

FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION
GENETIC POLICY
FOR THE RELEASE OF FINFISHES IN FLORIDA

By the Florida Fish and Wildlife Conservation Commission Genetics Policy Committee:

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EXECUTIVE SUMMARY

- It is the policy of the Florida Fish and Wildlife Conservation Commission that all activities involving the release of finfish shall be undertaken with full consideration of their impact on natural biological diversity and in ways that do not threaten the state's natural biological heritage.
- This policy applies to activities involving the intentional or unintentional release of cultured finfish into waters of the state, including activities conducted by the Commission, and where authorization is required for the importation of live fish into Florida.
- Released, cultured fish may come into contact with wild members of their species or with other species through a variety of aquaculture-based activities, including:
 - Restoration or enhancement of a natural stock,
 - Establishment and maintenance of 'put-and-take' fisheries,
 - Other purposeful introductions, and
 - Accidental or inadvertent introductions of commerce aquaculture.
- Genetic concerns related to these activities may be grouped into four categories:
 - Impacts from translocations of non-indigenous genes,
 - Impacts from propagation-related genetic effects,
 - Impacts from excessive genetic input into natural stocks, and
 - Indirect genetic impacts.
- The potential impacts stemming from these genetic concerns include the loss of wild population fitness and viability, altered natural genetic diversity within and among wild populations, and the reduced long-term adaptive potential of wild populations.
- Specific, actionable guidelines (policy standards) and associated management practices that facilitate appropriate and reasonable management of these genetic concerns include the following:
 - Releases activities that require authorization from the Commission shall undergo a Genetic Risk Assessment; a decision chart is provided for this assessment.
 - Risks for protracted release activities shall be re-evaluated at specified time intervals.
 - When indicated via the risk assessment, policy standards to be invoked shall include the following:
 - Develop a plan for genetic management of the aquaculture activity,
 - Identify geographic boundaries of relevant natural stocks,
 - Prevent the translocation of non-indigenous genomes,
 - Minimize potential impacts from propagation-related genetic changes in cultured fish,
 - Manage the proportion of cultured fish in cultured fish + wild fish admixtures,
 - Monitor recipient populations when genetic risks are deemed moderate to high, and
 - Undertake action to eliminate detected genetic hazards.
 - Suggestions and guidance are provided for accomplishing these policy standards.
- This policy shall be adaptive. Amendments and modifications shall be made as necessary, as genetic theory and analytical capabilities advance and fishery management and fish conservation needs change.
- This policy represents a consensus of opinion from the FWC Genetics Policy Committee.

MISSION STATEMENT AND SCOPE OF THE POLICY

It is the policy of the Florida Fish and Wildlife Conservation Commission (FWC) that all activities involving the release of finfish shall be undertaken with full consideration of their impacts on natural biological diversity and in ways that do not threaten the state's natural biological heritage. Within the context of the Florida Fish and Wildlife Conservation Commission's stated mission of "managing fish and wildlife resources for their long-term well-being and the benefit of people", the specific management goals addressed by this policy are the preservation and maintenance of the genetic diversity and prevention of genetic threats to the viability of Florida's natural populations of marine, estuarine, and freshwater finfishes.

The FWC genetic policy for the release of finfishes in Florida applies to all activities involving the intentional or unintentional release of cultured finfish into waters of the state, including activities conducted by the FWC, and where authorization is required for the importation of live fish into Florida. This policy shall serve as a basis for incorporating genetic concerns into the appropriate FWC rules, permits, and special activity licenses. Information is presented herein to provide justifiable and actionable guidelines that will assist entities involved in these activities. Activities involving ESA-listed species under the Endangered Species Act of 1973 shall also conform to all federal regulations and permitting requirements. The theoretical and empirical bases for the genetic concerns addressed in the policy are explained in Appendix A. A glossary of terms is provided in Appendix B.

1. BACKGROUND

In Florida, fish have been cultured and released for at least 100 years. In fresh water, the stocking of fish has long been used as a fisheries management tool. Over time, interest in the stocking of sport fishes has increased and state-sponsored captive-breeding and enhancement programs are well underway. In recent years, the conservation of genetic diversity has become a focal point associated with responsible stock enhancement in fisheries science. A number of needs and concerns that developed in association with stocking activities in Florida focused attention on the need for a statewide stock enhancement genetic policy.

Soon after the formation of the Florida Fish and Wildlife Conservation Commission in 1999, a workshop on freshwater and marine fish stock enhancement was held at Cedar Key, Florida. At this workshop, both freshwater and marine fisheries scientists voiced concerns about fish genetic stocks in Florida. Although the former Florida Game and Fresh Water Fish Commission's Division of Fisheries had developed a policy entitled "Rationale and Policy on Importation, Exportation, Genetic Alteration and Stocking of Fishes" in 1988, FWC freshwater biologists were concerned that the existence of this policy was not well known and that the guidelines it contained were not consistently applied. These scientists were especially concerned about the genetic integrity of the native subspecies of largemouth bass (*Micropterus salmoides floridanus*), the introduction of non-native fish into Florida freshwater environments, and the widespread transference of native freshwater fish among water masses. FWC and Mote Marine Laboratory marine biologists had already developed genetic management practices for red drum (*Sciaenops ocellatus*) and common snook (*Centropomus undecimalis*) enhancement programs. These and other scientists were becoming increasingly concerned about marine stock enhancement efforts that were in various stages of development throughout the state. Finally, FWC marine fisheries staff who wrote Special Activity Licenses for the capture, rearing, and

release of fish needed to incorporate population genetic considerations into their permitting process for these types of activities. Thus, to promote the responsible use of cultured fish, the FWC is formally incorporating the consideration of genetic concerns into its research and regulatory processes. The need for a genetic policy that addresses the release of cultured fish was recognized and, in 2001, an *ad hoc* Genetics Policy Committee was appointed to formulate it.

Regulatory and statutory authorities currently exist that pertain to the release of non-native freshwater and marine species into waters of the state. Rules 68A-4.005 and 68A-23.008, F.A.C., set forth regulations and permitting conditions for the transportation, introduction, and possession of non-native freshwater species in state waters. Title XXVIII, Ch. 370.081 of the Florida Statutes prohibits the release of any non-indigenous saltwater species into waters of the state and regulates the importation and possession of non-indigenous marine species that may endanger the state's marine resources or pose a human health hazard. Notwithstanding existing rules and statutes, activities leading to the intentional or unintentional release of any non-native finfish species into waters of the state shall be considered as within the scope of this genetic policy; genetic concerns associated with these activities are addressed explicitly in the following sections.

2. RATIONALE

A policy is necessary because genetic diversity within and between Florida's wild fish populations is a valuable resource. In a particular environment, genes can gain a range of functionality through mutation. Mutation, natural selection, genetic drift, mating systems, and the transport or dispersal of gametes by natural processes all combine as forces that influence genetic variation within individuals and genetic diversity among populations. The actions and interactions of the genes carried by individuals strongly influence their morphological, physiological, and life history traits and these traits influence the transmission of genetic material to future generations. Genetic influences acting on individual fish within groups result in the numerous, diverse populations and species of fish that have adapted to the environments they inhabit.

When there is excessive genetic input from cultured fish, the genetic diversity of natural populations can be compromised by losses in genetic variation and by alterations in genetic composition. If the cultured fish are non-native, the problem may be confounded by the introduction of non-indigenous genes into the gene pool of a local population, which presumably contains genes and gene complexes adapted to the local environment. Theoretical analyses indicate that, as a consequence of any of these processes, the average fitness in wild populations or species may be reduced or those populations or species may lose the genetic diversity needed to adapt over time to changing environments.

Unfortunately, the outcomes of population genetic processes are, by nature, uncertain. Established genetic theory can be used to forecast the most likely outcomes from a given series of events, but specific outcomes cannot be guaranteed. Genetic impacts, particularly those stemming from introductions of non-indigenous genes, are difficult if not impossible to reverse. In addition, some genetic hazards set into motion in the present may not become detectable until many generations have past. Responsible management of genetic resources therefore requires a precautionary approach.

Genetic concerns arising from the release of fish may be addressed through management policies and practices. Despite uncertainties, captive breeding and release programs can proceed without undue immediate harm to the genetic diversity of recipient populations, if population genetic concerns are managed throughout the activity (Epifanio et al., 2003). For example, scientists in Florida are working cooperatively to test various strategies for red drum stock enhancement in an effort to devise a plan for responsible stock enhancement of this species (Bert et al., 2003). Genetic management is also practiced in other Florida-based marine enhancement programs involving the common snook (Tringali and Leber, 1999) and the red snapper *Lutjanus campechanus* (K. M. Leber, pers. comm.). Genetic concerns have long been in the forefront of many conservation plans for endangered fish species such as the Pacific salmonids (Flagg et al., 1995; Hedrick et al., 2000) and sturgeons (Lowie, 2000; Ireland et al., 2001).

3. ACTIVITIES INVOLVING THE RELEASE OF CULTURED FISHES

Released, cultured fish may come into contact with wild conspecifics or other species through a variety of aquaculture-based activities. The potential for and nature of genetic impacts usually differ with respect to the type of activity, the cultured species, and the species composition of the recipient fish community. These activities may be broadly categorized as follows:

3.A. Restoration or enhancement of a natural stock

Cultured fishes are often released for the purpose of augmenting imperiled, depleted, over-harvested, or under-producing populations. These activities are generally conducted within the population's historical range. We delineate these management efforts into two types – stock *restoration* and stock *enhancement*. Stock restoration, or “conservation aquaculture” (*c.f.* Utter and Epifanio, 2002), applies to natural populations whose present demographic status ranges from non-self-sustaining (inviable) to locally extirpated. The goals of stock restoration are to maintain the demographic stability and biological diversity of a non-viable population through captive breeding and release until such time that naturally self-sustaining stock can be reestablished. Unless the root cause for the population's threatened or endangered status is also addressed, these goals are not likely to be accomplished and the potential for genetic damage to the remnant wild population is high. In extreme cases, when the native ecosystem is incapable of supporting the restoration efforts, an imperiled stock is sometimes maintained in captivity or outside of its historical range.

Stock enhancement, also known as stock supplementation, targets naturally reproducing populations that are demographically viable (*i.e.*, self-sustaining) and usually subject to fishery harvest. This activity represents an attempt to offset harvesting pressure or to capitalize on a presumed underutilized carrying capacity of the environment to expand stock size. The goal of this activity is generally to reinforce, through the release of many cultured conspecifics, the number of breeders in the reproductive population.

For both restoration and enhancement activities, the transfer of genetic material from cultured organisms into natural populations is expected, and, when it is not detrimental, is consistent with the goals for those activities.

3.B. Establishment and maintenance of 'put-and-take' fisheries

As implied in the name, put-and-take aquaculture is the artificial propagation and release of harvestable-sized fishes, frequently into a population of wild fish of the same species and with the objective of subsequent recreational or commercial harvest of all released fish. Closely related to this activity is put-grow-and-take aquaculture, in which the released fish are of sublegal size and must attain legal size in the wild. Because the goal is to provide fish for harvest within a single generation, the level of genetic input from cultured fish into recipient populations might be inversely related to the program's success. By definition, put-and-take and put-grow-and-take fisheries are maintained by continued stocking rather than by subsequent reproductive contribution of the released fish. However, some released fish might escape harvest and form self-sustaining feral populations or interbreed with native wild fish, leading to the mixing of cultured fish and wild fish genomes.

3.C. Other purposeful introductions

Some purposeful introductions involve the release of cultured fish into new habitats or locations outside the normal range of the source population from which those fish were derived. Sometimes these releases are used to create self-sustaining, fishable populations in man-made water bodies or severely altered habitats. In other instances, introduced fish are used as "natural" control measures for undesirable fish, plants, or invertebrates. Fish can also be introduced by well-meaning humans. Examples include the following:

- (1) Sport fish (e.g., largemouth bass, bluegill, channel catfish) are stocked into newly created or renovated impoundments to create recreational fishing opportunities.
- (2) Hybrid or other non-indigenous sport fish are stocked into severely altered or degraded waters to provide recreational fishing opportunities and/or control undesirable fish species.
- (3) Grass carp and mosquitofish are introduced into waterways to control aquatic weeds and mosquitoes, respectively.
- (4) Prey species are intentionally introduced into lakes and streams to provide food for carnivorous sport fish.
- (5) Baitfish are discarded from bait buckets into waterways by fishermen who, for some reason, do not use those fish.
- (6) Rather than destroy them, non-native aquarium fish are released into open waterways by well-meaning hobbyists.

Even when such activities occur outside of the natural habitats or ranges of natural populations (e.g., in man-made lakes or ponds), transference of genetic material from these fish into native populations may occur through interbreeding with native species. Spread of exotic genes can be exacerbated if the introduced fishes immigrate or are translocated by some extrinsic process (e.g., flooding, hurricanes, human transport).

3.D. Commerce Aquaculture

Commerce aquaculture refers to the production of fish sold for food, bait, aquarium commodities, or *ex situ* research or educational purposes. Whereas any releases resulting from this type of commerce aquaculture will usually be unintended, an operation's proclivity for escapement of its fish must, nevertheless, be considered by the appropriate regulatory entities. Such escapement may range

from chronic (recurring, e.g., a through a regular activity such as fishing or harvesting) to sporadic (occasional or unique) and from low-level to massive.

4. GENETIC CONCERNS

For each activity identified in 3.A-3.D, the potential for genetic impact will vary among species and depends, in part, on the abundances of the species in particular populations and on the species' life history and genetic diversity. There are numerous ways in which released, cultured fish can have direct or indirect genetic impacts on natural stocks. Direct impacts are those within- or among-stock genetic changes that occur following the transfer of genetic material from cultured fish into wild-population gene pools. Indirect impacts are defined here as the genetic changes in native stocks that are either caused by released fish or are related to their presence, but that occur in the absence of genetic exchange. Listing all potential direct and indirect genetic concerns for native stocks would encompass a broad range of possibilities. However, relevant concerns can be categorized so that specific, actionable guidelines can be developed that facilitate appropriate and reasonable management of genetic risks. That is, genetic concerns are grouped herein according to their proximate cause, because it is the proximate cause that will be the focus of prescribed best management practices. For this policy, the majority of genetic concerns may be grouped into the following four categories. These concerns are more fully explained and justified with empirical evidence and supporting information in Appendix A.

4.A. Impacts from translocations of non-indigenous genes

Genetic concerns within this category apply when released fish are derived from source natural populations that are genetically divergent from the recipient natural population. Unless cultured fish are made sterile prior to their release, the potential for genetic exchange between them or their descendants and wild individuals of the same or closely related species must be considered. When members from genetically divergent stocks interbreed, the ability of their progeny to survive and reproduce may be negatively impacted such that the fitness of the admixed population may decline. This reduction in fitness, which is caused by the disruption of genetic adaptation to the local environment, is known as "outbreeding depression." Relationships between non-native translocations and associated harmful genetic effects are diagrammed in Figure 1. Supportive scientific information is presented in Section 7A.

4.B. Impacts from propagation-related genetic changes in cultured fish

Genetic hazards in this category are associated with genetic changes in released fish (themselves) that have resulted from the process of artificial breeding and/or captive rearing. For example, because of intentional (trait-specific) selection or unintentional (domestication) selection, cultured fish may have genes or genomes that are not suitable for the environmental conditions into which they will be released. If the cultured fish and wild fish interbreed, the maladapted cultured individuals may trigger outbreeding depression in the recipient natural population. The potential for genetic impact from outbreeding depression will be influenced in part by the relative proportion of cultured fish in a cultured fish/wild fish admixture. Transgenic organisms may be especially dangerous if the genetic modification affords a mating advantage over wild conspecifics. Relationships between propagation-

related genetic changes and associated harmful genetic effects are diagrammed in Figure 2. Supportive scientific information is presented in Section 7B.

4.C. Impacts from excessive genetic input into natural populations

Genetic hazards in this category are associated with the magnitude and duration of release activities – i.e., when the proportion of cultured fish in an admixed population is too high and this condition exists for too long. Genetic swamping is the condition in which artificial genetic input exceeds the level at which a population can maintain its natural genetic state. Because the genetic compositions of hatchery populations rarely match those of wild populations, genetic swamping can compromise the genetic diversity and alter the fitness of natural populations even when inter-stock translocations have not occurred and when propagation-related effects, such as domestication selection, have been minimized. Possible outcomes of genetic swamping include the replacement of wild alleles with those derived from cultured fishes, the loss of fitness due to accumulation of deleterious mutations (genetic load), and the loss of potential for adaptation to environmental changes due to reduced genetic variation and altered genetic composition. Relationships between genetic swamping and associated harmful genetic effects are diagrammed in Figure 3. Supportive scientific information is presented in Section 7C.

4.D. Indirect genetic impacts

The transference of genetic material from cultured fish to wild fish may not be required for a detrimental genetic impact. It has been suggested (Waples, 1991) that the presence of cultured fishes may alter the natural selection regimes of the recipient populations, leading to genotypic and phenotypic modifications in the natural population. In addition, release practices that cause fragmentation or large reductions in the abundance of natural populations via competition, predation, or disease transmission may lead to concomitant reductions in the genetically effective sizes of those populations (N_e) to problematically low levels. Removing fish from threatened or endangered populations for use as broodfish may also reduce abundance and thus N_e . Large, sustained reductions in N_e may result in reduced variability, deleterious mutation accumulation, and inbreeding in the natural population. Thus, indirect processes merit consideration with respect to their potential for long-term genetic harm. However, it is anticipated that concomitant and more proximate non-genetic impacts associated with indirect genetic effects will be appropriately managed. This committee, by consensus agreement, will make no specific policy recommendations regarding indirect effects. Supportive scientific information is presented in Section 7D.

5. STANDARDS FOR THE RELEASE OF CULTURED FISHES

This Commission recommends and encourages the appropriate state agencies to set forth and enforce specific policies and management practices to prevent escapement of all non-native live products from commerce aquaculture activities. The remainder of this section establishes standards for those stock enhancement and restoration activities, put-and-take activities, and purposeful introductions that require authorization from the Commission. Because the evolutionary history, current demographic status, extrinsic influences, and intrinsic characteristics of each population are unique, activities involving the release of cultured fish shall be evaluated via risk assessment and managed on a case-by-case basis. Risk

assessments shall be conducted by qualified staff members of the FWC. Policy standards shall be consistently applied. The Decision Chart that shall be used to evaluate the genetic risk of proposed activities is provided in Figure 4. Upon evaluation, management practices shall incorporate the following policy standards where appropriate.

5.A. Develop a plan for genetic management of the aquacultural activity.

Based on the results of the risk assessment, program managers may be required to develop a Genetic Management Plan (GMP) for an aquacultural activity. A template for this plan is provided in Appendix C. The principal goal of the genetic management plan shall be the preservation and maintenance of the genetic diversity and fitness of potentially impacted natural populations. Genetics concerns raised in Sections 4.A-4.C of this policy shall be addressed in the plan. The range of scopes, goals, and criteria for success of the various types of aquacultural activities is broad and diverse. Programs that serve multiple purposes (e.g., commercial production and stock enhancement) may have complex objectives and be subject to greater genetic challenges. Program objectives and expected benefits shall be clearly stated in each plan. For stocking programs (activities 3.A-3.B), the maximum numbers of fish to be released per unit time and all locations of release shall be provided.

5.B. Geographically delineate natural stocks

During the risk assessment, boundaries of “natural populations” or, equivalently, “natural stocks” of potentially impacted wild fish shall be identified. Program managers may assist in the process by supplying relevant information. A natural population (stock) shall be defined as a genetically or biologically distinct group of fish whose members naturally interbreed in the wild to produce subsequent generations of young fish. Consistent with federal policy (Fay and Nammack, 1996), biological, morphological, ecological, or behavioral differences between groups of fish shall be presumed to have an underlying genetic (heritable) basis unless reasonably concluded otherwise by a preponderance of evidence. When genetic and biological data are unavailable or inconclusive, a precautionary approach is warranted; conservative geographic limits shall be evoked for stock boundaries. For example, fish within a given aquatic system, watershed, or 500-yr floodplain may be considered *a priori* to be members of a common, discrete gene pool. An estimate of the size (abundance) of each stock affected by the proposed activity will comprise a necessary element for the evaluation of the proposed activity.

5.C. Prevent the translocation of non-indigenous genomes

Program managers shall employ appropriate aquacultural practices to ensure that genetic material is not transferred between different stocks. For stock enhancement programs, broodfish shall originate from the appropriate stock. For stock restoration programs, broodfish shall come from the appropriate stock or, when this is not possible, from the most closely related wild stock. Likewise, for put-and-take and purposeful-introduction activities, when there is a possibility that some cultured fish could reproduce with wild conspecifics, broodfish shall be obtained from appropriate stocks. Sterilization of hatchlings (e.g., by generating triploid broods), to the extent that it is effective, represents a means of containing exogenous genomes for some put-and-take or purposeful-introduction activities.

5.D. Minimize impacts from propagation-related genetic changes in cultured fish

Cultured fish that have been genetically modified by exogenous gene insertion (i.e., transgenic fish) shall not be released if natural conspecific populations exist in state waters. The remainder of this policy standard applies to all enhancement/restoration programs and to programs of put-and-take and introduction where interactions between cultured and wild fish are likely. When percentages of cultured fish are expected to exceed 5% of the stock abundance in cultured fish/wild fish admixtures for at least one generation interval, in part or entirely due to the proposed release activities, program managers shall employ breeding protocols that minimize genetic changes in cultured fish stocks. The rationale for the 5% limit is explained in Section 7C. Specifically, breeding protocols shall strive to achieve the following:

- (1) Incorporate broodfish that collectively encompass a suitable range of adaptive genetic variation available in the natural stock;
- (2) Avoid the production of inbred fish (see, e.g., Bartley et al., 1995; Toro et al., 1999; Wang and Hill, 2000; Fernández and Caballero, 2001; Bert and Tringali, 2001);
- (3) Use sufficiently high effective numbers of breeders (~50-200 per generation interval); and
- (4) Avoid artificial and domestication selection.

5.E. Manage the proportion of cultured fish in cultured-fish + wild-fish admixtures

Potential escapement rates and planned stocking rates shall be evaluated for all proposed activities. When percentages of cultured fish are expected to exceed 5% of the stock abundance in cultured fish/wild fish admixtures for at least one generation interval, in part or entirely due to the proposed release activities, program managers shall assess risks relating to genetic swamping. The following analytical treatments are available for forecasting cultured-to-wild fish ratios that minimize genetic risk and may be useful for evaluating proposed activities:

- (1) Allelic replacement models (Chakraborty and Leimar, 1987), to manage genetic composition;
- (2) Population-admixture models (Ryman and Laikre, 1991; Ryman et al., 1995), to avoid damaging reductions in N_e of the admixed population; and
- (3) Demographic and evolutionary models (Lynch and O'Hely 2001; Tufto, 2001), when there may be fitness differences between cultured and wild fish, to avoid detrimental increases in deleterious alleles.

These models may be used to forecast expected changes in genetic diversity and population fitness. They are based on input that describes the following: breeding strategies (number of broodfish, sex ratios, variance in family size); selective differences, if any, between cultured and wild fish; ratios of cultured fish to wild fish; and expected levels of interbreeding between those two components.

5.F. Monitor potentially impacted populations

For each stock enhancement and restoration program, genetic risks shall be re-evaluated within a period of time that does not exceed one generation interval of the target species. During the initial risk assessment or subsequent evaluation of a proposed activity, it may be determined that genetic risks are not negligible and that

a Genetic Monitoring and Evaluation Plan is necessary. If so, the project manager shall develop a program of post-release monitoring to detect genetic effects, if they occur, in the recipient wild population. Monitoring may require the use of physical tags (coded-wire tags or other implanted marks) or genetic markers to identify cultured fish or a sufficient portion of them after release. Admixture proportions and survival rates can be estimated by physically tagging stocked fish (or a portion of them) prior to release. Using genetic markers, monitoring can be used to accomplish the following tasks:

- (1) Compare levels of genetic diversity in wild stocks versus cultured stocks,
- (2) Estimate levels of genetic divergence between cultured and wild stocks, and
- (3) Directly or indirectly estimate the relative proportions of cultured and wild fish in the admixed population.

5.G. Undertake action to eliminate detected genetic hazards

The Commission shall deny or recommend denial of any proposed activity where the intended release or potential unintended escapement of finfish presents an unacceptably high risk of genetically related damages to native finfish stocks. Additionally, the Commission shall require that any ongoing activity involving the intentional or unintentional release of finfish cease if genetically related damages caused by that activity are detected or forecasted.

6. CLOSING REMARKS

Although stock enhancement and restoration are frequently used to compensate for decreases in fish population sizes, these measures should not be used to the exclusion of other methods of replenishing or restoring stocks—that of reducing fishing pressure, restoring essential habitat, and providing for natural recolonization and population expansion. It is widely recognized that protection and natural restoration of populations are more likely to provide long-term stability and perpetuity to those populations and that short-term measures, such as hatchery-based stock enhancement and restoration, should be only interim measures.

Nevertheless, the risk of genetic impact from the release of cultured fish can be greatly reduced or negated by adopting management practices that adhere to standards set in this policy. The elements of concern in this policy are consistent with established genetic theory and, in nearly all cases, are justified by empirical evidence from natural and cultured fish populations. The policy provides for a science-based, adaptive management approach in that activities shall be re-evaluated at specific time intervals and managed accordingly. This policy represents a consensus of opinion from the FWC Genetics Policy Committee and shall be reviewed periodically to ensure that guidelines are effective and in keeping with current knowledge. This policy should be adaptive; modifications should be made as necessary, as genetic theory and analytical capabilities advance and fishery management and fish conservation needs change. The success of policies such as this depend on the close interaction of scientists who study population biology and population genetics of the relevant species, user-group awareness and sensitivity to the importance of preserving the genetic integrity and diversity of the stocks that they fish, and the ability of fishery managers to recognize the importance of conserving genetic diversity and to establish rules and practices that ensure the preservation of this valuable resource.

7. APPENDIX A – JUSTIFICATION FOR GENETIC CONCERNS

The following information is based on a survey of numerous published empirical studies or review articles pertaining to genetic effects of cultured-fish + wild-fish interactions. These studies are described as they relate to the categories of genetic concern described in Sections 4.A-4.D of the policy.

7.A. Impacts from translocations of non-indigenous genes

Genetic impacts to native local populations can occur when non-native fish are introduced. Growing evidence shows that many fishes are highly adapted to local environmental conditions and that natural selection operates at various life history stages (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, Hendry et al., 2000, Secor et al., 2000). 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 that decrease the probability 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 (Templeton, 1986). When members from genetically divergent stocks interbreed, the viability of their progeny may be adversely impacted such that mean fitness (and, therefore, population maintenance or population growth rate) may decline. The loss of fitness from maladapted alleles or disruption of genomic coadaptation is referred to as “outbreeding depression” (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 (which is usually manifested as morphological deformities) in hybrid crosses (Graham and Felley, 1983; Clarke, 1993). The degree of instability and, thus, the potential for disruption of genome coadaptation, generally increases with increased genetic divergence of the two interbreeding stocks (Leary et al., 1985). Because of possible heterosis and because gene complexes generally remain intact in the F₁ hybrid generation, the effects of disrupted coadaptation are expected to be more obvious in later (backcrossed) generations (Lynch, 1991). Unfortunately, data for these generations are often not available.

Aquaculture-mediated inter-stock genetic transfers and concomitant outbreeding effects are well documented, particularly for salmonids. 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). In rivers in western Ireland, the progeny of wild (native) × farmed (non-native) *Salmo salar* generally had reduced performance in survival and incidence of parr maturity compared to wild conspecifics (McGinnity et al., 1997). *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 (Skaala et al., 1996).

Lastly, in Norway, escaped, farmed Atlantic salmon and farmed-salmon/wild-salmon hybrids show decreases in a number of fitness-related characteristics, including important components such as growth, reproduction, and survival (Hindar and Fleming, in press).

7.B. Impacts from propagation-related genetic changes in cultured fish

Cultured fish may be individually compromised when breeding protocols foster mating between related broodstock. Like most sexually reproducing organisms, fish populations contain significant amounts of hidden genetic variation in the form of rare recessive alleles (Nevo, 1978; 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 they are present in the heterozygous state in individuals. Inbreeding in a hatchery setting can bring deleterious recessive alleles together in homozygous genotypes, thereby exposing them to stronger selective forces. This may lead to a form of reduced fitness known as 'inbreeding depression' (Lynch, 1991; Crnokrak and Roff, 1999). Among cultured fishes, inbreeding depression has been manifested as lethal or harmful morphological deformities such as missing operculi (*Brachydanio rerio*, ; Mrakovic and Haley, 1979; *Cichlosoma nigrofasciatum*, Winemiller and Taylor, 1982) or caudal deformities (*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 (Kincaid, 1983; Kinghorn, 1983; Leary et al., 1985; Chilcote et al., 1986; Ferguson and Drahushchak, 1990, Leider et al., 1990). Any of these problems could be transmitted to offspring that are the hybrids of the cultured fish and wild fish. Importantly, in vertebrate animals, inbreeding depression may be manifested more as altered life history traits (e.g., growth, reproductive potential, survival) than altered morphological traits (DeRose and Roff, 1999), unless the morphological changes are severe.

Potentially detrimental genetic changes in cultured fish or within cultured stocks also may arise through targeted selection and 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) (Lynch and Walsh, 1998). In aquaculture, selection may be intentional and have as its objective the achievement of a targeted response for a particular trait such as faster growth or earlier maturity (Tave, 1993). In contrast, domestication may occur (usually unintentionally) and is characterized by selective changes in multiple traits that are adaptive in captive environments; for example, changes in reproductive biology (Chilcote et al., 1986; Crandell and Gall, 1993; Danzmann et al., 1994; Chebanov and Ridell, 1998), growth, survival (Reisenbichler and McIntyre, 1977; Crandell and Gall, 1993), or behavior (Ruzzante and Doyle, 1991; Berejikian, 1995; Berejikian et al., 1996). In many cases, multiple, correlated responses to intentional selection or domestication 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. Often, crosses between farm-raised and wild fish lead to intermediate levels of performance in F₁ progeny (Einum and Fleming, 1997), although heterosis is occasionally

observed (Thornhill, 1993). As with translocation of non-indigenous genes, propagation-related divergence in fitness-related traits between farmed and wild fish can result in outbreeding depression if the farmed and wild fish successfully interbreed (Reisenbichler and McIntyre, 1997; Currens et al., 1997; Reisenbichler and Rubin, 1999).

Transgenic strains now exist for several fishes. In addition to the immediate threat they pose to Florida's natural biological diversity, when transgenic fish have a size-related reproductive advantage, they may pose a direct genetic threat to the viability of wild conspecific populations when released. For example, Muir and Howard (1999, 2002) showed that transgenic male medaka modified with salmon growth hormone genes possessed a considerable mating advantage over wild medaka. Offspring of transgenic medaka, however, possessed a survival disadvantage. When both of these fitness components were considered in forecast models, the transgene was predicted to spread in wild populations because of the associated mating advantage and ultimately cause population extinction because of the associated survival disadvantage.

Finally, in cultured fishes for which sex is determined or influenced by environmental factors (e.g., the channel catfish *Ictalurus punctatus* [Patiño et al., 1996], the Japanese flounder *Paralichthys olivaceus* [Yamamoto, 1999], the sockeye salmon *O. nerka* [Craig, 1996], and the tilapia *Oreochromis niloticus* [Lester et al., 1989]), a risk may ensue 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 can 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 variable species, which could lead to the extinction of the Y gene in natural populations.

7.C. Impacts from excessive genetic input into natural populations

Short-term risks from genetic swamping must be considered in light of the potential impact of cultured fish on the balance between migration, genetic drift, and selection in the recipient wild population; assessment of long-term risks also necessitates considering spontaneous mutation rates. Possible outcomes of swamping include: 1) replacement of wild alleles with those derived from cultured fishes, 2) loss of adaptive potential due to reduced levels of genetic variation 3) loss of fitness due to increased genetic loads, and 4) loss of fitness due to inbreeding.

Cultured fish may be viewed as a novel class of immigrants (entering via stocking, straying, or escapement) that, at a minimum, will have different levels of genetic diversity and inter-relatedness compared with individuals in the recipient stocks and, at a maximum, will have inbred or genetically divergent, maladapted genomes. Typically, the numbers of breeders selected to generate captive-broodstock fish populations represent a small percentage of the available breeders in source wild populations. Cultured stocks can also be comparatively impoverished in genetic variability if the initial sampling for broodstock fails to capture a sufficient range of heritable phenotypic variability available within the source wild population (Leary et al., 1986). When small numbers of founding breeders are used, cultured stocks can have large random differences in allele and genotype frequencies (Taniguchi and Sugama, 1990; Hansen et al., 1997; Koskenin et al., 2002), reduced

levels of genetic variation (Taniguchi et al., 1983; Ferguson et al., 1993; Bartley et al., 1995; Clifford et al., 1998), and higher levels of coancestry (Tringali, 2003) compared to the source or recipient wild stock. Cultured fish derived from limited numbers of broodstock may not suffer individual fitness deficits after their release, but as a group, they do not contain the genetic information expected from a similarly sized wild population.

Immigration rates of cultured fish (m_c ; the rates at which cultured fish are introduced into and successfully interbreed with the recipient wild population) can be used to predict replacement rates in wild populations of alleles that are assumed to be unaffected by selection (Felsenstein, 1997; Withler et al., 2003). For example, given a 5% immigration rate per generation, approximately 10% of wild alleles will be replaced by alleles from immigrants in only two generations (Chakraborty and Leimar, 1987). 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 replaced in approximately 10 generations. Empirical evidence confirms that allelic replacement can occur quickly. In Trinidad, the genotypes of cultured *Poecilia reticulata* individuals that were introduced into 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), ~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).

Here it should be noted that a low occurrence of cultured fish in a natural spawning population does not always translate to an immediate or significant impact on genotype frequencies. Marshall et al. (2000) determined through genetic monitoring that the reproductive contribution of a small number of hatchery-derived, adult, fall-run, Columbia River chinook salmon that strayed into the Snake River had little reproductive contribution to the out-migrating juvenile Snake River population. 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.

The process of allelic replacement may operate on alleles that have adaptive importance as well, and can lead to reduced levels of genetic variance and the consequent loss of the potential to adapt to changing environments (Lynch, 1996). Felsenstein (1977; 1997) has shown that stocking over concurrent generations will overwhelm the process of natural selection when the immigration rate exceeds the average selective difference between genotypes. Many adaptive traits are polygenic and the forces of selection are distributed over these loci. Thus, most adaptive alleles probably have very small individual selective effects ($s < 0.05$; Keightley, 1994; Keightley and Caballero, 1997). If so, immigration rates exceeding 5% could be sufficient to negate the selective retention of these alleles.

Lastly, levels of inbreeding in population admixtures of cultured and wild fish may be artificially inflated when family sizes in the cultured stock are significantly larger than those in the recipient population. Inbreeding depression may result because deleterious recessive traits may be exposed through homozygosity, if near or distance relatives mate. Ryman and Laikre (1991) provided a method for predicting post-immigration changes in levels of inbreeding via a simple model for inbreeding effective sizes (N_{ei}) in admixed populations (Wright, 1978). This method has been employed for particular species within the context of stock enhancement (Waples

and Do, 1994; Tringali and Bert, 1998; Tringali and Leber, 1999; Hedrick et al., 2000; Rieman and Allendorf, 2001).

7.D. Indirect genetic impacts

Several mechanisms for indirect genetic effects in recipient populations have been proposed (reviewed in Utter 1998). Following are some typical examples:

- (1) Because it may reduce the overall number of wild breeders, supplemental stocking can be counterproductive genetically when it stimulates increased fishing pressure on the stocked population (Nehlsen et al., 1991).
- (2) Endangered, threatened, and demographically at-risk populations may not be able to support the removal of even a small number of breeders for aquaculture or enhancement activities without risk to the population's demographic stability and viability (FDACS, 2000), which in turn lowers N_e .
- (3) The release of cultured fishes may introduce or exacerbate viral or bacterial diseases or parasites in recipient populations, thereby increasing mortality rates and reducing population sizes. (Johnsen and Jensen, 1986; Fries and Williams, 1996; Bakke et al., 1990; Kirk et al., 2000).
- (4) Competition between released cultured fish and wild fishes for limited biotic and abiotic resources can lead to higher mortality rates in wild populations (McGinnity et al., 1997).
- (5) Through some advantage (e.g., size), cultured fish may prey upon conspecific wild fish.
- (6) Gamete wastage and subsequent population declines may occur when the progeny of cultured \times wild fish are sterile. This process, termed 'non-introgressive' hybridization (Utter, 1998), is an interspecific process known to have affected endangered and threatened salmonid species such as the bull trout (*Salvelinus confluentus*) via mating with brook trout (*S. fontinalis*) (Leary et al., 1995). Atlantic salmon and brown trout are also subject to the effects of non-introgressive hybridization (Verspoor and Hammar, 1991).

8. APPENDIX B – GLOSSARY OF GENETIC TERMS AND PHRASES

allele – one of the alternative forms of a given gene.

adaptation – any response to a given environment that improves the chances that an organism will survive and leave descendents. Local adaptation refers to adaptation to a restricted set of environmental conditions. Maladapted individuals have genes or genomes not suited to their present environment.

adaptive potential – a genetic concept based on a premise that populations must possess a sufficient amount of genetic variance to respond to changing environments.

additive genetic variance – the component of genetic variance associated with the average additive effects of alleles. This component is related to the rate of response to selection on quantitative traits.

admixed population – a single population of fish that includes individuals from separate source populations; in this case, wild individuals and hatchery-bred (released, cultured) individuals.

captivity – when eggs or live organisms are held in a controlled or selected aquatic environment that has boundaries designed to prevent such eggs or live organisms from entering or leaving the controlled environment.

coancestry – F , an index of relatedness; the probability that two randomly chosen homologous genes from two individuals were derived from the same gene in a

previous generation. It is a measure of gene correlations among two individuals within a group. It equates to the inbreeding coefficient (f) for any offspring that may be produced by these two individuals.

coadaptation – a result of selection in which harmoniously interacting genes accumulate in the gene pool of a population. Genomic coadaptations are disrupted when the introduction of one or more maladapted genes elicits a detrimental phenotypic response.

conspecific – member of the same species.

cultured – any organism that has spent some phase of its life cycle in captivity or that has been transported > 25 km from its site of capture.

domestication selection – non-targeted selection that results in genomes suited to the particular conditions of culture.

demography – the statistical science dealing with the vital statistics (e.g., reproductive rate, mortality rate, age-structure, distribution, density) of a population.

demographic stability – a condition characterized by a protracted period, i.e., several generations, of relatively unchanging population abundance.

effective population size (N_e) – the hypothetical 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 the actual number of individuals. Here, two types of effective population numbers are relevant – the inbreeding (N_{ei}) and the variance (N_{ev}) effective numbers. N_{ei} describes the size of an ideal population that would have the same expected rate of loss of heterozygosity as the observed population. N_{ev} describes the size of an ideal population that would have the same amount of random gene-frequency drift as the observed population. These numbers will generally be equivalent unless population abundance is not constant over time or when there is population subdivision. For example, $N_{ev} > N_{ei}$ in growing populations whereas $N_{ei} > N_{ev}$ in declining populations.

estuarine – the part of a river or stream or other body of water having unimpaired connection with the open sea, where the sea water is measurably diluted with fresh water, and extending upstream to where ocean-derived salts measure less than 0.5 parts per thousand.

exogenous – non-native, derived from a genetically divergent stock or different species.

fitness – **1.** the ability of an individual to survive and leave descendants. **2.** rate of increase in size (abundance) of a population.

gamete – germ cell, i.e., sperm and egg.

gene – a segment of DNA that occupies a specific position (locus) on a chromosome. A gene is heritable unit that may have one or more specific effects on the phenotype of an organism.

gene pool – the total genetic information possessed by members of a population unit.

generation interval – the length of time it takes to replace the members of one generation in a natural stock. In age-structured stocks, it is estimated by calculating the average age of the breeders in that stock.

genetic composition – a component of genetic diversity that describes allele frequencies within a group of fish.

genetic diversity – the characteristic of individuals or populations that describes genetic heterozygosity (at the individual level) and polymorphism (at the population level). Genetic diversity has two components: genetic variation (or variability) and genetic composition.

genetic drift – a random process whereby fluctuations in gene frequencies occur as a result of error during the reproductive sampling of gametes.

genetic variability – (genetic variation) the component of genetic diversity that describes the numbers of alleles at polymorphic loci.

genetic variance – the component of phenotypic variance attributable to genetic interactions.

genome – all of the genes carried by a single gamete or single individual.

genotype – the combination of alleles at one or more loci in an individual.

homozygous – having two copies of the same allele at a particular locus.

heterosis – a product of hybridization in which the offspring display greater fitness-related characteristics (e.g., vigor, size, disease resistance) than the parents

heterozygous – having different alleles at a particular locus.

inbreeding – mating between related individuals.

inbreeding coefficient – the inbreeding coefficient, f , is the probability that two alleles at a locus in an individual were derived from the same gene in a common ancestor. It is also the proportion of loci in an individual that are homozygous.

inbreeding depression – decreased growth, survival, or fertility resulting from inbreeding.

indigenous – native, occurring naturally, not imported or introduced.

introgression – the incorporation of genes from one distinct gene pool into another distinct gene pool.

locus – the physical location of an allele on a chromosome for DNA in a cell's nucleus. Because it is transmitted clonally, the entire mitochondrial DNA genome is considered to be a single locus.

marine (finfish) species – a species of fish, including anadromous fish, capable of having any portion of its life cycle occurring in marine or estuarine waters, excluding striped bass (*Morone saxatilis*).

non-introgressive hybridization – interbreeding between members of two species or divergent populations that results in sterile offspring.

outbreeding depression – a reduction in individual fitness that results from the interbreeding of genetically divergent individuals.

phenotype – (phenotypic variance) the observable traits (e.g., shape, color, size) or properties (e.g., salinity tolerance, habitat usage, behavior) of an organism that are produced by genotypic and environmental interactions.

polygenic – under the control of multiple gene loci.

propagation-related – a condition in offspring (in this case, a genetic change) that is a byproduct or intended consequence of captive breeding and/or rearing.

quantitative trait – phenotypes that are quantitative (measurable) in nature and continuously distributed.

release – the intentional or unintentional introduction, reintroduction, or relocation of eggs or organisms that have been held in captivity into waters of the state, including municipal waters.

region – geographic area having context dependent boundaries.

selection – a deterministic process governing the fate of alleles and allocation of different genotypes in a population. Natural selection represents the differential fecundity in nature between individuals possessing different adaptive traits or genes. Artificial selection represents the differential fecundity between individuals possessing different adaptive traits or genes because of anthropogenic culling.

selection coefficient (s) – represents a measure of the strength of selective fitness differentials among genotypes. Notably, it reflects the strength of selection on a given locus, not on an expressed trait, which may be under the influence of many loci.

selective effect – (of a gene) the impact of a gene on the probability of successful reproduction for its carriers.

stock (natural) – a genetically or biologically distinct group of fish whose members naturally interbreed in the wild to produce subsequent generations of fish.

transgenic – organisms whose genomes have been modified by the introduction or deletion of specific genetic material. Organisms created by hybridization or polyploidy techniques do not fall under this definition.

translocation – a unnatural change in geographic location because of human activity such that genetic stock boundaries are transgressed.

9. APPENDIX C – GENETIC MANAGEMENT PLAN (GMP) TEMPLATE

SECTION 1. GENERAL PROGRAM DESCRIPTION

- 1.1. Indicate name of hatchery and program.
- 1.2. Identify responsible organization and individuals:
 - 1.2.1. Name (and title)
 - 1.2.2. Agency
 - 1.2.3. Address
 - 1.2.4. Telephone
 - 1.2.5. Fax
 - 1.2.6. Email
 - 1.2.7. List other agencies, collaborators, or organizations involved, and describe their extent of involvement in the program.
- 1.3. List funding sources, staffing level, and annual hatchery program operational costs.
- 1.4. Identify location(s) of hatchery and associated facilities.
- 1.5. List all species subject to propagation (note: a separate GMP shall be completed for each species under consideration).
 - 1.5.1. Specify Endangered Species Act-listing status, if applicable, of each species (available from www.nmfs.noaa.gov/pr/species/fish/).
- 1.6. Indicate type of program (e.g., stock enhancement, restoration, put-and-take, mitigation, commercial aquaculture).
- 1.7. Identify specific performance goals and quantitative success criteria of the program.
- 1.8. Describe current program performance if the program is ongoing (indicate the source of these data).
- 1.9. Provide the date release activities started or are expected to start.
- 1.10. State the expected duration of program.

SECTION 2. RELATIONSHIP OF PROGRAM TO OTHER MANAGEMENT OBJECTIVES

- 2.1. Describe the alignment of the program with any management or recovery plan or other regionally accepted policy. Explain any proposed deviations from the plan or policy.
- 2.2. Identify existing cooperative agreements, memoranda of understanding, memoranda of agreement, mitigation requirements or other management plans or court orders under which program operates.
- 2.3. Describe the relationship of the program to harvest objectives:
 - 2.3.1. Identify fisheries that will benefit from the program.
 - 2.3.2. Provide harvest levels of those fisheries for the last ten years, if available.

SECTION 3. FACILITIES

- 3.1. Provide detailed descriptions, supplemented with diagrams, of the following:
 - 3.1.1. Broodfish holding and spawning facilities.
 - 3.1.2. Incubation facilities.
 - 3.1.3. Rearing facilities.
 - 3.1.4. Acclimation/release facilities, if applicable.

SECTION 4. BROODFISH SOURCE

- 4.1. Indicate the geographic source of broodfish (include GPS coordinates of capture site for each fish or for the area encompassed by all broodfish captures).
- 4.2. If possible, provide supporting information for the validation of natural stock boundaries, including:
 - 4.2.1 Accepted geographic boundaries for natural stocks of target species, if available.
 - 4.2.2. Genetic and/or biological information relevant to natural stock structure of the proposed recipient population (include literature citations and other sources), if available.
 - 4.2.3 Estimated current adult abundance or spawning stock biomass of each natural stock that will receive fish, if available. Indicate source of information.
 - 4.2.4 Estimated generation interval (average age of female breeders) for the natural stock, if possible. Indicate source of information.

SECTION 5. BROODFISH COLLECTION

- 5.1. Describe the collection methods and sampling design for broodfish.
- 5.2. Describe methods to make individual broodfish identifiable and/or to segregate discrete spawning groups of broodfish.
- 5.3. Specify the proposed number of broodfish to be collected from each natural stock, by general life-history stage (adults, eggs, or juveniles).
- 5.4. If broodfish are currently on hand, specify the number of these collected from each natural stock, by year and life-history stage.
- 5.5. Describe the intended disposition of fish collected in surplus of broodfish needs.
- 5.6. Describe broodfish transportation and holding methods.

SECTION 6. MATING PROCEDURES

- 6.1 Provide confirmation that no transgenic modifications will be performed to any fish and that no such fish on the premises will be involved in the proposed program.
- 6.2. Provide confirmation that there will be no attempt at genetic improvement or other intentional trait-specific selection during production.
- 6.3 Describe the timing of production in comparison to natural production and recruitment.
- 6.4 Provide a detailed mating scheme, including:
 - 6.4.1 If broodfish are not wild, the number of generations they are removed from the wild (F1, F2, etc.).
 - 6.4.2. Description of controlled fertilization procedures (e.g., paired mating, strip-spawning), if applicable.
 - 6.4.3. Description of uncontrolled fertilization procedures (e.g., pond- or tank-spawning), if applicable.
- 6.5. With supporting information, provide an estimate of variance in family size and effective number of breeders or provide the following:

- 6.5.1. Minimum number of male and female breeders to be used to produce each progeny group.
- 6.5.2. Expected number of progeny groups to be released.
- 6.5.3. Estimated average progeny group size and variance in progeny group size (at the time of release).
- 6.6. Describe your plan for tracking information in 6.5.1 – 6.5.3 during operation of the program.

SECTION 7. INCUBATION AND REARING

- 7.1. Describe incubation procedures, including:
 - 7.1.1. Incubation conditions.
 - 7.1.2. Ponding.
 - 7.1.3. Number of eggs taken and survival rates to eye-up and/or ponding, if known.
 - 7.1.4. Causes for, and disposition of surplus eggs, if any.
- 7.2. Describe your plan for tracking information in 7.1.3 – 7.1.4 during operation of program.
- 7.3. Describe rearing procedures, including:
 - 7.3.1. Fish rearing conditions.
 - 7.3.2. Provide survival rate data by hatchery life stage (fry to fingerling; fingerling to advanced size) for the most recent five years, or for years that dependable data are available.
 - 7.3.3. Indicate weekly or monthly fish growth information, including length, weight, and condition factor data collected during rearing, if available.
 - 7.3.4. Rates of cannibalism, if applicable.
- 7.4. Describe your plan for tracking information in 7.3.2 – 7.3.4 during operation of program.

SECTION 8. RELEASE PROCEDURES

- 8.1. Indicate proposed numbers and average sizes of fish to be released for the program, by age class, release year, and natural stock (size data not required for eggs, larvae, and unfed fry).
- 8.2. Indicate specific location(s) of proposed release(s) by natural stock, including the following information:
 - 8.2.1. For freshwater releases – Name of stream, river, or waterbody and GPS coordinates for each release point.
 - 8.2.2. For marine/estuarine releases - Name of estuary or description of coastal region and GPS coordinates for each release point.
- 8.3. Describe your plan for tracking actual dates of release, release numbers, average sizes of released fish, and release locations.
- 8.4. For any fish released to date, list actual numbers and average sizes of fish released, by age class, release year, and natural stock (size data not required for eggs, larvae, and unfed fry).
- 8.5. Describe tags or marks applied, if any, and the proportions of the total hatchery cohort marked, to identify released individuals in subsequent captures.
- 8.6. Describe the disposition of fish that may be produced in excess of approved release levels.
- 8.7. Describe emergency release procedures in response to flooding or other failure that may result in unintended fish release.

SECTION 9. BEST MANAGEMENT PRACTICES

Provide a description of the precautionary approaches, procedures, and practices that will be implemented to mitigate each of the three genetic concerns (i.e., impacts from translocation of non-native fish, propagation-related genetic changes, and genetic swamping) identified in sections 4.A.-4.C. of the genetic policy.

SECTION 10. ATTACHMENTS AND LITERATURE CITATIONS

Please include all supplemental material and literature citations to support statements provided in sections 1-9 above.

SECTION 11. SIGNATURE OF RESPONSIBLE PARTY

Name, Title, and Signature of Applicant:

Submitted by (print) _____ Title _____

Signature _____ Date _____

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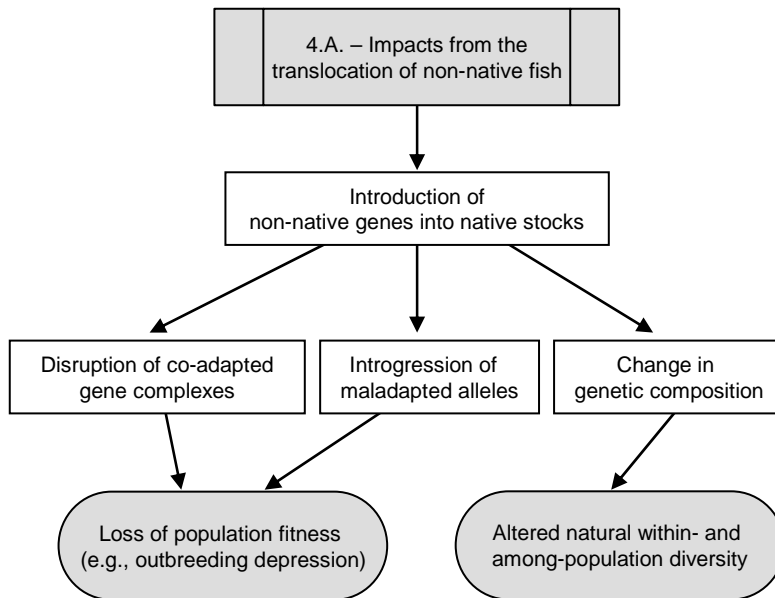


Figure 1. Cause-effect relationship between translocations of non-native fish and associated harmful genetic risks by way of intermediate processes. This relationship is summarized in section 4.A. and supported with background information in section 7.A.

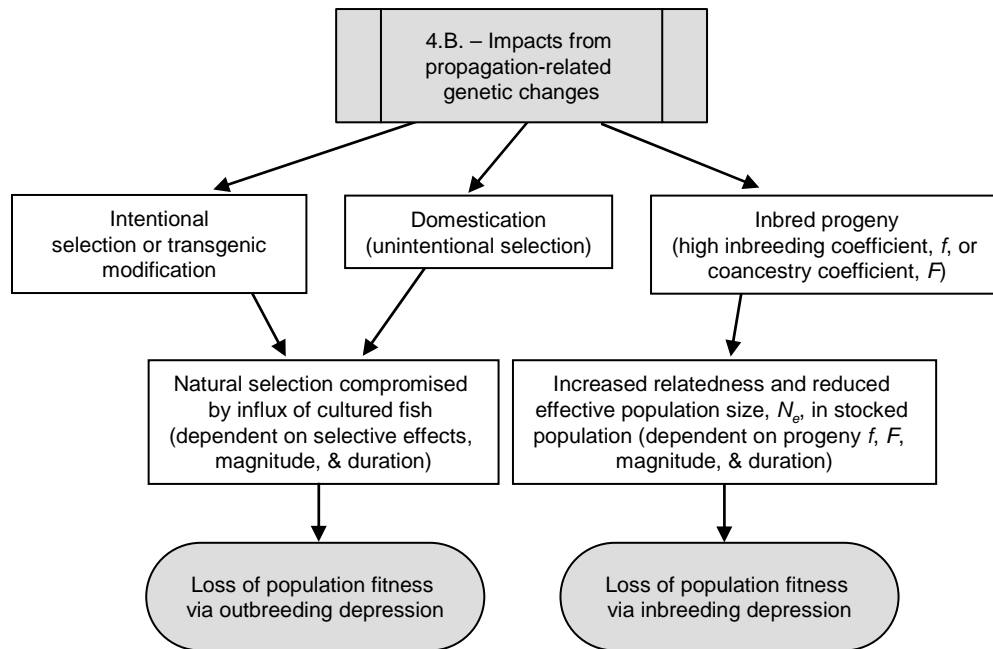


Figure 2. Cause-effect relationship between propagated genetic changes in released fish and associated harmful genetic risks by way of intermediate processes. This relationship is summarized in section 4.B. and supported with background information in section 7.B. Definitions for N_e , f , and F may be found in the glossary (section 8).

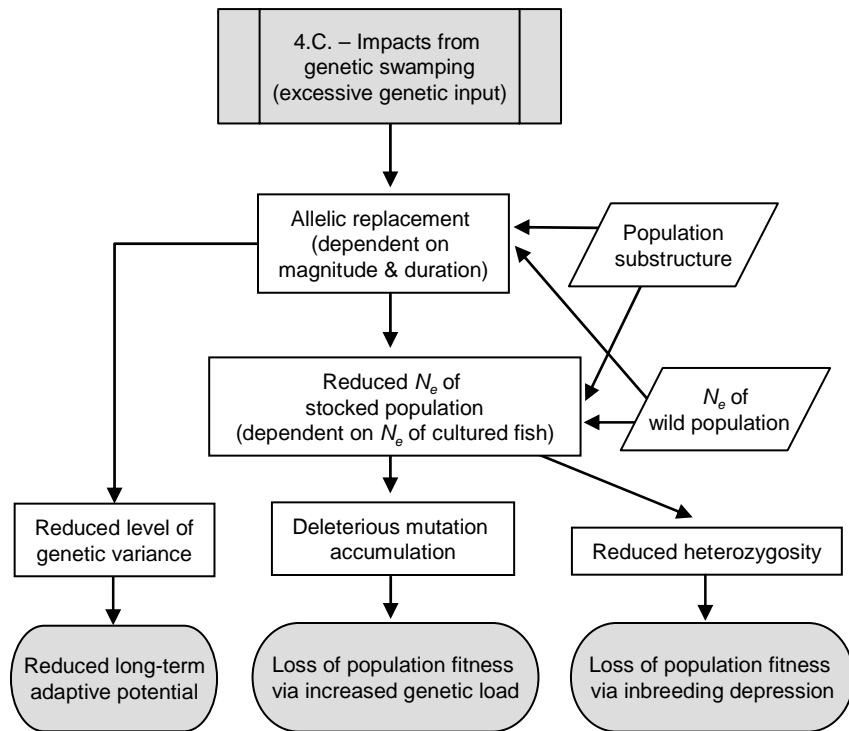


Figure 3. Cause-effect relationship between genetic swamping (excessive hatchery input) and associated harmful genetic risks by way of intermediate processes. This relationship is summarized in section 4.C. and supported with background information in section 7.C. Definitions for N_e may be found in the glossary (section 8).

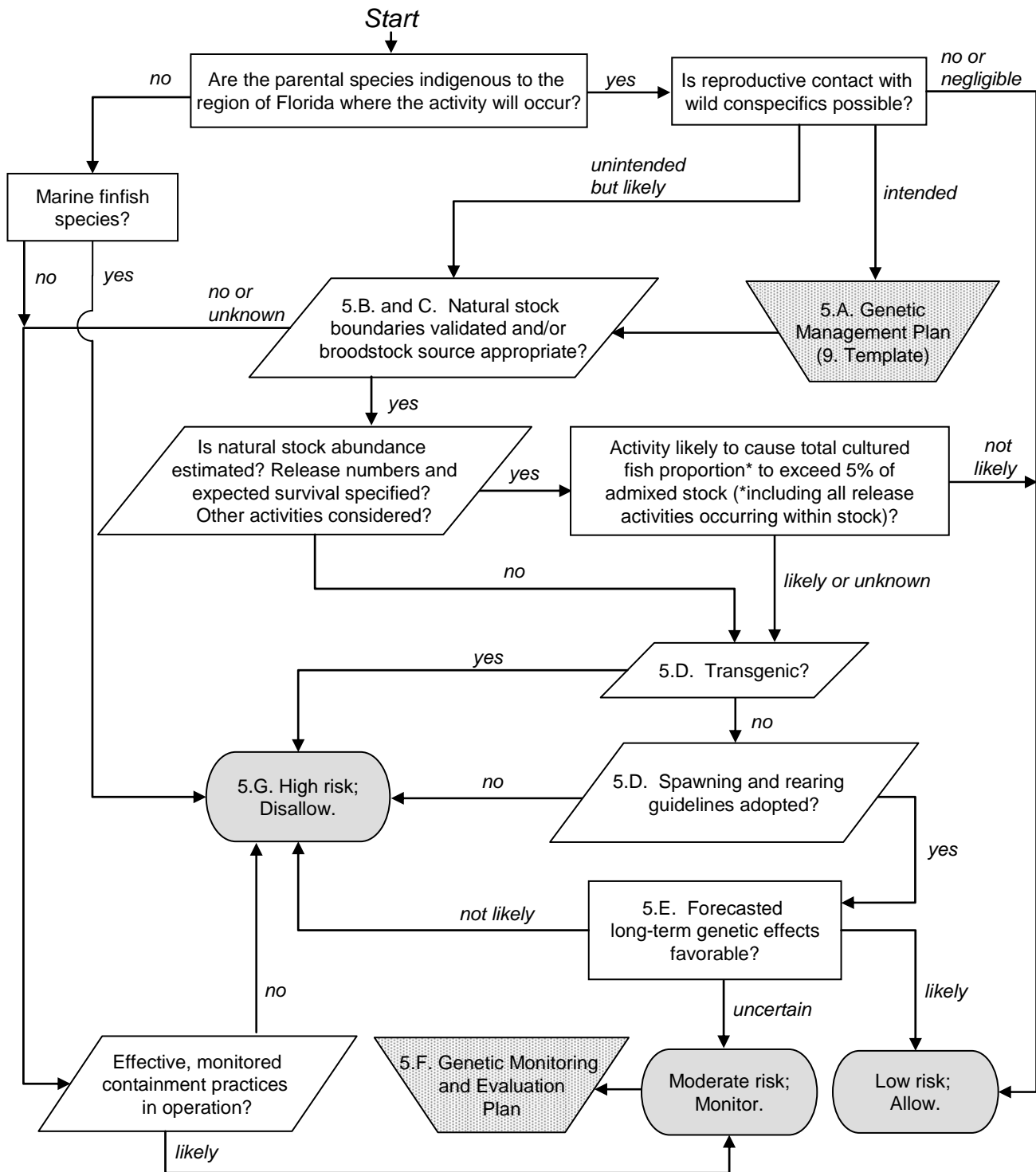


Figure 4. Diagrammatic representation of the decision process for genetic risk assessment. Where appropriate, applicable section numbers from the policy are indicated. Parallelograms identify applicant-supplied information; rectangles represent assessment decisions; grey polygons identify requisite plans; ovals depict assessment recommendations.