of polygyny as a function of increasing maternal variance in offspring production ($\sigma^2_k$). The graph was produced using values of $n = 3$, $m = 1$, and $N_S = 1000$. The dotted line depicts predicted values from Equation 13. The dashed line depicts values from the expression of Wang (1996). Plotted points depict the mean and standard deviations of $N_{el}$ from simulated values of $P$ (Simulations 5-8); some error bars may be too small to be visible. The $x$ axis was scaled using the common logarithm.
Figure 4-4. Effect of population growth on inbreeding effective number ($N_{el}$). The graph was produced using values of $n_j = m_j = 10$ and $\sigma^2_{k,j} = \sigma^2_{h,j} = 0$. Line plots were based on equations described in the text: the dashed line depicts predicted values from Equation 20 (from Wang 1996); the dotted line depicts predicted values from Equation 9 (from Crow and Denniston 1988). Plotted points depict the mean and standard deviations of $N_{el}$ from simulated values of $P$ (Simulations 10-16).

Discussion

The objective was to identify a valid expression for the instantaneous inbreeding effective number for a cohort of organisms bred in captivity under genetically complex circumstances. The mathematical arguments given here, supported by stochastic
simulation, show that equations 4-31 and 4-32 fulfill the objective providing that progeny comprising the cohort are pooled and survive randomly upon release and that sexes of progeny are determined randomly. Inspection of equation 4-32 reveals that the following six cohort-wide reproductive parameters $n_C$, $k_C$, $\sigma^2_{k_C}$, $m_C$, $h_C$, and $\sigma^2_{h_C}$ are sufficient to estimate $N_{el,C}$. Importantly from a practical standpoint, data pertaining to internal (spawning-group) structure may not be necessary for the estimation under the stated assumptions if the cohort is randomly and sufficiently sampled (parents determined) following pooling. Equation 4-31, however, offers the ability to specifically consider reductive effects on $N_{el,C}$ that may result from pooling progeny groups of ultimately disparate abundance. This may be an important issue for supplemental stocking programs in which husbandry practices (e.g., repeated use of some spawning groups, differential treatment/handling of lots or progeny groups) rather than differences in individual reproductive potential are responsible for the largest variance in family sizes.

Levels of kinship and inbreeding may be readily quantified with the appropriate molecular markers (e.g., Gerlach et al. 2001). When it is determined that broodfish are not inbred or closely related, the terms $F_{P_1}$ and $\theta_{P_1}$ can be ignored and equation 4-33 simplifies to the general expression of Crow and Denniston (1988). This condition might be common for stock enhancement programs involved with large, outcrossing populations (e.g., some marine fishes). In contrast, inbreeding coefficients for individuals in the parental generation may be relatively high in highly structured, fragmented, threatened, or endangered populations. When attempting to restore these stocks via captive breeding and release, it could be unwise to ignore $F_{P_1}$ and $\theta_{P_1}$. This is especially true if stocking has occurred over multiple generations or if broodfish are
drawn from subsequent generations of hatchery populations. For example, in a study of endangered winter-run chinook salmon, Hedrick et al. (2000) assessed potential genetic impacts upon wild conspecifics from supplemental stocking based on Ryman/Laikre (1991) considerations. In that study, they used Crow and Denniston’s (1988) expression to compute an expected value for the inbreeding effective number for a Fall 1994 cohort of captive-bred chinook salmon. Unfortunately, Hedrick and coworkers failed to consider that the broodfish, collected from a very small population (\(N_{el}\) putatively ranged 34 to 73), were inbred and related. Assuming that \(F_p \approx \theta_p \approx 1/(2NeI)\), the inbreeding effective numbers for the Fall 1994 captive-bred cohort were probably 30-50% lower than the unadjusted values used by the authors. Still, to the degree that their estimate of captive-bred salmon in the admixed population (40%) is accurate, release of the Fall 1994 cohort may have increased the overall (post-supplementation) effective number of chinook salmon, although not as much as reported by the authors.

In evaluating potential differences in \(N_{el}\) under conditions of single and multiple paternity, valid comparisons can only be made when all reproductive factors except \(l\) and \(b\) are identical for the two cases being compared. It is evident that the number of male mates per female and the number of female mates per male exert no independent effects on the inbreeding effective number of a population or cohort. Accordingly, a separate probability for multiple paternity (\(\phi_{w,j}\) or \(\phi'_{w,j}\)) is unnecessary and the estimation of \(N_{el}\) depends only on properly specified terms for the probabilities that two random progeny in a spawning group shared dams and sires. For monogamous mating structures and for all levels of polygyny, polyandry, and polygynandry, these terms are given with reasonable accuracy by \((\sigma^2_k + k(k-1))/(k(kn-1))\) and \((\sigma^2_h + h(h-1))/(h(hm-1))\),
respectively, and are contained in the general expression of Crow and Denniston (1988) and in equation 4-32 above.

We can conclude from the above analyses that there is no difference in expected levels of $F$ between otherwise similar monogamously and randomly mating dioecious populations under conditions of selective neutrality. However, actual levels of inbreeding vary among loci within individuals and among individuals within a population. For unlinked loci, Weir et al. (1980) showed that within-population variance in inbreeding will be greater for monogamously mating populations in comparison to randomly mating ones. This occurs because a full-sib mating contributes twice as much to $F$ as does a half-sib mating (except in very small populations, see Table 4-1) but four times as much to the variance of $F$. Specifically, Weir and coworkers determined that, after 4-6 generations, squared coefficients of variation of $1 - F$ reach asymptotic values of approximately $1/(6N_eI)$ for monogamously mating populations and approximately $1/(12N_eI)$ for randomly mating populations. Whether or not this translates to a proportionately greater amount of homozygous genotypes of deleterious recessive alleles affected by selection in a monogamously mating population compared to that in a randomly mating population is an interesting and perhaps important question. It may be that small monogamously mating populations are more susceptible to fitness reductions from inbreeding depression compared to otherwise similar but randomly mating populations.

The issue of population growth and its influence on $N_eI$ is particularly relevant to stock enhancement, as considerable gains in abundance can be achieved in or within a single generation interval via captive breeding and release. The expressions of Crow and
Denniston (1988) and Wang (1996) yield similar values under conditions of zero reproductive variance and equal sex ratios only when population abundance is constant. Whereas simulations indicate that the expression of Crow and Kimura (1988) sufficiently predicts the single-generation probability of identity by descent for growing populations, the expression of Wang (1996) failed in this regard. It is worth noting here that Crow and Denniston’s model simplifies to $N_{el} = 2n$ when mating is random (i.e., reproductive variances are binomial and the sex ratio is 1:1, algebra not shown), regardless of population growth rate; therefore, concordance with Wright’s (1969) model is maintained. When population structure and dynamics are constant over time, inbreeding effective numbers reach an asymptotic value (Caballero and Hill 1992, Wang 1997). Migration rates and population sizes, however, are rarely constant in natural populations. When supplemental stocking occurs within a randomly mating population or subpopulation and new broodfish are obtained from the recipient unit each generation, the genetic effect as it relates to inbreeding can be conceptualized *prima facie* as an increase, albeit artificially induced, in the family sizes of certain individuals. The single-generation impact on $N_{el}$ can be significant (see Chapter 2), but it is transient. The representative inbreeding effective number, i.e., that which describes the expected rate of change in heterozygosity, is approximately the harmonic mean of generational effective numbers over a specified time interval (Wright 1969).

The results presented here are relevant to other investigations of the temporal and spatial dynamics of genetic variance in populations and to evolutionary differences between diploid, matrilinear, hologynic, and holandric traits. Extending models of Chesser et al. (1993) and Sugg and Chesser (1994), Chesser and Baker (1996) developed
expressions for asymptotic effective numbers for uniparentally inherited genes. In that study, the term for the probability that two random progeny in a spawning group shared a father was amended a fourth time ($\Psi_m$ their equation 4). The authors concluded that $\phi_{f,j}$, $\Psi_m$, $\phi_{w,j}$, population structure, and rates of male and female dispersal were all relevant in comparisons of uniparental versus biparental gene flow and diversity maintenance. Nevertheless, because $l$ has no effect on the probability that random pairs of progeny born within a spawning group share a father, their new term $\Psi_m$ is incorrect except when $n = m$ and mating is monogamous. Thus, the issue of effective population numbers with holandric inheritance merits further investigation. Additionally, considering the results described herein as they pertain to diploid models, issues pertaining to differences among effective population numbers for mtDNA versus biparentally inherited genes in structured populations also should be reexamined.
Chapter 5

Maternal Reproductive Variance and Inbreeding Effective Number in a Cohort of Red Drum (Sciaenops ocellatus) Bred for Stock Enhancement

Introduction

Under genetically ideal circumstances, selection, migration, and mutation are negligible, and sex ratios of parents and progeny are 1:1 and do not co-vary. When we seek to determine the inbreeding effective number ($N_{eI}$) for a hatchery cohort, we really wish to know the number of breeders in a hypothetically ideal parental group whose offspring can be expected to accrue the same amount of gene correlation (Wright 1931) as that of the actual cohort. Individuals in a cohort having a larger inbreeding effective number will be less related, on average, than one with a smaller number. Thus, a desirable circumstance in captive breeding for stock enhancement might occur when the ratio of effective number achieved per total number of breeders used ($N_{eI}/N_{BT}$) is maximized. Unfortunately, evaluating $N_{eI}/N_{BT}$ ratios in aquaculture programs involved with undomesticated animals can be difficult, especially when direct control is not exercised over fertilization. When multiple breeders of each sex occupy spawning tanks or ponds and spawning occurs en masse or in an essentially synchronous fashion, determining levels of relatedness within a progeny group can be difficult because it is generally not known which of the potential breeders contribute gametes. The difficulties
are magnified when sex ratios of participating breeders within tanks differ from 1:1 and when multiple lots having different abundances are produced by multiple breeding groups and pooled to form a cohort. Moreover, post-release recruitment to the breeding population may differ among related and non-related progeny because of intrinsic and extrinsic factors. In this complex scenario, the mating system is non-random and there is no reasonable expectation of binomial variance in family sizes. Thus, the uniting of gametes, for biological and practical reasons, is an unpredictable process and describing gene correlations among offspring in such groups is both theoretically and technically challenging.

Theoretical considerations pertaining to the inbreeding effective number were examined in the preceding chapter and appropriate mathematical models for estimating $N_{eI}$ were identified. Here, I use the theory specifically to elucidate potential impacts of maternal variance in offspring production and skewed sex ratios among breeders under conditions relevant to the husbandry practices commonly employed in fish stock enhancement programs. Definitions and abbreviations of variables follow those given in Chapter 4; terminology is explained in Appendix A. Assuming broodfish are not inbred/related, no covariance in sexes of progeny, and parental sex ratios are 1:1, we can consider the following circumstances: 1) $\sigma^2_h$ is zero, 2) $\sigma^2_h$ is binomial, 3) $\sigma^2_h$ is of the same magnitude as $\sigma^2_k$, and 4) $\sigma^2_h$ is it’s maximum value. For given probabilities of $\phi'_m$ the inbreeding effective number of a cohort should generally increase with increasing numbers of potentially contributing dams ($n$) and decrease with increasing levels of maternal variance in offspring production ($\sigma^2_k$). Minimum values of $\phi'_m$ occur when all sires contribute equally to numbers of offspring within cohorts ($\sigma^2_h = 0$). Thus when $\sigma^2_h$
= 0, broodfish are not inbred or related, and the sex ratio of potential breeders is 1:1, then
\[ m = n, \ h = k, \] and equation 4-33 reduces to

\[
N_{el} = \frac{4k(kn-1)}{\sigma^2_k + 2k^2 - 2k}. \\
5-1
\]

In keeping with the empirical data for the studied red drum cohort (see below), the mean
number of offspring per parent was assigned a value of 285 for a numerical analysis of
equation 5-1. From Figure 5-1A, which represents a graphical depiction of that analysis,
it can be seen that maternal variance has very little effect on the inbreeding effective
number of a cohort produced by a small number of breeders unless \( \sigma^2_k \) is extremely high.
Considering the case in which the variance in male reproductive contribution is binomial,
broodfish are not inbred or related, and the sex ratio of breeders is equal, equation 4-33
simplifies to

\[
N_{el} = \frac{4kn(kn-1)}{\sigma^2_k n + 2k^2 n - kn - k}. \\
5-2
\]

Values of \( N_{el} \) predicted using equations 5-1 and 5-2 differ substantially only when \( k \) is
less than a few individuals (see also Chapter 4). Thus, the plots of \( N_{el} \) represented in
Figure 5-1B are essentially identical to those represented in Figure 5-1A and the above
Figure 5-1. Joint effects of the number of dams and maternal variance on inbreeding effective numbers. Contour lines represent values of $N_{el}$ as computed using equations 5-1 through 5-4. All plots were generated using $k = 285$. In plots A-D, paternal variances were modified as follows: (A) $\sigma^2_h$ was zero; (B) $\sigma^2_h$ was binomial; (C) $\sigma^2_h$ was of the same magnitude as $\sigma^2_k$; (D) $\sigma^2_h$ took its maximum value.
conclusion pertaining to the effect of $\sigma^2_k$ on $N_{el}$ also holds conditionally when paternal variance is binomial. We can also consider the case in which paternal reproductive variances are equivalent in magnitude to maternal reproductive variances. Thus when $\sigma^2_h = \sigma^2_k$, broodfish are not inbred or related, and the sex ratio is 1:1, then $\phi_f = \phi'_m$ and equation 4-33 becomes

$$N_{el} = \frac{2k(kn-1)}{\sigma^2_k + k(k-1)}.$$ \hspace{1cm} 5-3

In this case, the combined effect of paternal and maternal variance influence the value of $N_{el}$ (Figure 5-1C). It can be seen that these combined effects, although greater than that of the above cases, remain relatively minor unless variances are extremely large.

Maximum values of $\phi'_m$ occur when a single male sires all offspring within a cohort. In this case, $\phi'_m = 1$ and, under the usual assumptions, equation 4-33 reduces to

$$N_{el} = \frac{4kn(kn-1)}{\sigma^2_k + k^2 - 2k + k^2 n}.$$ \hspace{1cm} 5-4

As depicted in Figure 5-1D, the lag in the effect on $N_{el}$ by $\sigma^2_k$ remains. In this case, the fact that all offspring share a sire is responsible for the low values for the inbreeding effective cohort number.

The impact of an unequal broodfish sex ratio can also be depicted graphically in terms of its independent effect on $N_{el} / N_{BT}$ ratios. Assuming binomial variance in sire
and dam offspring contributions, equation 4-33 reduces to Wright’s (1969) classical equation (see Chapter 4, equation 4-10). The relationship between $N_{el} / N_{BT}$ and sex ratios is not linear (Figure 5-2).

![Figure 5-2](image_url)

Figure 5-2. The relationship between $N_{el} / N_{BT}$ and sex ratio ($n/m$ or $m/n$), as described by Wright’s (1969) classical equation.

The results of this numerical analysis should be seemingly comforting to culturists trying to maintain high $N_{el} / N_{BT}$ ratios. In general, when $k \gg 2$ (larger and smaller values of $k$ were analyzed, not shown) and spawning groups are small, extremely large male and/or female reproductive variances (on the order of $k^2$) are necessary for a significant reductive impact on $N_{el}$. Moreover, reasonably good $N_{el} / N_{BT}$ ratios (up to 88%) can be obtained with sex ratios as high as 2:1 (dam:sire or sire:dam).
In the remainder of this chapter, I apply the above theoretical considerations to an actual cohort of a fish (red drum, *Sciaenops ocellatus*), bred for the purpose of stock enhancement. My objectives were as follows. First, I sought to estimate with reasonable accuracy the inbreeding effective number for a discrete group of fish produced at a hatchery that employs unstructured, mass-spawning methods. Second, I sought to examine factors influenced by breeding practices that might reduce $N_{el} / N_{BT}$ ratios, in particular, factors related to family size variance, broodfish sex ratios, and disproportionate contributions from different spawning groups. Third, because it can exacerbate differences in family sizes, I sought to test for differential survivorship among related and non-related hatchlings during extended captive rearing. Indirect genetic approaches for determining $N_{el}$, such as the “heterozygote excess” method (Pudovkin et al. 1996), may be inappropriate for complex, unstructured breeding programs because they are based upon the assumption that gametes unite randomly. They are also inaccurate unless ~25 highly variable genetic loci and at least 60 individuals are assayed (Luikart and Cornuet 1999). Pedigree relationships are typically determined when gene correlations need to be accurately quantified. When families (or breeding pairs) are not physically separated, genetic data are required to determine parentage (e.g., Herbinger et al. 1995; Garcia De Leon et al. 1998). However, it was shown in Chapter 4 that only the relative reproductive contributions of sires and dams, respectively, to progeny groups or to cohorts are required to estimate $N_{el}$. Lacking suitable bi-parental markers at the inception of this study, only female reproductive contributions were quantified. I exploited a technical shortcut as I examined maternal reproductive variance and estimated inbreeding effective numbers for captive-bred red drum. Of particular interest
is the portion of $\frac{N_{el}}{\sigma^2_k}$ parameter space that progeny groups at in the studied cohort typically occupy – are they in the region where the impact of maternal reproductive variance on $N_{el}$ is negligible or significant?

**Material and Methods**

*Sampling of Broodstock and Progeny.* Study specimens were obtained from the Florida Marine Research Institute’s (FMRI) Stock Enhancement Research Facility (SERF) in Ruskin, Florida during the course of hatchery production for an attempted red drum stock-restoration program in Biscayne Bay, Florida. Lot *phase* is a non-standard term used by SERF personnel to categorize size-at-release classes of hatchlings: phase I refers to hatchlings ranging 25-65 mm standard length (SL); phase-II hatchlings range 66-135 mm SL; phase-III hatchlings are those greater than 135 mm SL. All broodfish were identifiable via “passive-integrated-transponder” (PIT) tags. For Fall-1994 (F94) hatchery production, male and female broodfish were subdivided among four spawning tanks (2-4 individuals of each sex per spawning group). Prior to spawning, unfertilized eggs were obtained from all fourteen F94 female broodfish via ovarian biopsies when SERF personnel inspected the progress of reproductive maturation; these were used to genotype potential dams. After spawning events, fertilized eggs were maintained in larval incubators for approximately 60 hrs and then stocked into 1-acre rearing ponds. Fifteen F94 lots were produced by these females (Table 5-1); however two lots, T9P2 and T9P6a, were terminated shortly after pond stocking because of poor survival. From the remaining thirteen lots, approximately 399,200 hatchling red drum were raised to phase-I
size and harvested for release. Remaining individuals for some F94 lots were reared for extended periods prior to release. Samples of hatchlings from F94 lots (approximately 65 whole fish per collection) were obtained at various times during rearing (Table 5-1), including near the time of phase-I harvest/release. Hatchlings were collected from ponds by arbitrarily dip-netting a few individuals at a time from a presumably well mixed lot following consolidation by a haul seine. Hatchling specimens were measured using digital calipers (mm, standard length), flash-frozen whole in liquid nitrogen, and stored at -80°C until processing. Stomachs and viscera were removed prior to DNA extraction.

Isolation of Markers for Female Parentage Testing. For each dam, total cellular DNA was isolated by standard Proteinase K/SDS lysis and organic extraction procedures (Sambrook et al. 1989). A 425-base-pair (bp) portion of the mtDNA control region was amplified by polymerase chain reaction (PCR) using "red drum-specific" light-chain (5’-GTAAACCGGATGTGGGGGTTAG-3’) and heavy-chain (5’-GGAACCAGATACCAGAATAGTTCA-3’) primers (see Seyoum et al. 2000). Amplifications were performed in 50 µl reactions with 32 cycles of denaturation (94°C), annealing (55°C), and extension (72°C). PCR amplicons were gel-purified in 1.5% low EEO agarose (Fisher Biotechnologies, Pittsburg, PA) and cleaned using the Strata Prep
Table 5-1. Fall 1994 phase-I production of red drum (*Sciaenops ocellatus*) at the Florida Marine Research Institute’s (FMRI) Stock Enhancement Research Facility (SERF).

<table>
<thead>
<tr>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Spawning Date</th>
<th>Age (days)</th>
<th>Number stocked&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number released</th>
</tr>
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<tr>
<td>T1P4</td>
<td>09/23/94</td>
<td>42</td>
<td>192,309</td>
<td>0</td>
</tr>
<tr>
<td>T1P5a</td>
<td>09/27/94</td>
<td>38</td>
<td>208,780</td>
<td>161,824</td>
</tr>
<tr>
<td>T7P1</td>
<td>09/30/94</td>
<td>35, 143</td>
<td>264,295</td>
<td>60,980</td>
</tr>
<tr>
<td>T7P11</td>
<td>10/5/94</td>
<td>35</td>
<td>223,816</td>
<td>19,984</td>
</tr>
<tr>
<td>T7P3</td>
<td>10/10/94</td>
<td>30</td>
<td>159,770</td>
<td>3,612</td>
</tr>
<tr>
<td>T7P6b</td>
<td>10/10/94</td>
<td>40</td>
<td>9,530</td>
<td>5,155</td>
</tr>
<tr>
<td>T7P7</td>
<td>10/12/94</td>
<td>35</td>
<td>156,130</td>
<td>0</td>
</tr>
<tr>
<td>T7P9</td>
<td>10/20/94</td>
<td>49, 125</td>
<td>149,780</td>
<td>0</td>
</tr>
<tr>
<td>T7P10b</td>
<td>11/3/94</td>
<td>27</td>
<td>154,200</td>
<td>0</td>
</tr>
<tr>
<td>T7P5b</td>
<td>11/29/94</td>
<td>30</td>
<td>97,800</td>
<td>0</td>
</tr>
<tr>
<td>T9P8</td>
<td>08/24/94</td>
<td>27</td>
<td>156,520</td>
<td>72,162</td>
</tr>
<tr>
<td>T8P10a</td>
<td>09/2/94</td>
<td>23</td>
<td>153,964</td>
<td>30,027</td>
</tr>
<tr>
<td>T8P12</td>
<td>09/8/94</td>
<td>20</td>
<td>154,964</td>
<td>45,447</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations follow the form T#P# where T = spawning tank ID; P = rearing pond ID.

<sup>b</sup> into 1 acre rearing ponds.
DNA Gel Extraction Kit (Stratagene, La Jolla, CA). DNA in each reaction was quantified by UV-spectrophotometry. One µl of the purified PCR product (∼ 5 ng) served as template for Big Dye™ Terminator Cycle Sequencing Ready Reactions (total volume of 5 µl); remaining reaction components were AmpliTaq FS DNA polymerase, the appropriate primer, and water. The resultant DNA fragments were ETOH precipitated, suspended in 20 µl of Template Suppression Reagent (TSR), and denatured according to the manufacturer’s instructions (PE Biosystems, Foster City, CA). For each female red drum, DNA sequences were generated from both directions using the red drum-specific primers on an ABI Prism™ 310 Genetic Analyzer and were aligned and edited using the AutoAssembler™ DNA Sequence Assembly Software (PE Biosystems, Foster City, CA). For female parentage testing, diagnostic markers consisted of mtDNA sequence haplotypes that were not shared by female broodfish in a given spawning tank.

Estimation of Individual Maternal Contribution to Lots. I assayed a total of 904 red drum hatchlings in this study. For each specimen, DNA was extracted and the 425-bp segment of mtDNA control region was amplified, gel-purified, and cleaned as described above. Aliquots of DNA preparations were stored at -80°C for future analyses. One of two types of sequenced-based maternity assay was employed, depending on whether individual (specimen) haplotype assignments or sample haplotype frequencies were required for specific analyses (see Results). When individual haplotype assignments were required, sequencing reactions were analyzed on the ABI 310 as described for maternal broodfish, but sequence was obtained from a single direction by using the light-chain primer. When only sample haplotype frequencies were to be
obtained, a screening approach that has been referred to as “diploid sequencing” (e.g., Hare and Palumbi 1999) was tested and subsequently employed. Because it is applied to mtDNA templates herein, I refer to this approach as “dual-target” sequencing. In this format, template preparation and sequence reaction components and conditions were the same as those described above except that 0.5 µl of purified PCR amplicon (2-3 ng) from each of two individuals were pooled in a single sequencing reaction, again utilizing the light-chain primer. DNA fragment arrays from dual-target reactions were analyzed on the ABI 310. Using this approach, the haplotype(s) present in a given sequence reaction can be inferred from expected “heterozygote” patterns provided that the amplifications of both DNA targets in a pooled reaction are faithful. Testing of this approach involved sequencing a subset of individuals (n = 110) both individually and by using the dual-target format and subsequently computing the error rate in sample haplotype frequency estimation.

*Statistical Analyses of Maternal and Phase-I Sample Haplotype Frequencies.*

Using the Mega2 program (Kumar et al. 2001), I computed the number of nucleotide differences between pairs of maternal haplotypes and performed a cluster analysis of the pairwise haplotype distance matrix based on the Neighbor-joining algorithm (Saitou and Nei 1987). Using Arlequin 2.0b2 (Schneider et al. 1999), I estimated haplotype diversity and sequence diversity (Nei 1987) for the sample of 14 female broodfish. For each phase-I sample of a F94 lot, the null hypothesis (H₀) that specific maternal (mtDNA) haplotypes were present in equal proportions was tested by using the single-classification goodness-of-fit procedure (Sokal and Rohlf 1985), as implemented in BIOMstat, version
3.0 (Rohlf and Slice 1995). Williams’ correction was applied to G statistics. Depending on the number of females spawning per tank, the maximum number of different haplotypes among offspring in a lot was three or four (see Results); thus, expected frequencies for each haplotype in a lot were 0.333 or 0.250, respectively. Single-classification G-tests were corrected for multiple tests of a given hypothesis using the sequential Bonferroni method (Rice 1989) to obtain experiment-wise significance levels (Weir 1996). I defined the “minimum detectable haplotype frequency” (MDF) as the frequency at which I could be ((1 - $\alpha$) × 100)% certain of sampling at least one offspring from a particular mother in a sample of size $n_s$ if it was present in a lot. Offspring bearing haplotypes that were present in frequencies below the MDF were at statistical risk of not being detected. Based on binomial sampling theory,

$$MDF = 1 - \exp\left[\frac{\ln(\alpha)}{n_s}\right].$$

where $\alpha = 0.05$ for $MDF_{95}$ and $\alpha = 0.20$ for $MDF_{80}$.

**Inbreeding Effective Numbers for Progeny Groups and Cohort.** Based on empirical data, the female inbreeding effective number for each spawning group ($N_{eI,f}$) was computed from $1/\phi_f$ (see Chapter 4). The inbreeding effective number for each spawning group ($N_{eI,j}$) was determined by using equation 4-25. With known values of $n_j$ and $m_j$, the variables $k_j$ and $h_j$ were determined from $N_{S,j}$ and the variable $\sigma^2_{k,j}$ was determined from estimates of $N_i$ after correction for a hypothesized pre-recruitment mortality. Because the parameter $\sigma^2_{h,j}$ was not determined empirically, the full range of
possible values of $\sigma^2_{h,j}$ was examined. The inbreeding effective number for the phase-I Fall 1994 cohort ($N_{e,C}$) was determined by using equation 4-32. With known values of $n_C$ and $m_C$, the variables $k_C$ and $h_C$ were determined from $N_C$ and the variable $\sigma^2_{k,C}$ was determined from estimates of $N_i$ after correction for pre-recruitment mortality. The parameter $\sigma^2_{h,C}$ was not determined empirically; therefore, the full range of possible values of $\sigma^2_{h,C}$ was considered.

*Analysis of Progeny Survival within Lots during Extended Rearing.* The null hypothesis that sample haplotype frequencies remained unchanged within lots during extended rearing was tested by using an $R \times C$ exact test of population differentiation (Raymond and Rousett 1995). Contingency tables were based on the distributions of “C” different haplotypes among “R” samples. Tests were conducted using Arlequin. The $R \times C$ exact test is analogous to Fisher’s $2 \times 2$ exact test but employs the Markov chain method (Metropolis et al. 1953) to estimate the exact probability for rejecting the independence of an $R \times C$ contingency table. Significance levels of $R \times C$ contingency tests were corrected for multiple tests of a given hypothesis using the sequential Bonferroni method as appropriate (Rice 1989). Component tests were not considered independent and a test criterion of $P_i \leq \alpha/(1 + r – i)$ for the $i$th test was adopted, where $r$ was defined as the number of tests performed and the experiment-wise $\alpha$ was 0.05.
Results

Suitability of the Genetic Marker for Maternity Assignment. Tank assignments, PIT-tag identifiers, and nucleotide sequence at variable sites for F94 female broodfish are given in Table 5-2. Overall, female breeders had a haplotype diversity of $0.934 \pm 0.061$, which was slightly lower than the range of values reported for native red drum (0.95 to 1.0; Seyoum et al. 2000). Nucleotide diversity for the 14 female breeders was $0.031 \pm 0.017$, which was within the range reported for native red drum samples (0.025 to 0.037).

Within tanks, not all females were identifiable by way of at least one unshared substitution. The 30 variable sites between nucleotide positions 100-286, however, provided sufficient polymorphism for within-tank maternity assignment of all offspring (Table 5-2). Maternal haplotype diversity within spawning tanks was 100% (Figure 5-3). Among tanks, female 197047 (Tank 1), female 1c3359 (Tank 7), female 054829 (Tank 8), and female 055e3a (Tank 9) shared a common haplotype. This haplotype corresponds to “haplotype 83” of Seyoum et al. (2000) and was the most frequently observed haplotype (17%) among a sample of wild Florida Atlantic red drum – the source of the broodfish for the Biscayne Bay stocking program. In Seyoum et al. (2000), haplotype 83 was observed infrequently (2%) among 104 Florida Gulf of Mexico red drum.

For individual and dual-target sequencing assays, a minimum of 340 bases beyond the light-chain primer could be routinely and unambiguously scored. For dual-target sequencing, the expected “heterozygous” genotypes based on sites 100-286 for all
Table 5-2. Mitochondrial DNA haplotypes of female red drum (*Sciaenops ocellatus*) used in the Fall of 1994 for hatchling production in the Biscayne Bay restocking program. Variable nucleotide sites between positions 100-286 (identified by italicized numbers in header) are listed. A dot (.) indicates identity with the uppermost sequence at a site. The unique substitutions between haplotypes within tanks (bolded in sequences) are tabulated in parentheses.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>PIT-tag #</th>
<th>Tank</th>
<th>Nucleotide Sequence (No. of Unique Substitutions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>173f59</td>
<td>1</td>
<td>CACCTTGTT CGCTTAAGTT ACCGAACAG (4)</td>
</tr>
<tr>
<td>B</td>
<td>197047</td>
<td>1</td>
<td>T.T.A..C T.............. .T... (2)</td>
</tr>
<tr>
<td>C</td>
<td>34414d</td>
<td>1</td>
<td>...TCCAACC T.C.T.ACC ..TAGG.GG. (15)</td>
</tr>
<tr>
<td>B</td>
<td>1c3359</td>
<td>7</td>
<td>T.T.A..C T.............. .T... (3)</td>
</tr>
<tr>
<td>E</td>
<td>107e2a</td>
<td>7</td>
<td>..T...A.A.C T.A...... GA........ (5)</td>
</tr>
<tr>
<td>F</td>
<td>356c21</td>
<td>7</td>
<td>.C....A..C T.............. (1)</td>
</tr>
<tr>
<td>B</td>
<td>054829</td>
<td>8</td>
<td>T.T.A..C T.............. .T... (3)</td>
</tr>
<tr>
<td>G</td>
<td>1d4440</td>
<td>8</td>
<td>......A..C T.T....T.. GT..G.... (3)</td>
</tr>
<tr>
<td>I</td>
<td>19750a</td>
<td>8</td>
<td>......A..C T..C...... .T........ (0)</td>
</tr>
<tr>
<td>J</td>
<td>430e38</td>
<td>9</td>
<td>.....CA.CC T.............. A (1)</td>
</tr>
<tr>
<td>K</td>
<td>05686b</td>
<td>9</td>
<td>.....CA.CC T.............. (0)</td>
</tr>
<tr>
<td>B</td>
<td>055e3a</td>
<td>9</td>
<td>T.T.A..C T.............. .T... (3)</td>
</tr>
</tbody>
</table>
Figure 5-3. Neighbor-joining cluster of pairwise haplotype distances among Fall 1994 SERF female broodfish. Distance estimates were based on the number of nucleotide sequence differences along a 425 base-pair segment of the mitochondrial DNA control region.

Possible within-tank maternal combinations are given in Table 5-3. A total of 110 offspring (38 from T7P1W20, 40 from T7P9W17, 6 from T8P12W3 and 26 from T8P10W3) were assayed twice – individually and in the dual-target format. In all cases, dual-haplotype scores for pairs of individuals that were sequenced together were consistent with the haplotypes of the same two individuals sequenced separately. Therefore, it is unlikely that misreading of sequence in the dual-target format contributed significantly to error in the determination of relative maternal contributions to lots. Only
Table 5-3. Expected heterozygous genotypes. Polymorphic sites were manifested as doublet chromatogram peaks. Based on maternal haplotypes (Table 5-2), offspring heterozygous genotypes could be predicted and are shown below. Different types of doublets are denoted by \( R = A \leftrightarrow G; \ Y = T \leftrightarrow C; \ W = A \leftrightarrow T; \ M = A \leftrightarrow C; \ K = G \leftrightarrow T; \ S = G \leftrightarrow C. \)

|    | A/B | A/C | B/C | B/D | B/E | B/F | D/E | D/F | E/F | B/G | B/H | B/I | G/H | G/I | H/I | B/J | B/K | J/K |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | YACYTTRGTY | YGYCTTARCT | YACCTTARCT | YACYTTAGTY | YACCTTARCT | YACCTTARCT | YACYTTRGTY | YACCTTARCT | YACCTTARCT | YACYTTAGTY | YACYTTRGTY | YACCTTARCT | YACCTTARCT | YACCTTARCT | YACYTTAGTY | YACYTTAGTY | YACYTTAGTY |
|    | YGYCTTARCT | YACYTTAGTY | YACYTTRGTY | YGYCTTARCT | YACYTTRGTY | YACYTTRGTY | YGYCTTARCT | YACYTTRGTY | YACYTTRGTY | YGYCTTARCT | YACYTTRGTY | YACYTTRGTY | YACYTTRGTY | YACYTTRGTY | YACYTTRGTY | YACYTTRGTY | YACYTTRGTY |
|    | ACCGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG | ACMGAAYAAG |

1111111111 1112222222 2222222222 0023555677 8890011222 2223334478 0805357034 1553907235 6786895746
haplotypes J and K differed from each other by a single nucleotide polymorphism. All other pairs of haplotypes potentially occurring for pairs of offspring differed at three or more sites. Of the 904 individuals that contributed to the data set for sample haplotype frequencies, 782 individuals were assayed from lots sampled prior to phase-I harvest and a total of 122 individuals were assayed from subsequent collections from two lots (T7P1 and T7P9) that had been reared for extended periods (Table 5-1).

**Individual Female Contributions to Lots.** Sample haplotype frequencies for all lots sampled prior to phase-I harvest are reported in Table 5-4. Female 356c21 expired after production of cohort T7P1. Of the 10 lots having three potential mothers, a reproductive contribution from a single female was detected on three (30%) occasions, from two females on two (20%) occasions, and from all three females on five (50%) occasions. Of the three lots having four potential mothers, two females contributed on one occasion (33%) and three females contributed on two occasions (67%). Often, females spawned successfully in consecutive days. Goodness-of-fit testing revealed that a significant level of heterogeneity in among-female offspring production existed within tanks. That is, after correction for multiple tests by adopting an experiment-wise $\alpha$ of 0.05, the null hypothesis (equal maternal contribution) was rejected in 12 of 13 (92%) lots (Table 5-4). Within-sample (lot) variance ranged 0.0244 to 0.3333.
Table 5-4. Sample Haplotype Frequencies.

<table>
<thead>
<tr>
<th>Samplea</th>
<th>n</th>
<th>Haplotype Frequencies (♀)</th>
<th>MDF&lt;sub&gt;♀ var&lt;/sub&gt;</th>
<th>G-statistic (df)</th>
<th>Signif.&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(A) (B) (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1P4</td>
<td>58</td>
<td>0.0 1.0 0.0</td>
<td>0.050 0.3333</td>
<td>125.99 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T1P5a</td>
<td>45</td>
<td>0.778 0.222 0.0</td>
<td>0.064 0.1606</td>
<td>50.45 (2)</td>
<td>***</td>
</tr>
<tr>
<td></td>
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<tr>
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</tr>
<tr>
<td>T7P1</td>
<td>64</td>
<td>0.344 0.0 0.656 0.0</td>
<td>0.045 0.0996</td>
<td>93.86 (3)</td>
<td>***</td>
</tr>
<tr>
<td>T7P11</td>
<td>62</td>
<td>0.322 0.081 0.597 -- na</td>
<td>0.0667</td>
<td>27.30 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T7P3</td>
<td>56</td>
<td>1.0 0.0 0.0 --</td>
<td>0.052 0.3333</td>
<td>121.60 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T7P6b</td>
<td>62</td>
<td>0.403 0.194 0.403 -- na</td>
<td>0.0244</td>
<td>5.93 (2)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>T7P7</td>
<td>64</td>
<td>0.578 0.250 0.172 -- na</td>
<td>0.0464</td>
<td>16.80 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T7P9</td>
<td>63</td>
<td>0.222 0.190 0.588 -- na</td>
<td>0.0489</td>
<td>16.95 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T7P10b</td>
<td>62</td>
<td>0.597 0.048 0.355 -- na</td>
<td>0.0757</td>
<td>33.90 (2)</td>
<td>***</td>
</tr>
<tr>
<td>T7P5b</td>
<td>60</td>
<td>0.766 0.233 0.0 --</td>
<td>0.049 0.1549</td>
<td>65.91 (2)</td>
<td>***</td>
</tr>
<tr>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8P10a</td>
<td>64</td>
<td>0.031 0.094 0.875 0.0</td>
<td>0.045 0.1751</td>
<td>118.68 (3)</td>
<td>***</td>
</tr>
<tr>
<td>T8P12</td>
<td>60</td>
<td>0.200 0.0 0.800 0.0</td>
<td>0.049 0.1433</td>
<td>104.85 (3)</td>
<td>***</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9P8</td>
<td>62</td>
<td>0.0 1.0 0.0</td>
<td>0.047 0.3333</td>
<td>134.78 (2)</td>
<td>***</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations for samples are described in Table 5-1.

<sup>b</sup> Female identifiers and haplotype information given in Table 5-2.

<sup>c</sup> MDF values are described in text; “na” = values not applicable.

<sup>d</sup> Asterisks denote probabilities of G-tests for rejection of H₀; (*** = P < 0.001.)
Projected Female Contributions to Progeny Groups. The MDF₉₅ for individual lots ranged 0.045 to 0.064 for sample sizes that ranged 64 to 45, respectively (Table 5-4). Thus, up to three individuals bearing alternate haplotypes may not have been detected in any single sample of a lot and that, in turn, could lead to upward biases on estimates of \( D_A \) and \( \sigma^2_k \) and slightly downward (conservative) biases on estimates of \( N_{el} \). For example, individuals bearing haplotype D were not detected in sample T7P1 but were present because they were detected in a subsequent collection of that lot (see below). To account for potential biases in progeny-group projections that may have been induced by sampling error, I adjusted the observed haplotype frequencies for lots that had non-detected haplotypes as follows. To samples T1P5a and T8P10a, I added the non-detected haplotype at a frequency equivalent to MDF₉₅ and subtracted proportionally from the frequencies of the detected haplotypes. To samples T7P1, T7P3, T8P12, and T9P8, I added both of the non-detected haplotypes at frequencies equivalent to MDF₈₀ and subtracted proportionally from the frequencies of the detected haplotypes. Because hatchlings from T1P4 and T7P5b were not included in the phase-I F94 cohort, no adjustment was necessary for these samples. Following the adjustments, I recomputed all estimated contributions. This approach is expected to be liberal on an experiment-wise basis and, when compared to estimates based on unadjusted frequencies, provide a qualitative means of assessing the possible effects of sampling error. Hereafter, estimates based on adjusted sample haplotype frequencies are denoted with the subscript \( adj. \).
Individual female contributions to the F94 phase-I cohort at the time of release are given in Table 5-5. Contributions based on observed haplotype frequencies ranged 0 to 125,737 offspring. Contributions based on adjusted haplotype frequencies ranged 1,876 to 117,808 offspring. Based on observed frequencies, the two most successful females accounted for 50\% of the total number of offspring in the F94 cohort; based on adjusted frequencies, these two females accounted for 47\% of the overall production. Projected individual female contributions to the F1 breeding cohort \( (N_i \text{ and } N_{i, \text{adj}}) \) are also given in Table 5-5; these assume 99\% mortality between time at release and time at entry to the breeding population.

Table 5-5. Estimated and adjusted offspring contributions, relative haplotype frequencies, and summary statistics\(^a\) for the F94 phase-I subpopulation. Parameter notations – see text for explanation of the \((\text{adj.})\) subscript. Projected contributions assume 99\% mortality prior to spawning stock recruitment.

<table>
<thead>
<tr>
<th>( \varphi ) ( ^b )</th>
<th>( N_i ) ( ^c )</th>
<th>Projected ( N_i ) ( ^d )</th>
<th>( N_{i, \text{adj}} )</th>
<th>Projected ( N_{i, \text{adj}} ) ( ^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>173f59</td>
<td>125,737</td>
<td>1,257 (0.315)</td>
<td>117,808</td>
<td>1,178 (0.295)</td>
</tr>
<tr>
<td>197047</td>
<td>35,925</td>
<td>359 (0.090)</td>
<td>33,659</td>
<td>337 (0.084)</td>
</tr>
<tr>
<td>34414d</td>
<td>0</td>
<td>0 (0.0)</td>
<td>10,358</td>
<td>104 (0.026)</td>
</tr>
<tr>
<td>1c3359</td>
<td>33,131</td>
<td>331 (0.083)</td>
<td>31,863</td>
<td>319 (0.080)</td>
</tr>
<tr>
<td>19786f</td>
<td>2,633</td>
<td>26 (0.007)</td>
<td>4,244</td>
<td>42 (0.010)</td>
</tr>
<tr>
<td>107e2a</td>
<td>54,060</td>
<td>541 (0.135)</td>
<td>52,099</td>
<td>521 (0.131)</td>
</tr>
<tr>
<td>356c21</td>
<td>0</td>
<td>0 (0.0)</td>
<td>1,524</td>
<td>15 (0.003)</td>
</tr>
</tbody>
</table>
Table 5-5 (Continued)

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<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10,040</td>
<td>100 (0.025)</td>
<td>8,552</td>
<td>86 (0.024)</td>
<td></td>
</tr>
<tr>
<td>1d4440</td>
<td>2,822</td>
<td>28 (0.007)</td>
<td>3,884</td>
<td>39 (0.009)</td>
<td></td>
</tr>
<tr>
<td>155327</td>
<td>62,681</td>
<td>627 (0.157)</td>
<td>60,506</td>
<td>605 (0.151)</td>
<td></td>
</tr>
<tr>
<td>19750a</td>
<td>0</td>
<td>0 (0.0)</td>
<td>2,533</td>
<td>25 (0.006)</td>
<td></td>
</tr>
<tr>
<td>430e38</td>
<td>0</td>
<td>0 (0.0)</td>
<td>1,876</td>
<td>19 (0.003)</td>
<td></td>
</tr>
<tr>
<td>05686b</td>
<td>72,162</td>
<td>722 (0.18)</td>
<td>68,409</td>
<td>684 (0.172)</td>
<td></td>
</tr>
<tr>
<td>055e3a</td>
<td>0</td>
<td>0 (0.0)</td>
<td>1,876</td>
<td>19 (0.006)</td>
<td></td>
</tr>
</tbody>
</table>

Summary Statistics

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>399,191</td>
<td>3,992 (1.000)</td>
<td>399,191</td>
</tr>
<tr>
<td>$k_C$</td>
<td>28,513.6</td>
<td>285</td>
<td>28,513.6</td>
</tr>
<tr>
<td>$\sigma_{k_C}$</td>
<td>36,830</td>
<td>368.3</td>
<td>33,731</td>
</tr>
<tr>
<td>$\sigma^2_{k_C}$</td>
<td>$1.36 \times 10^9$</td>
<td>$1.36 \times 10^5$</td>
<td>$1.14 \times 10^9$</td>
</tr>
</tbody>
</table>

$a$ Variables: $k_C$, $\sigma_{k_C}$ and $\sigma^2_{k_C}$ represent the overall mean, standard deviation, and variance in offspring contribution.

$b$ Female identifiers and haplotype information given in Table 5-2.

$c$ Number of offspring produced by the $i$th female.

d value in parenthesis is the frequency of offspring produced by the $i$th female ($N_i/N_C$).

Estimation of $N_{el,f}$ and $N_{el}$ for SERF Red Drum. For progeny groups, parameter values used to estimate the female inbreeding effective numbers are reported in Table 5-6. Within-group values of $k$ (and $k_{adj}$) ranged 189 to 539. Observed within-group maternal variances were very high, ranging $5.0 \times 10^4$ to $2.8 \times 10^5$. Adjusted within-group
Table 5-6. Spawning-group breeding values used in the estimation of female inbreeding effective numbers for the phase-1 F94 hatchery progeny groups. The parameter $x$ represents the relative contribution of progeny from the specified brood to the cohort. The remaining parameter notations are described in the text.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tank 1</th>
<th>Tank 7</th>
<th>Tank 8</th>
<th>Tank 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n/m$</td>
<td>3/3</td>
<td>4/2</td>
<td>4/3</td>
<td>3/3</td>
</tr>
<tr>
<td>$k$ and $k_{adj}$</td>
<td>539</td>
<td>225</td>
<td>189</td>
<td>241</td>
</tr>
<tr>
<td>$\sigma^2_k$</td>
<td>$2.8\times10^5$</td>
<td>$5.0\times10^4$</td>
<td>$6.5\times10^4$</td>
<td>$1.2\times10^5$</td>
</tr>
<tr>
<td>$\sigma^2_k_{adj}$</td>
<td>$2.1\times10^5$</td>
<td>$4.3\times10^4$</td>
<td>$5.8\times10^4$</td>
<td>$9.8\times10^4$</td>
</tr>
<tr>
<td>$\phi_f$</td>
<td>0.654</td>
<td>0.498</td>
<td>0.707</td>
<td>1.000</td>
</tr>
<tr>
<td>$\phi_{f, adj}$</td>
<td>0.577</td>
<td>0.464</td>
<td>0.658</td>
<td>0.898</td>
</tr>
<tr>
<td>$N_{e,f}$</td>
<td>1.530</td>
<td>2.008</td>
<td>1.414</td>
<td>1.000</td>
</tr>
<tr>
<td>$N_{e,f(adj)}$</td>
<td>1.732</td>
<td>2.154</td>
<td>1.520</td>
<td>1.112</td>
</tr>
<tr>
<td>$x$</td>
<td>0.4050</td>
<td>0.2250</td>
<td>0.1892</td>
<td>0.1808</td>
</tr>
</tbody>
</table>

Maternal variances ranged $4.3 \times 10^4$ to $2.1 \times 10^5$. Within-group estimates of $\phi_f$ ranged 0.498 to 1.0 and 0.464 to 0.898 based on observed and adjusted values, respectively.

Within-group estimates of $N_{e,f}$ ranged 2.008 to 1.0 and 2.154 to 1.112 based on observed and adjusted values, respectively. Dams occupying spawning tank 1 provided the greatest relative contribution to the cohort (40%) whereas dams from tank 9 provided the smallest contribution (18%).
Maximum values of $N_{el}$ occur when $\phi'_m$ is at a minimum whereas minimum values of $N_{el}$ occur when $\phi'_m$ is at a maximum. Maximum and minimum values of $N_{el}$ were determined for each group by using available maternal data and assuming best- and worst-case circumstances for individual sire contributions. Minimum values of $\phi'_m$ were obtained by assuming that $\sigma^2_h \leq h(1 - 1/m)$ within progeny groups. Thus, maximum values of $N_{el}$ ranged 4.05 to 3.0 for progeny groups and maximum values of $N_{el,adj.}$ ranged 4.39 to 3.21. Maximum values of $\phi'_m$ (i.e., 1.0) were obtained by assuming that only a single male in a given spawning group sired progeny within that group. Minimum values of $N_{el}$ ranged 2.68 to 2.0 for each group; minimum values of $N_{el,adj.}$ ranged 2.72 to 2.09. For each group, Figure 5-4 depicts values of $N_{el}$ when $\sigma^2_h \leq \sigma^2_k$, and also minimum and maximum values of $N_{el}$.

The likely range of the total inbreeding effective number for the F94 phase-I cohort was estimated based on the summary statistics reported in Table 5-5 and maximum/minimum values of $\phi'_{m,C}$. The minimum and maximum values of $N_{el,C}$ were 8.5 and 13.6, respectively. The minimum and maximum values of $N_{el,C,adj.}$ were 8.8 and 14.6, respectively. Thus, the $N_{el,C}/N_{BT}$ ratio for hatchling production for the F94 cohort ranged 34% to 58%. Because differences between estimated values using observed and adjusted data were minor, sample sizes were likely adequate for making reasonable estimates of $N_{el}$. The estimated range applies to the overall inbreeding effective size of the cohort, despite the absence of data pertaining to paternity.
Figure 5-4. Estimates of $N_{elj}$ for each progeny group over a range of $\sigma^2_h$. Solid lines depict $N_{elj}$ values for observed values of $\sigma^2_k$; dotted lines depict these values for adjusted values of $\sigma^2_k$. Plotted circles depict observed (closed) and adjusted (open) point estimates assuming $\sigma^2_h$ was equivalent to $\sigma^2_k$. Ranges bounded by the maximum possible values of $N_{elj}$ occur to the left of dashed vertical lines.

Progeny Survival within Lots during Extended Rearing. For one of the progeny groups, I assayed samples from two lots collected subsequent to phase-I harvests (Table 5-1). In addition to T7P1W4, sample T7P1W20 was assayed; in addition to T7P9W6, sample T7P9W17 was assayed. The latter collections were composed of phase-III hatchlings.
both cases, haplotype frequencies shifted significantly between early and late collections. Figure 5-5 depicts phase-I and phase-III haplotype frequency distributions and associated binomial standard deviations for these two lots. Significant differences were detected in T7P1 between the early and late collections (Figure 5-5A; \( P < 0.0001 \)) as well as in T7P9 between the early and late collections (Figure 5-5B; \( P < 0.043 \)). After correction, both tests retained statistical significance with an experiment-wise \( \alpha \) of 0.05, confirming that differential offspring survivorship had occurred within these lots. In both lots, maternal half-sibs of dam 19786f increased in frequency at the expense of maternal half-sibs of dams 1c3359 and 107e2a.

**Discussion**

*Applicability of the Models.* Gene correlations should be investigated at meaningful hierarchical levels. Two models presented in Chapter 4, the *progeny-group* model (equation 4-31) and the *cohort* model (equation 4-32), use the same raw data and will provide the same estimate of \( N_{el,C} \) in studies such as this. In this case, however, it was necessary not only to quantify \( N_{el}/N_{BT} \) ratios in hatchling production but also to identify potential improvements in husbandry practices for optimal use of broodfish. Both models allow partitioning of the reductive influences that unequal sex ratios and maternal and paternal variance may have on \( N_{el,C} \). However, the model specified in equation 4-31 further allows examination of the role played by differences in progeny-group abundance.
Figure 5-5. Early and late maternal frequency distributions for two Fall 1994 lots derived from the Tank 7 spawning group. (A) samples from P1. (B) samples from P9. Sample abbreviations and information is given in Table 5-1. Error bars represent standard deviations for the observed frequencies.

The progeny-group and cohort models are generally applicable to captive-breeding and stocking programs but there remain several theoretical considerations. These caveats are not necessarily exclusive to these models; they apply to all approaches for estimating $N_{el}$. First, a simplifying assumption was made that gene correlation among individuals in the P1 generation was negligible. This represents a valid assumption if a relatively small number of broodfish were randomly sampled from a reasonably large,
outcrossing population (Rasmussen 1979). It presumably holds for the red drum broodfish used in the present study (Gold et al. 1994, Seyoum et al. 2000) and for many marine fishes. Nonetheless, the assumption should be evoked cautiously for studies involving small/fragmented populations or large populations subject to recurrent inbreeding (e.g. sessile marine organisms).

Second, I assumed that sexes of progeny were determined randomly. In this case, the assumption was necessary because hatchlings were released prior to sexual maturity. It is not known generally if this represents a significant source of bias in cultured fishes. However, it may be possible that one dam, for example, may tend to produce mostly sons whereas another may tend to produce mostly daughters. To the extent that covariance in progeny sex occurs, the estimates of NeI reported here may be biased upwardly. This potential source of bias may be examined by determining parentage and sexes of F1 progeny, in the case of red drum, via recapture of released, mature individuals. Numerical and simulation analyses of the potential effects of covariance in the number of male and female progeny per parent on NeI under the general circumstances considered here might also be useful.

Third, there is an implicit assumption that gene correlations in the hatchery cohort remain constant over time. In reality, intrinsic non-random effects relating to maternity (see Bernardo 1996, Chambers and Leggett 1996) and genotype viability are likely. For example, empirical data from this study indicate that there is a significant maternal component to offspring viability in red drum during extended rearing at SERF. During high density rearing in ponds, larger red drum feed on smaller red drum (J. C. Young, pers. comm.). The maternal effect may be caused by cannibalism if early growth is
related to maternity in red drum. At present, it is unknown whether the observed non-random survivorship resulted from differential genotype fitness or from maternal influences. Undoubtedly, the selective pressures experienced by red drum hatchlings in their natural environment will differ from those experienced in rearing ponds (see Kohanne and Parsons 1988, Fleming 1995). Nonetheless, there is no reason to assume that post-release genotypic and/or maternal effects will be negligible (e.g., Geiger et al. 1997).

Fourth, F1 progeny may not be well mixed upon release or survivorship may be affected extrinsically. In the present study, various red drum phase-I lots were considered to be a “single” cohort in the sense that individual lots were all released into a single estuary within a limited time-span (in conformance to the natural breeding season). It is possible, however, that factors (biotic or abiotic) specific to different release habitats within the estuary may have resulted in site-specific survival effects. In addition, effects relating to handling stress during harvest, overland transport, and release may have varied significantly among different lots (pers. obs.). Finally, release densities varied (SERF, unpublished data), potentially increasing variance in survival among lots.

Uncertainties related to the second, third and fourth assumptions directly lend themselves to applied study, provided that a suitable system of fish-marking can be used. Specifically, hatchery-derived red drum would need to be sampled after recruitment into the breeding population and their parentage established. For red drum, a genetics-based marking system has been recently developed (Tringali in press) involving the use of microsatellite DNA markers (Turner et al. 1998, Chapman et al. 1999). High-resolution parentage assays of red drum sampled in Tampa Bay are being conducted to identify
hatchery progeny produced at SERF. For each hatchery progeny identified, male and female parents are being recorded. If sufficient numbers of hatchery progeny can be recaptured from the fishery (statistical power is a consideration), then covariance in sexes of progeny can be examined, differential survival within and between families can be assessed, and levels of genetic change following release can be compared to those exhibited during rearing.

Lastly, the progeny-group and cohort models were designed to estimate the inbreeding effective size of a discrete hatchery cohort. In many cases, however, populations being stocked are age-structured and/or stocking may continue in one location over the course of several breeding seasons. In the present study, the released cohort comprised a portion of individuals in one generation interval in an age-structured red drum population, where a generation interval equates to the mean age of parents in the breeding population (Nunney 1993). For age-structured populations such as red drum, generation effective sizes can be approximated if post-release age distributions are measured and if offspring from early cohorts are not recaptured within a generation interval and used to produce later cohorts. Fishery data indicate that the mean age of parents in the red drum breeding population, corrected for age-specific fecundity, may be approximately 6 yrs (Turner et al. 1999).

Culture Strategies. The mathematical components of the progeny-group model can be especially useful in forecasting effects on $N_{el}$ caused by modifications to breeding protocols. Mindful of the practical constraints that characterize particular captive-breeding programs, protocols can be designed to reduce gene correlation between
hatchlings within the progeny group and increase the effective numbers of breeders. Because disproportionate contributions to the cohort from individual spawning groups exert a reductive effect on the inbreeding effective size of the cohort, culture strategies that minimize differences in progeny abundance among spawning groups may lead to higher effective sizes. High $N_{el}/N_{BT}$ ratios can only be achieved when sex ratios in spawning groups do not deviate greatly from approximately 2:1. Modifications pertaining to reducing variance in individual male or female contributions can greatly impact the inbreeding effective sizes of hatchery cohorts when that variance is high.

Numerical results (see Figure 5-1) depicted an extended lag-phase effect on $N_{el}$, even as variance in family size increases beyond binomial expectations. Indeed, for relatively small, prolific spawning groups ($N_{B,j} < 20, k \gg 2$), intra-group $N_{el,j}/N_{B,j}$ ratio is not greatly reduced by family size variance until family size variance increases beyond $k^2$.

Once behavioral mating tendencies (e.g., levels of paternal and maternal variance in offspring production) have been determined, spawning group size and composition can be optimized. For example, if male dominance occurs within spawning groups, its reductive effect on the overall $N_{el}$ could be minimized, in theory, by increasing the degree of subdivision. However, mating behaviors in small groups could differ from those of large groups or genetic factors (e.g., genetic incompatibilities among certain pairs) may be significant. Therefore, the particular effect of subdivision should be considered on a case-by-case basis in each breeding program. Alternatively, an empirical determination of an average $N_{el}/N_{BT}$ ratio that is characteristic of a particular set of breeding circumstances may suffice in some cases. Once the ratio is established, culturists may
simply be able increase the actual number of breeders and/or spawning groups, in lieu of modifying breeding procedures, to achieve a targeted effective number.

\[ N_{el,j}/N_{B,j} \text{ Ratios for Red Drum Production at SERF.} \]

Obtaining empirical estimates of \( N_{el} \) and \( N_{el,j}/N_{B,j} \) for the red drum breeding program I studied required the application of genetic markers. Here, diagnostic mtDNA markers allowed for a direct estimation of \( \phi_f \) for each spawning group. Because Sugg and Chesser’s (1994) parameter “\( \phi_w \)” was found to be superfluous (see Chapter 4), relative dam contributions to individual lots, rather than specific maternity assignments, were sufficient. In lieu of sequencing all progeny individually, the dual-target assay developed for this study represented a considerable reduction in laboratory effort and expense. Despite the lack of paternity data, the \( N_{el}/N_{BT} \) ratio for the F94 cohort could be reasonably estimated – it ranged 34%-58%. Therefore, SERF personnel used 2-3 actual breeders for every 1 genetically effective breeder. For restoration and large-scale enhancement programs, it is typically recommended that at least 100 genetically effective breeders be used (Hynes et al. 1981). At the observed level of efficiency, SERF personnel would need to employ 6 discrete spawning groups for 6 consecutive years to incorporate 100 genetically effective breeders per generation in a large-scale stocking effort.

According to the general models, \( N_{el} \) and thus \( N_{el}/N_{B} \) decrease with higher values of \( \phi_f \). Within a spawning group, \( \phi_f \) increases when the number of dams decreases and when variance in maternal offspring production increases. Despite the production of multiple lots from most spawning tanks during F94 production, reproductive contributions from some of the available female broodfish were below the threshold of
detection and within-spawning group variances in individual maternal contributions were extremely high – on the order of $k^2$. Thus, for a given red drum progeny group, the probability that two randomly chosen F1 hatchlings shared a dam was relatively high. Moreover, the progeny group having the highest level of maternal variance also had the greatest relative contribution to the cohort (Table 5-6). Overall, maternal variance accounted for approximately 37% of the reduction in genetic efficiency for the cohort. In this study, there was no indication that increasing the number of lots stocked from each spawning group effectively reduced the overall maternal variance in its progeny group. Although the coefficient of variation ($CV$) for maternal contribution in the spawning group that produced 8 lots (Tank 7) was lower than $CV$s for the groups that produced one or two lots (Tanks 1,8, and 9) based on numbers stocked (not numbers released), overall maternal variance for Tank 7 was still much higher than $k^2$.

To make full use of $N_{el}$ models, both maternal and paternal contributions to progeny should be determined. For this study, in the absence of explicit paternity testing for F1 offspring, the potential effects of paternal variance on $N_{el}$ were evaluated over a range of likely values, drawing on observed values of $m$ and $h$. Again, I note that only relative sire contributions need be determined, not individual paternity. I employed numerical analyses to examine the behavior of $N_{el}$ over the full range of $\phi'_m$ – i.e., its lower ($\sigma^2_h = 0$ or $\leq$ binomial) and upper ($\phi'_m = 1$) bounds. This approach allowed a limited comparison between variance in paternal contributions and other causative factors relating to the accrual of gene correlation among progeny. At most, i.e., if there were total male dominance within each group, the variance in individual male contribution to family size would have accounted for a 28% (cohort-wide) reduction in genetic
efficiency. The actual percent reduction attributable to paternal variance was undoubtedly lower.

The effect that disproportionate contributions from individual spawning groups exerts on genetic efficiency in the red drum breeding program can be quantified by comparing observed values of $N_{el}$ to a hypothetical case in which the four spawning-group contributions were equivalent. For the F94 cohort, a maximal 5% reduction in genetic efficiency could be attributed to this factor. Notably, the impact on genetic efficiency was relatively minor in comparison to (possible) effects relating to individual dam (and sire) mating success. This may not always be the case, particularly should a numerically dominant contribution of offspring be received from a spawning group that has a low $N_{el}$ relative to the other groups.

Concluding Remarks. Of the 14 potential female breeders studied here, two females were responsible for up to 50% of the offspring, several females made no detectable contribution to the phase-I cohort, and the overall variance in maternal contribution was extremely high. Nonetheless, the $N_{el}/N_{BT}$ ratio estimated at the time of release was likely on the order of 40-50%. In general, when spawning groups are small, extra-binomial variance in family sizes need not lead to significant declines in genetic efficiency within progeny groups. In many stock-enhancement scenarios, when some level of reproductive contribution is obtained from the majority of breeders, $N_{el}/N_{BT}$ ratios can be fairly good even when breeding protocols are unstructured, sex ratios in breeders are somewhat skewed, and little or no effort is made to minimize family size
variance. When appropriate and reasonable, exerting some degree of control over family sizes within very large cohorts would likely lead to very high $N_{el}/N_{BT}$ ratios.
Chapter 6

Do Temporally Fluctuating Population Sizes and Partially Recessive Selective Effects Hasten the Approach to Genetic Inviability?

Introduction

Three genetic challenges to the maintenance of population viability – inbreeding depression, the loss of adaptive variation, and the loss of population fitness due to accumulating deleterious mutations – are thought to occur principally because populations become small or fragmented (Frankham 1996, Lynch 1996). It has been posited (Lande and Barrowclough 1987) that inbreeding depression and lost adaptive potential may be avoided entirely under certain circumstances. That is, ‘threshold’ variance effective population numbers ($N_{eV}$) presumably exist beyond which populations will behave as if they were infinitely large with respect to those genetic processes. The cumulative damage caused by the fixation of numerous mildly deleterious alleles may also be a threshold process. In this case, the time-scale over which the damage occurs is, in theory, inversely and asymptotically related to $N_{eV}$ (Lande 1994). When the genetic load attributable to fixed deleterious alleles reaches a critical mass, the population is expected to enter into a progressively degenerative state known as a “mutational meltdown” (cf. Lynch et al. 1995b), which has the likely consequence of extinction.
In Chapter 2, post-immigration inbreeding effective numbers \( (N'_{eI}) \) in population admixtures of cultured and wild fish were shown to be highly sensitive to the proportion of cultured fish in the mix. \( N_{eI} \) and \( N_{eV} \) are convergent in most cases, an exception being when population size (abundance) changes over time (Crow and Denniston 1988). Therefore, to the extent that cultured fish augment wild fish instead of replace them, \( N_{eI} \) and \( N_{eV} \) will differ in population admixtures. The reductive effect on the variance effective number for a population admixture \( (N'_{eV}) \) is describable \textit{prima facie} by the expression

\[
N'_{eV} = \frac{1 - \frac{1}{2N}}{N' + \frac{N'_e}{N_c} \left[ \frac{N'_e - \frac{1}{2}}{2} \right] + \frac{N'_w}{N_w} \left[ \frac{N'_w - \frac{1}{2}}{2} \right] - \frac{1}{N}},
\]

where \( N \) = the total number of individuals in the parental generation, \( N' \) = the total number of individuals in the offspring generation, \( N_c \) = the number of parents in the captive spawning group, \( N_w \) = the number of parents in the wild spawning group, \( N'_e \) = the number of offspring in the admixed population that were produced in captivity, and \( N'_w \) = the number of offspring in the admixture that were produced by wild breeders (Ryman et al. 1995). For example, we may consider a population that increases in size from 10,000 in one generation to 13,000 in the next due to the addition of a cultured fish cohort (derived from \( N_c = 100 \) breeders [collected randomly from the 10,000]). If cultured fish comprised 30% of the population admixture, the variance effective number
for the admixture, according to equation 6-1, would be ~1080, i.e., nearly a ten-fold reduction. Indeed, the reduction would likely be greater; i.e., equation 6-1 underestimates the actual variance effective number when variance in the number of progeny per breeder is extra-binomial in captive and/or wild spawning groups (as is likely, see Chapters 4 and 5). Nevertheless, the point is clear – the practice of propagating large families from comparatively few breeders and releasing them into natural populations will lead to significantly increased rates of random gene-frequency drift in the population admixtures, at least transiently. Because the long-term \( N_{eV} \) may be approximated by the harmonic mean of generation effective population numbers (Crow and Kimura 1970), extreme values exert a disproportionate effect on the rate of drift over time scales relevant to the fixation process. To what extent does this disproportionate effect extend to cases where selection is operating to cull deleterious alleles?

In order to more fully understand the impact that cultured fish may exert on mean fitness and population viability, the general effect that transiently reduced effective population numbers have on accumulating mutational loads must be considered. Unfortunately, all models describing the process of mutation meltdown have been developed thus far under the assumption that population sizes (and hence variance effective numbers) remain constant over time. Moreover, these models may be biased toward underestimating the risk of meltdown because they fail to adequately account for effects introduced by biologically realistic modes of gene action. In this chapter, I extend mutation meltdown theory (MMT) in order to investigate the effects of fluctuating population sizes and recessive gene action.
Background

*Mutational Meltdown in Sexual Populations.* Kimura (1962) used a diffusion approximation to determine the probability of fixation, \( u \), for alleles under selection. For the general case of a mutant allele having an initial frequency \( q \) in a diploid population of size \( N \), the formula for fixation probability reduces to

\[
\int_0^q e^{-2N_e s (2h-1)x (1-x) - 2N_e s} dx
\]

where \( N_e \) is the variance effective population number, \( x \) is the frequency of the mutant during segregation, \( s \) is the selection coefficient of the allele, and \( h \) is the dominance coefficient. The quantities \( s \) and \( sh \) represent the selective advantage (or disadvantages) of the mutant homozygote and heterozygote, respectively.

Mutational meltdown has been described as a three-phase process (Lynch et al. 1993). Phase 1 corresponds to the brief period after the founding of a population (~ \( 4N_e \) generations) during which equilibrium is attained between mutation, drift, and selection. During phase 2, the stochastic fixation of spontaneously occurring deleterious alleles leads to a gradual erosion of the intrinsic rate of increase \( (r_t) \) per unit time until the population reaches genetic inviability \( (r_t \leq 0) \). When \( r_t \) becomes negative (phase 3), population extinction becomes likely because of synergistic interactions between declining abundance, genetic drift, and growing mutational pressure. Phases 1 and 3
should be extremely short compared to phase 2 (Lynch et al. 1993), at least in cases where the segregation load is negligible (Lynch et al. 1995a). Consequently, the mean time to extinction ($t_e$) may be approximated by the expected duration of phase 2 (Lande 1994).

Gabriel et al. (1993) first noted that the relationship between $t_e$ and $s$ is not monotonic over all values of $N_e$; for populations larger than a few individuals, there is an intermediate value of $s$, denoted $\hat{s}$, that minimizes $t_e$. Lande (1994) extended that concept to diploid populations, for which the reduction in mean fitness is approximately $(1 - 2s)$. Using a counting approach based on the Poisson point process and considering a uniform selection coefficient, Lande (1994) showed that $\hat{s} \approx -0.4/N_e$ for diploid populations. In natural populations, however, alleles in the pool of newly arising mutations do not have identical selective effects – a fact that substantially affects expectations for extinction time. To analyze the case of non-uniformity in selective effects, Lande (1994) adopted an Ornstein-Uhlenbeck diffusion approach, modeling the rate of change in the Malthusian fitness parameter ($r$) during phase 2. Assuming an initial pool of mutants having exponentially distributed selection coefficients with a mean and standard deviation of $-0.025$, Lande predicted that the average selection coefficient of fixed mutations, denoted $\hat{s}_f$, will be close to values of $\hat{s}$ for a given effective population number. Indeed, because the individual selective effects of a large portion of deleterious mutations are expected to occur within the range of effective neutrality ($|s| < 1/2N_e$; Wright 1938), Lande concluded that populations having long-term effective sizes on the order of $10^3$ face a substantial risk of extinction from their growing mutational loads. Lynch et al. (1995a), while agreeing in principle with the impact of variance in $s$, questioned the accuracy of Lande’s
diffusion approach for moderate to large population abundances (>10^2), in part, because the Kimura’s diffusion approximation putatively tends to overestimate fixation probabilities of deleterious mutations as N_e and s increase (Bürger and Ewens 1995).

*Fluctuating Populations.* In nature, of course, population sizes change over time, a fact that may greatly influence the dynamics of allele segregation and fixation (Kimura and Ohta 1974). The issue of fluctuating population size was not addressed directly by Lande (1994) – i.e., his estimates of t_e were modeled under the assumption that populations were randomly mating and of constant size. Incorporation of the parameter N_e into the mathematical approximation for t_e (via the term for u), however, implicitly allows for extension of that approximation to populations of changing sizes (and to other non-ideal conditions). For fluctuating populations, Ewens (1967) found that the fixation probability for a mutant allele originating in generation t is approximately 2s\(\bar{N}/N_t\), where \(\bar{N}\) denotes the harmonic mean of population sizes and \(N_t\) denotes the actual size in generation t. Because \(N_e \approx \bar{N}\) in randomly mating, fluctuating populations (Kimura 1970), the harmonic mean of \(N\), if it can be known, should be interchangeable with the effective population number in the approximation for \(t_e\). However, Otto and Whitlock (1996) examined fixation probabilities in cyclical populations with uniform selection coefficients and showed that the harmonic mean approximation begins to break down in the diffusion equation for u when selection is strong and/or the period or the amplitude of the oscillation in population size is large. It may therefore be of interest to examine the effect of fluctuating size on fixation probabilities and mutation loads for populations.
subject to a heterogeneous pool of deleterious mutants having predominantly weak selective effects.

*Mutation Parameters U, ŝ, and h.* Empirical studies indicate that deleterious mutations occur frequently and that most are partially recessive and have individually small homozygous effects (Deng and Lynch 1996, Drake et al. 1998, Vassilieva and Lynch 1999). The average (diploid) genomic rate \( U \) of mildly deleterious mutations per generation undoubtedly varies among taxa, likely a consequence of genome size and complexity and of differential life histories. For example, in *Drosophila*, mildly deleterious mutations are thought to occur randomly at a minimum rate of \( U = 1.5 \) (Deng and Lynch 1996). In contrast, the genomic rate of the nematode *Caenorhabditis elegans* appears to be substantially lower, perhaps two-fold to twenty-fold (Keightley and Caballero 1997, Vassilieva and Lynch 1999).

The distribution of selection coefficients appears to be well approximated by the negative exponential distribution

\[
p(s) = \frac{1}{s} e^{(-s/\hat{s})}
\]

where \( \hat{s} \) denotes the mean value for the distribution (Ohta 1977; Mackay et al. 1992, Keightley 1994). For example, in studies of *Drosophila*, selection coefficients of mutant alleles appear to be exponentially distributed with \( \hat{s} = -0.015 \) (reviewed by Deng and Lynch 1996). The manner in which dominance coefficients are distributed is less clearly
understood, especially for mildly deleterious alleles, but the *Drosophila* distribution appears to have a mean of 0.36. Both empirical mutation data and biochemical considerations are generally suggestive of an inverse relationship between the homozygous effect of a mutation and its dominance coefficient (Kacser and Burns 1981; Crow and Simmons 1983). Notwithstanding the large sampling error associated with empirical measurements of $h$ for mildly deleterious alleles, it is reasonable to consider dominance coefficients that are widely and uniformly scattered below an upper bound that is related to $s$ (e.g., Caballero and Keightley 1994).

In *MMT*, the treatment of recessive mutational effects is not a trivial exercise. Partially recessive genes should have higher fixation probabilities than additive genes with equivalent selection coefficients (Crow and Kimura 1970). Consequently, deleterious alleles having low dominance coefficients and low to intermediate selection coefficients, although they may comprise a small portion of the pool of *de novo* mutations, may significantly increase the mutational load. Unfortunately, for partially recessive genes, there is no simple solution to the diffusion formula for $u$ (Equation 6-2 above; Kimura 1962). This has hampered mathematical analyses of the effects of recessive gene action on the accumulation of mildly deleterious mutations. Lynch et al. (1995a and b) applied a transition-matrix approach to model the expected number of recessive mutant alleles that ultimately become fixed in populations of various sizes. Unfortunately, selection and dominance coefficients were necessarily predefined (uniform) in their treatment. Nonetheless, the authors concluded that the effect of non-additive gene action on declining population fitness was likely to be negligible in populations larger than ~50 individuals unless $s$ was small. However, mindful of the
significant reduction in $t_c$ related to variance in $s$, it remains possible that, upon simultaneous consideration of variable distributions for both $s$ and $h$, the impact of recessive mutants could be different than that predicted by Lynch et al. (1995b).

In order to investigate the influences of fluctuating population sizes and partial recessivity on the duration of phase 2 of mutation meltdown, a new analytical approach was devised. First, the probability distribution for the time in generations at which $r_t \leq 0$ (Lande 1994; Equation 2, page 1462) was generalized and parameterized as a renewal process. Then, Monte Carlo sampling was used to approximate the expectation for that distribution under relevant conditions.

Methods

Stochastic Model and Assumptions. Maintaining consistency with models used by Lande, Lynch, and their respective coworkers, the following general assumptions are applied here: 1) mutations randomly enter the population at a genomic rate of $U$, 2) the process of fixation acts independently across freely recombining loci, 3) upon origination of a mutant allele at a locus, no further mutation occurs at that locus as long as it is polymorphic, and 4) the selective effect of a particular mutation remains constant and unconditionally deleterious, although selective effects of individual mutations may differ. For this study, I also assume that populations are diploid, randomly mating, closed to immigration and, generations are discrete. Lastly, I assume for convenience that when population size fluctuates over time, the oscillations occur with a definable periodicity.
Herein, I extend Lande’s (1994) original application of the counting approach to the cases of fluctuating population sizes, and non-uniform, non-additive selective effects. The counting approach is sometimes referred to as a renewal process (Parzen 1962). Briefly, for a random sequence of events on the interval \( t \) from 0 to \( \infty \), let \( W_n \) be a random variable denoting the waiting time to the \( n \)th event in a time series that occurs as a (generalized) Poisson process with a mean rate \( \nu \), where \( n \) is an Erlang variable. Accordingly, \( W_n \) will obey the gamma probability law for parameter values \( n>0 \) and \( \nu>0 \). For \( t>0 \), the lengths of intervals between two events are distributed according to the density function

\[
f_{W_n}(t) = \nu e^{-\nu t} \frac{(\nu t)^{n-1}}{(n-1)!}.
\]

Therefore, the waiting time distribution function is

\[
F_{W_n}(t) = 1 - e^{-\nu t} \left( 1 + \nu t + \cdots + \frac{(\nu t)^{n-1}}{(n-1)!} \right),
\]

and the characteristic function is

\[
\phi_{W_n}(u) = \left( 1 - i \frac{u}{\nu} \right)^{-n};
\]
thus, the expectation and variance, respectively, are

\[ E[W_n] = \frac{n}{\nu}, \quad 6-7 \]

and

\[ Var[W_n] = \frac{n}{\nu^2}. \quad 6-8 \]

From equation 6-7, one may infer that the expected waiting time (in generations) for satisfaction of the condition \( r_t \leq 0 \) (genetic inviability) is a simple function of the parameters \( n_f \) (the total number of fixed mutations that will drive mean Malthusian fitness to zero) and \( \nu_f \) (the average rate at which those mutations become fixed per generation).

Therefore, extension of the renewal process to a wide range of cases for MMT is straightforward, provided that parameter estimates of \( n_f \) and \( \nu_f \) can be obtained and that the interval lengths (i.e., successive times between fixations of individual alleles) are mutually independent and roughly exponentially distributed. The latter two assumptions appear to hold for several well-known models of selection such as the overdominance, SAS-CFF (Stochastic Additive Scale-Concave Fitness Function), and TIM (named for its founders, Takahata, Iishi, and Matsuda [Takahata et al. 1975]) models (Gillespie 1994).

Notably, Gillespie (1993) showed that the behavior of higher-order moments under directional selection against mutations having exponentially or gamma distributed deleterious effects causes the fixation processes for these cases to be indistinguishable from a renewal process.
The total number of fixed deleterious alleles \((n_f)\) necessary to drive a population to genetic inviability is directly related to the maximum rate of population increase per generation and inversely related to the magnitude of the selective effects of those alleles that become fixed. When selective effects are uniform (no variance in \(s\)), Lande (1994) noted that \(n_f = r_o T/(-2s)\), where \(-2s\) is the fractional reduction in viability caused by a fixed mutation, \(r_o\) is the maximum intrinsic growth rate at low population density and under suitable ecological conditions, and \(T\) is the average age of parents in the population. However, when deleterious alleles have non-uniform mutant effects (variance in \(s\) solely or variance in \(s\) and \(h\) simultaneously), \(n_f = r_o T/(-2 \hat{s}_f)\). Use of the arithmetic mean of the selection coefficients of fixed mutations (\(\hat{s}_f\)) in this case is justified in Crow and Kimura (1970, pgs.5-9). Because \(\hat{s}_f\) is conditioned on allele fixation, I hereafter refer to it as the conditional mean selection coefficient. Lande (1994) provides an asymptotic approximation for \(\hat{s}_f\) that is inversely scaled to values of \(N_e\) and \(\hat{s}\); however, the accuracy of his mathematical approximation is uncertain and is not expected to hold for \(-4N_e \hat{s} < 1\).

For a renewal process, the average fixation rate \((\nu_f)\) that applies to \(n_f\) deleterious mutations is a function of the origination frequency (number of new mutations arising per generation) and the associated fixation probability. Recalling that \(U\) denotes the diploid genomic rate of mildly deleterious mutations and assuming that it remains constant, the origination frequency is simply \(NU\) when population abundances are constant. Thus, \(\nu_f = NUu\) and equation 6-2 reduces to \(u = (1 - e^{-2s})/(1 - e^{-4Ns})\) for additive, uniform \(s\) (Lande 1994). When mutational effects are non-uniform, however, fixation probabilities for \(de novo\) mutations are governed by initial frequencies and by individual selective behaviors.
When population size fluctuates over time, mutations originate at various frequencies and their fixation probabilities may depend on future population sizes (Otto and Whitlock 1996). Consequently, the fixation process on the time scale of $W_n$ can be considered to be the superposition of numerous, independent Poisson processes from which events may be summed over a given time interval to derive the overall fixation rate (Çinlar 1975). If the superposition of the independent Poisson processes approximates a renewal process (see above), the overall fixation rate for this process becomes $\hat{N}Uu_f$, where $u_f$ represents the overall fixation probability for the suite of fixed mutations.

Substituting the above expressions for $n_f$ and $\nu_f$ into equations 6-7 and 6-8, the expected waiting time to genetic inviability and its variance, respectively, are

$$E[W_{n(f)}] = t_e = \frac{r_o T}{-2\hat{s}_f \hat{N}Uu_f},$$  

6-9

and

$$Var[W_{n(f)}] = \sigma^2_{t_e} = \frac{r_o T}{-2\hat{s}_f (\hat{N}Uu_f)^2}.$$  

6-10

Equation 6-9 can be further rearranged as follows to yield an estimate of the mean time to extinction that is scaled with respect to population growth rate and genomic mutation rate as in Lande (1994):

$$\left(\frac{U}{r_o T}\right)_{t_e} = \left(-2\hat{s}_f \hat{N}u_f\right)^{-1}.$$  

6-11
Accordingly, the scaled “waiting time” to entry into phase 3, as modeled here, is a stochastic process that can be described using three interrelated variables – $\hat{s}_f$, $\hat{N}$, and $u_f$. Whereas mathematical description of this process for the interesting cases of fluctuating population sizes and non-uniform $s$ with partial recessivity would be extremely complex, the blackbox variables in question ($\hat{s}_f$ and $u_f$) can easily be cast as measurable quantities in microcomputer simulations.

*Computer Algorithm.* Monte Carlo simulations were conducted using a single-locus, two-allele, Wright-Fisher model for randomly mating, diploid populations. The computer program was written in PASCAL. For populations of constant abundance $N$, a single copy of a mutant allele was introduced at the start of each trial with an initial frequency of $1/2N$. For fluctuating populations, $N_t$ individuals were deterministically obtained each generation according to a sine wave population growth model (see applicable section in Results). For populations whose sizes cycled over a given interval (period) of $\tau$ generations, a mutation event was initiated in a generation ($t_r$) that was randomly selected from within a cycle according to the uniform distribution $U[0, \tau]$ so that initially $q = 1/2N_{t(r)}$.

For a given trial, an introduced allele was assigned a selection coefficient ($s_f$) that had been cast as a random variate from the exponential distribution with a mean and variance of $\hat{s}$ and $\hat{s}^2$, respectively. When additive effects were considered, each new allele was assigned a dominance value of $h = 0.5$. Details regarding the assignment of $h$ in simulations involving recessive gene action are given in the appropriate section below.
Allele frequencies were adjusted for viability selection using the recursion \( q_t = q_{t-1}(p_{t-1}w_{12} + q_{t-1}w_{22})/ w_{\text{aver}} \), where \( w_{12} = 1-2h\rho \), \( w_{22} = 1-2s\rho \), and \( w_{\text{aver}} = p_{t-1}^2 + 2p_{t-1}q_{t-1}w_{12} + q_{t-1}^2w_{22} \). The quantity \( 2N_t \) was converted to the nearest integer value and new alleles were sampled each generation with a binomial probability of \( B(q_{t}, 2N_t) \). For larger population sizes and allele frequencies, the binomial probability was approximated from a Gaussian distribution for program efficiency.

For each trial, a population was followed until the introduced allele was absorbed (either fixed or lost). By conducting multiple, independent trials, the variables \( u_\rho \), \( \hat{s}_\rho \) and \( \hat{N} \) were computed. For alleles that were lost, the mean time to loss (in generations), \( t_{\rho_{\text{L}}} \) was computed. For alleles that became fixed, the mean time to fixation, \( t_{\rho_{\text{F}}} \) was computed. For each simulation, a sufficient number of trials (ranging 2 million-500 million) were conducted such that standard errors for \( u_\rho \) were at least three orders of magnitude below the estimated value. This generated a minimum of 10,000 fixation events per simulation from which to compute \( t_{\rho_{\text{L}}} \), \( t_{\rho_{\text{F}}} \), and \( \hat{s}_\rho \) and their standard deviations. The scaled mean time to extinction was then approximated by using equation 6-11.

In the sections below, I first extend the computer-simulated counting approach to the case of diploid populations of constant abundance \( (N = N_e) \) for which mutations have additive, exponentially distributed selective effects. Adopting parameter space investigated by Lande, I analyze the behavior of the random variable \( \hat{s}_f \) and compare simulated estimates of \( t_e \) (counting approach) to predicted values (diffusion-approach). I then employ the Monte Carlo simulation to estimate extinction times in populations whose abundances change over time for various cycle periodicities to identify parameter
space in which the diffusion-based approach loses accuracy. Finally, sampling from illustrative distributions of selection and dominance coefficients, I examine the influence of recessive gene action on $t_e$ over a range of population sizes.

Results

**Constant Population Abundance and Variable, Additive Selective Effects.** For this case, simulations were conducted for the following parameter set: $N = 2, 5, 10, 20, 50, 100, 200, 500, 1000, \text{ and } 2000$, respectively; additive selective effects distributed as in equation 6-3 with $\hat{s} = -0.025$. Population sizes were kept constant over time; thus, $N = \tilde{N} = N_{eV}$. Simulated estimates of $u_p$, $\hat{s}_p$, and $t_p$; simulated values of $t_e$ computed from equation 6-11, and Lande’s (1994) predicted values of $t_e$ are reported in Table 6-1. Simulated estimates of mean time to extinction were in very good agreement with those predicted by diffusion theory, especially for small population numbers. As population numbers approached $10^3$, there appeared to be a slightly increasing tendency for diffusion-based predictions to overestimate the mean time to extinction. As predicted by Lynch et al. (1995a), this overestimation was likely due to decay in the diffusion approximation for $u$ [equation 6-2] at larger population sizes. Fortunately, the deviation between approaches was not dramatic ($<2\%$) over the range of $N$ considered here. At larger population sizes (e.g., $>10^4$), the decay in deterministically obtained values of $u_f$ may lead to significant underestimates of $t_e$. 

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Table 6-1. Simulated \((t_{e,S})\) and predicted \((t_{e,P})^a\) mean times to extinction for constant \(N\) and non-uniform \(s\) \((\hat{s} = -0.025)\).

<table>
<thead>
<tr>
<th>(N)</th>
<th>(u_f)</th>
<th>(\hat{s}_f^b)</th>
<th>(t_f^b)</th>
<th>(t_{e,S})</th>
<th>(t_{e,P})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.232</td>
<td>0.023 (0.022)</td>
<td>6 (6)</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>0.0807</td>
<td>0.0195 (0.0192)</td>
<td>17 (20)</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>0.0326</td>
<td>0.0155 (0.0150)</td>
<td>37 (42)</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>20</td>
<td>0.0119</td>
<td>0.0109 (0.0104)</td>
<td>76 (86)</td>
<td>194</td>
<td>193</td>
</tr>
<tr>
<td>50</td>
<td>0.00257</td>
<td>0.00577 (0.00537)</td>
<td>191 (218)</td>
<td>674</td>
<td>677</td>
</tr>
<tr>
<td>100</td>
<td>0.000725</td>
<td>0.00322 (0.00300)</td>
<td>384 (436)</td>
<td>2136</td>
<td>2149</td>
</tr>
<tr>
<td>200</td>
<td>0.000194</td>
<td>0.00170 (0.00155)</td>
<td>767 (870)</td>
<td>7674</td>
<td>7690</td>
</tr>
<tr>
<td>500</td>
<td>0.0000324</td>
<td>0.000713 (0.000650)</td>
<td>1908 (2168)</td>
<td>43,232</td>
<td>44,000</td>
</tr>
<tr>
<td>1000</td>
<td>0.00000828</td>
<td>0.000368 (0.000338)</td>
<td>3806 (4312)</td>
<td>164,134</td>
<td>166,375</td>
</tr>
<tr>
<td>2000</td>
<td>0.0000203</td>
<td>0.000188 (0.000179)</td>
<td>7480 (8534)</td>
<td>652,737</td>
<td>665,500</td>
</tr>
</tbody>
</table>

\(^a\) Predicted values from Lande (1994); both scaled to \(U/r_0 T\).

\(^b\) Values in parentheses denote the standard deviations of \(\hat{s}_f\) and \(t_f\).

The selection coefficients of fixed mutations, like those of the pool of mutations from which they originated, were approximately exponentially distributed \((\hat{s}_f \equiv \sigma_{s(f)})\) (Table 6-1). For all values of \(N\), mutations that became fixed were, on average, effectively neutral; that is, \(|\hat{s}_f| < 1/2N\). For populations for which \(N > 100\), the average
The selection coefficient of fixed mutations was quantitatively close to \( \hat{s} \), as predicted by Lande (1994). At large effective population numbers, simulated values of \( \hat{s}_f \) were nearly equivalent to the diffusion-based approximations for \( \hat{s}_f \) (i.e., -0.3655/\( N \)). For \( N < 100 \), however, \( \hat{s}_f \) did not scale (inversely) to \( N \). Below effective population numbers of 100, the proportion of fixed mutations that were effectively neutral increased rapidly as \( N \) decreased (Figure 6-1). Although \( \hat{s}_f \) was considerably lower than \( \hat{s} \) for small populations, estimates of \( t_e \) were only slightly higher than the theoretical minima.

![Graph](image)

Figure 6-1. Simulated mean values of selection coefficients of mutations that segregated to fixation (\( \hat{s}_f \)). Data shown for \( N = 2, 5, 10, 20, 50, \) and 100. Black circles – simulated values of \( \hat{s}_f \); solid line – Lande’s (1994) diffusion approximation for \( \hat{s}_f \); dotted line – theoretical values of \( s \) above which mutations are effectively neutral.
Cyclically Varying Population Abundances and Variable, Additive Selective Effects. For this case, I considered populations whose abundances fluctuated sinusoidally over time according to

\[ N_t = \bar{N} + \alpha \sin\left(\frac{2\pi t}{\tau}\right), \]

where \( \bar{N} \) is the arithmetic mean of population abundances over time and \( \alpha \) is the amplitude of the population fluctuations. Again, modeled selective effects were additive and distributed as in equation 6-3 with \( \delta = -0.015 \) or 0.025. The deterministic population-growth parameters (equation 6-12) are given in Table 6-2. Simulations were conducted for each pair of \( \bar{N} \) and \( \alpha \) for ranges of \( \tau \) (Table 6-2). The ranges of \( \tau \) were selected such that, for the largest value of \( \tau \), a single cycle approached the expected time scale of \( t_e \).

Because of differences in rounding over given values of \( \tau \) during the selection of \( N_{(\tau)} \), slight modifications to \( \alpha \) were required to obtain comparable values of \( \bar{N} \) for each series of \( \bar{N} = 50, 200, \) and 500, respectively (Table 6-2). In other simulations (not shown), estimates of \( u_f \) and \( \delta_f \) were unaffected by variations in amplitude considerably larger than those given in Table 6-2.

Overall fixation probabilities for cycling populations (Table 6-3) were similar to those computed for stable populations (constant \( N \)) when \( \tau \) was small but were increasingly higher when \( \tau \) reached a threshold value at \( |\delta_f \tau| > 1 \). In contrast, a more pronounced effect with \( \tau \) was observed for the conditional mean selection coefficient –
Table 6-2. Parameter set for simulation analyses of cyclic population sizes

<table>
<thead>
<tr>
<th>Set of cycle periods,</th>
<th>(in generations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tilde{N}$</td>
<td>$\tilde{N}$</td>
</tr>
<tr>
<td>50</td>
<td>112</td>
</tr>
<tr>
<td>200</td>
<td>447</td>
</tr>
<tr>
<td>500</td>
<td>1118</td>
</tr>
</tbody>
</table>

$a\tilde{N} = \sqrt{\tilde{N}^2 - \alpha^2}$, where $\tilde{N}$ is the arithmetic mean of population size over time and $\alpha$ is the cycle amplitude.

Values of $\hat{s}_j$ decreased as $\tau$ increased. However, the decrease in $\hat{s}_j$ was gradual until fixation probabilities began to rapidly increase, at which point, conditional mean selection coefficients began to rapidly decrease over the range of cycle periodicities investigated (Table 6-3). The contrasting dynamics of $u_j$ and $\hat{s}_j$ are illustrated in Figure 6-2. Extinction-time estimates were relatively sensitive to these decreases. Accordingly, all waiting-time durations were affected to some degree by populations fluctuations. The relative mean extinction time ($T_e$) was taken to be the quotient of $t_e$ simulated under cyclically fluctuating $N$ and $t_e$ simulated constant $N$. The overall effects of cycle
Table 6-3. Fixation probabilities \( u_f \), conditional mean selection coefficients \( \hat{s}_f \),
and mean fixation times \( t_f \) for simulated cyclical population sizes \( \hat{N} = 50 \) and \( 200 \).

<table>
<thead>
<tr>
<th>( \hat{N} )</th>
<th>( \hat{s} = -0.015 )</th>
<th>( \hat{s} = -0.025 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( u_f )</td>
<td>( \hat{s}_f )</td>
<td>( t_f )</td>
</tr>
<tr>
<td>10</td>
<td>0.00371</td>
<td>-0.00522</td>
</tr>
<tr>
<td>50</td>
<td>0.00375</td>
<td>-0.00516</td>
</tr>
<tr>
<td>100</td>
<td>0.00377</td>
<td>-0.00531</td>
</tr>
<tr>
<td>200</td>
<td>0.00390</td>
<td>-0.00593</td>
</tr>
<tr>
<td>300</td>
<td>0.00409</td>
<td>-0.00661</td>
</tr>
<tr>
<td>400</td>
<td>0.00426</td>
<td>-0.00709</td>
</tr>
<tr>
<td>( \hat{N} = 200 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.000310</td>
<td>-0.001648</td>
</tr>
<tr>
<td>100</td>
<td>0.000310</td>
<td>-0.001653</td>
</tr>
<tr>
<td>500</td>
<td>0.000325</td>
<td>-0.001926</td>
</tr>
<tr>
<td>1000</td>
<td>0.000362</td>
<td>-0.002637</td>
</tr>
<tr>
<td>2000</td>
<td>0.000446</td>
<td>-0.003453</td>
</tr>
<tr>
<td>2500</td>
<td>0.000476</td>
<td>-0.003650</td>
</tr>
</tbody>
</table>
Figure 6-2. Effects of cyclically fluctuating population abundance on fixation probabilities ($u_f$), conditional mean selection coefficients ($\hat{s}_f$), and relative mean times to extinction ($T_e$). Open circles represent data points for simulations involving $\hat{s} = -0.015$; closed circles represent data points for simulations involving $\hat{s} = -0.025$. The dashed vertical line indicates $|\hat{s}_f \tau| > 1$. (A) Harmonic mean population abundance ($\bar{N}$) = 50. (B) Harmonic mean population abundance ($\bar{N}$) = 200.

Periodicity on extinction times can also be seen in Figure 6-2. For all harmonic mean population numbers, $T_e$ declined as the cycle periodicity increased. As values of $\tau$ approached the time-scale of the extinction-time estimate, the magnitude of the decrease in $T_e$ was larger for larger values of $\bar{N}$. 

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To further illustrate the dynamics of fixation for different cycle periodicities, I plotted the distributions of fixed mutations with respect to population sizes at the time of their origination \( (N_{o(t)}) \) for simulations involving \( \bar{N} = 500 \), noting whether mutation entry occurred during a growing or declining phase (Figure 6-3). When \( \tau \) was 100 (\( \left| -\hat{s}_f \tau \right| < 1 \)), fixation probabilities depended principally upon the population abundance at the time of origination, irrespective of whether the population was growing or declining. Approximately 75% of the probability mass could be attributed to fixed mutations originating at sizes \( \leq \bar{N} \). In contrast, when \( \tau \) was 1000 (\( \left| -\hat{s}_f \tau \right| > 1 \)), the probability mass of fixations increased substantially (compared to \( \tau = 100 \)) for mutant alleles introduced during the latter portion \( (N_{t(r)} < \bar{N}) \) of the declining phase and during the very early portion of the recovery phase. For larger \( \tau \), there was a relative decrease in the probability mass of fixed mutants introduced during the middle and latter portions of the recovery phase, but this was minor compared to the increased probability mass incurred during the declining/low abundance phase. In this case, approximately 85% of the probability mass was attributable to fixations for which \( N_{o(t)} \leq \bar{N} \) and the magnitude of \( \hat{s}_f \) was \( \sim 11\% \) greater compared to that for \( \tau = 100 \).

**Recessive Gene Action.** The impact of gene action on mutational loads was examined by incorporating recessive effects, i.e., co-varying selection and dominance coefficients, into the allele recursion of the simulation. Here, the relative mean extinction time \( (T_e) \) was taken to be the quotient of \( t_e \) simulated under recessive-effect conditions and \( t_e \) simulated under additive-effect conditions. Because biologically representative
Figure 6-3. Phase shift in probability mass of fixed mutations as a result of increasing cycle periodicity (\( \tau \)). \( N_{\alpha(\ell)} \); Population size at the time of mutant origination. \( n_f \); number of mutants fixed at a given \( N_{\alpha(\ell)} \) during the depicted portion of the cycle (shown for \( \tau = 100 \) and 1000, respectively). Relative \( n_f \); the difference between \( n_f \) for \( \tau = 100 \) and 1000 at a given \( N_{\alpha(\ell)} \) during the depicted portion of the cycle.

distributions of \( h \) with respect to \( s \) are not well known, I addressed the issue of variance in \( h \) by bounding potentially meaningful parameter space rather than employing speculative distributions. To develop high and low estimates of \( T_e \) for given population sizes, I assumed the following two generalities: individual dominance coefficients in the population distribution are widely scattered about a mean value of \( \approx 0.36 \), and, for a given
mutant, the magnitude of $h$ tends to be inversely related to $s$. As before, for each trial, selection coefficients ($s_r$) were drawn from a negative exponential distribution (equation 6-3) where $\hat{s} = -0.025$ or -0.015, respectively. The value of $h$ was then assigned by drawing the variate $h_r$ from a random distribution $U[0, s_{max}]$ where $s_{max} = e^{\hat{s}} / b$ and where $b = 1$ for the upper bound $h$ distribution and $b = 2$ for the lower bound. The resulting joint sample distributions of $s$ and $h$, which were employed in simulations, are depicted in Figure 6-4. The mean value of $h_r$ was 0.49 in both joint sample distributions having the upper-bound $h$ distribution; the mean value was 0.24 in both distributions having the lower-bound $h$ distribution. Most biologically meaningful cases should occur within the parameter space bounded by high and low $T_e$ estimates.

Simulations were conducted for the following parameter set: $N = 5, 10, 20, 50, 100, 200, 500, 1000,$ and $2000$, respectively; selective effects were as described above. For both $s$ distributions, simulations involving the upper-bound $h$ distribution produced mean extinction times that were only slightly lower (<2%) than those produced under additive conditions, irrespective of population abundance (Figure 6-5). However, in simulations involving the lower-bound $h$ distribution, values of $T_e$ declined precipitously at low population sizes ($N < 50$) before leveling off to an apparent asymptote whose magnitude was governed by the $s$ distribution (Figure 6-5). Importantly, reductions in $t_e$ caused by the lower-bound $h$ distribution did not exceed 16% and 18% for $\hat{s} = -0.015$ and -0.025, respectively. Based on the current state of knowledge regarding mutational effects, reductions in $t_e$ caused by recessive gene action should not exceed those levels in most animal populations.
Figure 6-4. Upper- and lower-bound sample distributions of modeled selection and dominance coefficients, $s$ and $h$, respectively. See text for a description of random variables $h_r$ and $s_r$ for upper- and lower-bound distributions. Shaded areas in the plots of the modeled dominance coefficients represents the probability distribution from which $h_r$ and $s_r$ were drawn. (A) Distributions for simulations involving $\hat{s} = -0.015$. (B) Distributions for simulations involving $\hat{s} = -0.025$. 
Figure 6-5. Effect of recessive gene action on relative mean times to extinction ($T_e$).

Data for the following sequence of $N$ values were plotted: 5, 10, 20, 50, 100, 200, 500, 1000, and 2000. Triangles represent comparisons between simulation results for additive selective effects and simulation results for upper-bound dominance effects ($b=2$; mean $h_r = 0.49$). Circles represent comparisons between simulation results for additive selective effects and simulation results for lower-bound dominance effects ($b=2$; mean $h_r = 0.24$).

(A) Relative mean extinction times for simulations involving $\hat{s} = -0.015$. (B) Relative mean extinction times for simulations involving $\hat{s} = -0.025$.  

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Discussion

To forecast and manage genetic risks, genetic processes should be considered with the best available models and under realistic assumptions. In this study, I developed and used a mutation meltdown model that was more general than those used by previous investigators. To overcome difficulties that restricted previous analytical treatments, computer simulations were used to estimate values of latent parameters under relevant conditions. In my approach, the conditional mean selection coefficient was parameterized and shown to be of practical use for examining the influence of fluctuating population sizes and modes of gene action on mean times to extinction.

Lande’s (1994) diffusion-based approach for modeling $t_e$ under the limited set of conditions set forth in his study – i.e., constant $N$, additive, variable $s$, and singleton mutation entry – was largely supported in my stochastic simulations. In contrast, when population size was allowed to fluctuate over time, Lande’s (1994) approximation for $t_e$ was shown to be inaccurate for potentially important areas of parameter space. It was not surprising that there would be an observable decay in estimates of $t_e$ over increasing values of $\tau$. Using a branching process (Haldane 1927), Otto and Whitlock (1996) showed that the fixation probability for cycling populations becomes somewhat sensitive to future population dynamics when the product of $s$ and $\tau$ exceeds unity. Because they considered only uniform $s$, however, it was unclear whether $u_f$ would scale to $\hat{s}$ or $\dot{s}_f$ for a population of a given $\bar{N}$. From my simulation results, it is clear that $u_f$ scales to the conditional mean value and increases most rapidly when $|\dot{s}_f \tau| > 1$. What perhaps was
unexpected was the observed increase in the magnitude of $\tilde{s}_f$ as $\tau$ increased. This increase is an unfortunate consequence of the shift in probability mass of fixed mutations relative to origination abundance (Figure 6-3), and ultimately leads to synergy from $u_f$ and $\tilde{s}_f$.

That is, not only do more deleterious alleles become fixed when values of $\tau$ are large but, because most of the additional probability mass comes from alleles originating at low population abundance, conditional mean selection coefficients also increase.

It should be noted that cycle periodicity must be fairly large to cause a substantive loss in accuracy for diffusion-based estimates. The decrease in accuracy increases asymptotically with $\tau$ until $\tau$ approaches a time-scale that is on the order of the extinction time estimate. Unfortunately, in some regions of large-$\tau$ parameter space, the statistical requirements for evoking a renewal process are likely to be violated. That is, for populations with low numbers of broad cycles during phase 2, it is less likely that the successive times between fixations will be exponentially (randomly) distributed.

Because they are based on the same underlying statistical processes, inaccuracy in renewal-approach parameter space will translate to similar inaccuracy for the diffusion approach. Fixation times (in generations) for each mutation are traceable in the simulation procedure; thus, the above method could be extended to quantitatively investigate the relationship between cycle periodicity and renewal-process criteria. How might this issue affect real populations, whose sizes may be highly and stochastically irregular over time? Because the overwhelming portion of fixed mutations originate during generations at which population sizes are lowest, ultimately, it may turn out that the accuracy of these approaches (and the estimate of $t_e$) depends in large part on the temporal nature (periodicity) of the most severe population declines during phase 2.
Whereas Lande (1994) speculated that partial recessivity may significantly alter estimates of $t_e$, Lynch et al. (1995b) countered that an effect would be *de minimus* at population numbers greater than ~50. Unfortunately, because of mathematical difficulties, they were unable to resolve cases for which selective effects were non-additive and non-uniform. Monte Carlo simulation provides a means to examine those cases. Mindful of the fixation dynamics of partially recessive alleles, I had two *a priori* expectations: 1) alleles with lowest selection and dominance coefficients would be preferentially fixed, and 2) if there was a sufficient number of low $s$/low $h$ alleles in the pool of mutants, $t_e$ would be lower compared to the additive case. Of interest was how much lower. To investigate this, I modeled two pools of deleterious mutations. In one pool, dominance was presumed to exert an exaggerated effect compared to what may be experienced in most animal species and, in the other pool, dominance was presumed to exert a lesser-than-expected effect. Note, in its present form, equation 6-11 does not account for the additional reduction in mean Malthusian fitness caused by segregating mutations (although it could be modified accordingly; see Lynch et al. 1995b). Thus, like Lande’s diffusion approximation, this expression is expected to overestimate mean times to extinction for very small populations ($N_{ev} < 50$). Nonetheless, for larger populations (where segregation loads are negligible [Crow 1992]), simulation results indicate that a reduction in $t_e$ attributable to partial recessivity is not likely to exceed ~20% for most species and, as Lynch and coworkers speculated, could conceivably be negligible, depending on the true distribution of dominance coefficients and their relationship to $s$.

The foregoing analyses clearly illustrate that populations of sexual, diploid organisms having undergone long periods of low abundance in the past are at risk, even if
current abundance is high. Fortunately, prior demographic events (i.e., population expansions and contractions) often leave genetic signatures encoded in DNA sequence (reviewed in Harpending et al. 1998). Thus, examination of patterns in gene genealogies in population samples, combined with long-term estimates of $N_e$ (Kuhner et al. 1999), may provide useful information to resource managers striving to maintain the long-term genetic fitness of natural populations.

An important conclusion can be drawn from the above analyses. For populations having effective numbers that fluctuate over time, the overwhelming majority of deleterious mutations that ultimately become fixed are those which originate during relative periods of lowest $N_e^V$, even though effective numbers may recover during the period of their segregation. Moreover, those fixed mutations have a higher selective cost on average than other fixed mutations. In the context of current theory, populations have a finite lifespan; mutational attrition and meltdown can only be delayed by consistently maintaining large effective population numbers over sufficiently long periods of time. Thus, when large decreases in $N_e^V$ accompany admixtures of cultured and wild fish, the natural dynamic between selection and drift is altered and a genetic debt is very likely incurred, albeit one that may take many generations to become payable. Notably, incursion of the debt is not predicated upon the existence of fitness differentials among cultured and wild fish.
Chapter 7
Summary and Conclusions

I concluded the introductory chapter with the conjecture that aquaculture policy and management might be overly focused on broodfish-source and propagation-related effects, sometimes to the exclusion of other genetic concerns. Therefore, I return to a key and admittedly counterintuitive question in this closing chapter – do released, cultured fish pose a genetic risk for recipient natural populations even when they are no more inbred or less fit (on average) than their wild counterparts and carry only indigenous genes? This question was of particular interest to me because I have, for the most part, followed the activities of stocking programs for marine organisms. It is common in these programs to collect 50-200 unrelated broodfish directly from the target recipient population, mate them, and release their progeny after very brief periods of rearing, avoiding (or at least trying to avoid) any direct form of selection or inbreeding. Can programs operating in this fashion release unlimited numbers of cultured fish with impunity? Hopefully, some light will be shed on this question as we review chapters 2-6.

Throughout this dissertation, I have devoted a considerable amount of attention to potential post-release changes in inbreeding and variance effective sizes of recipient populations, recognizing that these parameters represent indicators of expected rates of population-level change in inbreeding and drift variance, respectively. Chapter 2
originates from a publication entitled “Risk to effective population size should be an important consideration in fish stock enhancement programs” (Tringali, M. D. and T. M. Bert. 1998. Bull. Mar. Sci., 62:641-660 [Abstract omitted]). In this study, Dr. Theresa Bert, of the Florida Marine Research Institute, St. Petersburg, FL, and I adopted a mixture model developed by Ryman and Laikre (1991) to evaluate the effects of stocking upon the inbreeding effective sizes in hatchery-fish/wild-fish admixtures. Examining the sensitivity of the model’s parameters, we showed that the parameter for relative hatchery contribution, which is related to the scale of stocking, wild population demographics, and degree of population substructure, exerts the greatest influence on the effective sizes of stocked populations. When 100 effective hatchery breeders were assumed (a commonly recommended amount), no recipient population maintained inbreeding effective sizes >500 when the hatchery component of the admixture exceeded 45%, regardless of the pre-stocking $N_{el}$. Therefore, reductions in $N_{el}$ appear to be an unavoidable, although perhaps temporary, consequence of supplemental stocking programs, unless the stocked population is very small. To emphasize the effect of genetic structure on genetic risk, we developed case studies for red drum ($Sciaenops ocellatus$) and Gulf sturgeon ($Acipenser oxyrhynchus desotoi$), species that differ greatly in biology and demography and are both subjects of proposed or ongoing aquaculture programs designed for supplementation of wild stocks. Both case studies were conducted assuming that broodfish were obtained from native stocks and that released fish were themselves neither inbred nor less fit than native fish. If carried out as proposed, the stocking program for Gulf sturgeon would likely lead to inbreeding effective population sizes well below generally recommended threshold values. Overall, the analyses in this chapter suggested that we should consider
long-term genetic risks of stocking and also that the answer to the question posed above might be “yes”, if it could be demonstrated that transient decreases in the effective sizes of stocked populations lead to adverse genetic effects.

The effort in Chapter 3 involved a stock enhancement program for the common snook (*Centropomus undecimalis*). Dr. Kenneth Leber of Mote Marine Laboratory, Sarasota, FL, and I published this chapter in the Proceedings of the United States/Japan Cooperative Program in Natural Resources 27th Aquaculture Panel Symposium, *Goals and Strategies for Breeding in Fishes* (Tringali, M. D. and K. M. Leber. 1999. Bull. Natl. Res. Inst. Aquacult., Suppl. 1:109-119 [Abstract omitted]). We began by noting that certain genetic risks, especially those involving genetic swamping, may be lower in new (experimental) programs that are of limited scope and scale. We conveyed the idea that genetic management should be adaptable as a stock-enhancement program evolves from the experimental to the expanded phases of production and release. Relevant genetic, demographic, and biological data for wild snook stocks were then used to develop genetic-management recommendations regarding broodfish source and breeding/rearing protocols. A caveat – because the assumption of marker neutrality was not explored in our genomic-mtDNA restriction fragment analyses, AMOVA results and estimates of $N_{e,w}$ for snook should be interpreted cautiously; the program would benefit from a rigorous multi-locus analysis of population structure. Applying lessons from Chapter 2, the potential reductive effects of stocking on the post-release effective sizes of wild snook populations were considered. Assuming that at least 50 effective breeders will be used per generation interval to cultivate snook, we recommended that total hatchery contributions to Atlantic or Gulf stocks, respectively, should not exceed 31% in the first
generation of stocking in order to maintain inbreeding effective sizes above 500. Conservatively estimating hatchling survivorship and wild spawning stock abundance, we proposed stocking guidelines that would satisfy that recommendation.

Empirically, one of the objectives was to estimate the pre-release inbreeding effective size of a hatchery cohort – a Ryman-Laikre model parameter – in an actual captive-breeding circumstance. During the course of this activity, I learned that estimated values of $N_{el}$ can differ considerably for a given set of data, depending upon which family of mathematical models is used. Puzzled by the apparent incongruence over seemingly basic issues, I examined several contemporary $N_{el}$ models, using mathematical analyses and stochastic simulation (Chapter 4). The effort was directed explicitly toward evaluating the effects of breeding-group structure/mating systems on the accrual of gene correlations among F1 progeny (hatchlings). In the models of Dr. Ronald Chesser and coworkers, $N_{el}$ was putatively shown to increase with increasing levels of multiple paternity. Maintaining strict attention to kinship probabilities at appropriate hierarchical levels, however, my mathematical analysis showed that the number of male mates per female or the number of female mates per male exerts no independent effect on $N_{el}$. Stochastic computer simulations yielded values of $N_{el}$ in agreement with my mathematical result. Of the models studied, only the model of Crow and Denniston (1988) yielded values for $N_{el}$ that were consistent with simulated values for all cases considered (monogamy, polyandry, polygyny, and polygynandry) and for various rates of P1 to F1 population growth. I rederived this model using path analysis and also extended it to the more general case in which P1 parents may have any level of inbreeding or relatedness. The extension yields more precise (instantaneous) estimates of
$N_{el}$ via a discretized expression (equation 4-33) that may be used to track average inbreeding coefficients, coancestry, and $N_{el}$ over time. This feature will be important in the consideration of the effects of long-term (multi-generation) stocking.

With a $N_{el}$ model in hand, I returned to my empirical goals (Chapter 5). In many captive-breeding situations, especially those employed in aquaculture, direct control is not exercised during fertilization and thus the uniting of gametes is an unpredictable process; an accurate description of gene correlations among progeny under these circumstances usually requires empirical study. My objectives were to estimate $N_{el}$ for a cohort of red drum bred via an unstructured, mass-spawning method and to partition the causes for reductions in $N_{el}/N_B$ ratios (where $N_B$ is the actual number of breeders). A maximal $N_{el}/N_B$ ratio, i.e., one as close to unity as possible, is desirable in these situations. I also tested for differential survivorship among related and non-related hatchlings during extended rearing in captivity. First, using numerical analyses based on expressions derived in Chapter 4, I showed that $N_{el}/N_B$ ratios should not be significantly reduced for small spawning groups unless family size variance exceeds (approximately) the square of the mean number of contributing parents and/or unless parental sex ratios are highly skewed. I used this theoretical exercise to underscore that if culturists equilibrate broodfish sex ratios and maintain reasonable levels of family size variance, then $N_{el}/N_B \approx 1$ is possible. I then used mitochondrial DNA markers to empirically determine maternity for progeny from a cohort produced by four discrete spawning groups, finding that the two most successful dams accounted for 50% of the total number of offspring in the cohort. The estimated variance in maternal contribution for the cohort was extremely high – greater than the square of the mean number of offspring per female. Allowing for
maximum and minimum values of paternal variance, $N_{el} / N_B$ ratios for the cohort ranged 34%-58%. Extra-binomial maternal variance accounted for approximately 37% of the reduction in the $N_{el} / N_B$ ratios. At most, i.e., if there were total male dominance within each spawning group, the variance in individual male contribution to family size would have accounted for a 28% reduction in $N_{el} / N_B$. A maximal 5% reduction in $N_{el} / N_B$ could be attributed to disproportionate offspring contributions from spawning groups. Significant differences were observed in the relative frequencies of maternally related offspring between early and late collections, suggesting that a maternal component, either heritable or phenotypic, influenced hatchling survivorship during rearing. Perhaps the most important aspect of the work in this chapter was the illustration that, when spawning groups are small, $N_{el} / N_B$ ratios approaching unity should be readily achievable in unstructured hatchery breeding programs with modest attention to sex ratios in spawning groups and individual variance in offspring production within and among progeny groups.

Finally, I addressed a potential long-term consequence of hatchery-induced reductions in effective population size (Chapter 6). In a theoretical process commonly referred to as “mutational meltdown”, the cumulative damage caused by mildly deleterious alleles is thought to threaten the viability of natural populations. The rate of approach to mutational meltdown is putatively inversely and asymptotically related to $N_{ev}$. Cultured fish, when they are propagated in very large families and by using comparatively few breeders, can significantly reduce $N_{ev}$ in population admixtures, at least transiently, and could conceivably hasten the approach to mutational meltdown. Unfortunately, prior mutation-meltdown models were developed with the assumption that
$N_{eV}$ remains constant over time and have failed to account for biologically realistic modes of gene action. I used renewal theory to generate the probability distribution for the expected time ($t_e$) at which mean Malthusian fitness ($r$) ≤ 0 and Monte Carlo sampling to approximate the expectations for that distribution under relevant conditions. This approach overcomes several mathematical limitations inherent in prior theoretical analyses of mutational meltdown, e.g., it can accommodate fluctuating population sizes and recessive gene action. Estimates for $t_e$ made via the diffusion-based formula of Lande (1994) were generally consistent with those generated in simulations when population size remained constant over time and exponentially distributed selective effects ($s$) were additive. When population size fluctuated cyclically over time, however, the diffusion formula was shown to be increasingly inaccurate when $|\hat{s}_f \tau| > 1$, where $\hat{s}_f$ was the average selection coefficient of fixed mutations and $\tau$ was cycle periodicity. In general, for cycling populations, the great majority of deleterious mutations that become fixed were those originating during (relative) periods of low $N_{eV}$, even though effective sizes may have recovered rapidly. Because of their prevalence and higher selective cost (on average), $t_e$ is greatly influenced by low-$N_{eV}$ fixations. Thus, the accuracy of the diffusion- and renewal-based approaches likely depends principally on the temporal distribution of the most severe population declines. When $N_{eV} > 50$, reductions in $t_e$ attributable to partial recessivity are expected to be lower than 20% for most animal populations, depending on the true distribution of dominance coefficients and their relationship to $s$. In the context of current theory, it is expected that when large decreases
in $N_{eV}$ accompany admixtures of cultured and wild fish, the genetic lifespan of the population (time to inviability) will be significantly reduced.

From all of this, I draw the following general inference – cultured fish may indeed pose a genetic risk to recipient populations even when broodfish originate from the recipient stock and artificial selection, domestication, and inbreeding have been minimized during propagation. The mechanism associated with this risk is the transient but (usually) considerable post-release reduction in effective population size expected when cultured fish are derived from comparatively few parents and released in large numbers relative to the abundance of the recipient stock. Fortunately, a small but number of growing researchers (i.e., Waples and Do 1994, Utter 1998, Hedrick et al. 2001, Phillip et al. 2002, Hedgecock and Koykendall, in press) have recognized and considered this mechanism. In addition to broodfish source and breeding tactics, resource managers should be more mindful of both the scale and duration of interactions between stocked fish and wild fish. It would be prudent also to recognize that the potentially damaging consequences of greatly reduced effective sizes in stocked populations might not become apparent for many generations.

In closing, I point out that a comprehensive theory to address the genetic impacts that cultured fishes may have on natural populations still does not exist. Such a theory would necessarily incorporate the major genetic processes – drift, migration (between the hatchery and wild stocks and among wild stocks), selection, and mutation in light of the temporally and spatially heterogeneous environments that fish occupy. Many have studied these processes independently in the context of cultured fish/wild fish interactions; some components (e.g., selection and migration) have been addressed
jointly. Herein, I modeled jointly but in a general manner the processes of mutation, selection, and drift, noting potential connectivity with the issue of released, cultured fishes via effective population sizes. Although I extended current mutational meltdown theory to accommodate more realistic single-locus fixation dynamics, I, like others, was forced to neglect the genetic background in which new deleterious mutations arise, the influences of temporally/spatially heterogeneous environments, and potentially confounding issues stemming from the use of single-locus model. Until all relevant genetic processes are explored jointly and in a robust manner, it will be difficult to predict the extent to which cultured fish may genetically impact natural populations, and whether the expectations for those impacts will be that they are detrimental, negligible, or, in some cases, beneficial.
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Appendices
Appendix A: General Definitions and Terminology for Chapters 4 and 5

In hatcheries and in the wild, some organisms are serial breeders; each female may breed (e.g., spawn) multiple times during a given breeding season. *Broodfish* are all potential hatchery parents (sires and dams, generation P1) that collectively comprise the overall “breeding group” for hatchling production during a given breeding season. The total number of broodfish in the breeding group is denoted $N_{BT}$. A *spawning group* is composed of all potentially interbreeding male and female broodfish, e.g., those within a spawning tank. The total number of broodfish in the $j$th spawning group is denoted $N_{B,j}$.

*Hatchlings* are the captive-bred offspring (progeny, generation F1) of broodfish. In most biological disciplines, the term *brood* refers to the offspring of a single female during a lifetime of production, single breeding season, or single breeding event. However, in some aquaculture sectors, the term brood may be used casually to describe the progeny from a single (synchronized) “spawning event” to which multiple females may have contributed. For clarity, I adopt the standard biological usage and define a brood as all progeny produced by a particular female within a spawning group, in this case, during a season of hatchery production. I employ an alternative term – *clutch* – to denote offspring from a single female from a single spawning event. When multiple females contribute in a group-synchronous spawning event, their clutches collectively form a *lot* (not a brood). All hatchlings produced by a spawning group collectively form a *progeny group*. All hatchlings collectively produced by the breeding group during a specified unit of production form a *cohort*. Some level of genetic *polygyny* occurs when at least one male successfully mates with more than one female within the spawning group. *Harem
Appendix A (Continued)

*Polygyny* occurs when multiple females maintain breeding fidelity with a single male. *Polyandry* occurs when multiple males maintain breeding fidelity with a single female. *Polygynandry* occurs when multiple males mate with multiple females. In contrast to *single paternity* (including *monogamy*), *multiple paternity* occurs when more than one male successfully mates with a given female.
Appendix B: Estimation of $N_{el}$ by Monte Carlo Simulation

A probabilistic approach to determining $N_{el}$ follows directly from the definition of coancestry and may be accomplished via stochastic simulation. That is, Monte Carlo computer simulations can be used to estimate the probability ($P$) that two randomly chosen, homologous genes from two individuals (generation F1) within a progeny group were derived from the same parent (generation P1). In conducting such simulations, I considered a single locus and assigned to each parent two copies of a unique allele at that locus. Progeny distributions were derived by assigning genotypes to each offspring in the progeny group based on the breeding dynamics of the parents in that group. In simulations, a trial consisted of two processes. First, two individuals were randomly selected with replacement from the progeny distribution. Second, from each of the two selected individuals, one allele was randomly selected. For each trial, there were two possible outcomes; i.e., the two chosen alleles from the chosen individuals were derived from the same parent (Outcome 1) or from different parents (Outcome 2). Thus, the probability $P$ that two randomly chosen homologous genes were derived from the same parent was the number of trials having Outcome 1 / number of trials.

In addition to providing an estimate of $N_{el}$, I used the simulations to simultaneously provide direct estimates of the probabilities $\phi_f$, $\phi'_m$, and $\psi_{FS}$. That is, for each trial, the two randomly chosen individuals could have been products of the same mother (full sibs or maternal half sibs; Outcome 3) or of different mothers (paternal half sibs or unrelated; Outcome 4). Accordingly, $\phi_f$ was the number of trials having Outcome 3 / number of
Appendix B (Continued)

trials. Similarly, the two randomly chosen individuals could have been products of the same father (full sibs or paternal half sibs; Outcome 5) or of different fathers (maternal half sibs or unrelated; Outcome 6). Accordingly, $\phi_m'$ was the number of trials having Outcome 5 / number of trials. Lastly, the two randomly chosen individuals could have been products of the same mother and the same father (full sibs; Outcome 7) or not from the same mother and same father (not full sibs; Outcome 8). Accordingly, $\psi_{FS}$ was the number of trials having Outcome 7 / number of trials.

For each progeny-group, 20 simulations, each consisting of 1000 trials, were performed. The means and standard deviations of $P$, $\phi_f$, $\phi_m'$, and $\psi_{FS}$ for each progeny group were determined. The inverse of $P$ equates to the initial inbreeding effective size (sensu Chesser et al. 1993) based on intragroup gene correlations among F1 progeny.
About the Author

Michael Tringali received a Bachelor of Science degree in Biology from the University of Florida in 1986 and a Masters degree in Marine Science from the University of South Florida in 1991. He also began his Career-Service Employment with the Florida Marine Research Institute in 1991 and remains as an Associate Research Scientist, conducting and overseeing grant-funded research on the molecular phylogenetics of fishes, fisheries stock identification, and the genetic management of stocking programs. Michael has authored/coauthored 15 peer-reviewed journal articles and one book chapter. He has also reviewed numerous article submissions for scientific journals and grant proposals for state and federal agencies, including NSF, NOAA, and SeaGrant. Most recently, he has participated on the Genetics Stocking Policy Committee for Florida’s Fish and Wildlife agency and has coauthored the associated policy statement.