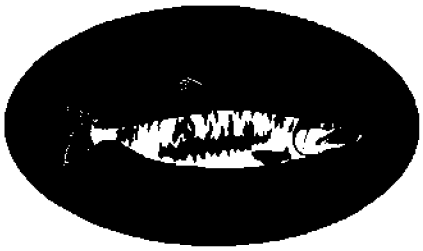

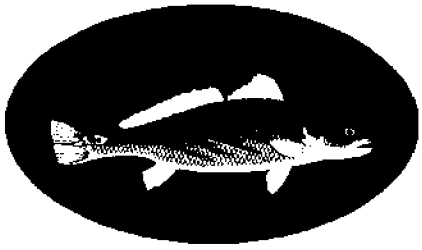

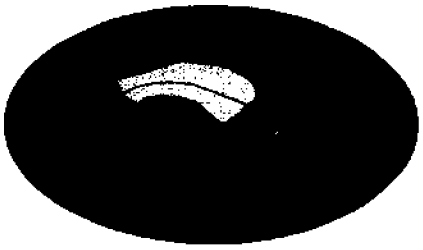

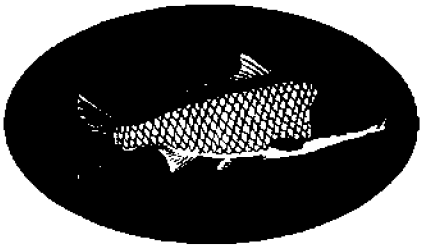

Selective Breeding of Fishes in Asia and the United States

Edited by
Kevan L. Main
and
Elizabeth Reynolds

The Oceanic Institute

Selective Breeding of Fishes in Asia and the United States

Proceedings of a Workshop in Honolulu, Hawaii May 3-7, 1993

Edited by
Kevan L. Main and Betsy Reynolds
THE OCEANIC INSTITUTE

Published by
The Oceanic Institute
Makapuu Point
P.O. Box 25280
Honolulu, HI 96825

Sponsored by
The National Oceanic and
Atmospheric Administration
United States Department
of Commerce

For additional copies, please contact:
The Oceanic Institute
Makapuu Point
P.O. Box 25280
Honolulu, HI 96825

ISBN 0-9617016-8-4

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Workshop Participants: *Front row (from left)*: Alcian Choy, Logan Duong, Cheng-Sheng Lee, Remedios Bolivar, Violet Phang, Supattra Uraiwan, Dequan Xia, William Wolters, Sudarto, Nobuhiko Taniguchi, Kevan Main, Elizabeth Reynolds, Sifa Li, Lynette Shi, (Missing: Trygve Gjedrem); *Back row (from left)*: William Smoker, Ernest Tresselt, James Kenney, James Parsons, Su-Lean Chang, Graham Gall, Eric Hallerman, William Hershberger, Chingjiang Wu, Kenneth Leber, James Shaklee, Tran Mai Thien

Preface

The Asian Interchange Program was founded at The Oceanic Institute in 1989. The program's purpose is to facilitate the exchange of applied aquaculture information and technology between the United States and Asia. This is accomplished through international workshops and distribution of workshop results to information networks throughout the United States and Asia.

This is the fourth workshop proceedings issued by the Asian Interchange Program. This years conference focused on three aspects of selective breeding of fishes: selective breeding to improve fish performance for aquaculture, genetic resource management for stock enhancement and conservation of natural genetic resources. Previous conferences and proceedings addressed culture of cold-tolerant marine shrimp, culture of rotifers and microalgae as a live feed for cultured fishes, crustaceans and molluscs and diseases of cultured marine shrimp.

The Workshop

The Selective Breeding of Fishes in Asia and the United States workshop took place in Honolulu, Hawaii from May 3-7, 1993, and included experts from eight countries in Asia (Indonesia, Japan, the Philippines, the People's Republic of China, Singapore, Taiwan, Thailand, Vietnam), the United States (Alaska, California, Hawaii, Idaho, Maryland, Mississippi, Virginia, Washington) and Norway (see photo). Formal papers were presented during morning sessions and in the afternoons participants shared information and ideas about selective breeding of fishes during informal discussion groups. Japanese, Vietnamese and Chinese interpreters were present to facilitate communications and simultaneous interpretation services were provided during the presentation and discussion group sessions.

The Proceedings

This volume is divided into three parts: the introduction, contributed papers and discussion group summaries. The introduction reviews and defines topics and terminology used throughout the proceedings. The discussion group summaries are divided into two sections: Aquaculture Production and Conservation, and Stock Enhancement and Conservation. They provide a group perspective on key topics related to selective breeding of fishes. The first section concludes with guidelines for selective breeding to improve fish performance and the second section concludes with guidelines for genetic resource management in stock enhancement.

The contributed papers are grouped into three sections: Aquaculture/Conservation, Enhancement/Conservation and Country Reviews.

In the *Aquaculture/Conservation* section, Graham Gall presents three strategies that have been used to conserve natural genetic resources and suggests the only viable conservation strategy is the management of specific populations. Strategic implementation is discussed. Trygve Gjedrem discusses the potential to improve performance in fishes through selective breeding. Genetic gains of 10-20% per generation have been demonstrated for Atlantic salmon. He briefly reviews breeding programs for several fishes, including Atlantic salmon programs in Norway, Iceland, Canada, Sweden and the United States; rainbow trout programs in the United States and Norway; channel catfish programs in the United States; carp programs in Israel, Japan and Russia; and the tilapia program in the Philippines. A mechanism to initiate a breeding program is also presented. Eric Hallerman reviews the potential economic benefits and environmental risks of biotechnology, as well as present and future public policies that will regulate the development and use of genetically-modified aquatic organisms. Sifa Li presents an example of genetic resource deterioration in cultured and natural fish species in China. The need to implement a genetic conservation program in China is discussed. Violet Phang reviews breeding program methods for ornamental fishes in Asia. Ornamental fish breeders continually introduce new genetic variation into their stocks to develop novel strains and avoid inbreeding depression. Traditional and modern genetic technologies for ornamental fish production are discussed. Nobuhiko Taniguchi et al. follows up on this topic with a review of Japan's research on genetically-modified fish and proposed policies to regulate the use of these fishes. Breeding and selection programs for channel catfish in the United States are presented by William Wolters. Research has shown potential for improved catfish stocks through traditional animal breeding, but these techniques are not presently applied by commercial catfish farmers.

In the *Enhancement/Conservation* section, William Hershberger discusses the importance of natural genetic resource conservation for future finfish aquaculture needs. The potential for aquaculture to enhance natural stocks and to produce genetically improved stocks for intensive aquaculture are discussed. James Shaklee et al. reviews the Washington State Department of Fisheries' conservation programs that protect Pacific salmon genetic resources. In Washington, natural salmon stocks are enhanced through hatchery production. The importance of genetic resource management is recognized and formal policies for genetics, stock management and conservation are in practice. William Smoker reviews the history of Pacific salmon stock enhancement and ocean ranching efforts in Alaska. As in Washington, Alaska's resource managers have been concerned about salmon genetic resource losses and have implemented a program to prevent overfishing of wild stocks.

In the *Country Reviews* section, Remedios Bolivar describes development of the Philippines national breeding program, which is to genetically improve tilapia stocks. The program, initiated in 1988, is modeled after the Norwegian salmon breeding program. In the Philippines, wild African Nile tilapia stocks were introduced to serve as a base population for the selective breeding program. I.C. Liao et al. reviews the history of Taiwanese fish genetic research, stock enhancement efforts and the need for conservation of genetic resources. Genetic research in Taiwan has been fragmented. Some successes have been

documented in tilapia hybridization and selective breeding, and more recently, in the production of genetically-modified fish. The effectiveness of releasing fish fry to enhance depleted fishery populations is not known, although enhancement has been practiced since the 1970s. Sudarto reviews genetic research on carp, tilapia and other species used to resolve Indonesia's inbreeding problems. The most commonly cultured fish in Indonesia is the common carp. Tran Mai Thien discusses the history of fish breeding research and deterioration of genetic quality in cultured Vietnamese fish populations. Fish breeding research has focused on carp and tilapia hybridization. Supattra Uraiwan reviews genetic research and conservation issues in Thailand, and discusses selective breeding programs for tilapia, walking catfish, common carp and Java carp. Thailand is concerned about conserving the genetic diversity of natural fish stocks and is in the process of setting up a conservation program. Traditional selective breeding practices in the People's Republic of China are reviewed by Chingjiang Wu. As in other Asian countries, traditional breeding research has focused on hybridization of the carp species. The use of genetic markers or phenotypic differences, such as color patterns, to determine genetic differences is presented. Finally, Dequan Xia and Ting-ting Wu review the potential of biotechnology-based research to genetically improve fish stocks in the People's Republic of China.

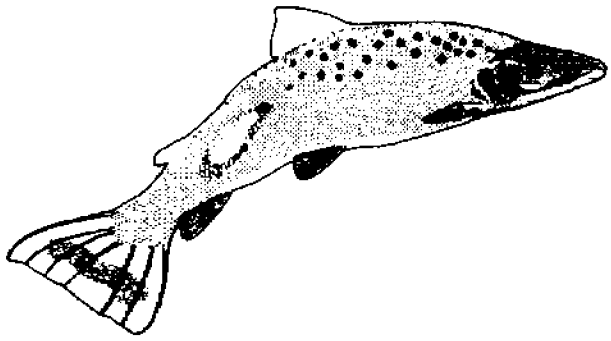
Acknowledgments

The editors thank the University of Hawaii Sea Grant College Program for its administrative support throughout this project. The Asian Interchange Program is funded by the National Oceanic and Atmospheric Administration, United States Department of Commerce (Grant #NA90AA-D-SG483), which is administered by the University of Hawaii Sea Grant College Program.

A number of individuals contributed to this work. Most importantly, we would like to thank the authors who participated in the workshop and prepared the papers included in this volume. Graham Gall provided valuable guidance and technical assistance in the development of the meeting agenda and workshop details. Cheng-Sheng Lee, I.C. Liao, Roger Doyle and Graham Gall helped to identify and select workshop participants. Graham Gall, Kenneth Leber, William Smoker, Trygve Gjedrem, Eric Hallerman and James Shaklee reviewed the discussion group summaries. We thank Cheryl Rosenfeld for editorial assistance throughout the production of this proceedings. Esma Harper, Rose Marie Norton, Lana Pigao and Paula Steib proofread the manuscript. We also acknowledge our capable interpreters, Logan Duong, Hongja Harrison, James Kenney, Lynette Shi, Taeko Wellington and Shugiang Zhang.

The introduction and discussion group summaries were written by Kevan Main, with editorial support from Cheryl Rosenfeld and Elizabeth Reynolds. The final production of the proceedings and the cover design was done by Cheryl Rosenfeld, with assistance from Alcian Choy. A special mahalo to Jan Dill for his support of the Asian Interchange Program.

Part I: Introduction



Introduction

Successful commercial fish farming depends on the operator's understanding of genetics and selective breeding principles. Selective breeding of fish stocks can improve growth rates and disease resistance, and prevent inbreeding or other negative genetic consequences. Stock enhancement programs can use these principles to manage resources and maintain genetic variation, while conservation programs can use the principles to evaluate and maintain genetic diversity of wild stocks.

Three aspects of selective breeding of fishes are reviewed in this volume: selective breeding to improve fish performance for aquaculture, genetic resource management for stock enhancement and conservation of natural genetic resources. The important themes that emerged during the workshop and the terminology and topics discussed throughout the proceedings are explained below.

Important Themes

Selective Breeding Improves Production Efficiency

The potential for commercial fish farmers to improve production efficiency with selective breeding is significant and may be greater in fish than in terrestrial farm animals. Using a combination of mass selection and family selection, Hershberger et al. (1990) averaged a 10.1% increase in coho salmon body weight per generation and Gjedrem (this volume) reports that genetic gains of 10-23% have been achieved in fish.

The development of successful breeding programs for aquacultured species requires commitment to a long-range permanent improvement program, industry acceptance and direct industry experience with the improved stocks. Implementation of new selection programs requires government support during the early years, followed by on-site demonstration projects under conditions familiar to farmers.

Genetic Conservation and Enhancement of Natural Stocks

Stock enhancement has been effective in some locations at augmenting existing fish populations and restoring fish populations in environments where they previously existed. Enhancement programs must consider gene conservation. Enhancement will affect the genetics of wild stocks when gene flow occurs between cultured and wild stocks.

Monitoring is essential for long-term success of stock enhancement. A stock enhancement program should include an initial inventory of the natural population's genetic resources and periodic characterization to evaluate any changes in gene frequencies following hatchery releases.

Need for Conservation of Genetic Resources

The importance of natural genetic diversity for future aquaculture production, enhance-

ment efforts and conservation is recognized worldwide. Declines in natural fish populations, of cultured and natural species, have been documented in many countries. Overfishing and environmental degradation have been principally responsible for the declining abundances. Direct evidence of negative genetic effects of cultured fishes on natural populations is lacking; however, decisions are being made by individual countries about conserving genetic diversity of natural stocks. Which species to conserve, how to store genetic material and costs of conservation must all be considered.

Potential Breeding Benefits and Risks

There are both benefits and risks posed by developments in biotechnology. Potential benefits include the production of sterile fish for farming and commercial fisheries, and the ability to preserve reproductive cells for future use. Potential risks include negative ecological and genetic interactions between natural and genetically engineered stocks. Transgenic fish could potentially breed with wild fish and transfer transgenes into wild populations. Various countries and international organizations are presently developing guidelines on the use of genetically modified fish in aquaculture systems (Hallerman, this volume).

The genetic risk posed by stock enhancement on natural stocks varies depending on the protocols used for broodstock collection and hatchery production. Two approaches have been used to enhance fish stocks around the world: relocation of broodstock from one region to another, and collection of local broodstock to produce juveniles for release within the same region. Movement of broodstock between regions has a greater potential to affect the genetic variation of the local population than collection and production of local stocks. Use of local broodstock for production of juveniles is needed to prevent loss of adaptive genes.

Terminology

Breeding for Aquaculture Production

Few genetically improved stocks have been developed for commercial finfish aquaculture, although several methods are available to domesticate those stocks. They include mass selection, family selection, cross-breeding and new or biotechnology-based techniques.

Mass selection or individual selection is considered to be the simplest, oldest and most effective method of genetic improvement for some species. It consists of selecting individuals according to their phenotype or individual performance. Each individual, regardless of family, is compared to all others, and the best performers are selected to produce the next generation. To determine the effectiveness of mass selection, two generations (parents and their offspring) must be grown to maturity. Mass selection is not efficient for traits with low heritability. For fish, it is therefore best applied to growth rate and to some extent to selection for age and sexual maturity (Gjedrem 1985).

Family selection is based on the performance of families rather than individuals. Specific pairs of brood fish are mated and the offspring are reared separately in individual family groups (Tucker and Robinson 1990). More extensive facilities are required for

family selection than for mass selection, because a relatively large number of families are raised and tested to achieve a reasonable selection intensity and reduce inbreeding (Wohlfarth and Hulata 1989). Family selection is preferred when the selected character is difficult to measure on individual fish, cannot be measured without sacrificing the fish, or has additive genetic variance with low heritability. Family selection is an efficient selection technique for the following traits: survival, age at maturation and feed conversion efficiency (Gjedrem 1985).

Cross-breeding or hybridization is a process where new combinations of alleles are created in the offspring by mating fish with different genetic backgrounds. Parents can be from different strains within the same species (intraspecific hybridization) or from different species (interspecific hybridization) (Tucker and Robinson 1990).

Often, **inbreeding** occurs in an attempt to genetically improve a fish population. Inbreeding within a large population is defined as the mating of individuals that are more closely related to each other than to randomly-mated individuals. Although inbreeding reduces genetic variation and productivity, inbreeding is sometimes used to create inbred lines that breed true for a specific character (Tave 1993). Inbreeding depression results when there is a reduction in the expected performance of the affected trait.

A number of new biotechnologies have been developed to genetically improve fish stocks, including polyploidy, gynogenesis and transgenics. **Polyploids** are individuals with more than the normal (2N) number of chromosome sets, which were induced in the fish by temperature or pressure shocks to eggs soon after fertilization. It is relatively easy to change the chromosome number in fish because fertilization is external for most fish species. Triploids (3N) are created to increase growth and produce sterile fish. Tetraploids (4N) are created to cross with diploid fish and produce triploid offspring. This eliminates the need to produce triploid fish manually (Tave 1993).

Gynogenesis is a genetic engineering procedure that pairs both sets of chromosomes from a single female parent and results in the production of all female fish. Because female fish tend to be larger than males, producing all female offspring results in production of larger fish for market.

Genetic engineering has also been used to transfer genes from one animal into another. If successful, the gene is expressed in the parental fish and transmitted to the offspring. The fish are then referred to as transgenic or genetically modified fish. Genes can be transferred between different species or different types of organisms. Genetic engineering has the potential to improve growth rates or increase disease resistance in fish.

Enhancement of Natural Stocks

Stock enhancement is the release or stocking of hatchery-reared juvenile organisms into the natural environment to supplement the existing population and thereby, expand opportunities for harvesting, rebuilding declining populations or establishing new populations (National Research Council 1992). The need for stock enhancement has principally been attributed to two factors, habitat degradation and overfishing.

Marine stock enhancement was first practiced in the United States in the late 1800s, when thousands of unmarked eggs and newly

hatched larvae of several species of commercially important marine fish were released to supplement natural stocks (National Research Council 1992). Early enhancement efforts ignored the genetic effects of escape or release of hatchery-reared fish on wild populations (Pullin 1992).

More recently it has been recognized that unintentional selection can change the gene pool of natural breeding populations and potentially affect their survival and reproduction. The **gene pool** is defined as the sum total of all the genetic information within a population of interbreeding or reproducing individuals (Oldfield 1989). Thus, for enhancing natural stocks, the hatchery manager's goal is to **avoid selection** in the hatchery.

Today many enhancement programs have taken a new approach that incorporates genetic resource management together with a monitoring and evaluation program (Discussion Group Summaries and Shaklee et al., this volume). Marking systems (i.e., coded wire tags, genetic markers) are used to evaluate the impact of enhancement efforts.

Conserving Genetic Resources in a Natural Environment

Conservation is defined as the wise use of natural resources (Oldfield 1989). Conservation does not imply that every species or every form of genetic resource must be preserved in perpetuity, but that genetic resources need to be carefully managed against exploitation or habitat destruction. **Genetic resources** are the economic or societal value of the genetic materials contained within or among species (Oldfield 1989). Ecologists and evolutionary biologists generally agree that species diversity and genetic variability are necessary for the long-term maintenance of stable populations, species and complex ecosystems (Smith and Chesser 1981).

Preservation and conservation of genetic resources differ. **Preservation** is a short-term static process that involves removing a species or their reproductive parts for management or preservation to a storage facility (i.e., gene bank). Alternatively, conservation is a long-term dynamic process involving management of genetic resources under natural conditions, allowing evolution to continue (Frankel 1970, Oldfield 1989, Gall, this volume).

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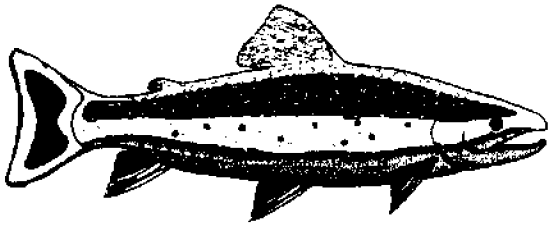
Part II:
Aquaculture/Conservation



Development of Fish Breeding and Conservation Programs

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Abstract

The development of effective conservation programs for fish is only in the initial stages of discussion. One of the most perplexing aspects of the discussion has been identifying a basic framework for the development of effective strategies. There are three basic elements to any conservation program; the objective (target stock or species), the time scale and the management protocol of the program. It also is necessary to distinguish between preservation, a short-term static approach; and conservation, a long-term dynamic approach that ensures the continuing evolution of the program target. There are three basic conservation strategies: provide sufficient resources so all species can continue to evolve; accept extinction and preservation of some species in zoological gardens; and provide special management for specific populations of species requiring protection. The third, management of specific populations, appears to represent the only viable strategy.

Genetic management of wild species can take many tenants from programs for domestic crops and livestock. Breeders of domestic species have programs to conserve the genetic resources essential for recombination and selection of genetic material so future generations can respond to changes in the physical, biological and economic environment. Species in nature require the same reservoirs of genetic diversity to adapt and survive. Based on this experience, it is clear that management strategies for natural species must address the basic question of whether the use of nature reserves is likely to facilitate the continuing evolution of the target (ecotype, species, ecosystem). Secondly, management discussions must address alternative opportunities, the viability of managing the general landscape and the impact of human activity so that a multitude of species can prosper.

Conservationists have suggested the "metapopulation" concept of population structure for wild species, based on population genetic theory and the kinds of activities used by domestic crop and livestock breeders. The concept is very appealing and offers a framework for utilizing the general landscape. It assumes a species naturally is subdivided into a large number of subpopulations with some local adaptation of subgroups, but with continuous or intermittent gene flow among subgroups. Implementation of this type of conservation strategy requires a careful inventory of the target, an assessment of the causes of numerical decline of the target, the current status of the target and common sense application of population genetic principles.

Introduction

There are three basic elements to any conservation program, the objective of the program, the time scale of the program and the management protocol of the program (Frankel 1983). The objective specifies the target and purpose with regard to the specific biological material considered by the program. The target can be anything from a group of subpopulations of a species, a subspecies, all members of a species, a community of species, an ecosystem or a geographic region. The time scale of the program is critical, as it defines the expected degree of intervention. For cases where the causes of concern are few in number and readily amenable to corrective action, the time required to ensure survival of the target may be no more than one or two generations. However, for critical cases with no apparent solutions to the causal factors, the conservation program is likely to be required into perpetuity.

Many believe all biological resource management activities should be considered conservation programs and so continuous input and control are essential in all cases. Thus, the management element of a conservation program is generally an ongoing activity. Direct attention to the genetic nature of the target resource and whether management activities impact the target, positively or negatively, has often been absent in the past. It also must be recognized that all management activities affect both the abundance and genetic status of species. Even the absence of specific management plans can affect the genetic status of target resources exposed to human activities.

The three basic elements of a conservation program are not independent, but are interactive. Programs with broad objectives generally have a longer time scale than programs designed to remedy a narrowly focused objective. Long-term conservation programs are generally faced with major habitat disturbances and greatly reduced abundance of a specific species. Thus, the objective almost always must include the ecosystem, either as a direct or implied target. A constant limitation to many conservation programs is that management inevitably focuses on the present with little regard for long-term implications. Long-term management goals must be defined in policy that focuses attention on the future, be based on biologically sound principles and provide specific objectives that are realistic and manageable. Management's focus on the present, in the absence of well established policy, can have many unexpected consequences. Experience has provided numerous examples, including the negative impacts of policies for the prevention of forest fires, the complex aspects of fishery enhancement and the impacts of selective harvest management on single species.

Conservation Strategies

The concepts of genetic resources conservation are not the same as those generally associated with the preservation of genetic material. Although both ideas can apply to genotypes and species, preservation implies a short-term static process while conservation denotes a long-term dynamic process, one that provides "continuing evolution" (Frankel 1970). Collections of genetic material placed in storage, or a few individuals of a specific genotype held in a genetically closed group, are examples of preservation management. Conservation programs maintain the target genetic resource in a dynamic

interbreeding state and in an environment either typical for or native to the target. Under such conditions, the target is free to evolve as the biological and physical environments change.

There are three conservation strategies available to managers of species-specific genetic resources. One is to provide sufficient nature reserve space of diverse types to ensure the continuing evolution of all species. The second is to accept extinction as inevitable for some species, and to attempt the preservation of a sample of some species by moving remnant material to zoological or botanical gardens. The third strategy is to manage the population size, the population structure and the relevant ecosystem to offer protection to selected, presumably "threatened" species. The first strategy is an abstraction, not a reality. The number and size of nature reserves necessary to effectively conserve at-large biodiversity is beyond the capacity of available habitat given the size and continued expansion of the human populations (Gall and Orians 1992). The second strategy has become the only alternative for some species but should be avoided if the species in question can be maintained in its natural environment and is compatible with human presence. Gall and Orians (1992) include a discussion of the issue of compatibility. The third strategy of utilizing the general landscape probably is the only realistic approach (Gilpin et al. 1992). Such an approach requires that all resource utilization activities (urban and industrial development, farming, fishing, forestry, recreation, etc.) be managed in an integrated way as to insure a place for all species. A benefit of this approach, in addition to conservation of native species, is the development or maintenance of a landscape that is aesthetically pleasing to humans, and thus has redeeming social value (for example, an attractive agricultural landscape). However, for this approach to represent a viable alternative, management protocols must embrace the integration of conservation with resource exploitation and utilization.

Genetic Management

One of the central issues in assessing conservation strategies should be genetic management of biological resources. This is particularly critical when the target of the conservation program is a species or subpopulation within species. The notions and requirements of genetic management for wild and domesticated fish species have close affinities to genetic management tools employed for domestic crops and livestock. The notions used for the genetic resource conservation of domestic crops and livestock arose from a need to conserve genetic material for future breeding programs.

Two ideas, genetic resources and genetic diversity, are given careful attention in genetic management by crop and livestock breeders. The genetic resource of interest is the genetic diversity available to animal and plant breeders for future genetic recombination and selection. Genetic diversity, the sum total of all the genetic variation within a species, is essential for the breeder to respond to changes in the physical, biological or economic environment.

Natural populations require the same reservoirs of genetic diversity for adaptation and survival as crop and livestock breeders require for successful breeding programs. The most frequent response to the need for genetic conservation of wild species is the development of nature reserves. As Frankel

(1983) has pointed out, the central questions concerning the value of nature reserves as a viable conservation strategy are: do nature reserves facilitate, restrict, or inhibit the continuing evolution of species; and can the general landscape be managed to ensure the continuing evolution of species? As a general rule, populations in nature reserves are small and fragmented so genetic management must deal with the associated problems of inbreeding, genetic drift, and the random loss of alleles. If these forces cannot be controlled satisfactorily, maintenance of a species in nature reserves can result in populations that are gradually weakened and genetically impoverished. In addition, it is essential to assess the probability of long-term stability of large complex nature reserves within the framework of global and local politics.

The solutions that have evolved in crop and livestock breeding programs involve the systematic management of fragmented populations. The genetic health of domestic genetic resources has been achieved through maintaining sources of material for manipulation in the form of germplasm collections, propagation of special genetic stocks, the development and maintenance of specific varieties and breeds, and the development of inbred lines. In the case of crop plants, effort also has been placed on understanding the genetic nature of wild relatives. The best example of the use of inbred lines has been the development of a broad array of inbred lines of the mouse and their deliberate manipulation to provide the genetic diversity of research material essential for biological and medical research. Specific breeds of livestock, although maintained in fragmented herds, have retained a healthy genetic status through the constant exchange of germplasm among herds (the genetic equivalent of gene flow).

Conservation genetics requires a framework that encompasses the same issues and factors of concern to crop and livestock breeders. One common theme of this framework is that populations do not exist as a single group of breeding individuals kept in isolation. Rather, the total population is made up of many smaller units with significant gene flow among the subunits. In contrast, nature reserves represent spatial conditions that restrict population size so survival and long-term genetic adaptation are restricted. Thus, conservation of genetic diversity should be viewed as a space-demanding process.

The metapopulation concept has been proposed as an appropriate framework for understanding and describing the population structure of natural populations (Levins 1971). The concept considers populations of a species to exist as a continuum of a large number of subgroups with gene flow among the subgroups. There is potential for local adaptation within subpopulations or groups of subpopulations. The gene flow may be continuous or intermittent and the rate of gene flow among subgroups may be uneven. The level of differentiation between any two subpopulations is determined by the balance between the effects of random and selective forces driving allele frequencies apart and gene flow pulling gene frequencies together. Theoretical models of population dynamics under the metapopulation concept are being developed (Soulé 1987). However, it is clear that this notion of population structure is consistent with the need to use the general landscape for conservation programs (Henry et al. 1991). Fragmentation of habitat can be beneficial if partitioned appropriately and

if barriers to movement of individuals or gametes are not excessive.

Implementation for Wild Species

Understanding and implementing the metapopulation concept to a particular species will require special effort to describe population structure. Thus, an inventory of the genetic resource is essential. At a minimum, the inventory should establish which species are present in the ecosystem and critical to the target species, the status of the species including any that appear to be threatened with extinction, the population structure of the target species, what constitutes the principal breeding or evolutionary unit within the population structure and the distribution of genetic variability within and among various subdivisions in the population structure.

When threatened species are identified, the causes of the threatened condition, such as habitat loss, overexploitation, or forced hybridization, must be delineated. For species that are threatened, the remaining populations generally are small in size, and subpopulations are usually disconnected and occupy fragmented habitat. Such conditions bring into play two central concepts of population genetics: fitness and how it is affected by inbreeding; and genetic variance and its importance to adaptation. Both are population size dependent (Frankel 1983) and have received preliminary attention. For example, it has been suggested that an effective population size of 50 is sufficient to restrict inbreeding to an acceptable level. On the other hand, Frankel (1983) suggested that an effective size of 500 would be minimal for the maintenance of additive genetic variance. Both of these numbers are little more than educated guesses and neither considers the importance of non-additive genetic variance or the effects of selection and gene flow. In addition, it is unlikely that any single set of values will be appropriate for all species. Thus, intensive work is needed to determine the population size parameters that will ensure viability for any species under conservation (genetic) management.

Stock Enhancement and Conservation

Enhancement of fisheries through hatchery propagation represents a special case. It involves genetic manipulation of a species in a culture environment, as well as the potential disruption of the natural population structure and rates of gene flow. In addition, stock enhancement is often proposed as a conservation strategy. Thus, it is worthwhile to consider briefly, as a final section of this paper, the issues involved in integrating stock enhancement and natural production of a species.

There are four major genetic issues involved in establishing and maintaining hatchery populations for enhancement of natural production or as a conservation strategy: sampling broodstock from the natural population, domestication of the broodstock, management and operation of the hatchery and defining the genetic and breeding goals of the program. Establishing a protocol for sampling the natural population should consider potential founder effects (poor or modified performance caused by limited sampling of the genetic material in the natural population), the range of phenotypic variation present, and the importance of sampling the full range of genetic variation. Thus, attention must be given not only to the total number of individuals sampled, but to the spatial and temporal range over which the genetic variation may exist. It is likely the total number of individuals sampled will be large if an

honest effort is made to identify and sample the full range of phenotypic variation present in the target population.

The extent of domestication of the broodstock due simply to hatchery culture, and the effect of some degree of domestication on the future genetic health of the natural population have not received extensive attention (Doyle 1983). However, a few affects are likely to be common to all stock enhancement programs. There will be some selection, even under management conditions designed to select future parents randomly. The selection pressure will come from the physical nature of the culture system and the adaptability of the sampled genotypes to the hatchery environment. Restrictions on physical facilities available for hatchery culture almost always limit the population size that can be managed within the facility. Thus, it is likely there will be some loss of genetic variability. Finally, hatchery reproduction most generally requires some degree of manipulation, either environmental or behavioral, so change due to selection is likely as a result of differential responses of individuals to induced spawning or artificial removal of gametes.

The type of management and the operational protocols of a hatchery program represent major sources of affects on the genetic variation among individuals produced by the program. It is suggested by some that the protocols must be benign with regard to genetic effects. However, this is not realistic since any manipulation of the natural life-cycle carries the potential of causing genetic change. Some obvious management options can at least be minimized. Discarding (culling) of individuals to improve production efficiency should be avoided since it could impose size selection. On the other hand, sorting by size may be a viable method of reducing cannibalism of smaller individuals for some species, but will probably result in higher survival than would be expected in nature. Taking care not to restrict the reproductive period should reduce the likelihood of loss of genetic variation for season of spawning. However, achieving successful reproduction over the full reproductive period may be difficult due to distorted sex ratios among mature individuals or limited numbers of individuals maturing at any given time, particularly at the extremes of the reproductive season. The mating scheme can be controlled, at least within the limits of the maturation schedule of the broodstock. Generally, it is preferable to use single-pair matings to maximize genetic recombination and genetic variability rather than to mix gametes prior to fertilization. This is due to the fact that some gametes are likely to be dominant in a mix and cause a reduction in effective population size (Withler 1988).

Conclusion

Defining genetic and breeding goals for the program is critical to the outcome of the program. These must be established in concert with the objectives of the enhancement or conservation program. The major concerns are: is there a need to maintain any subpopulation structure within the broodstock? How will parents of the next generation be chosen? Is there a need to optimize genetic differences? And is artificial selection to be minimized or used as part of a genetic improvement program? The choice

of answers to these questions must be determined on a case-by-case basis. There are situations where choice of parents could utilize non-random selection as a means of increasing among family variability. An enhancement program could adopt selection as an integral component to improve survival to the fishery. In other cases, conservation of natural variability may have a higher priority than improving performance, so the goal would be to minimize selection. Some cases may involve enhancement of several subpopulations within the target species so that strict management of mating and the rearing of progeny would be essential to maintaining the genetic integrity of the subgroups; gene flow would occur only among naturally reproducing segments of the overall population. These are a few examples, but clearly there will be many factors influencing decisions for each enhancement or conservation situation.

The most important effort in any enhancement or conservation program is obtaining the proper data of the requisite quality. Only then can intelligent decisions be made regarding the genetic needs and implications of the program. Past experience suggests that most programs have been defined on an *ad hoc* basis with little attention to the long-term genetic implications of the program. Consequently, many hatchery enhancement programs, and natural conservation efforts have come under attack not for lack of good intentions on the part of the program managers, but due to a lack of planning and evaluation based, at a minimum, on common sense and good genetic rationale. Few past programs have had the requisite data available, often because political pressure was impatient or the need for a conservation effort was so critical, time would not permit proper planning. We must try at every opportunity to increase our understanding, and that of fisheries managers, of the genetic implications of human intervention in natural lifecycles and the genetic significance of management options.

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International Selective Breeding Programs: Constraints and Future Prospects

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Abstract

Internationally, only a few efficient breeding programs for fish are in operation. In Norway, a national breeding program has been running since 1975 for Atlantic salmon and rainbow trout. Iceland has just started a breeding program for farming and sea ranching Atlantic salmon. Breeding programs in Canada and Sweden for farming Atlantic salmon and rainbow trout, respectively, and Israel runs a cross-breeding program for carp. Some private companies also claim to run breeding programs. The knowledge necessary to develop efficient breeding programs is available for just a few species. This includes salmonids, channel catfish, tilapia and carp. For most of the species it is not possible to carry out controlled mating and reproduction because their reproduction biology is not yet satisfactorily understood. Prospects for genetic gain in fishes are very good and are even better than in other farm animals. A genetic gain of 10 to 20% per generation has been shown to be obtainable. Because breeding programs in fish can be centralized, it is expected that the cost will be relatively low compared to most farm animals. The profitability for fish breeding programs is also likely to be very high. The most serious constraint to running a breeding program is the risk of spreading diseases from breeding centers to the farming industry, and the potential infection of wild populations. Domesticated stock may escape and spawn with wild populations, thus reducing the genetic variation and fitness within the wild strains. An interesting challenge is how to start a breeding program. All present breeding programs in Norway were initiated and implemented by research institutions. In the farm animal industry, however, private companies and farmers' cooperatives dominate. To stimulate interest and initiate breeding programs it is necessary to demonstrate potential for genetic gain in each species, and then show the benefits and economic potentials to researchers, farmers, farmers' organizations and officials.

Introduction

The purpose of a breeding program is to change the average performance of a population in a defined direction to the benefit of industry and the consumer market. A breeding program is more complex than a breeding research project, which usually studies just a part of a breeding program and examines how to make the program more efficient and more applicable.

The main elements of a breeding program include:

- Definition of the breeding goals,
- Description of the breeding and selection methods to be used,
- Detailed information of how the animals should be tested in order to estimate breeding values,

- Detailed description of how and when each trait is measured or judged,
- Economic value of each trait,
- Selection indexes to be used,
- Guidelines for selection of broodstock,
- Expected genetic change per generation.

The theoretical basis for breeding and selection was primarily conceived during the first three decades of this century. Scientists central to the development of animal breeding theory were Sewall Wright and Jay L. Lush. The first efficient breeding and selection programs in plants and farm animals were started in the mid-1930s. Since then, breeding and selection programs have been developed for plants and farm animals in most countries. The increase in productivity and product quality has been tremendous. Today one can not really think of using animals and plants not developed through breeding and selection for food production.

Conditions for a Breeding Program

It is essential that the entire reproduction cycle for the species in question be controlled in captivity to control fertilization, hatching and first feeding.

An inexpensive marking or tagging system is a great advantage and is a necessity for a breeding program that tests families.

It must be possible to measure and record traits of interest on many animals at a reasonable cost and within a certain time interval.

The industry must have interest in buying broodstock/milt/eggs/fry or fingerlings.

The economic traits included in the breeding goal must show additive and/or non-additive genetic variation.

Breeding Methods

Since inbreeding should be avoided in any breeding program there are two methods available for practical use:

- Cross-breeding - crossing strains or inbred lines and
- Pure-breeding - mating animals less related than the average in the population.

Choice of a breeding method depends primarily on the type of genetic variation present in the traits of interest.

Cross-breeding may be used if non-additive genetic variation is considerable, while selection and pure-breeding should be used to exploit additive genetic variation. Additive genetic variation is due to the additive genetic effects (intermediate) while non-additive genetic variation is due to dominance and epistacy effects. It is not always easy to decide which breeding method to use, however, Gjedrem (1985) concluded that if there is additive genetic variation, one should always apply pure-breeding combined with selection. And if the component of non-additive genetic variance is considerable, pure-breeding should be combined with cross-breeding. As a rule, cross-breeding should be part of a breeding program when the heterosis effect is larger than the expected genetic gain per generation in a pure-breeding program.

Methods of Selection

Individual selection is the most widely used method of fish selection. It is easy to practice, the cost for testing and recording to estimate breeding values is low compared to other methods of selection and it does not extend the interval between generations. However, individual selection has limitations. The efficiency is low for traits with low heritability, can hardly be used for all or none traits such as dead or alive and age at maturation, and it cannot be used for carcass quality traits. In fish breeding this usually limits individual selection to growth rate.

Since within-family selection and progeny testing have shown less efficiency, an alternative selection method family selection, can be used. (Falconer 1961; Gall and Huang 1988 a, b; Gjedrem 1985). Family selection is relatively efficient compared to individual selection for traits with low heritability, it can be applied for all or none traits, meat quality traits and it does not the interval between generations.

Family selection is of particular interest in fish because of high fecundity; large numbers of half- and full-sib groups can be produced. Since it is impossible to mark newly hatched larvae or fry, each family must be reared in separate tanks for the first months of their lives. This type of rearing will, however, introduce some environmental effects common to families.

Generally, a combination of individual and family selection will be more efficient than using just one method (Falconer 1961).

Breeding and selection in fish

- **Rainbow trout**

In their natural habitat rainbow trout are spring spawners. In 1936, Lewis (1944) started to select for fall spawning at Hot Creek Hatchery, California. Early spawning and faster growth rate was obtained by using individual selection. Selection for early spawning has been applied since then, and Siitonen and Gall (1989) observed a median spawn date of 15 August for the 1980 and 1981 year-classes. They estimated a response to selection for early spawning of seven days per generation during each of six generations.

Donaldson and Olson (1955) and Donaldson (1970) presented results from a selection experiment based on individual selection that started in 1932 at the College of Fisheries, University of Washington, Seattle. Growth rate and number of eggs per spawn increased markedly and the age at first maturation was reduced. Since there was no control group, the results were phenotypic values such as genetic response and environmental change. The selection was not continued when Donaldson retired but the Donaldson strain of rainbow trout has been distributed to many countries for fish farm use. In Sweden, Sylven and Elvingson (1992) compared Donaldson's strain with three other strains of rainbow trout and found that it ranked lowest and was 54% below their best strain, which had been selected for five generations for growth rate.

Kato (1978) selected rainbow trout for spawning twice a year in and obtained a response. After one generation of selection 40% of its progenies spawned twice a year. During three generations of individual selection for body weight at 147 days in a fall

spawning stock of rainbow trout, Kincaid et al. (1977) obtained a 67% increase in body weight and a correlated response in increased hatching percentage and fry survival.

At the Rainbow Trout Symposium held at the University of Stirling, Scotland in 1990, Gjedrem (1992) discussed breeding plans for rainbow trout and described the national breeding program for rainbow trout in Norway. This program was started in 1972 and has now completed six generations of selection. In each generation about 120 full-sib families within about 30 half-sib families were tested. The selection has been on high body weight at harvest after 1.5 years in seacages (at 2.5 years of age) and low frequency of early maturity during their first winter at sea. A weak selection for survival in the freshwater period was practiced. Family selection was applied for all traits and combined individual selection and family selection was used for growth rate. Gjerde (1986) reported a genetic gain of 13% per generation for body weight in the first two generations of selection.

In Sweden, breeding programs have been developed for rainbow trout and arctic char with a similar design of that used for rainbow trout in Norway (Elvingson personal communication).

Cross-breeding of rainbow trout has given varying results. Ayles and Baker (1983) found significant heterosis for both growth and survival of some but not all crosses. Gjerde (1988) studied the heterosis effect in a diallele cross among six inbred groups. Total heterosis was significant for all traits studied. Total heterosis for weight at slaughter ranged from 17% below to 18% above the non-inbred control groups. Hørstgen-Schwark et al. (1986) found that the mean growth performance of all the crossbred fish were not significantly different from that of the purebreds.

- **Salmon**

In 1949 an individual selection program for chinook salmon under sea ranching conditions was started at University of Washington, Seattle by Lauren R. Donaldson (Donaldson and Menasveta 1961). According to Donaldson (1968), the selection response was significant for growth rate and fecundity from 1960 to 1967.

A Nordic research project in Atlantic salmon ranching was started at the Institute of Freshwater Fisheries, Iceland in 1987 (Jonasson 1993). The purpose was to study the genetic variation in return frequency and growth rate. For three year-classes a significant genetic variation in return frequencies have been obtained (Jonasson, personal communication). This project has now been transformed into a family selection breeding program in order to increase return frequencies and growth rate. Each year 100 full-sib families within about 30 half-sib groups are tested by releasing them into the sea. A corresponding number of families are used in a breeding program for fish farming. So far, records of response to selection have not been available (Jonasson, personal communication).

Hershberger et al. (1990), using combined individual and family selection for increased body weight in coho salmon during four generations, obtained an average of 10.1% gain per generation in weight over an eight month period. Realized heritabilities were estimated to be 1.22 ± 0.32 for the odd-year line and 0.81 ± 0.30 for the even-year line, while the heritability determined by ANOVA remained at a level of 0.20. The experiment was terminated after a period of

10 years when an improvement of 60% had been reached.

In Norway, AKVAFORSK started a selection program in the fall of 1971. Since the generation interval is four years in Norwegian Atlantic salmon, four year-classes were formed by sampling broodstock from 41 river strains. Selection began in the fall of 1975. Data from the first generation were used to estimate phenotypic and genetic parameters and to develop a breeding program (Gunnes and Gjedrem 1978). At present, four generations of selection have been completed in all four year-classes; selection for increased growth rate in the first two generations and for increased growth rate and low frequencies of early sexual maturation in the following generations. Since 1990, the families have been tested for resistance against furunculosis in challenge tests with one-year-old fingerlings (Gjedrem et al. 1991). Each year 120 full-sib families are tested. Selection is based on family selection for low frequency of early maturation, disease resistance and combined selection for growth rate (Refstie 1990).

Response to selection for growth rate was estimated to be 14.6% in the first generation for growth rate (Gjerde 1986). In later generations, the genetic gain has most likely been about 10% for growth rate and 8% for age at early maturation. In the third generation of the four year-classes, the selection differential for body weight was estimated to vary between 10.6 and 14.2% per generation (Gjerde, personal communication).

In 1982 AKVAFORSK approached the Fish Farmers Association to develop a national breeding program based on the AKVAFORSK stock for Atlantic salmon and rainbow trout in Norway. The national breeding program was realized in 1986 and new breeding station and multiplier stations were established. Today about 75% of all Atlantic salmon farmed in Norway comes from the national breeding program.

The Atlantic Salmon Federation, St. Andrews, Canada, has started a breeding program for the Canadian salmon farming industry. Family selection is applied for the traits; length at harvest, percentage non-grilse (salmon that do not mature their first year at sea), and percentage S^1 smolt (Friars, personal communication).

There are few reports on cross-breeding in salmon. Gjerde and Refstie (1984) crossed five strains of Atlantic salmon and estimated generally low non-additive genetic variance, particularly for traits recorded in the later stages of life.

Gjedrem et al. (1991) reviewed the literature concerning genetic variation in survival in Atlantic salmon and found it to be rather low. However, applying challenge tests Gjedrem et al. (1991) found a high heritability for resistance against furunculosis while Gjedrem and Gjøen (1993) found a moderate heritability for resistance against furunculosis, BKD and cold-water vibriosis.

Rye and Refstie (1993) estimated genetic parameters for carcass quality traits in Atlantic salmon. They found that heritability estimates were medium to high for the different traits studied. Similar results were obtained by Gjerde and Schaeffer (1989) in rainbow trout. It can, therefore, be concluded that the carcass quality traits show considerable genetic variation.

• **Carp**

A long term selection program was conducted in Israel and it was concluded that individual selection alone was not an effective method for improving growth rate in domesticated common carp (Moav and Wohlfarth 1976). However, heterosis for growth rate and disease resistance have been shown (Moav et al. 1975). Comparisons between different crosses led to the identification of two parental strains. These are practically the only brood stocks in commercial use today (Wolhfarth et al. 1987).

Suzuki and Yamaguchi (1980) studied the heterosis effect by crossing Chinese, Japanese and European races of common carp. Significant heterosis was found in seven of 12 F_1 hybrids. Of the F_1 hybrids the cross between yamato and mirror carp had the highest growth rate.

In 1963 in Russia, V.S. Kirpichnikov started selecting for resistance to dropsy disease in common carp by applying challenge tests. Individual selection within breeds produced varying results. Five generations of selection in mirror carp and Ukrainian Ropsha hybrid carp gave higher gain than seven generations in Ropsha carp. Maximum gain was found among Ukrainian Ropsha hybrid carp. After the fifth generation of selection, the selected line had a mortality of 10.7% compared to 51.0% in the control group (Ilyassov 1987).

• **Channel catfish**

Dunham (1987) reported that the domesticated strain grew faster than wild strains. One generation of individual selection for growth rate in channel catfish gave genetic change of 12 to 18% in all populations evaluated. Bondary (1983) used a combination of family and individual selection for high and low growth rate at 40 weeks of age. Body weight changes, measured as deviations from control line, were about 20% in both directions. Realized heritability for body weight was estimated to 0.10.

Cross-breeding among strains of channel catfish selected for improved body weight has also been studied. Fifty-five percent of the crosses resulted in positive heterosis for growth rate (Dunham and Smitherman 1983). They conclude that heterosis declined over time. Dunham (1987) showed that cross-breeding can also improve disease resistance.

• **Tilapia**

Jarimopas (1988) reported a response to individual selection for body weight in red tilapia of 15.7% after the second generation. However, Hulata et al. (1989); Teichert-Coddington and Smitherman (1988) and Huang and Liao (1990) found no response to individual selection for body weight in Nile tilapia.

In the Philippines, a breeding experiment with Nile tilapia called Genetic Improvement of Farmed Tilapia (the GIFT project) has been carried out since 1988 (Pullin et al. 1991). The first generation was a comparison of seven tilapia strains. In the second generation a full diallele cross between eight tilapia strains was completed and a weak selection for growth rate was practiced among the pure and cross-bred groups produced in the third generation. In the fourth and fifth generations selection for body weight took place based on combined family and individual selection. The response to selection was 23% in body weight in the fourth generation and the fish in the fourth generation grew 70% faster than the most commonly produced strain in the Philippines (Eknath, personal communication).

The selected fish from the GIFT project will be used to start a national breeding program in the Philippines this year. International Center For Living Aquatic Resources Management (ICLARM) is building up an international network of ten countries in Asia and Africa with the purpose of starting national breeding programs and disseminating fish from the GIFT project (Eknath, personal communication).

Genotype - Environment Interaction

When planning a breeding program it is important to know if genotype-environment interactions are present. If there is no interaction, the breeding plan can concentrate on the best strain or combine the best strains into a synthetic population. On the other hand, if a significant genotype-environment interaction exists, the response to selection will be reduced and consequently, it may be desirable to develop strains for different environments.

In rainbow trout, Atlantic salmon, tilapia and selected lines of channel catfish negligible genotype-environmental interactions have been found (Gunnes and Gjedrem 1981; 1978; Eknath, personal communication; Dunham 1987). In common carp and cross-bred channel catfish genotype-environment interaction has been shown (Moav 1976; Dunham 1987). The magnitude of the interaction must be determined before deciding how many strains will be used in a district or a country.

Prospects for Genetic Gain

The conclusion from the review of different species is that there exists considerable additive genetic variation in most of the economic important traits studied. This means that selection programs will give response. Results from cross-breeding experiments vary from one experiment to another and consequently it is not possible to conclude whether cross-breeding should be used in a breeding program.

In the large scale selection programs that use a combination of family and individual selection carried out in Atlantic salmon (Gjerde 1986), coho salmon (Hershberger et al. 1990), rainbow trout (Gjerde 1986) and tilapia (Eknath, personal communication) the genetic gain in body weight per generation varied between 10.1% and 23.0%. Considerable genetic gain has also been obtained for age at maturation (Gjerde 1986) and disease resistance (Ilyassov 1987; Gjedrem et al. 1991).

The genetic gain obtained in fish is higher than in farm animals. The main reason might be that it is possible to have a higher selection intensity in fish because of high fecundity. The genetic variation of growth rate is very high and is usually much higher than in farm animals.

The profitability for a breeding program in fish is therefore likely to be very high. Gjerde and Olsen (1990) estimated the economic value of a genetic gain of 10% in body weight and 8% in reduced frequency of early maturation equal to US$0.20 per kg produced per generation in Atlantic salmon. For a production of 100,000 tons, the economic gain will be in the order of US$20 million per year while the yearly cost to run the breeding program will be around US$2.5 million.

Constraints for a Breeding Program

A breeding goal is likely to remain constant for traits like growth rate and disease resis-

tance. However, for traits like meat quality, consumers may change their opinion and prefer a quality that differs from the one selected. This type of risk is difficult to avoid. However, it is very important for a breeding organization to carefully study market preferences particularly concerning product quality.

When a breeding program is centralized, as discussed above, there is a risk that infectious diseases can be transferred from breeding centers to the industry and cause problems. To avoid this it is extremely important that breeding centers and multiplier stations work within strict hygienic standards, carefully control the importation of new fish and do everything possible to produce healthy fish.

Genotype-environment interaction must be investigated at the start of a breeding program. In the GIFT project the strain-environment interaction was negligible for body weight and survival when the eight strains were tested in several very different environments (Eknath et al. 1993). In Norway, genotype-environment interaction represented between 1.2% and 5.5% of the variation in body weight in Atlantic salmon and rainbow trout studied under a variety of environmental conditions (Gunnes and Gjedrem 1978; 1981). As a result, selection can be carried out in a single population for each year-class.

When reproducing species with high fecundity, inbreeding can occur rapidly. Since inbreeding reduces fitness and growth, it must be kept at a minimum. In a breeding program based on tagged families it is quite easy to avoid mating close relatives. When individual selection is used without fish identification, it may be difficult to avoid inbreeding. However, it should be possible to reduce the inbreeding problem by dividing the breeding population into at least two subpopulations. Broodstock should be selected and produced within each subpopulation and fish for farming should be produced by crossing subpopulations.

Fish selected for farming often become domesticated. These fish are considered a potential problem as some of them may escape and mix with wild stocks of the same species. In particular, there are two potential problems:

> The escapees may be disease carriers. If these fish mix with wild fish they may spread the infection,

> The domesticated fish may interspawn and reproduce or cross with the wild population. It is argued that this type of hybridization will reduce the genetic variation between the wild strains and reduce their fitness.

The transfer of diseases between farmed and wild fish may be a problem and should be taken seriously. Reduction of escapees and preventing disease is the best way to reduce this problem. Concerning the genetic influence from domesticated fish, fish farmers should do their utmost to reduce escapement. This is also in their own economical interest. So far it has been shown that escaped domesticated Atlantic salmon will spawn and the eggs will hatch in rivers. However, little is known about how the fry of domesticated parents may compete with wild fry in the river. Since domesticated fish have been selected for a life in captivity, it is not likely that they can compete in the wild. If they are not well adapted, natural selection will reduce their frequency in the next generation. It should be taken into account that all se-

lected traits in a breeding program are quantitative traits, which are regulated by a large number of genes and that each generation of selection will lead to minor change in gene frequencies.

Another constraint mentioned in connection with selection programs is a possible reduction of genetic variation. According to Falconer (1961) inbreeding will reduce genetic variance. The effect of selection on the genetic variation has been discussed by several authors. Fimland (1979) showed that a small reduction in the genetic variation is expected during the first generations of selection until stabilization is reached. The level of this stabilized state depends on selection intensity of parents and the accuracy of selection. If the accuracy of selection does not exceed 0.6 the stabilized level will not be lower than 80%. The true variation will be reestablished to the initial variation if selection is terminated. Enfield (1974), selecting for pupa weight in tribolium in 120 generations, found no reduction in genetic or phenotypic variation during the selection period. Long term breeding programs of large populations of farm animals, normally do not result in reduction of genetic variation.

Recommendations

Family information should be used in a breeding program for fish because:

- Combined selection is more efficient than individual selection,
- For economic traits like disease resistance, age at maturation and meat quality, individual selection is inefficient and partly impossible,
- In order to identify the fish and keep pedigree records to avoid inbreeding, families must be reared separately until tagging. Family information will then be available,
- A combination of family and individual selection is more efficient than each of them alone.

It is possible to produce large numbers of eggs and sperm from a few selected fish. Consequently, the breeding work can be centralized and only a few breeding centers are needed. A breeding center should be able to test a large number of half- and full-sib families each year. The breeding center must be able to control the entire life-cycle of the fish and testing should be done under environmental conditions similar to those found in the farming industry.

When testing, estimations of breeding values and selections are centralized. The breeding work, therefore, can be conducted in a more sophisticated way by applying available theoretical and practical knowledge. The fish farmer should receive egg/fry/fingerling or broodstock. The farmer needs no new technology and no new equipment to grow improved fish. Introduction of breeding programs in aquaculture is of interest particularly in developing countries because of needs for increased protein production.

Families breeding values should be based on test results from several private farms under commercial conditions. If family testing is conducted in an experimental environment, there is the risk of developing a population unsuitable in some commercial environments, although the genotype-environmental interaction is low.

If the genotype-environment interaction is considerably more, then one population should be developed. The number of populations needed will depend on the magnitude of the interaction. Thus, to avoid an environment where the improved fish do not adapt well, it is important to carefully study the genotype-environment interaction in a breeding program.

The improved fish will grow faster and the farmer can chose to grow the same size fish using a shorter production time, or grow bigger fish using the same production time and improved fish. The increased survival of the improved fish will result in a larger biomass production and the reduced use of antibiotics. If meat quality is included in the breeding goal, the farmer will be able to market a better quality fish at a higher price. The end result for the farmer should be higher production of better quality fish and a reduction in production costs.

By applying selection, the fish farmer creates an advantage over fisheries. Farmers can produce a quality product when the consumer wants it any time of the year.

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Public Policies Regulating the Use of Genetically-Modified Aquatic Organisms: Current and Future Needs Internationally

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Abstract

The development and use of genetically-modified organisms (GMOs) pose both economic benefits and environmental risks. To optimize the mix of benefits and risks, a number of countries and international organizations have implemented policies regulating activities with GMOs. The applicability of these policies to activities with *aquatic* GMOs is, however, limited. The United States, the Organization for Economic Cooperation and Development, and the Food and Agriculture Organization of the United Nations have initiated efforts to develop regulatory instruments specifically addressing concerns posed by development and use of aquatic GMOs. Additional scientific information and well directed public policies will be needed before the commercialization of aquatic GMOs can go forward with minimal environmental risk.

Introduction

Genetic improvement of aquatic species has long encompassed classical breeding practices, such as domestication, selection, crossbreeding, and hybridization. In recent years, breeding experiments involving aquatic organisms have come to encompass biotechnology-based techniques, such as chromosome set manipulation and gene transfer. The development and evaluation of genetically-modified aquatic organisms (aquatic GMOs) is a highly active area of research (Hallerman et al. 1990; Ihssen et al. 1990).

Biotechnology-based methods of genetic improvement pose important implications for aquaculture, fisheries management and conservation. Against the background of the potential benefits posed by aquatic GMOs and the ecological concerns posed by utilization of GMOs in the aquatic environment, the objectives of this study are:

> to review existing public policies regulating the development and use of aquatic GMOs, and

> to identify areas of concern that should be addressed through development of appropriate public policies.

Technical Background

- **Benefits Posed by Genetically-Modified Aquatic Organisms**

» Chromosome set manipulation

Chromosome set manipulation of animal genomes involves suppression of normal

meiosis or mitosis to retain additional haploid sets of chromosomes, prevention of the genetic contribution of sperm or egg nuclei, or both. A large number of experiments have evaluated chromosomally-manipulated aquatic species for aquaculture, sport fishery or aquatic weed control purposes. Interest in the various types of chromosomally-manipulated aquatic species stems from their reproductive sterility, the possibility of rapid growth, eased production of interspecific hybrids, rapid production of inbred lines, and possible production of trophy fish (Thorgaard and Allen 1987; Ihssen et al. 1990).

To date, technical factors have limited the use of chromosome set manipulation of many species for aquaculture and fisheries management purposes. Studies of chromosome set manipulation have just recently emerged from the technical demonstration stage for a wide variety of species and treated groups of eggs have often been too small to commence field trials of performance (Thorgaard and Allen 1987). For example, whether adult triploids can provide trophy fish or more economical aquaculture production is still unclear, because results to date are inconsistent, especially among species (Ihssen et al. 1990). Still, some chromosome set manipulated aquatic organisms, such as triploid grass carp (*Ctenopharyngodon idella*) and Pacific oyster (*Crassostrea gigas*), have proven utility and are available commercially.

» Gene transfer

To effect transfer of a gene, a recombinant DNA construct is introduced into recently fertilized eggs, usually through microinjection. Of animals developing from eggs so treated, generally 20% will have incorporated the introduced gene construct into their own chromosomal DNA. A large research and development effort has been focused on transgenic fish, with 14 species subject to gene transfer experimentation in 13 countries (Hallerman et al. 1990). Most work aimed at practical applications has addressed transfer of growth hormone genes for purposes of growth enhancement or of antifreeze polypeptides for freeze resistance. Development of genetic lines of fish bearing introduced genes is at an early stage. The first field tests of transgenic fishes are now underway in the United States (NBIAP 1992a), China (Perry Hackett, University of Minnesota, personal communication), Israel (Boaz Moav, Tel Aviv University, personal communication) and other countries. The results of field performance tests have not yet been published. However, data on performance in laboratory systems have indicated significantly improved growth rates among fish expressing introduced growth hormone genes and a minor degree of freezing point depression among fish expressing an introduced antifreeze polypeptide gene. Reliable quantitative estimates of the degree of performance enhancement should become available within the next two years.

In general, the potential of biotechnological means of genetic improvement for the enhancement of aquacultural productivity and profitability will become clearer over the next decade

• Concerns posed by genetically-manipulated aquatic organisms

Although the potential economic benefits posed by use of aquatic GMOs are clear, there is also a considerable likelihood that such organisms pose significant ecological impacts (Kapuscinski and Hallerman 1990a, 1991; Hallerman and Kapuscinski 1992a, 1992b, 1993; Gregory 1992). Many geneti-

cally-modified organisms are not greatly altered from the wild type, suggesting that were they to escape from confinement, they would be likely to persist, reproduce, and disperse. Because they express novel phenotypes, the entry of aquatic GMOs into natural ecosystems poses environmental impacts of unknown type and magnitude.

As the basis for ecological impacts of a GMO, neither the source of the transferred gene nor the presence or absence of a homologous gene in the host genome will prove important, but rather the type and magnitude of phenotypic alteration of the modified organism (Tiedje et al. 1989; Kapuscinski and Hallerman 1990). Although we cannot predict the full range of phenotypic or performance changes that might be expressed among GMOs, broad classes of phenotypic alterations that could give rise to ecological impacts include: (a) metabolic rates, (b) tolerances to physical factors, (c) behavior, (d) resource use, or (e) resistance to predators, parasites and pathogens. Predicting the types and magnitudes of phenotypic alterations consequent to transfer of a given gene is complicated by the possibility of several types of unintended and uncontrollable genetic effects. These effects include expression of the transgene outside the control of normal homeostatic mechanisms, novel pleiotropy and insertional mutagenesis. These possibilities refute the assertions of some molecular biologists that the phenotypic effects of transfers of particular, well-characterized genes can be predicted with confidence.

Different phenotypic alterations would be expected to form the basis for different mechanisms giving rise to ecological impacts. It is possible that GMOs might prove able to adapt to new ecological niches or to a wider range of ecosystems. Given the complex and poorly understood inter-relationships of organisms within natural ecosystems, it is difficult to predict the range of mechanisms by which altered phenotypes among GMOs might perturb biological communities. Further, it is impossible to predict the long-term responses of conspecific populations or of biological communities to a perturbation, and whether such responses would jeopardize the self-perpetuation of community structure or function. Thus, consideration of the implications of release of a given GMO involves not only the salient qualities of the GMO, but also of the receiving ecosystem.

Should aquatic GMOs reproduce within natural systems, any ecological or genetic impacts that they pose would be perpetuated. In determining the extent of such impacts, the key issue is the fitness of such fish in natural systems. Key unknowns affect the reproductive success and viability of aquatic GMOs. The impacts of reproduction of aquatic GMOs on the viability of conspecific natural populations cannot now be anticipated.

Public Policies Regarding Genetically-Modified Organisms and GMOs

Given that risks are posed by release of GMOs with altered phenotypes into the environment, there follows a need for policies that will lead to realization of economic benefits while minimizing environmental risks. The perception that development of GMOs might pose risks to human health or to the environment led a number of governments and international institutions to promulgate guidelines regulating research and development of GMOs as a broad class. Al-

though several regulatory schemes explicitly concede that GMOs are not intrinsically dangerous (e.g., Office of Science and Technology Policy 1992), the continuing focus on GMOs is due to the limited experience with their application and the potential for the novel roles that they might play in the environment. The design of safety procedures for GMOs might prove useful for other classes of organisms, such as non-modified pathogens or non-indigenous species (Working Group I 1991).

Reviews of existing safety procedures, regulations and guidelines from various countries and international organizations (Working Group I 1991; Hallerman and Kapuscinski 1992b; Custers and Sterrenberg 1992) indicate many common features. Contained laboratory use of GMOs is regulated on the basis of the classification of risk categories and safety procedures that have evolved after 20 years of laboratory experience (Working Group I 1991). Releases of GMOs into the environment are reviewed on a case-by-case basis and conducted in a step-by-step manner, because, in general, there are insufficient data to permit classification of risk.

Although aquatic organisms present a unique collection of potentials and concerns, no nation or international institution has yet implemented public policies regulating the development and use of *aquatic* GMOs. In this section, biotechnology policies of key countries and international organizations are reviewed with regard to their applicability to aquatic GMOs.

- **United States**

To some degree, biotechnology policies adopted by the United States affect the subsequent development of biotechnology policies in other countries. A detailed description of U.S. biotechnology policies is, therefore, useful for purpose of this review.

Federal biotechnology policies in the United States are based upon the Coordinated Framework for the Regulation of Biotechnology (Office of Science and Technology Policy 1985, 1986). The Coordinated Framework is based on the premise that no special legislation is needed to regulate biotechnology, i.e., that through promulgation of new regulations, the scope of existing statutes could be extended to effectively cover concerns raised by the development and commercialization of GMOs. Several agencies were directed to promulgate the relevant regulations, notably, the U.S. Department of Agriculture (USDA) to cover multicellular GMOs and the Environmental Protection Agency (EPA) to cover microbial GMOs.

Under the terms of the Coordinated Framework, regulatory authority over development and release of aquatic GMOs is incomplete (Hallerman and Kapuscinski 1990a):

> At institutions receiving federal funding, compliance with guidelines specified by the Coordinated Framework is *required*. Among institutions not receiving federal funding, voluntary compliance with appropriate guidelines is merely *expected*, leaving the private sector effectively unregulated. Although little work with aquatic GMOs currently occurs in the private sector, the proportion will grow larger with time.

> USDA regulates GMOs using regulations promulgated under the Plant Pest Act (USDA-APHIS 1987), even for organisms that could not

conceivably be considered plant pests. Because aquatic GMOs do not come under the legislative purview of the Plant Pest Act, they are not subject to direct legislative authority (NBIAP 1992a).

> Because the Constitution did not specifically reserve the power for the federal government, the states have the authority to regulate activities affecting fishery resources within their borders. The federal government has regulatory authority over fisheries only on federal lands or for migratory species. Although charged with oversight responsibilities for multi-cellular GMOs under the Coordinated Framework, the USDA has no statutory authority over aquatic organisms.

> Because the Coordinated Framework utilized authorities granted under statutes regulating interstate commerce, the regulatory authority of USDA or EPA over work with GMOs *within* particular states was limited (Stern 1986; Fanning 1988).

> The Coordinated Framework was at best sketchy on regulations for administering outdoor releases of GMOs and provided only minimal direction for regulating the commercialization of GMOs.

The Office of Science and Technology Policy (1990) subsequently modified the Coordinated Framework by adopting the draft "Scope" document. "Scope" modified U.S. regulatory policy in two key ways. First, it declared that regulatory purview was to be based, not on the *process* by which an organism was modified (e.g., through gene transfer), but on the *characteristics* of the organism itself. However, using the modified phenotype as the criterion for regulation complicates the issue of what is regulated by the body of evolving public policy. Second, the Scope Document declared that regulation of the products of biotechnology would be risk-based. The degree of regulatory oversight would be a direct function of the risks that the product poses to human health or to the environment. Yet, the risks posed by fish expressing an introduced growth hormone gene, for example, cannot be quantified on the basis of present knowledge. The Office of Science and Technology Policy (1992) subsequently announced a final "Scope" policy on exercise of federal oversight of introductions of biotechnology products into the environment.

As required under the Coordinated Framework and following the direction of the draft Scope Document, federal agencies promulgate draft biotechnology guidelines, USDA for multicellular organisms and EPA for microbes. The USDA guidelines (USDA-CSRS 1991) are designed to be used as research aids to assess the risk posed by a given proposed release of a GMO and to set appropriate confinement levels for designing a protocol to minimize the risk.

The development of policies on the environmental release of aquatic GMOs has been conducted on an *ad hoc* basis, to a large degree driven by requests for environmental release permits. The first was a request by Rex Dunham of Auburn University to release transgenic common carp (*Cyprinis carpio*) into a facility at the university's Agricultural Experiment Station. Controversies surrounding the permit request centered on

the degree of confinement offered by the pond complex and on the lack of an explicit policy on environmental releases of aquatic organisms. The permit request was granted, but a second, more secure facility was also built. A subsequent request to release transgenic channel catfish (*Ictalurus punctatus*) in the new, state-of-the-art facility was handled more smoothly (NBIAP 1992a), although again, on an *ad hoc* basis.

Anticipating further requests for release permits, the USDA Office of Agricultural Biotechnology, through a working group under its Agricultural Biotechnology Research Advisory Committee (ABRAC), is working to develop performance standards for safely conducting research with genetically-modified fishes, molluscs and crustaceans. Development of the performance standards is ongoing, and the draft document should be ready for publication by late 1993.

» State biotechnology regulations

Because federal regulatory authority over activities within particular states is limited and because of the limited scope and slow pace of development of biotechnology regulations at the federal level, nine states have enacted legislation regulating biotechnology (Committee on Biotechnology 1990; Biotechnology Working Group 1993). Most of these regulatory instruments go beyond the provisions of the Coordinated Framework and subsequent federal regulations to address key loopholes or procedural ambiguities. However, none of these effectively address concerns unique to genetically-engineered aquatic organisms. Regulatory authorities in one state, Minnesota, have actively solicited the advice of fisheries professionals in order to address this shortcoming. Additionally, the author is working with aquatic resources management agencies in Virginia to develop guidelines for safe development and use of aquatic GMOs.

• **Organization for Economic Cooperation and Development**

The Organization for Economic Cooperation and Development (OECD) is a group of 25 industrialized nations. An *ad hoc* group of technical experts was commissioned by the OECD to identify scientific criteria for the safe use of recombinant DNA-bearing organisms in industry, agriculture and the environment. Their report (OECD 1986) influenced development of biotechnology policies worldwide, and proved an important step toward international harmonization of biotechnology regulation. However, neither the 1986 OECD report, nor a subsequent one (OECD 1992) offered guidance on safe development or field testing of aquatic GMOs.

At an OECD-sponsored symposium on Aquatic Biotechnology and Food Safety in June 1992, the need was recognized for additional study of a set of related problems. Hence, an OECD workshop on Environmental Impacts of Aquaculture Using Aquatic Organisms Derived Through Modern Biotechnology was held in June 1993 in Trondheim, Norway. The output of the workshop will be a state-of-the-art report on aquaculture biotechnology, which will identify gaps in knowledge and further needs for priority attention. The report will help define the work needed to promote the sustainable development and use of aquatic organisms derived through modern biotechnology.

• **European Community and its member nations**

The European Community (EC) is an organization of 12 countries aimed at achieving high level economic and political coopera-

tion. With the drive for political integration came the need to coordinate biotechnology policy among all the EC countries. Against the background of diverse existing biotechnology regulations among member countries, directives on regulations for contained use (90/219) and for deliberate release (90/220) of GMOs were adopted by the European Commission. The directives set out general regulatory guidelines, and member countries have some degree of discretion regarding how to implement the directives in national regulations. The respective countries are at different points in the process of adopting legislation and developing administrative procedures for regulating research and field release activities with GMOs (Custers and Sterrenberg 1992). Policies addressing concerns particular to aquatic GMOs have not yet been addressed at the national level.

- **United Nations**

The program of the United Nations Conference on Environment and Development (UNCED), held in Rio de Janeiro in June 1992, included a component on environmentally sound management of biotechnology. The Preparatory Committee for UNCED (Working Group I 1991) requested that the Secretary General of UNCED follow the work of relevant international organizations on safety in biotechnology, with a view to expediting the elaboration of basic guidelines and facilitating the preparation of an international Code of Conduct. The Committee also asked the Secretary General to prepare a report on the methods that could be used internationally to assess biotechnology risks to human health and the environment and the impact of biotechnology on socioeconomic conditions. A conceptual plan for an integrated program on the environmentally sound management of biotechnology was described, which included a component for freshwater and marine aquaculture.

The biotechnology policy so commissioned was adopted as Chapter 16 of Agenda 21 (UNCED 1992), a document committing signatory nations to strive for environmentally sustainable development, which was adopted at the UNCED. The program areas set out in Chapter 16 seek to foster application of internationally agreed principles to ensure the environmentally sound management of biotechnology to engender public trust and confidence, to promote the sustainable applications of biotechnology, and to establish appropriate enabling mechanisms for these purposes. Chapter 16 specifically stated that government and non-government entities should evaluate the use of various biotechnology techniques to improve the yields of fish, algal and aquatic species.

As the U.N. body concerned with agriculture, forestry and fisheries, the Food and Agriculture Organization (FAO) is the lead agency for implementing the broad mandates of Agenda 21 into specific programs. FAO is planning to produce a publication stating its policy on biotechnology (H. De Haen, UN-FAO, personal communication). The purpose of the publication is to inform policy makers, research managers and technology managers at national and international levels of FAO's perception and approach toward increasing national capabilities, especially of developing countries, for rational and balanced exploitation of biotechnology.

To support its efforts in this area, the Fishery Resources and Aquaculture Service of FAO has asked the author to produce a technical review of aspects of biotechnology as they relate to aquaculture, fisheries management

and conservation. Now in preparation, the review addresses the potential benefits and risks associated with development and use of aquatic GMOs and the policy options available regulating activities with aquatic GMOs. The document is intended for distribution to fisheries departments, field projects, FAO professionals and local and regional governments, who will be faced with policy decisions regarding application of biotechnology in the aquatic sector.

- **Norway**

It is expected that a legislative proposal regulating the use of GMOs will be acted upon by the national assembly during the spring 1993 session (Jarle Mork, Trondheim Biological Station, personal communication). The purpose of the proposed Norwegian Law for Development and Use of Genetically-Modified Organisms is to insure that production and use of genetically-modified organisms will be carried out in an ethically and socially proper manner in accordance with the principle of sustainable development and without health or environmental damage (Helge Klungland, Agricultural University of Norway, personal communication). The proposition is very similar to the EC and OECD directives, although it emphasizes ethical and social concerns to a greater degree.

Key definitions in the proposed Norwegian law imply that the scope of coverage will be limited to organisms derived from recombinant DNA or cell fusion techniques, i.e., that the law might not apply to ploidy manipulated organisms. The proposed law regulates commercialization of GMOs, addressing conditions of environmental release, product labeling, product liabilities and consequences for non-compliance.

Regarding aquatic GMOs, concerns about escape of fish from floating net-pens have led Norway to adopt a policy not to use organisms modified by genetic engineering as production animals in the aquaculture industry (Maryln Cordle, USDA, personal communication).

- **Japan**

Responsibility for regulating laboratory production of GMOs in Japan is divided among several public agencies, depending on where it is carried out (McCormick 1987). Research at universities is subject to guidelines promulgated by the Ministry of Education, at other agencies to guidelines of the Science and Technology Agency, and in industry, to guidelines of the Ministry of International Trade and Industry. Regulatory authority over agriculture-related environmental releases of GMOs lies with the Ministry of Agriculture, Forestry and Fisheries (McCormick 1987).

Only a single field trial of a recombinant DNA-bearing organism, a plant, has been carried out in Japan (Miller 1993). The biotechnology regulatory climate in Japan has been criticized (Miller 1993) for not providing clear, predictable, risk-based regulation to those contemplating field trials.

- **Canada**

The regulatory approach taken in Canada is to apply existing legislation to cover concerns posed by biotechnology. Laboratory production of GMOs is regulated under guidelines promulgated by the Medical Research Council (MRC). Weaknesses in existing regulatory authority (Kapuscinski and Hallerman 1990b) include the requirement to follow the guidelines only among projects funded by the MRC or by the Natural Sciences and Engineering Research Council,

leaving the private sector effectively unregulated.

Although the applicability of legislation such as the Canadian Food and Drug Act, the Quarantine Act, and the Animal Disease and Protection Act to the testing of veterinary biologics, food, or drugs produced through biotechnology seems straightforward, products such as genetically-modified animals, which are intended for use in the environment, are not well covered. The Canadian Environmental Protection Act may be applied in situations where regulatory coverage under existing legislation is absent or unclear (Government of Canada 1988). Draft regulations promulgated under the Act, including those for confinement of transgenic animals and for assessing permit applications for environmental releases, are in development.

- **Other countries**

Research addressing the genetic modification of aquatic organisms is going forward in a number of countries where there are no public policies mandating safe laboratory practices or restricting environmental release of GMOs (Table 1). Outdoor releases of transgenic fishes have taken place in at least two of these countries (China and Israel).

A number of issues complicate development of public policies regulating development of GMOs in developing countries. Such countries often lack the relevant technical expertise and management experience (Working Group I, 1991). Further, public awareness or concern about activities regarding development of GMOs, may not be high enough to drive development of regulatory public policies.

In a pro-active approach to addressing the lack of relevant expertise in developing countries, the Stockholm Environmental Institute held a Biosafety Workshop in December 1990 to consider the organization of an independent international biosafety panel to provide advice on request with respect to the release of transgenic organisms into the environment (Working Group I 1991). Participants suggested that the concept be broadened to cover agricultural biotechnology, and made recommendations on panel structure, organization and implementation.

Future Policy Needs Regarding Aquatic GMOS

Despite the readiness of many lines of aquatic GMOs for field testing, there is a general lack of relevant and explicit guidelines for expeditious, but environmentally sensitive field testing. Some researchers have complained that the lack of explicit field testing guidelines has constrained the progress of their projects. The need for field testing guidelines is being addressed by certain countries and international groups (United States, Norway, Organization for Economic Cooperation and Development, UN-Food and Agriculture Organization). The development of detailed guidelines regulating the field testing of aquatic organisms remains a clear and present need. However, the lack of guidelines for field testing aquatic GMOs is but one issue that will need to be addressed in the foreseeable future. In this section, other policy issues are identified that will need to be resolved before aquatic GMOs can be utilized broadly for their intended applications.

- **Targeted research to support policy development**

The justification for biotechnology regulatory policy is the maximization of benefits

Table 1. Examples of countries where research on genetic modification of aquatic organisms is conducted, and presence or absence of regulations over research with, or environmental release of, genetically-modified organisms (after Hallerman and Kapuscinski, 1992b).

Country	Source of regulation[1]		
	National	EC Member[2]	OECD Member[3]
Canada	yes	no	yes
France	yes	yes	yes
Germany	yes	yes	yes
Hungary	no	no	no
India	yes	no	no
Indonesia	no	no	no
Israel	no	no	no
Ireland	no	yes	yes
Japan	yes	no	yes
Malaysia	no	no	no
Mexico[4]	yes	no	no
Norway	yes	no	yes
People's Rep. of China	no	no	no
Thailand	no	no	no
Russia	no	no	no
United Kingdom	yes	yes	yes
United States[4]	yes	no	yes

[1] In some cases, there may be regulation of research involving recombinant DNA at particular institutions.

[2] Compliance with European Community (EC) directives implies prompt enactment of regulations.

[3] Suggests voluntary compliance with recommended Organisation for Economic Co-operation and Development (OECD) guidelines.

[4] Member of Inter-American Institute for Cooperation on Agriculture (IICA).

accruing from use of GMOs and the minimization of associated ecological risk. Public policies must be science-based so that a demonstrably justifiable balance can be struck between commercial and environmental protection interests. In the aquatic sector, there is a lack of quantitative information upon which sound regulatory policies can be based.

Faced with a large number of unknowns, it is clear that only through field testing will quantitative data be assembled which (1) characterize phenotypic modification in manipulated lines, (2) quantify the fitness of genetically-modified lines under aquaculture and more natural conditions, and (3) identify and assess the likelihood of various ecological impact mechanisms. Experimental designs for quantifying phenotypic modification, in terms of both targeted performance traits and other traits, are relatively straightforward. However, practical experimental designs for quantifying fitness and likelihood of environmental impacts are not well established. Data from such experiments will provide the quantitative input for the process of

risk management, the process of using scientific data to reach decisions maximizing the benefits of using GMOs while minimizing risk (Gregory 1992).

- **Policies on commercialization of GMOs**

The first commercialization of GMOs is underway. Rapid technical progress in the development of aquatic GMOs suggests that commercialization of many of them could be sought by the middle of the 1990s. Development of sound public policies on commercialization of biotechnology-derived products is supported by the industry because it is good business policy for the long term. By reducing the likelihood of undesired ecological impacts, it will engender greater investor and consumer confidence in the biotechnology industry. However, two issues relating to commercialization of the products of biotechnology are not yet fully addressed.

The first issue is the determination of environmental safety for commercial-scale production of GMOs. The collection and interpretation of relevant field testing data, are necessary pre-conditions for the determination of environmental safety for commercialization of a particular GMO. Requests for permits to go forward with commercial production of GMOs have been handled on a case-by-case basis. The first wave of biotechnology products, mostly for medical applications, has been licensed. In the United States, for example, 45 biotechnology products, 39 for diagnosis of disease and six gene-deleted vaccines, have been licensed for commercial production (Songer 1993). In contrast, the first biotechnology-derived food products are only now reaching the commercialization stage. In October 1992, the USDA announced that CalGene's Flavr Savr tomato was approved for commercial production, with the US Food and Drug Administration (FDA) expected to find the tomato marketable (NBIAP 1992c). In its proposed law, Norway asserts a high standard for environmental safety of GMOs. A product will not be approved for sales unless there is no possibility for adverse effects on human health or the environment. Assuming that many countries will require a finding of environmental safety, the commercialization of aquatic GMOs may be delayed because of difficulty in reaching such a finding.

A second issue for public policy formulation concerns the food safety of products of biotechnology. Consumer groups, particularly in developed countries, have raised the issue of food safety to a high level. For example, in the United States, major controversies have addressed the safety of milk from cows injected with bovine somatotropin (growth hormone) and of the Flavr Savr tomato to be marketed by CalGene. The occurrence of these controversies implies that segments of the American public will not be satisfied with the FDA's statement of policy that no special testing, labeling, or pre-market notification will be needed for foods derived from plant varieties developed through biotechnology (NBIAP 1992b). Taking the opposite approach, the proposed Norwegian law for development and use of GMOs provides for special labeling of products consisting of or containing GMOs. Of direct relevance to this workshop, a Symposium on Aquaculture Biotechnology and Food Safety was held by the OECD in June 1992 in Bergen, Norway.

- **International policy coordination**

Because the environment of all countries is interconnected and because of the special needs of developing countries, a degree of

international policy coordination is needed for environmentally sound management of biotechnology. Chapter 16 of Agenda 21, adopted by almost all countries at UNCED in 1992, sets out a number of program areas to foster internationally agreed principles to promote development of sustainable applications of biotechnology and to establish appropriate enabling mechanisms. Activities called for include: evaluation of the use of biotechnology techniques to improve the yields of fish, algal and aquatic species; development of education programs for decision-makers and the general public regarding benefits and risks of biotechnology; enhancement of human resources in developing countries; and development of mechanisms for international cooperation for information exchange and for adoption of technical guidelines and safety procedures. Chapter 16, and indeed all of Agenda 21, is but a framework for action; much work must follow in order to achieve the adopted goals.

- **International intellectual property protection**

The development of GMOs requires a rather long-term investment of human and physical resources. The distribution of GMOs through channels in the private sector will depend on the ability of private producers to recover the value of their investment. The commercialization of aquatic GMOs will, thus, depend on the development and institutionalization of relevant intellectual property protection. The applicability of patenting and other forms of intellectual property protection has emerged as a contentious public policy issue, raising legal, economic, social and ethical questions (Hallerman and Kapuscinski 1990b). The political importance of intellectual property protection was underscored when the United States refused to sign the UNCED Biological Diversity Convention, specifically because insufficient intellectual property protection was offered to the products of biotechnology.

Differences exist among countries regarding what intellectual property protection is offered for biotechnological inventions (OTA 1991). The key legal precedents establishing the patentability of GMOs took place in the United States (OTA 1989), where four genetically-modified animals have been patented to date. Many countries grant patents for novel microorganisms, and a small proportion do so for plants. It seems likely that other nations will issue animal patents in the future (Raines 1988).

Several international agreements have been reached regarding protection of intellectual property rights for biological inventions (OTA 1989). While virtually all developed nations and many developing nations are signatories to key treaties, some counties are not. This is likely to give rise to resistance on the part of those who have developed aquatic or other GMOs to share biological materials or methodologies with prospective counterparts in non-signatory countries. For example, while Chile has a rather well developed aquaculture industry, it is not a signatory of any patent-related treaty.

Perspective

Aquaculture, fishery management and conservation activities are practiced within the context of natural ecosystems. Results from laboratory and field tests suggest that use of aquatic GMOs poses considerable benefits. The goal of realizing the benefits posed by aquatic GMOs, while minimizing risk to natural ecosystems, will require concerted activity by a wide range of professionals:

- Production of aquatic GMOs will require the expertise of molecular geneticists and animal breeders,

- Development of risk assessment methods and data sets to support quantitative risk assessments will require interaction among ecologists, aquaculture scientists and risk assessment specialists,

- Other expertise will be needed for risk management, integrating benefit and risk information with input reflecting societal values to reach defensible policies for commercialization of aquatic GMOs,

- Environmentally responsible realization of the economic benefits posed by use of aquatic GMOs will require adoption of a long time horizon and sound judgement by public funding agencies and the private sector.

Taking these many functions into account, it is clear that realization of economic benefits with minimal environmental risk will depend upon the careful crafting of public policies to guide and expedite well safeguarded research and development activities with aquatic GMOs. The development of such policies is, therefore, timely and appropriate.

Acknowledgements

I gratefully acknowledge support of my public policy research by the Virginia Graduate Marine Science Consortium. This work is a result of research sponsored by NOAA Office of Sea Grant, U.S. Department of Commerce, under federal Grant No. NA90AA-D-SG045 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyrighted notation that might appear herein. I am also grateful to The Oceanic Institute for supporting my attendance of this workshop.

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A Review of Freshwater Fish Genetic Conservation Research and Practices in China

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Abstract

China's natural aquatic diversity and traditional inland fisheries are threatened by the need to feed the most populous nation on earth. As the world's leader in aquaculture production, conservation strategies are extremely necessary to protect China's genetic resources. In this paper, the status of fish genetic resources and a relevant study in China are presented. Efforts toward genetic conservation of the major Chinese carp are described. The impact of the Three Gorges Dam is also considered.

Introduction

For thousands of years plants and animals have been domesticated and adapted to agriculture by intentional and unintentional genetic changes. In contrast, a few fish species (common carp, rainbow trout, Atlantic salmon) have been domesticated in a rather short period.

Aquaculture, the so-called "blue revolution," is now at the stage where genetic erosion is beginning to impact fish populations. It is essential to foresee, and if possible to forestall, a major loss of aquatic genetic resources. The strategies used to maintain those genetic resources will be significantly different from those used for plant crops.

Genetic conservation studies of fish began only recently in China. As the leading aquaculture producing country in the world, it is necessary to develop a genetic conservation program for aquatic organisms.

Overview of Freshwater Fish Genetic Resources in China

- **Genetic resources and their role in Chinese aquaculture**

China has a rich freshwater fishery resource. There are more than 800 species distributed throughout China. Among them, about 760 are pure freshwater fishes (subspecies), 60 are migrant and 21 are exotic food fishes. Less than 200 species contribute significantly to food production. Table 1 shows fish species found in the major river systems in China. These fishes are essential genetic resources for aquaculture development in China.

Chinese carp are the primary aquaculture species in China and the world (Table 2). In 1990, fisheries production in China reached 12.37 million tons, of which freshwater aquaculture accounted for 4.4 million tons. The filter feeding species (silver carp and bighead carp) accounted for 2.5 million tons and the herbivorous species (grass carp and blunt snout bream) accounted for 1 million

Table 1. Incomplete statistics of number of fish species (Spp.) in the major river systems of China. (--) refers to those species for which data is not available.

River	Zhujiang (Pearl)		Changjiang (Yangtze)		Huanghe (Yellow)		Heilongjiang (Amur)	
Family	Spp.	%	Spp.	%	Spp.	%	Spp.	%
Cyprinidae	167	45.1%	141	49.8%	84	55.6%	59	52.2%
Bagridae	23	6.2%	19	6.7%	6	4.0%	4	3.6%
Gobiidae	17	4.6%	12	4.2%	8	5.2%	3	2.7%
Cobitidae	28	7.6%	19	6.7%	18	11.7%	--	--
Salmonidae	--	--	1	--	--	--	11	9.8%
Homalopteridae	22	6.0%	15	5.3%	--	--	--	--
Salangidae	--	--	5	1.8%	8	5.2%	--	--
Acipenseridae	--	--	3	1.1%	--	--	2	1.8%
Others	121	32.7%	68	24.0%	29	18.3%	34	30.0%
Total	378	100.0%	283	100.0%	153	100.0%	113	100.0%

Table 2. The position of Chinese carp in fish culture production in China and the world.

Species	Rank	
	In China*	In World **
Silver carp	1	1
Grass carp	2	3
Bighead carp	3	4
Common carp	4	2
Black carp	5	
Crucian carp	6	
Blunt snout bream	7	

Source: * Bureau of Aquatic Products, Agriculture Ministry of China, 1991. ** FAO, 1990.

tons of the freshwater aquaculture production.

- **Loss of genetic resources**

Loss of biodiversity throughout China will severely limit future options for sustainable development of aquaculture and fisheries. Genetic diversity of fishes in China is decreasing as a result of loss of species and loss of genetic variability within species.

- **Loss of species**

With the rapid development of the country and the ever increasing demand for fish as food, aquatic ecosystems are under constant stress. This stress has resulted in the loss of many species.

One preliminary estimate states that there are approximately 100 fish species (9 orders, 24 families and 80 genera) that are either extinct, endangered or are under threatened status in China (Li 1991, cited in Li 1992). Extinct examples include *Cyprinus yilongenesis*, *Anabarilius albumops*, and *A. ploylepis*; endangered examples include *Schizothorax taliensis*, *S. biddulphi*, *Cyprinus longipectoralis*, *C. crassilabris*, *C. megalophthalmus*, *Barbodes exigiua*, *B. coggili*, *B. exigua*, *Psephurs gladius*, *Aspiorhynchus laticeps*, and *Macrura reevesi* and threatened examples include *Myxocyprinus asiaticus* and *Acipenser sinensis*.

Compared to the ocean, inland rivers and lakes have simple fish faunas, lower biodiversity indices and a fish diversity that is very sensitive to ecosystem changes. The endemic fish species may suffer from the introduction of exotic species, destruction of specific habitats and overfishing.

The introduction of exotic species has impacted fish species in many areas of China. Introduction may also cause a loss of production. In Fuqiaohe Reservoir (2,000 ha), fry of the predatory fish *Elopichthys bambusa*, were accidentally introduced with major carp fry. The fish production decreased from 420 kg/ha in 1966 to 25 kg/ha in 1975. Certain species may be particularly susceptible to the introduction of exotics. In Buston Lake (960 km^2) in west China, *Perca fluviatilis* was introduced in the 1960s. They reproduced rapidly and preyed on the local species *Aspiorhychus laticeps*, which had previously dominated the lake's fish and are now rare.

Pollution from industry, habitat loss as a result of reservoir construction, building of facilities along shores of river and lakes, silt and sediment from land based forestry, agriculture and construction have affected fish resources throughout the world. Habitat loss on the Yangtze River, one of the most heavily exploited rivers in China, has significantly affected fish populations.

The Chinese sturgeon (*Acipenser sinensis*, Fig. 1) migrates between the ocean and river. The construction of Gouzhouba Dam in 1981 blocked its spawning migration route. This fish is no longer present in the upper reaches of the Yangtze River.

Chinese paddle fish (*Psephurus gladius*, Fig. 2) are distributed in the middle and lower reaches of the Yangtze River. Since the 1980s, its populations have sharply declined. The sampling data from June-August at the estuary of the Yangtze River is presented in Table 3.

Over-exploitation of many species is well-known and documented. The prized anadromous hilsa (*Macrura reevesi*, Fig. 3) provided 300,000-500,000 kg catch annually in Yangtze River before the 1970s. The highest

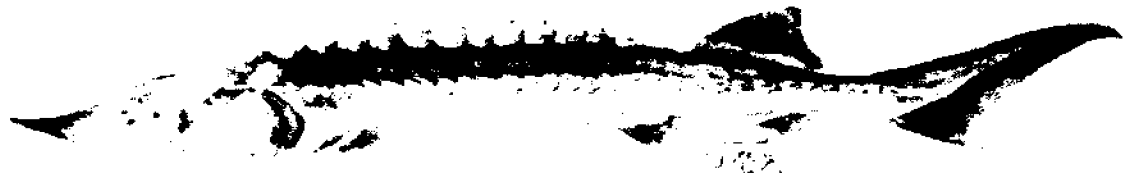

Figure 1. Chinese sturgeon (*Acipenser sinnensis*)

Table 3. Catch data for Chinese Paddle Fish in the Yangtze River Estuary (1983-1990)

Year	Number sampled
1983	587
1984	9
1985	84
1986	2
1988	5
1989	0
1990	0

yield reached 1,575,000 kg in 1974. It has now lost its significance in the Yangtze River fishery and has completely disappeared in the Qiantangjiang River (Zhou et al., Internal material, 1991).

Overfishing is the main reason for the reduction of fisheries resources in many lakes. For example, in catch composition small species and young age groups dominate the fishery. In Taihu lake (233,800 ha), the small fish, *Coila ectenes*, which has little market value, accounted for an average of 41.1% of the total aquatic production from 1980 to 1988. Another small but valuable species, ice fish, *Neosalanx tangkahkeii* accounted for 9.4% of the total production during the same period (Fig. 4, Cai 1990).

- **Loss of genetic diversity within species**

Genetic pools may be changed through selective fishing of the stock, selection and hybrid breeding and transplantation to new

Figure 2. Chinese paddle fish (*Psephurus gladius*)

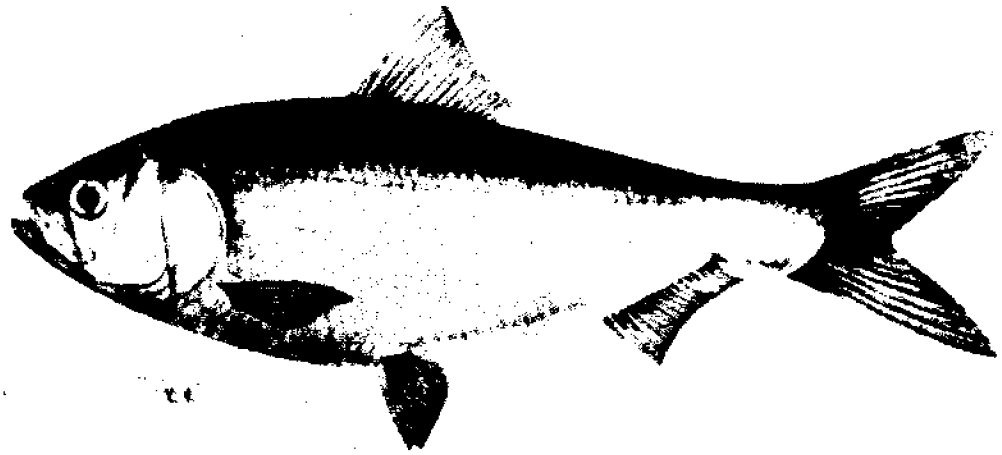

Figure 3. Hilsa (*Macrura reevesi*)

locations. Such changes may affect the resiliency of the species to environmental fluctuations, and finally, the production potential.

The Chinese have a tradition of using the productivity of the waters on a large scale through aquaculture. However, the decrease of the relevant gene pools due to intensification of aquaculture activities, extensive application of artificial reproduction and bioengineering techniques has posed a serious constraint to aquaculture development.

China produces more cultured freshwater fish than any other country. Aquaculture activities are being conducted in ponds, lakes, reservoirs and rivers. Due to stocking methods, escapes and unpredicted flooding, there are many opportunities for cultivated populations to enter natural waters. To meet the demand for aquacultured species in different areas of China, the transportation of fish fry and/or fingerlings is conducted on a large scale. There is concern that this may disturb local fish populations.

Since 1972, significant effort has been expended on fish hybridization, primarily for use of F_1 hybrid heterosis. Zhang et al. (1988) summarized crosses made among 25 fish species belonging to three orders. Emphasis was on use of F_1 hybrid heterosis in common carp. At least six crosses ("harvest carp,""Heyuan carp," "triple-cross carp," "Yue carp,""Furong carp" and "Ying carp") are widely cultured. However, it is not possible to estimate how many hybrids enter natural waters. The pure strain of common carp is difficult to find except in northeastern China.

The development of gene transfer as a means of improving cultured fish stocks is progressing rapidly. At least six research institutes in China are developing transgenic fish. Once these are released to farms, it will be virtually impossible to prevent them from escaping into natural systems. The subsequent impacts on native stocks and aquatic communities are presently unknown (Hallerman and Kapuscinski 1992).

Case Study of Genetic Variation and Conservation of Chinese Carp

- **Genetic evaluation of major Chinese carp**

Since the 1980s, studies on genetic resources and the conservation of important freshwater

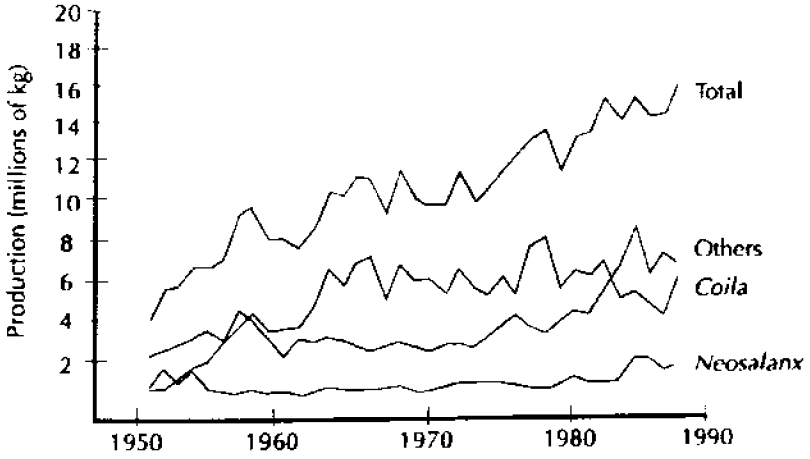

Figure 4. Production of the major species in Taihu lake

fishes have been conducted. Traditional farm fishes include the grass carp (*Ctenopharyngodon idellus*), black carp (*Mylopharyngodon piceus*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), and blunt snout bream (*Megalobrama amblycephala*). These fish are widely recognized as the most important species for farming in a wide range of aquaculture systems, including extensive and intensive, ponds, cages, pens, lakes and reservoirs.

Blunt snout bream is a herbivorous species. It is distributed in several lakes along the Yangtze River. Since the 1960s, it has become a major species in pond and cage fish culture as its production reached 150,000 tons in 1991. A study of the morphological and biochemical genetic variation among different populations of blunt snout bream, supported by IDRC, has been conducted since 1985 in Shanghai Fisheries University (SFU). The results from three lakes and one tributary indicated that the mean proportion of polymorphism was 13.3-20.0%, the average heterozygosity was 0.0549-0.0851, and the Nei genetic distance was 0-0.0219 (Li et al. 1993).

SFU started a study on genetic resources of Chinese carp in 1982 under the support of a national government program and the International Foundation for Science (IFS). The research team included the SFU; Institute of Hydrobiology (IHB), Academia Sinica; Heilongjiang (= Amur) River Fisheries Research Institute (HRFI), Changjiang (= Yangtze) River Fisheries Research Institute (CRFI) and Zhujinag (= Pearl) River Fisheries Research Institute (ZRFI) of the Chinese Academy of Fisheries Science.

The major findings from this study from 1982 to 1989 were:

(1) There was significant intraspecific divergence in morphometric characters among populations of Chinese carp from the three Chinese rivers. The number of scales on the lateral line of silver carp and bighead carp decreases from northern to southern China (Li 1990; Li et al. 1990).

(2) There was biochemical genetic variation among the different populations of Chinese carp from the three rivers. The range of the average heterozygosity was 0.0484-0.0511 for silver carp, 0.1042-0.1133 for bighead and 0.0454-0.1076 for grass carp. The range in proportion of polymorphic loci was 11.8-23.5% for silver carp, 29.4 % for bighead and 20.0-33.3% for grass carp. The population in the south had a higher proportion of polymorphic loci than those in the north (Li 1990; Li et al. 1990).

The proportion of polymorphic loci and the average heterozygosity of wild Chinese carp is much higher than that of the grass carp introduced in the United States (Utter and Folmar 1978; Brummett et al.1988).

(3) Under the same culture conditions, the growth rate of silver and bighead carp from the Changjiang River is 5-10% faster than those from the Zhujiang River (Li 1990; Li et al.1990). Since the annual production of these two species exceeds 2 million tons, this is a major finding and of immediate applicability. In addition, the growth of the wild population of silver and bighead carp from both river systems were shown to be 5-10% faster than hatchery populations. This shows there was a negative response to the domestication process, which requires more attention and further study. This study also showed that genetic factors had a considerable effect on growth variation.

(4) Under culture conditions, silver carp and bighead carp from the Changjiang and Jhujiang Rivers reached maturity at the same time. Environmental factors had a major effect on their gonadal development and age of sexual maturity (Li et al. 1990).

(5) Fishery resources of silver and bighead carp have decreased in three major rivers. In the early 1980s, catches of marketable-sized fish were half of the catch in the 1950s, and the catch of natural fry was one-quarter of the catch in the 1960s. In recent years this decline in fishery resources has accelerated. The catches of silver and bighead carp fry are now insufficient to meet the demand from the limited number of original brooder farms.

Establishment of Fish Gene Banks

Since 1985, there has been an effort to learn how to conserve the genetic material of important freshwater fish species at the population, individual and cellular level. A few different methods have been investigated.

- **Live gene conservation banks**

A gene bank pond farm with a 24 ha area was set up in the Yangtze River Fisheries Research Institute in 1989-1990. Ten species including silver crucian carp (*Carassius auratus gibelio*,), mirror common carp, tilapia nilotica, tilapia aurea, Xingguo red common carp (*Cyprinus carpio singuonensis*), red purse common carp (*Cyprinus carpio wuyuanensis*), silver carp, bighead carp, grass carp and black carp have been preserved in these ponds. The effects of environmental factors on growth, metabolism, gonad development and fecundity of the fish were studied. The optimum environmental parameters, as well as reasonable stocking and rearing techniques were proposed to improve the management of these ponds.

From 1986 - 1990, Xingguo red common carp and red purse common carp gene bank ponds have been set up with 4 ha and 20 ha ponds, respectively. Each bank can supply hundreds of brooders and thousands of fingerlings of red carp. These two common carp are local strains developed under specific natural environments. They have a high potential for heterosis in hybridization.

A gene bank for blunt snout bream was set up in 1990 in Yuli Lake, with an area of 2,000 ha area combined with 5.5 ha ponds. A 300 ha natural spawning ground has been rehabilitated, and a fish screen has been built to prevent fish from escaping. This bank can protect the bream at a population level, and can produce 1500 brooder pairs, 1900 kg of fingerlings and 100 million fry annually.

- **Cryogenic gene bank**

Parallel to live fish collection from the wild, a fish spermatozoa cryopreservation bank has been set up at the National Laboratory of Freshwater Fish Germplasm Resources & Biotechnology (NLFFGRB). The sperm from eight economically important species (black carp, grass carp, silver carp, bighead carp, blunt snout bream, Xingguo red common carp, mirror common carp and silver crucian carp) have been stored.

Although cryopreserved sperm do not undergo changes during storage in liquid nitrogen, it represents only half the genome. The banked sperm of a stock might be less useful if the female of that strain were to become unavailable.

Genetic Conservation Program of the Yangtze River and Evaluation of Impact of the Three Gorges Dam

• The importance of Yangtze River for fisheries

The Changjiang River is the third longest river in the world. It originates in the Qinghai-Tibetan plateau (4000m elevation), flows through nine provinces and has a total length of about 6300 km. It enters the East China Sea on the north side of Shanghai. Its catchment covers an area of 364,000 km^2, and with favorable monsoon climate and good ecological conditions, an abundant fish composition has developed in the Chinese and Asian river systems. Among them, about 100 species are native to the Chinese plain rivers including grass carp, black carp, silver carp, bighead carp, *Elopichthys bambusa*, *Coreius heterodon*, white amur bream (*Parabramis pekinensis*), black bream (*Megalobrama terminalis*), blunt snout bream, and mandarin fish (*Siniperca chuatsi*).

Fish production from the Changjiang River basin accounts for 60% of the total freshwater fish production in China. It is the major source of wild brooders for artificial breeding and ensures the genetic variability of cultured Chinese carp.

In the early 1960s, it was estimated that the annual fry production of the four major carp species was 115 billion and that 200,000 spawning brooders of these four species were available. But only 17.3 billion fry were produced in 1980 (Survey Team of Spawning Grounds of Domestic Fishes in Changjiang River 1982) and today, resources have decreased even further.

The sturgeons (Chinese sturgeon), Changjiang sturgeon (*Acipenser dabryanus*), and Chinese paddle fish are all ranked as protected fish species in China. To rehabilitate the Chinese sturgeon, a hatchery was established below the Gouzhouba Dam. Artificial reproduction has been successful and the hatched fry and fingerlings have recently been released into the river. As a result, the population has been increased. After construction of the Three Gorges Dam, they may face a new challenge.

• The construction of the Three Gorges Dam and an evaluation of its impact on fisheries resources

The decision to construct the Three Gorges Dam on the Yangtze River was made in 1992. The principle reasons are to produce electric power, control flooding and improve navigation. As a result, a huge reservoir 175 meters high and 500-600 km in long will be created. Many evaluations of impact on the environment, including work on fisheries resources, have been completed, but the impact on the genetic resources of major cultured fish species has been ignored.

Through changed water flows, elimination of seasonal flooding and water temperature changes that presently trigger spawning, construction of the Yangtze High Dam will obliterate the major spawning grounds (downstream of the Gouzhouba Dam, Fig. 5) for carp in the most important part of the river. More problematic will be a reduction of flooded backwater areas, which form the nursery and fattening areas for fry and fingerlings of Chinese carp. This may result in a significant loss of genetic diversity from the single most important source of these fish in the world.

After spawning below the Gouzhouba Dam disappears, spawning might move downstream and the Swan oxbow would lose its

function as a breeding and rearing area for Yangtze River fish.

The Yangtze River is the cradle of Chinese fish culture. It contains essential resources for the maintenance of Chinese carp genetic diversity. It is necessary to protect its resources so that China can maintain its position as the world's leader in aquaculture production, as well as maintain the most important freshwater fish genetic resource in the world.

Recently, a research study has been proposed by the author to the Ministry of Agriculture to address current issues that may affect the genetic diversity within the Yangtze River. The proposed research will focus on:

(1) Evaluating the potential impact of the Three Gorges Dam on the genetic resources of major Chinese carp,

(2) Maintaining the genetic diversity of major Chinese carp of the Yangtze River by establishing a series of reserves (Fig. 5) along the Yangtze River and a gene bank at SFU,

(3) Designing a sustainable management systems and ensuring preservation of the genetic resources that are available for production,

(4) Providing fish and germplasm to national and/or international research programs and to fish farms by distributing Yangtze stock of Chinese carp through the National Committee of Aquatic Varieties Certification (NCAVC) and the Yangtze River Wild Fish Stock Utilization Program (YRWFSUP).

- **Current research**

Since 1991, a national research program has been carried out under the title, "Natural Ecological Bank of Genetic Resources of Major Chinese carp in Yangtze River." This program has focused on three goals.

> Establish an isolated conservation area for major Chinese carp

Louheko oxbow is located at the middle reach of the Yangtze River and forms a

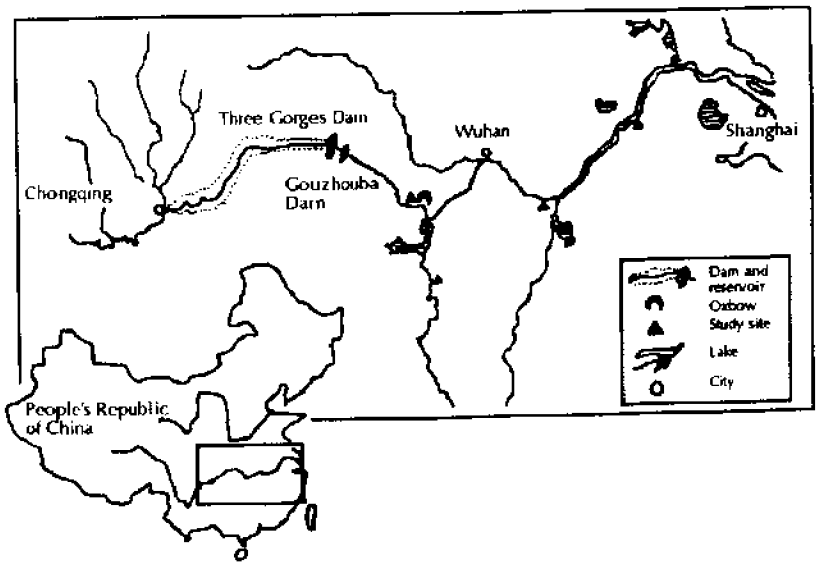

Figure 5. The middle and lower stream of the Yangtze River, Gouzhouba Dam, Three Gorges Dam, Swan oxbow and five study sites.

20,000-ha area. This oxbow is completely isolated from the Yangtze River. The wild fry are collected from the river and nursed in ponds in their first year and stocked into the oxbow in their second year. This conservation bank will support wild fish brooders for the hatcheries.

> Establish an open conservation area for major Chinese carp

The Swan oxbow was formed in the 1970s and remains open to the Yangtze River and has an area of 1200 ha. The major Chinese carp migrate between the oxbow and river. Swan oxbow will be used as a conservation area to keep a sufficient amount of fish to supply brooders to the hatchery. It will also be used as a window to observe the genetic variation of major Chinese carp affected by the changing environment of the Yangtze River, particularly after the damming of Three Gorges valley.

> Establish a genetic standard for major Chinese carp

In contrast to well-developed fish culture techniques, which date back thousands of years, genetic improvement of Chinese carp has so far remained almost untouched by recent biological advances. This is due to several reasons.

> Chinese carp have a long generation time (4-6 years) and require big ponds to grow and mature properly,

> The farming of Chinese carp was based primarily on the use of wild seed until the breakthrough of induced breeding in the 1960s,

> There has been a lack of genetic skills in China until recently,

> There has been a lack of sufficient funding to support long-term genetic research

In 1986, a national program was started to establish genetic standards for major Chinese carp. The propagation of fry by thousands of hatcheries in China reaching 140 billion a year, and the transportation of these fish throughout the country, prompted the study. Its goal is to establish genotypic and phenotypic criteria for the sustainable use of genetic resources and the healthy development of aquaculture in China. These standards are for government, scientists and managers to use in the different levels of hatchery and farm production, and for monitoring and controlling the quality of the product. This study, now under the direction of the Shanghai Fisheries University, should be completed by 1995

Responses from the Chinese Fisheries Community to the Genetic Conservation Issue

In China, there is a rich diversity of freshwater fishes and there are strong national movements both for genetic improvement and genetic conservation. It is well recognized that the application of genetic principles to aquaculture is far behind that of agriculture or animal husbandry. Conservation of genetic resources will bring long-term benefits to the development of aquaculture in China and the world. Due to rapidly changing socioeconomics and a changing environment (for example, the construction of the Three Gorges Dam and the expansion of industry in the countryside), genetic conservation strategies are necessary to protect genetic resources. Several relevant events are mentioned below.

In 1988, the National Laboratory of Freshwater Fish Germplasm and Biotechnique (NLFFGB) was established. It reflects a growing national concern for the conservation of aquatic genetic resources from the government and fisheries community.

The Yangtze River Wild Fish Stock Utilization Program (YRWFSUP) was organized in 1991. It is a collective organization formed by people interested in the utilization and protection of the wild Chinese carp stocks of the Yangtze River. Now it involves about 30 fish farms, research institutes and administrative extension agencies.

The National Committee of Aquatic Varieties Certification (NCAVC) was established in 1991 under the Ministry of Agriculture. The NCAVC is planning to set up 15 national farms for wild and domesticated fish and crab broodstock to protect and produce better strains under certification and authorization.

In 1992, the Aquatic Genetic Resources Laboratory(AGRL) at Shanghai Fisheries University was established. Its major targets are genetic variation of natural hatchery populations, conservation of genetic resources and international cooperation in aquatic genetic diversity and conservation.

Acknowledgments

I wish to express my gratitude to Drs. B. Ayles and J. Mathias for their constructive comments and English help with the manuscript.

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Breeding Programs for Ornamental Fish Production in Asia

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Abstract

All fish breeders aim to produce fish that are healthy, fast growing and viable. However, ornamental fish breeders face an additional challenge of providing diversity to, capture and stimulate the interests of fish hobbyists. Traditional genetic methods used to produce phenotypic variation include rigorous selection for rare spontaneous mutations, close inbreeding to fix desirable genes, selective breeding and hybridization. Although successful, it has taken decades, even centuries. Advances in molecular technology have enabled the unraveling and engineering of DNA. The challenge therefore, is to determine if the ornamental fish industry can harness biotechnological advancements, for example, transgenesis or induced mutagenesis and collaborate with scientists to develop diversity in a much shorter time frame. In addition, ornamental fish breeders need to select for new criteria such as, adaptability to transportation conditions and urban environments, such as indoor aquaria and small ponds. Since ornamental fish farms are almost entirely small family run businesses, the fish stocks are invariably kept as small, closed populations to preserve purity of the lines. The result is erosion of genetic variability and inbreeding depression despite outcrosses to introduce new genetic variation. With DNA fingerprinting, distant outcrosses between stocks could be planned based on DNA profiles. With the growing awareness of biodiversity, conservation problems of overfishing and continued destruction of the environment, there is need to take remedial measures. Captive breeding of wild-caught fishes needs to be stepped up and global alliance is required to designate more protected areas and sanctuaries. International gene banks can provide for ex-situ conservation of the valuable genetic diversity of domesticated and wild-caught aquarium fishes.

Introduction

The culture and keeping of fish as pets have their roots in Asia. Goldfish (*Carassius auratus*) have been kept as pets in the Southern Song dynasty in China since before 1000 A. D. (Li 1990). The Japanese Koi carp is another long-time favorite. The development of ornamental Koi from the common carp (*Cyprinus carpio*), which was introduced to Japan from Eurasia some 2000 years ago, can be traced back to the Tokugawa period in the 17th century (Tamadachi 1990).

Breeders of food and ornamental fishes share the common goal of improving the reproductive fitness of fish in terms of faster growth, higher fecundity, greater disease resistance and adaptability to fluctuating environmental conditions, by using selective breeding. Ornamental fish breeders face another challenge. Whereas consumers are notoriously conservative in their choice of food fish, the reverse is true for ornamental fish enthusiasts who have an insatiable craving for "novel fish." Thus, the ornamental fish industry is

compelled to continually provide diversity to captivate, sustain and enhance the interests of hobbyists. Diversity is achieved by developing new strains of cultured fish species through genetic methodology and introducing previously unknown species.

Traditional Methods

Traditional methods used to develop novel strains involve continual rigorous and tedious screening of thousands of fish stocks for rare spontaneous mutants. The desired mutant characteristics that appeal to and selected by fish hobbyists include: new or enhanced coloration and color patterns; improvement of body and fin shapes; unique body or fin shapes and sizes; and bizarre morphological structures. This is followed by several generations of close inbreeding of the selected fish to fix the desired gene(s) and development of a pure breeding line. Subsequently, the population size increases. Using carefully chosen parents, selective breeding is practiced on each generation to improve the quality of the stock, desired phenotypic characteristics, fitness and viability. Hybridization between different strains is also practiced to create new variation, and this is also followed by generations of intense selection to develop a new line.

These traditional methods have been successful as testified by three well-known favorites; goldfish, Koi and guppies. However, the selected characteristics are very specific for each species. Goldfish are famous for the numerous strains showing various colors. The body has been selected for short spherical shape and dorsal and tail fins for different shapes and forms. Goldfish are particularly noted for varieties with bizarre morphological structures on the head like lionhead, telescopic eyes and others. Koi are selected for a streamlined body showing different pure colors of silvery-white, gold, orange-red or black, as well as strategically placed color patterns. The guppy (*Poecilia reticulata*) is sexually dimorphic, with males of the different varieties displaying striking coloration on their bodies and large dorsal and caudal fins.

The ornamental fish industry has, and will continue, to depend on traditional selection methods to provide new forms that are essential for the trade. However, these methods are slow and laborious often taking decades or centuries to produce results. They are very dependent on rare and chance occurrences of suitable spontaneous mutations. Some basic research on the gene control of the different color varieties has been conducted on the guppy (Fernando and Phang 1990; Phang et al. 1989, 1990, 1991), the goldfish (Kajishima 1977) and Japanese Koi (Katosonov 1978; Cherfas et al. 1992). When the genes involved have been elucidated, crossing between different varieties can be planned on a scientific basis rather than in a haphazard manner to give new variation.

New Technology

Today all industries must update and utilize new technology to survive and progress in the twenty-first century. The ornamental fish industry is no exception. But what kind of new technology is available for the industry to harness? There has been rapid development of molecular techniques and DNA methodology, especially in the last two decades. In recent years, application of molecular technology has become increasingly important in the fields of medicine and agriculture. However, the aquaculture and fisheries community has done little in comparison

(Hallerman and Beckman 1988; Hadrys et al. 1992). The ornamental fish industry consists mostly of small firms and family-run farms that, unlike multinational organizations, do not have the capacity to maintain their own research and development programs. Hence, to latch on to new technology, they have to work with researchers in institutions and universities.

One challenge is whether it is possible to induce mutants rather than waiting for them to occur infrequently and randomly. Pioneering work on induced mutagenesis has been conducted in the zebrafish, *Brachydanio rerio*, using X-rays (Streisinger et al. 1981; Walker and Streisinger 1983). Shima and Shimada (1991) used irradiation and chemicals to induce mutations in the Japanese Medaka fish, *Oryzias latipes*. Induced mutagenesis will yield a higher frequency of mutations from which to carry out selection.

Production of transgenic fish could be a useful method of genetic manipulation. Recent work on the zebrafish, the Japanese medaka and other species have shown that fish are an excellent system for gene transfer because innumerable eggs can be laid by a single female and development is external and relatively short (Kimmel 1989; Vielkind 1992). Genetic engineering of freeze-resistant Atlantic salmon has been carried out by microinjection of the antifreeze gene of the winter flounder into salmon eggs (Fletcher et al. 1992). Studies by Zhu (1992) indicated that the human growth hormone gene accelerated growth rates of individual transgenic loach, crucian carp and common carp.

Cloning of useful genes in ornamental fish, like those that control color and morphological aberrancies, are important and will eventually entail application of gene transfer technology. As an example, the gene that produces tyrosinase, a key enzyme in the production of the black pigment, melanin, has been cloned in mouse and man (Shibahara et al. 1986; Kwon et al. 1988). Fish tyrosinase genes can also be cloned and used for transgenic work to produce new color variants. Recently, germ-line chimeras have been produced in the zebrafish by cell transplants from unhatched embryos of pigmented wild-type to those of the albino (Lin et al. 1992). A high percentage of chimeras (85%), with each fish having its own pigmentation pattern, was obtained. When fully developed, such techniques promise to be valuable in the production of ornamental fish variations. In the future, it may be possible for the industry to cater to the demands of fish hobbyists by providing novel varieties of fishes that have been genetically engineered using molecular techniques, which takes less time compared to traditional breeding methods.

Unique Considerations in Breeding Ornamentals

Farmers raising food fish most often concentrate on producing one or a few species. However, ornamental fish farmers, out of necessity to provide diversity for fish fanciers, have to practice either monoculture of a number of varieties of a single species, as in the case of goldfish, Koi carp and guppies (Fernando and Phang 1985) or polyculture of several less established fish species. Hence, ornamental fish farms characteristically have numerous small aquaria, small ponds and net-cages suspended in large ponds. Due to financial, labor and physical constraints, they can only maintain a small effective population size (N_e) for each of the stocks. Restriction of population size and genetic drift result in loss of genetic diversity

and fixation of undesirable genes within stocks, leading to increased inbreeding levels. The main symptoms of inbreeding depression are: susceptibility to disease, slow growth, low fecundity, high sterility, slow or no response to selection and an increase in abnormalities. Inbreeding depression may lead to complete loss of lines.

Designing a Breeding Program for Ornamentals

To improve the gene pool and minimize the undesirable effects of inbreeding, fish farmers must understand the importance of practicing genetic broodstock management. The N_e for each stock must be kept as large as possible, taking into account limiting factors. N_e depends on the total number of breeding individuals, sex ratio, mating system and variance of family size. The necessity to provide for variety/species diversity does not allow the farmer to keep large N_e. This problem is more serious in the case of large ornamentals like the Koi carp and less for small-sized species like the guppy. What would be a reasonable guide as to the number of male and female broodstock? Recommended numbers range from 50-1000 (see reviews by Ryman and Utter 1986; Tave 1993). Unfortunately, there is no recommended magic number to guarantee prevention of inbreeding and genetic drift. Since the founder population determines how much genetic variability exists, it is important for the farmer to avoid using only a few individuals, or large number of individuals, from a single spawn. However, with ornamental fish there is need to use highly selected broodstock to maintain and improve the quality of the stock. Thus, there is a trade-off between selection intensity and N_e.

Alternative ways to increase N_e are to control the sex ratio and variance of family size. A 1:1 male to female sex ratio maximizes N_e while a skewed ratio reduces N_e. A survey on guppy farms in Singapore showed that the sex ratio is usually 1:3, 1:4 or even 1:10 (Fernando and Phang 1985). There is more stringent selection on males for vivid coloration and large well-shaped fins. Using equal sex ratio for guppies means lowering selection intensity of males. Another recommendation is to spawn more fish than the farmer actually needs and to keep a sample from each spawn for the stock. From a short-term perspective this is a waste of effort, but from the long-term genetic viewpoint it is worthwhile.

Another way to increase genetic variability in fish stocks is to outcross fish of the same variety/species with other sources. This leads to an immediate increase in genetic variability. However, if this is again followed by restricting population size, the stock will deteriorate after some generations through erosion of genetic variability.

Future Considerations

DNA fingerprinting is assuming an important role in aquaculture and is becoming a standard technique for monitoring population and inbreeding levels, pedigree studies and genetic broodstock management (Hallerman and Beckmann 1988). The use of arbitrarily primed polymerase chain reaction, a simpler, faster and less costly method of fingerprinting DNA amplified products, has been developed. This allows the screening of larger numbers of samples (Welsh and McClelland 1990; Williams et al. 1990; Dinesh et al. 1992) and genetic outcrossing programs can be drawn up based on DNA profiles. This may be possible, for example,

in the case of guppies in Singapore, where there are a number of farms that culture ten to fifteen different color varieties. The DNA fingerprints allow recommendations as to the genetically most-distant source(s) of fish the farmer should acquire for outcrossing his own stocks.

Setting up gene repositories is recommended as a means of conserving the tremendous ex-situ genetic diversity of both cultured and wild-caught ornamental fish. At present, cryopreservation of sperm is the only technique available for conservation of fish germplasm. Androgenesis could be a method of reconstituting the organism from male gametes.

Almost all ornamental fishes are produced or wild-caught in tropical countries and air-freighted to temperate countries where the main markets exist. In the future, breeders should also focus on traits hitherto not considered before. The first is tolerance to conditions of transportation that include low pH and oxygen, high ammonia and carbon dioxide, and sharp drops in temperatures when the carrier is flying at high altitudes (Teo et al. 1989). This will reduce mortality rate during transportation. Another trait is tolerance to urban environments provided by consumers, which include indoor aquaria and small indoor or outdoor ponds.

Conclusion

A large percentage of ornamental fishes are wild-caught from tropical waters. Freshwater species come mainly from the Amazon basin, with the Neon tetra (*Paracheirodon innesi*) accounting for 80% of the millions of fishes exported from this region. Marine ornamentals consist almost entirely of tropical coral fishes. Overfishing, pollution and environmental destruction are the cause of diminishing biodiversity and possible extinction of rare species. According to McNeely (1992) the main cause of over-exploitation of biodiversity in tropical countries is the inequity between rich countries (consumers) and poor countries (producers). The global trade in ornamental fish undervalues natural resources, resulting in poor countries remaining in poverty.

There is urgent need for global cooperation where the industry, governments, fishermen and hobbyists work together. Measures like designation of national conservation parks, sanctuaries, controlled fishing and protection of endangered species should be implemented before it is too late. Captive breeding of ornamental fish that are presently obtained from the wild should also be stepped up. What is required is the conservation of biodiversity for sustainable use. This means controlled harvesting without depleting the overall diversity of the ecosystem.

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Use of Chromosome-Manipulated Fish in Aquaculture and Related Problems of Conservation of Wild Stock

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Abstract

Chromosome manipulation and breeding of gynogenetic diploids and clones are some forms of biotechnology applicable to the selective breeding process of aquacultured fish species. These technologies allow accelerated fixation of genetic variations hidden in target populations, and promote future goals such as the genetic improvement of cultured fish. If manipulated animals are reared in the appropriate culture system, isolated from natural environments, genetic variations can be regarded as an extension of conventional breeding and, therefore, do not create a critical problem (risk) in the management and conservation of a wild population. This paper presents an outline of chromosome manipulation technology and the genetic characteristics of induced fish, such as gynogenetic diploids and clones. As an example of regulation systems for the use of this technology, guidelines for chromosome-manipulated fish use proposed by the government and the Japanese Fisheries Department are briefly discussed.

Introduction

Chromosome manipulation is one form of biotechnology applicable to the selective breeding of fish. This technology allows hidden genetic variations in a target fish population to be fixed in a shorter period of time. In the near future, it is expected that chromosome manipulation will be used for genetic improvement of cultured fish. As chromosome manipulation utilizes existing genetic variations, it can be regarded as an extension of conventional selective breeding. Therefore, if the chromosome-manipulated animals are reared in the appropriate culture systems, isolated from natural environments, it does not pose risks in the management and conservation of wild stocks. This paper presents an outline of chromosome manipulation, genetic characteristics of induced fish, methods of application and related problems of conservation of wild stocks in Japan.

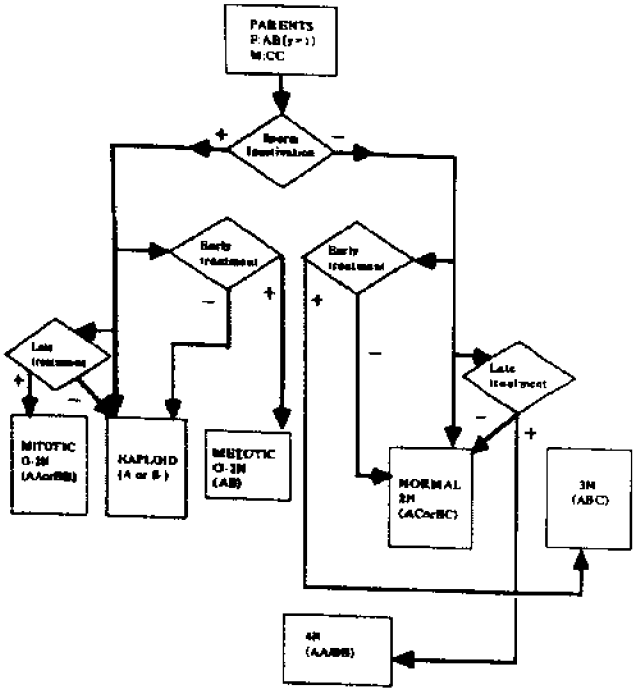

Figure 1. Method to induce ploidy using genetic markers.

Chromosome Manipulation: Theory and Results

- **How to induce chromosome manipulation in fish through gynogenesis**

Gynogenesis consists of two key components, (1) technology related to genetic inactivation of sperm, and (2) technology for suppressing the division of fertilized eggs. Figure 1 illustrates several methods for inducing gynogenetic haploids, diploids, triploids and tetraploids. Figure 2 illustrates the relationship between gametogenesis, oogenesis and fertilization.

The gynogenetic diploid can be induced by applying an early shock-treatment or late shock-treatment to the developing eggs inseminated with UV irradiated sperm. The sperm's

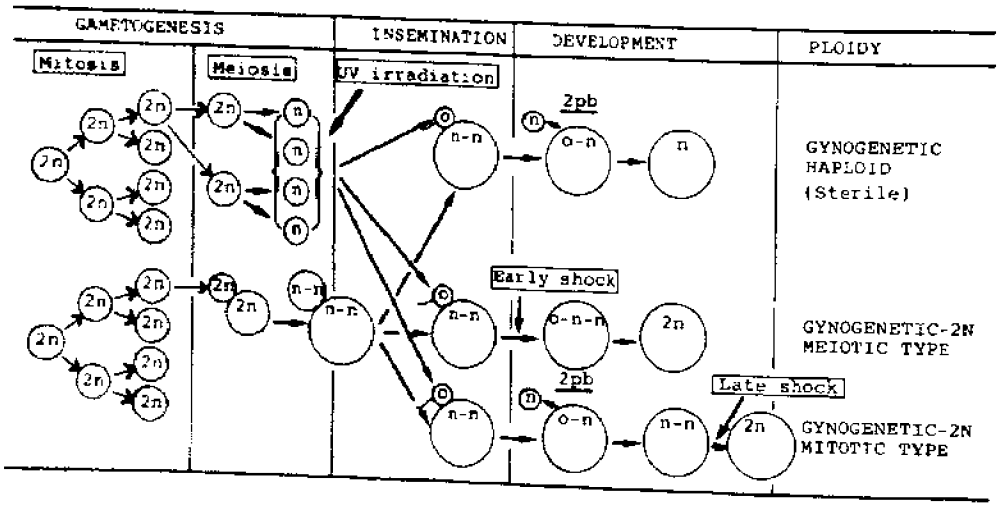

Figure 2. Method to produce gynogenetic offspring.

DNA is inactivated by ultraviolet (UV) rays, but they remain viable and can stimulate egg development. Division of egg cells can be suppressed by low water temperature or hydropressure. Optimal conditions differ among species, and should be clarified through experimentation (Purdom 1983; Suzuki 1989).

- **Theory of gynogenetic selective breeding method**

In theory, for quantitative traits such as fork length and body weight, the phenotypic value (P) of each individual can generally be indicated as the sum of genotypic values (G) and environmental effects (E). Selective effects become greater as the value of G increases. It is impossible, however, to identify the composition of P, G and E directly for each individual. Variations among large numbers of individuals are measured, and their respective composition of P, G and E can be estimated from variance (V) analyses. If the phenotypic variance of the original population is indicated as Vp = Vg + Ve, the variation will decrease within an inbreeding line, and will increase among lines which were induced by selective breeding. This can be indicated as Vp = Vg (1-F) + Ve within a line, and as Vp = Vg (2F) + Ve among lines (Kimura 1965), where F= the value of inbreeding coefficient.

The conventional method of genetic improvement through selection is shown in Figure 3. This figure assumes a typical model of selective breeding in which the selection response occurs in opposite directions. This method of selection requires at least twenty generations of sib-matings before a genetically pure line (F= 1) can be established (Allendorf and Leary 1984; Guyomard 1984). Because of difficulties in rearing, maintenance and reproduction over a long period of time, there are very few successful cases of selective breeding in fish using the conventional method.

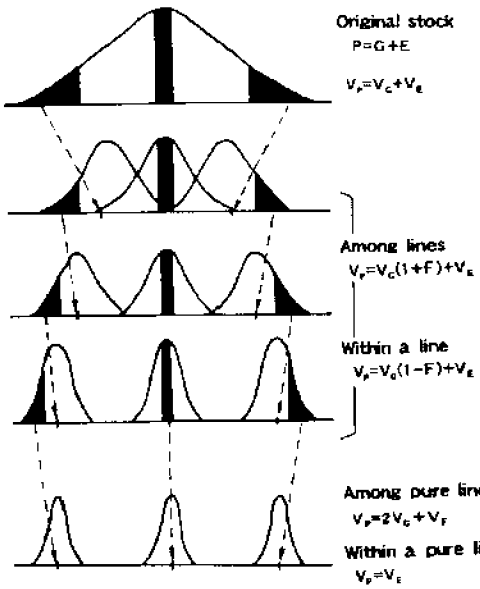

Figure 3. Genetic improvement of some quantitative traits by conventional selection methods.

The gynogenetic selective breeding method is a modification of the conventional selective breeding model in that it is combined with chromosome manipulation (Taniguchi 1989). Figures 4 and 5 show two types of gynogenetic diploids. The fundamental difference between these two types lies in the gap in values of the inbreeding coefficient F (Taniguchi et al. 1990).

In the first generation of the meitotic-gynogenetic diploid, the degree of individual variation increases in line with the inbreeding coefficient (F), which can be indicated as Vp = Vg (1+ F) + Ve. On the other hand, the inbreeding nature strengthens within the individuals of the first generation of the gynogenetic diploid. Therefore, the second gen-

eration manifests conspicuous effects of selection as well as a reduced range of variation (Taniguchi et al. 1990).

The meiotic type of gynogenesis is inappropriate for inducing clones because, depending on the locus, it cannot prevent the occurrence of recombinants (Thorgaard et al. 1983). This results in poor efficiency of genetic purification (Fig. 6).

In the mitotic type of gynogenesis, individual differences are enhanced in the first generation, but all loci are fully homozygous in each individual; the inbreeding coefficient F becomes 1 and the genetic variation is doubled ($Vp = 2 \times Vg + Ve$) (Fig. 5). The batch will become a copy of the mother with a meiotic type of induction to another gynogenetic diploid on the second generation. These are cloned fish that can achieve both improvement and fixation of traits without genetic variation as shown by $Vp = Ve$. If the clones are reared under the same environmental conditions in a communal rearing tank, genetic variance can be estimated. The Ve and Vg of the experimental fish group can be estimated using the Ve value of the clone.

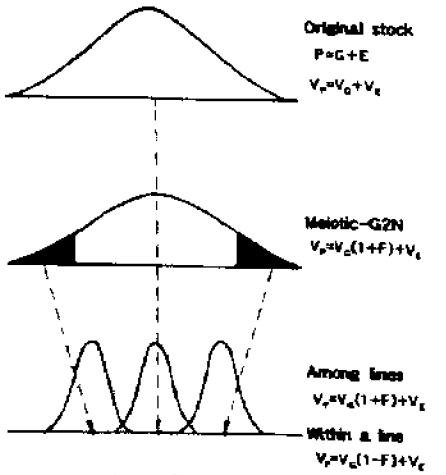

Figure 4. Genetic improvement of some quantitative traits using the meiotic-gynogenetic method.

The increase and decrease in variation in chromosome-manipulated fish is one of the most remarkable consequences of the gynogenetic selection method. The methods utilizing these genetic characteristics make it easier to perform genetic studies on quantitative traits by comparing the phenotypic variation of genetic groups produced by chromosome manipulation. This method has enabled researchers to shorten the number of generations of repeated matings from 20 using the conventional selection method, down to 2 using the chromosome-manipulated method.

- **Observation of traits**

In gynogenetically produced diploid fish, basic studies have been conducted on genetic characteristics using ayu fish (Taniguchi et al. 1988, 1990), varicolored common carp (nishikigoi in Japanese) (Taniguchi et al. 1986) and red sea bream (Sugama et al. 1990, 1992). In the case of ayu fish, both meiotic and mitotic gynogenetic diploids have been produced (Taniguchi et al. 1988).

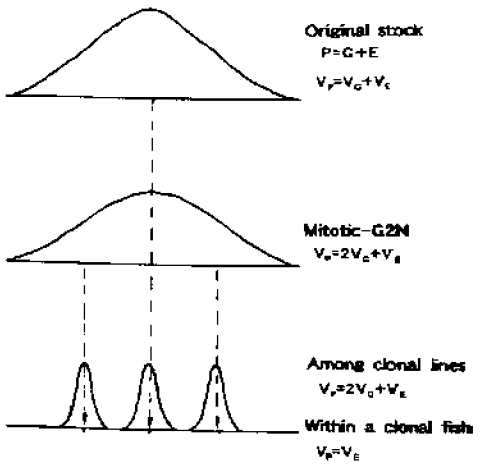

Figure 5. Genetic improvement of some quantitative traits using the mitotic-gynogenetic method.

The heritability of each trait can be estimated by comparing the phenotypic trait variation in two types of gynogenetic diploids with the control group. This heritability is used to predict the selection response of each trait.

The range of variation in the fork length and body weight of adult ayu fish is maximum in the mitotic type, minimum in the normal diploid and medium in the meiotic type (Table 1). The mitotic type of gynogenetic diploids always has the largest variation and that is consistent with the theoretical expectation of trait variation expansion in the first generation of gynogenetic diploids.

Figure 7 illustrates variation expansion in the body weight and vertebrae number of ayu gynogenetic diploids. In terms of body weight, it is observed that not only the variation of gynogenetic diploids is expanded, but also the mean value is lower compared to normal diploids (Fig. 8). This reflects the influence of the increased inbreeding coefficient on the gynogenetic diploids, as well as the expansion of genetic variation. Thus, the breeding performance of the first generation of gynogenetic diploids is generally poor in terms of growth and survival rates.

- **Traits of cloned fish**

Clones produced through mitotic-gynogenetic diploid ayu were compared with normal diploids and gynogenetic diploids (Fig. 8). Histocompatibility tests and DNA fingerprint analyses were performed to confirm the clonal nature of the fish (Han et al. 1991, 1992). Figure 9 shows the mini-satellite-DNA fingerprint pattern of three genetic groups. The clones produced through the mitotic type, G2N, shared all of the bands of the DNA fingerprint pattern. This indicated successfully cloned fish. While remarkable individual variations are observed in normal diploids and gynogenetic diploids, all cloned individuals show identical patterns.

Table 2 shows the reduction of variability in cloned fish as compared with out-bred controls. Similar results were observed in some morphological traits (Table 3). These results suggest that the phenotypic variance in the clones consists entirely of environmental variance (Vp+ Ve). It was concluded that genetic variance can be estimated, if the clones are reared under the same environmental conditions in a communal rearing tank. We can estimate the Ve and Vg of experimental fish groups using the Ve value of the clone.

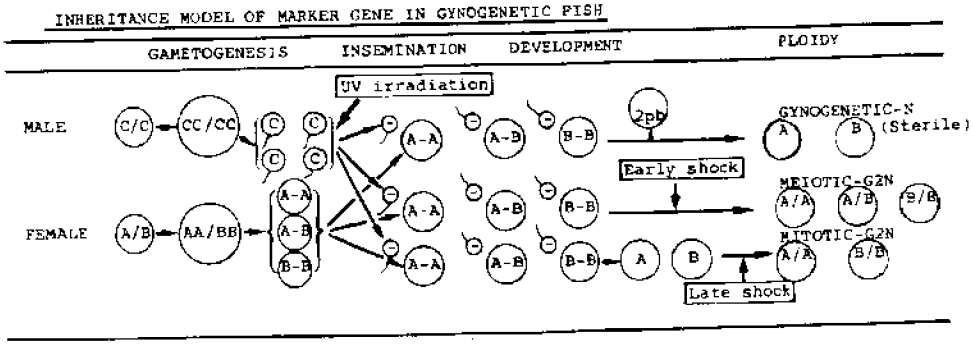

Figure 6. Inheritance of marker genes in chromosome-manipulated fish and variations in genotype.

Table 1. Expansion of variation in gynogenetic diploids in ayu fish, *Plecoglossus altivelis* (Taniguchi et al. 1990).

Traits		Normal - 2N	Gynogenetic diploids	
			Meiotic type	Mitotic type
Fork length (8 month old)	Mean (cm)	12.1	12.3	12.0
	SD	0.75	1.33	1.81
	Variance	0.56	1.76	3.27
Body weight (8 month old)	Mean (g)	21.7	22.6	19.2
	SD	4.26	7.48	8.97
	Variance	18.14	55.95	80.46

The composition of phenotypic variances in the control group, gynogenetic fish and clones are shown in Table 4.

• **Potential uses for cloned fish**

When evaluating useful traits for selective breeding, it is difficult to evaluate the heritability of physiological and morphological traits such as growth features, spawning period, territorial behavior and anadromous behavior. However, the heritability of these traits can now be estimated by comparing the variance between the cloned fish and the target group (Fig. 10 and 11). Each batch of cloned fish can be distinguished from another by highly heritable traits. Of the induced clones, good performers turn out to have genetic uniformity. In fact, the clones did not always show superior growth, but they were easy to breed and their survival rate was high. Clones are also used as broodstock to produce heterozygous, all-female, cloned diploids and triploids, which are useful for seed in aquaculture.

Cloned fish are also important experimental animals. They can serve as the control in an experiment, and as the test animal for fish diseases, medicines and feed pellets. They can be used to evaluate the genetic variations in traits within different fish strains. Highly reliable data can be obtained from using clones for these tests.

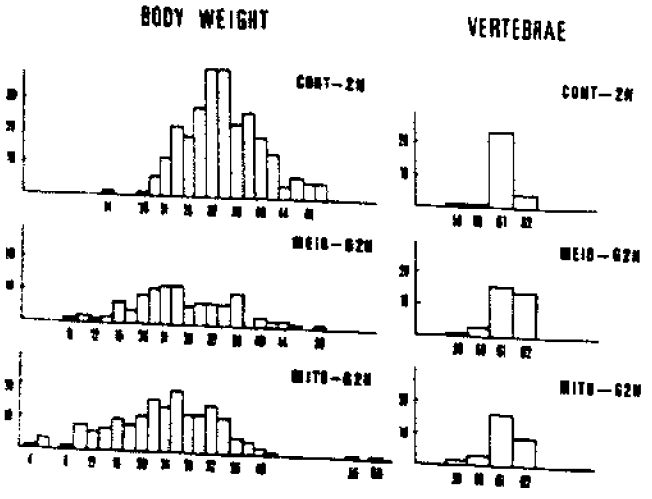

Figure 7. Difference in frequency distribution patterns for body weight and vertebral counts by different chromosome-manipulated groups in same-month-old fish reared in the same environmental conditions.

Table 2. Comparison of body length and weight in clonal and out-bred (control) ayu fish at six and nine months of age.

Age		6 month old		9 month old	
		Clones	Outbred*	Clones	Outbred*
Fork length	Mean (cm)	6.3	6.4	11.8	12.6
	SD	0.179	0.418	0.339	0.628
	Variance	0.03	0.18	0.16	0.39
Body weight	Mean (cm)	1.5	1.7	12.7	16.3
	SD	0.171	0.448	1.492	2.950
	Variance	0.03	0.20	2.23	8.70

* The outbred fish were produced by crossing the mitotic-G2N and the normal-2N.

Guidelines for Using Chromosome-manipulated Fish

In 1991, the Japanese Ministry of Agriculture, Department of Fisheries, Forestry and Fisheries, organized a committee to prepare guidelines for the use of chromosome-manipulated fish in Japan. Committee members consisted of scientists, university professors in genetics, ecology, physiology and environmental sciences, and a few director generals of fisheries institutes and experimental stations. The private sector was not represented on the committee.

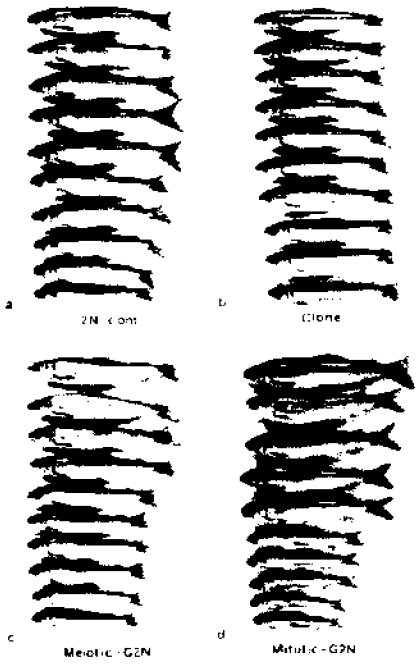

Figure 8. Size variations in chromosome-manipulated ayu fish by different genetic groups (control 2-N, clones, meiotic-G2N and mitotic-G2N)

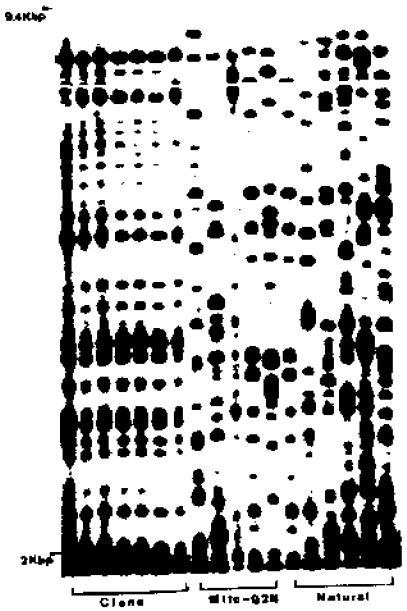

Figure 9. DNA fingerprinting of clones compared with normal-2n and mitotic-G2N ayu fish. The minisatellite DNA fragmented by the Hae-III restriction endonucleus were visualized by bateriophage M-13 DNA probes with 32P isotope.

Table 3. Comparison of mean values and variability in some measureable morphological traits of nine-month-old clones and their outbred controls reared in a communal tank.

Traits		Clones	Out-bred controls	t-statistics
Vertebrae number	N	54	41	
	Mean	62.50	62.02	2.560*
	SD	0.719	1.024	
	V	0.52	1.05	
	CV	1.2	1.7	
Dorsal fin rays	N	32	27	
	Mean	10.41	10.29	0.972
	SD	0.491	0.456	
	V	0.24	0.21	
	CV (%)	4.7	4.4	
Pectoral fin rays (Left)	N	31	27	
	Mean	12.68	12.82	1.243
	SD	0.468	0.381	
	V	0.13	0.15	
	CV (%)	3.7	2.9	
Anal fin rays	N	31	27	
	Mean	14.74	14.17	4.800***
	SD	0.438	0.456	
	V	0.19	0.21	
	CV (%)	2.9	3.2	
Gill rakers	N	31	26	
	Mean	31.07	32.44	7.815***
	SD	0.246	0.864	
	CV (%)	0.8	2.7	
Teeth (upper)	N	31	26	
	Mean	24.52	26.13	6.072***
	SD	0.911	1.053	
	CV (%)	3.7	4.0	
Teeth (Lower)	N	31	26	
	Mean	24.71	25.50	3.033**
	SD	0.770	1.118	
	CV (%)	3.1	4.4	

* Significant at $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$

Table 4. Composition of phenotypic variances in normal - 2N, meiotic-G2N, mitotic-G2N and clones.

Normal diploid	$V_p (1) = V_g + V_e$ ----------(1)
Meiotic-G2N	$V_p (2) = V_g (1 + F) + V_e$ ----(2)
Mitotic-G2N	$V_p (3) = 2 * V_g + V_e$ ---------(3)
Clones	$V_p (4) = V_e$ ----------(4)
Estimation of Heritability: $h^2 = V_g / V_p$	
For normal-2N: $h^2 = (V_p(1) - V_p(4)) / V_p (1)$	
For mitotic-G2N: $h^2 = (V_g(3) - V_p(4)) / V_p(3)$	

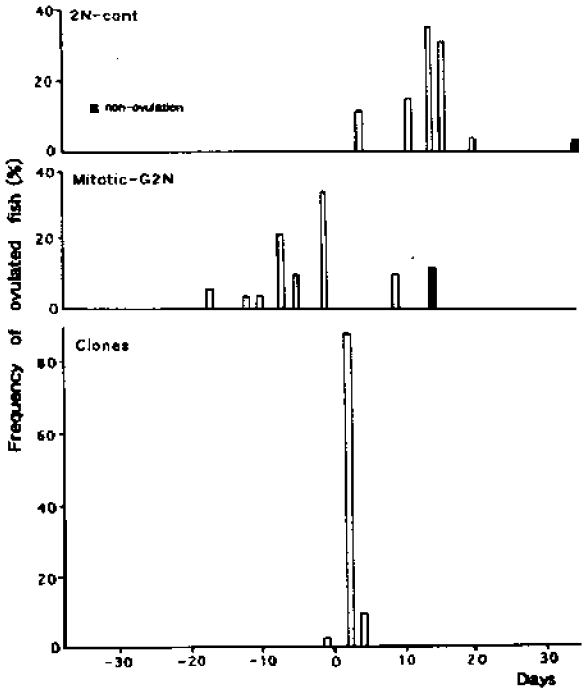

Figure 10. Comparison of spawning day and duration of clones with normal-2N and mitotic-G2N.

- **Principles of the guidelines:**

> Dealings and decisions are required to be based on careful scientific investigation,

> To protect wild stocks and existing animal communities, use of the animals should be limited to closed system aquaculture,

> Animals can be used only after an evaluation and risk assessment have been completed,

> Users must perform the evaluations and the risk assessments according to the guidelines,

> Users must submit their results to the director general of the Fisheries Agency of Japan to receive approval from the government,

> Guidelines will be modified following new developments in technology,

> Gene-manipulated fish (transgenic fish) are not covered in these guidelines.

- **Considerations for the use of chromosome-manipulated fish:**

> Objectives for using the animals should be classified into three categories: experiments, aquaculture and stock enhancement,

> Biological characteristics of the original species must be thoroughly investigated with concern for taxonomic situation, present distribution, mode of reproduction, genetic variability, habitat and environment demands, feeding habits and other physiological and ecological characteristics.

Characteristics of chromosome-manipulated animals also must be well investigated regarding:

> Methodology of manipulation applied,

> Characteristics such as reproductive performance, growth performance, genetic variability, environmental adaptability, feeding habits, physiological and ecological characteristics.

- **Environmental requirements for handling chromosome-manipulated animals**

Based on the assessment of biological characteristics, chromosome-manipulated animals are approved for use in one of four environmental situations:

> Application in a closed environment,

> Application in an aquaculture environment,

> Application in a semi-open environment,

> Application in an open environment.

Laboratories should be isolated from the open environment and rearing tanks, equipment and apparatus should be located inside the laboratory. The rearing tanks and other equipment should be carefully designed to isolate the animals from wild stocks.

Chromosome manipulation should be performed in a closed environment to prevent the escape of manipulated eggs and larvae and unused manipulated sperm and eggs should be destroyed. Manipulated animals should always be in a closed environment and the live animals should never be discarded. Contamination and escape have to be prevented during transportation.

In the aquaculture environment facility, rearing tank and cage locations should reflect the ecological and physiological characteristics of the animals produced. Preparation of the isolated facility should depend on the results of animal assessments. Normal diploid animals should never be reared where manipulated animals are reared and when the ma-

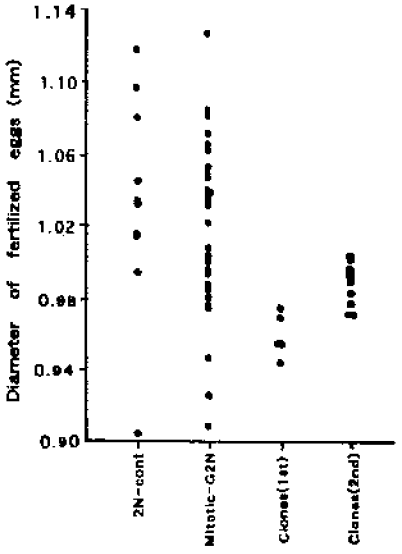

Fig.11. Variability in egg size for clones with normal-2N and mitotic-G2N of ayu fish. Each dot represents a mean value of eggs from one fish.

nipulated animals produce gametes, the animals should be prevented from having any influence on normal animal species. Movement and escape of animals from the aquaculture area to open areas must be prevented.

- **Regulating the use of chromosome-manipulated fish**

Regulations must be administered by farm owners and every farm must have a chief manager who is responsible for controlling production of manipulated animals. The chief manager gives instructions to workers about the guidelines; workers must know the characteristics of the manipulated animals and pay attention to the regulation of animals.

- **Permitting and reporting requirements:**

Owners who want to use manipulated fish in their farms must receive authorization from the director general of the Japanese Fisheries Agency. Approval is based on the owner's assessment, request and record keeping. Any new information obtained after receiving authorization should be reported to the director general of the Japanese Fisheries Agency.

- **Efficiency of the guidelines**

The guidelines have been in effect since 1992 and a few local governments have submitted requests to use manipulated fish. The use of all female diploid and triploid rainbow trout reared in the concrete ponds has been approved. But, a request to raise triploid oysters in Hiroshima Bay is still under consideration because they will be raised where normal diploid oysters are cultivated. These guidelines are expected to effectively regulate use of chromosome-manipulated animals.

Conclusion

Risk assessment of manipulated fish, in relation to conservation of wild stock, is needed. Although chromosome-manipulated fish are induced using existing variation, they are recognized as genetically modified from the original population and as having lost their genetic variation. To prevent adverse effects on wild stocks these fish should be dealt with carefully. Figure 12 shows principles of artificial propagation that should prevent unexpected genetic effects on wild stocks.

Fish that are modified by selective breeding or some other manipulation, usually show good performance and adaptation to the culture environment, but do not always show good performance and survival in the natural environment. On the other hand, the wild stock is expected to have maximum fitness in the natural environment, but when raised in a domesticated environment their fitness may be reduced. This suggests that the phenotypic values of both wild and domesticated fish includes genotypic differences. These fish may have different fitness under both culture and natural environments.

Although it is difficult to quantify the effects of genetically-manipulated fish on wild stocks, we should handle these fish carefully to conserve the wild stocks. The genetically modified fish should be used in an environment isolated from the natural environment. (Fig. 12). Using genetically modified animals should be based on the guidelines described herein.

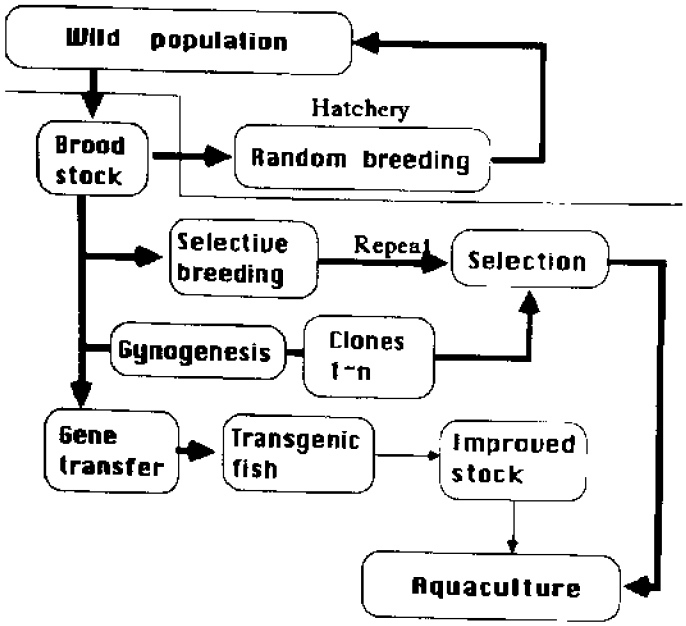

Figure 12. Artificial propagation for stocking wild populations and genetic improvement of fish races in aquaculture.

Acknowledgments

This research was supported partly by the grant-in aid from the Fisheries Agency, Japan and the grant-in aid of Ministry of Education, Japan (code no. 04454094).

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Channel Catfish Breeding and Selection Programs: Constraints and Future Prospects

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Abstract

Channel catfish, *Ictalurus punctatus*, culture is one of the most successful aquaculture enterprises in the United States. Applications of genetic improvement in channel catfish culture have not made a large impact on the industry when compared to other commercial livestock systems. Past research efforts in reproduction, qualitative and quantitative genetics, sex reversal and induced polyploidy have all made important contributions to the catfish genetics knowledge base and shown the potential for the development of improved germplasm utilizing traditional animal breeding approaches. However, additional research in these areas applied to a coordinated industry-research program will be necessary to realize the benefits to commercial culture from genetic research. Application of new biotechnologies such as gene transfer with traditional breeding programs will also be an important part of catfish breeding programs. Knowledge of procedures and protocols for conducting research with genetically modified organisms and the potential impacts from accidental releases will be required.

Introduction

Channel catfish, *Ictalurus punctatus*, farming began over 30 years ago and has become one of the most successful aquaculture enterprises in the United States. Production of channel catfish has surpassed other commercially cultured foodfish in the U.S. (Stickney 1993). Annual yields and production acreage have steadily increased because of shifts in per capita consumption toward fisheries products, development of an effective industry infrastructure, successful marketing and research support. Early research efforts in reproduction, nutrition and disease control laid the basis for the establishment of commercial culture (Tucker and Robinson 1990). The most recent survey showed 64,000 ha of ponds in production with 178 million kg of fish processed in 1991. Approximately 90% of the commercial production is located in the southeastern U.S. in Mississippi (60.0%), Arkansas (12.6%), Alabama (11.4%) and Louisiana (6.3%) (USDA 1992).

Research on channel catfish genetics and breeding began in the late 1960s and early 1970s (Dunham and Smitherman 1987), however, applications of genetic improvement in channel catfish culture fall behind genetic improvements made in other farm animal industries. These improvements are most apparent in the poultry, beef and dairy cattle and swine industries where genetic research, particularly in the areas of quantitative inheritance, has made major contributions to industry advancement and profitability.

In dairy cattle from 1930 to 1976, milk yield per cow increased 125%, from approximately

Table 1. Potential for increased efficiency of producing foods of animal origin in 2000 and 2030.[1]

Animal/Product	Unit	% Improvement 2000	% Improvement 2030
Beef	Live weight marketed per breeding female	25	60
Pork	Live weight marketed per breeding female	35	60
Dairy	Milk marketed per breeding female	30	65
Sheep	Live weight marketed per breeding female	35	70
Broiler chicken	Live weight marketed per breeding female	30	35
Turkeys	Live weight marketed per breeding female	40	40
Laying hens	Number of eggs	20	25
Fish (catfish)	Age to market weight (1 lb.)	50	200

[1]Smith, L.W. 1991

2,000 kg/cow to 4,500 kg/cow. Significant advances in pork production have also been made through genetic improvement. Since 1945, the time required to produce a marketable pig (100 kg) has been reduced from 200 to 160 days. In the poultry industry, broiler producers have reduced the time required to produce a marketable (1.7 kg) broiler from fourteen to seven weeks and doubled feed efficiency (Warwick and Legates 1979; Gall et al. 1988).

The successful breeding programs and increased production realized in these industries have been the result of a long-term effort in basic and applied research coupled with information transferred to industry. Animal products produced from these industries in the United States supply 53% of all food consumed and 69% of the protein. The potential for continued improvement and increased efficiency still exists in these animals and will result from further genetic improvements, increased reproductive efficiency and better nutrition. Projections for future increases in production efficiency are summarized in Table 1 (Smith 1991).

The possible increases in production efficiency from catfish breeding are projected to be larger than any increases in other animals. Improving production efficiency through the use of genetic improvement is possible, but will occur only through long-term genetic research programs integrated with improved culture technology. Constraints limiting the potential of channel catfish breeding and selection programs need to be identified.

The goal of a breeding and selection program in an agricultural system should alter or change the animal's characteristics so it is more profitable to raise and the production system is more efficient. The task facing the research geneticist is to determine the amount and type of genetic control over the animal's performance and then implement a system of breeding and selection to improve production efficiency. An undisputed need certainly exists for improving channel catfish production in aquaculture through planned breeding programs. Future breeding programs will be required to incorporate new biotechnologies and to address areas of qualitative and quantitative genetics, reproductive efficiency, molecular and cellular genetics.

Reproduction and Spawning

Control of reproduction and spawning is a necessary element of an effective genetic improvement program. Channel catfish repro-

duction in commercial culture is most commonly done by the open pond method during the natural spawning season. Male and female broodfish are allowed to mate randomly in large ponds supplied with spawning containers (Tucker and Robinson 1990). Spawning containers are checked periodically during the spawning season and eggs are usually removed and taken to a hatchery for artificial incubation. Newly hatched fry are also trained to accept formulated diets. The spawning season is protracted when this method is used, and generally 30-50% of the female broodfish spawn. The open pond method is extensive and the most practical method for obtaining large numbers of eggs.

Pen and aquarium spawning methods are more intensive, but give the culturist greater control over broodstock selection. Pen spawning is similar to pond spawning, but involves construction of spawning pens in outdoor ponds, and is used primarily for selecting and spawning particular pairs of broodfish. Aquarium or tank spawning is more intensive and involves pairing broodfish in indoor tanks or aquariums supplied with flowing water. Both of these methods require accurate sexing, determination of the stage of reproductive development and hormone injections to stimulate ovulation are often used.

All three methods have applications in breeding programs, however, pond spawning is suitable only for producing large numbers of families of unknown parentage. Pen spawning can be used to produce intraspecific hybrids and mate selected sires and dams to estimate heritabilities and genetic correlations from sib analyses, however, families will often not be contemporaneous and differential fish sizes or age will require statistical adjustment. Aquarium spawning is well-suited for factorial matings and can provide contemporaneous families. Both of the latter methods have been used in research studies to provide statistical estimates of heritabilities and genetic correlations. Facilities allowing the design and implementation of such studies are available at a few research institutions in the southeastern United States. (Figure 1). No commercial facilities for conducting genetic research have been constructed.

Gamete manipulation (manual stripping of eggs and sperm) is not easily done with channel catfish as compared with salmonids. Spawning of salmonids is commonly done in both commercial production and research by manually stripping sperm and ovulated eggs followed by artificial fertilization (Leitritz and Lewis 1980). Oviposition in channel catfish occurs over several hours and is a constraint to multiple matings between males and females (Clemens and Sneed 1957; Dupree and Green 1969). Artificial fertilization in channel catfish requires aquarium spawning to time and observe ovulation. Male gametes cannot be stripped and require dissection and maceration of testes to obtain sperm suspensions. Artificial fertilization has been used successfully to produce catfish hybrids and allow the induction of polyploidy (Dupree and Green 1969; Wolters et al. 1981).

Attempts to induce spawning outside the normal spawning season with temperature and photoperiod manipulations have had limited success. Culturists and breeders currently, can only obtain gametes and make matings during the natural spawning season (May-July). Future research on catfish endocrinology and control of reproduction could provide year-round spawning and a shorter generation interval, currently three to five years, to allow more rapid genetic progress.

Cryopreservation of gametes could be a useful tool in future breeding programs, however, only preservation of channel catfish sperm has been successfully reported (Guest et al. 1976). Long-term gamete storage would permit a reduction in the number of facilities needed for broodstock maintenance and probably increase the numbers of genetic stocks available, facilitate shipment of germplasm or reference stocks to widespread locations and provide a year-round supply of gametes for commercial production and research.

Qualitative Genetics

Most traits of economic importance such as growth rate, show continuous variation and are controlled by many pairs of genes (Warwick and Legates 1979; Falconer 1981). Qualitative traits either modify the appearance of an organism or can be precisely characterized as with color, presence/absence of a protein or a body modification, and are usually controlled by one to several genes.

Many physical deformities such as tailless, side-sprigs, triple-tailed, and stump-body have been described in channel catfish and most have been found to be detrimental to overall performance (Figure 2) (Bondari 1981; Dunham and Smitherman 1987). The frequency of these traits is highly variable and may be environmentally induced. The inheritance of deformities has not been studied or found to have a genetic basis. Deformities can lower processing percentages and processors can penalize producers if a high percentage of deformities are present.

The best known potentially valuable qualitative trait in channel catfish is albinism and is inherited as a single homozygous recessive gene (Figure 3) (Prather 1961; Bondari 1981). Fillets from processed albino catfish are lighter in color, appear to have a fresher quality, and are more appealing to consumers (Tucker and Robinson 1990). Studies comparing the performance of albino and normally-pigmented channel catfish have been contradictory (Dunham and Smitherman 1987). Growth of albinos is similar to normally pigmented fish, however, spawning success is usually lower and albinos possibly have more rigid temperature requirements for spawning (Bondari 1981; Goudie et al. 1992).

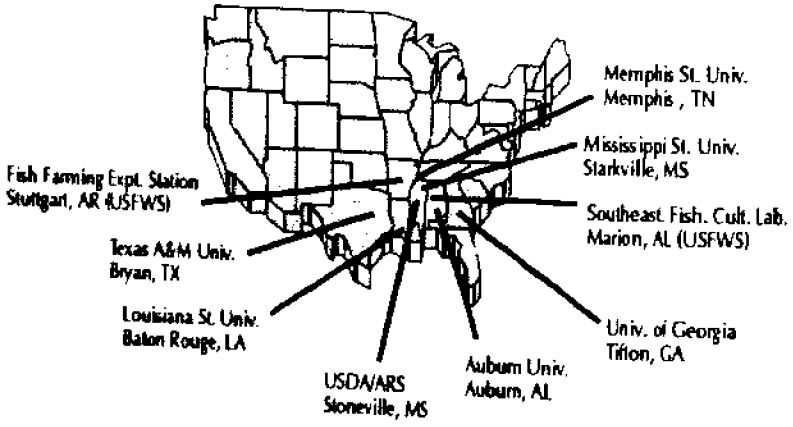

Figure 1. Institutions conducting research on catfish genetics and breeding in the southeastern United States from the 1960s through the 1980s.

Despite apparent difficulties raising albino catfish and a potentially higher incidence of bird depredation in ponds, many commercial producers are stocking larger percentages of albino fish in production ponds because of higher consumer appeal.

Electrophoretic analyses of enzyme and protein variation have been extensively studied in channel, blue (*I. furcatus*) and white catfish (*I. catus*) (Dunham and Smitherman 1984; Hallerman et al. 1986; Carmichael et al. 1992). These studies have demonstrated the ability to differentiate between species and stocks of catfish and measure changes in gene frequencies caused by selection. A total of 24 known polymorphic loci have been reported in channel catfish populations, and 15 fixed allelic differences are known between channel and blue catfish (Carmichael et al. 1992). Although these fixed and polymorphic loci could be used to determine unique genetic markers of populations and species, future research will undoubtedly focus on identifying unique DNA sequences between strains and populations (Lloyd et al. 1989; Turner et al. 1989; Turner et al. 1991). Probes specific for unique DNA sequences or markers will be used to maintain strain integrity and correlate with commercially important traits.

Figure 2. Normally pigmented channel catfish with normal and abnormal side-sprig tail morphology.

Figure 3. Comparison of normally pigmented and albino channel catfish.

Additional qualitative traits need to be identified in channel catfish. Except for albinism and protein variation, currently known qualitative traits modifying expression of the catfish phenotype have limited value. Future research linking gene frequencies or markers with performance, particularly disease resistance and polymorphisms of major histocompatibility complex loci, should be high priority (Chevassus and Dorson 1990; Stet et al. 1990; Kirpichnikov 1992).

Quantitative Genetics

As stated earlier, traits of economic importance such as growth, feed efficiency, disease resistance and processing characteristics show continuous variation and are controlled by many gene loci. Most previous research in this area has focused on improving growth rate with selection programs, hybridization, cross-breeding and strain evaluations.

Table 2. Production characteristics of channel catfish and its hybrid with the blue catfish[1].

Cross	Gain (grams)	Feed Conversion	Visceral Fat %	Fillet Dressout Percentage
Channel x Channel (Auburn strain)	482	1.36	3.5	43.9
Channel ♀ x Blue ♂	563	1.21	3.8	48.5
Blue x Blue	436	1.51	4.6	45.7

[1]Gain, feed conversion, and visceral fat% data adapted from Dunham and Smitherman, 1987. Fillet dressout percentage from unpublished data on the same crosses, W.R. Wolters, USDA/ARS Catfish Genetics Research Unit, Stoneville, MS.

Hybridization, particularly between channel catfish females and blue catfish males, provides significant increases in production (Table 2). Twenty-eight different interspecific hybrids have been produced from seven different channel catfish species (Dunham and Smitherman 1987). The three most commonly used catfish species in hybridization studies have been the channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and white catfish (*I. catus*) (Figure 4). However, the low rate of hybridization is the main constraint to the use of hybrids in commercial culture. Although some success in producing hybrids can be achieved with hormone injections using pen and aquarium spawning procedures, it is not practical in a large commercial operation. Future research needs to be conducted on developing procedures to increase hybridization rates for commercial culture.

Strains of channel catfish used in research and commercial culture are based on origin rather than performance records (Dunham and Smitherman 1984). Strains that have been cultured in hatcheries or used in commercial culture generally have faster growth than wild stocks. In addition to growth, strains have also been shown to differ in feed efficiency, disease resistance, dressout percentage, environmental tolerances and reproduction (Table 3) (Dunham and Smitherman 1987; Broussard and Stickney 1981; Tomasso and Carmichael 1991). Crossbreeding between specific strains has led to improved performance (Table 4) (Dunham and Smitherman 1985). Many of these strain evaluations were conducted more than ten years ago. Research needs to be repeated under current high-density culture conditions in several geographic locations.

Heritability estimates from sib analyses and realized heritabilities calculated from selection responses have been made for several production parameters in channel catfish (Tave 1986; Dunham and Smitherman 1983; Dunham and Smitherman 1987). Most estimates have been for body weight or length, and mass selection has been successful in increasing body weight (Table 5). Most selection programs developed at public research institutions have been hindered by the lack of long-term funding to sustain continuity. Because of a three to five year generation interval, a long-term funding commitment must be made for any significant progress to occur.

Coordinated Industry/Research Breeding Programs

No genetic improvement programs have been developed in the private industry on commercial farms. Although most commercial

Table 3. Least squares mean mortality (adjusted for weight) from environmental nitrite exposure in five channel catfish strains.[1]

Strain	Mortality (%)
Kansas x Red River	36.5
Red River	51.0
Marion	56.5
Mississippi	57.0
Norris	59.5
Marion x Kansas	73.5
Kansas	100.0

[1]Data adapted from Tomasso and Carmichael 1991.

Table 4. Effectiveness of cross-breeding on several traits in channel catfish.[1]

Trait	Marion x Kansas	Marion	Kansas
18 month weight (grams)	745	617	695
Spawning % - 3 yr	62	28	4
Spawning % - 4 yr	53	54	49
# eggs/lb female - 3 yr	3530	2320	3153
# eggs/lb female - 4 yr	3687	3673	3639
# fingerlings/lb female - 3 yr	1101	200	20
# fingerlings/lb female - 4 yr	818	684	798

producers probably recognize the value of genetic research to solve production problems, practical management considerations dictate and emphasize on short-term problems. Shultz (1986) outlined and discussed important elements in developing a commercial breeding program. These ten elements involve production assessment, establishing goals, recording data, determination of selection methods to be used, monitoring progress and continual evaluation. All should be considered in both research and commercial breeding programs.

The first element, industry assessment, involves an understanding of production systems, processing and marketing, and is particularly important when considering applications of genetic improvement in commercial channel catfish culture. The current production system used in commercial pond culture will be a major constraint to the implementation of breeding programs on commercial farms and involves raising multiple size-classes of fish in a single pond without a clean harvest (Tucker et al. 1992). In this management system, the average harvest size increases because the frequencies of fish in different size categories changes over time. A mass selection program with culling for weight above a certain size (Figure 4) would be difficult to implement in a multiple batch culture system because the age of fish selected is unknown and the phenotype will be measured inaccurately (Table 6).

Because of different management constraints, channel catfish genetic improvement

Table 5. Response to selection and realized heritabilities for body weight in three channel catfish strains.[1]

Strain	Mean Weight (g)	Response (g)	Selection Differential (g)	Realized Heritability
Rio Grande				
Select	431	63	263	0.24 ± 0.06
Random	368			
Marion				
Select	486	73	145	0.50 ± 0.13
Random	413			
Kansas				
Select	513	54	163	0.33 ± 0.10
Random	459			

[1] Data adapted from Dunham and Smitherman 1983.

programs will be most effective if located at federal or university research stations. However, a comprehensive program to transfer results of genetic research to private industry needs to be developed. Previous releases of catfish strains have been by research universities directly to commercial farmers (Dunham and Smitherman 1987). A protocol for release, testing, stock multiplication and stock verification needs to be developed for channel catfish stocks and could be patterned after well-established procedures for releasing and testing plant varieties (Poehlman 1959). A process of stock multiplication and release of germoplasm, independent of research or breeder organization and reviewed by industry, needs to be developed and followed by all cooperating agencies (Figure 5). The genetic identity of catfish germ plasm should be maintained and available. Technologies enabling identification of genetic identity should be standardized and could include protein or DNA polymorphisms unique to specific stocks. Without research and industry cooperation, minimal benefit will come from genetic research.

Figure 4. Three catfish species that have been used in hybridization studies to evaluate interspecific crosses for heterosis: channel catfish (top), blue catfish (middle) and white catfish (bottom).

- Sex reversal

Monosex female populations of channel catfish have been produced by feeding hormone-treated feed to newly hatched fry (Goudie et al. 1983). The sex-reversed XY females were mated with normal XY males to yield a 2.8 male: 1 female sex ratio. Male catfish are apparently heterogametic. YY males are viable and can be mated with XX females to produce monosex XY male populations. Because male catfish generally grow

Table 6. Percent weight distributions by size category for channel catfish in a multiple-batch production system in earthen ponds at 19,760 fish/ha over a three-year production season.[1]

Size Category	Year 1	Year 2	Year 3
<227 grams	0.10	0.05	0.93
>227 - 336 grams	11.26	3.56	6.00
>336 - 568 grams	39.27	30.09	15.91
>568 - 1135 grams	47.54	42.52	40.01
>1135 - 1816 grams	1.84	21.27	25.99
>1816 grams	0.00	2.51	11.16
Average Size (grams)	499	681	745

[1] Data adapted from Tucker et al. 1992.

faster than females in mixed sex populations, all-male culture would provide an instant increase in production efficiency (Simco et al. 1989). Progeny testing of sex reversed fish leading to the production and maintenance of YY male lines will be an important long-term activity of breeding programs.

- **Polyploidy**

Triploidy and tetraploidy have been induced in channel catfish by cold shocking or heat shocking fertilized eggs at appropriate times after fertilization (Wolters et al. 1981; Bidwell et al. 1985). Triploid fish were found to be sterile and exhibit faster growth than diploids in tank culture (Wolters et al. 1982). Subsequent studies showed no production improvement when triploid fish were grown in earthen ponds (Wolters et al. 1991). Because of the practical limitations on collecting large numbers of fertilized catfish eggs through artificial fertilization, it is unlikely that polyploid catfish will be used in commercial culture. The development of tetraploid broodstock would theoretically enable tetraploid females to spawn with diploid males using conventional pond spawning procedures; however, this accomplishment has not been demonstrated.

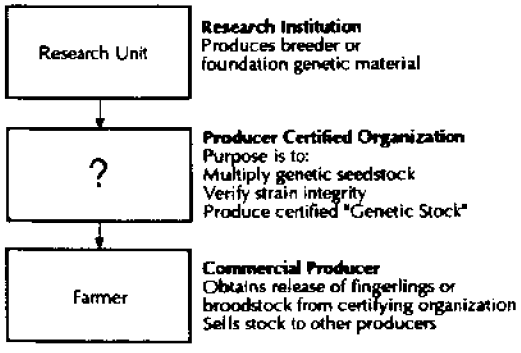

Figure 5. Proposed system for development, multiplication and release of improved catfish germplasm to commercial producers.

- **Gene Transfer**

Gene transfer technology is an established tool in molecular genetics. The ability to transfer individual genes from one organism to another has led to the development of many new biotechnology industries (Pursel et al. 1989). Over the past few years, the technology has been successfully applied to produce transgenic fish in several species (Maclean and Penman 1990). Foreign DNA has been successfully integrated into the channel catfish genome (Dunham et al. 1987). Research is currently underway to determine the contribution transgenic fish will make in aquaculture. Faster growth and increased disease resistance exhibited by transgenic fish would certainly improve production.

The use of transgenic fish, and also genetically improved hatchery stocks, in aquaculture has ecological implications (Kapuscinksi and Hallerman 1990; Hew and Gong 1992; Hallerman and Kapuscinski 1992). Production of channel catfish is almost exclusively in large earthen ponds, often built in areas prone to flooding. The accidental release of transgenic fish from commercial ponds is a possibility, and transgenic fish could breed with wild fish transferring transgenes into the native population. The genetic and ecological structure of native channel catfish populations has not been well studied as compared to salmonids. Guidelines on the use of transgenic catfish in aquaculture systems need to be developed to insure against accidental release. Future studies should focus on characterization of the total phenotype of transgenic catfish including all aspects of physiology, reproduction and behavior. Fish will be excellent models for transgenic animal research, however, considerable research is still needed on how introduced genes modify the phenotype of transgenic fish and the application of transgenic fish into production systems.

Impacts of Hatchery Fish on Wild Populations

Many state and federal management agencies in the U.S. have in the past and currently stock channel catfish into public waters for recreational fisheries enhancement. It is possible that the fitness of hatchery raised catfish is different than the wild populations. The hatchery raised fish may have been developed at local hatcheries and undergone some selection pressure from hatchery practices or they may have been transplanted from a different location. If possible broodstock used for supplemental stocking should be obtained from the same environment into which fish will be stocked (Krueger et al. 1981). Hatchery practices should maximize the size of broodstock populations and minimize the hatchery rearing period (Kapuscinski and Jacobson 1987).

Conclusion

Channel catfish genetic improvement programs offer potential for improvement to benefit commercial production. Research areas should incorporate traditional animal breeding approaches and new biotechnologies (Table 7). Programs that coordinate industry and research while addressing priority areas of importance to commercial production will have the greatest chance for success.

Table 7. Future priority areas of research and program development in catfish genetics and breeding.

Program Area	Specific Need
Reproduction and Spawning	1) Develop out-of-season spawning techniques to shorten the generation interval and provide year-round seedstock. 2) Cryopreservation of gametes for germplasm storage and continuous seedstock availability.
Qualitative Genetics	1) Identify protein and DNA markers unique to genetic stocks for validation of strain integrity. 2) Correlate markers with economically important traits.
Quantitative Genetics	1) Improve interspecific hybridization rate to increase the use of hybrids in commercial culture. 2) Conduct strain evaluations at diverse locations under current commercial culture conditions. 3) Estimate h^2 for many economically important traits and compare efficiency of different selection programs. 4) Develop long-term coordination of catfish genetics research with industry to insure program continuity and success. 5) Develop procedures for germplasm testing and release.
Sex Reversal	1) Test monosex lines in commercial culture systems. 2) Develop and maintain broodstock (YY males) to be used in commercial culture.
Gene Transfer	1) Evaluate phenotype of transgenic fish. 2) Develop guidelines for the use of transgenic fish in research and commercial culture.

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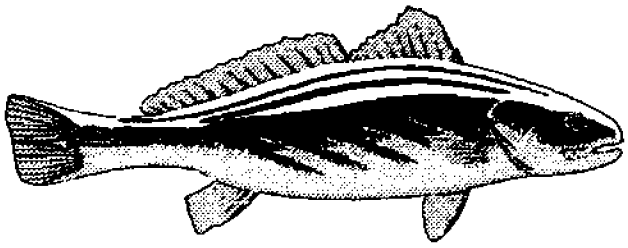

Genetic Resources for Future Finfish Aquaculture

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Abstract

As background for gaining some insights into the genetic resources for future finfish aquaculture, this report defines some of the major characteristics of the current industry and the genetic resources utilized. Based on the growth exhibited by aquaculture, the diversity of the industry and the resources utilized, and the identified need to genetically characterize and maintain the genetic resources, the current and future effects of various factors on the natural genetic resources are explored. In addition, the status and potential for stock development are examined. While the major impacts on genetic resources result from insults to the aquatic environment and overfishing, aquaculture will play a major role in restoration efforts, although some operational changes will be needed. Aquaculture will become increasingly involved in both *in situ* and *ex situ* genetic conservation efforts and will, perhaps, provide the impetus for development of techniques to facilitate these efforts. Intensive aquaculture is projected to need to develop more efficient production stocks through the increased use of selection and breeding approaches, as well as through biotechnological advances. Overshadowing all of the projections and needs for genetic resources for future finfish aquaculture is the lack of a coordinated genetic information base on which decisions can be centered. The challenge for the future is the wise utilization of available genetic resources and, at the same time, their effective conservation.

Introduction

Attempting to define the genetic resources needed for the future of any food production industry is difficult, if not impossible, with the rapid changes that are occurring in many sectors of the industry. Changes in areas such as market demand, price structures, governmental policies, global warming and biotechnology can change the characteristics of an industry very rapidly. Finfish aquaculture is equally, if not more, susceptible to such changes, making projections about the future very tenuous. However, since finfish aquaculture is a relatively new commercial endeavor that still relies to a large extent on natural resources, it would be wise to consider what directions should be taken to ensure the wise use and conservation of the available genetic resources.

Before looking into the genetic resources for future finfish aquaculture, some of the current features of the industry that could have an impact on these resources should be examined. First, finfish aquaculture is a rapidly growing industry. In 1990 the harvest of finfish from aquaculture was about 8.2 million metric tons (MMT) (FAO 1992a). When this is compared to the total worldwide harvest of aquatic species (excluding seaweeds) of 97.2 MMT (FAO 1992b), it does not seem to be a very major part. However,

considering that this is a 62% increase in production over a five year period (1985-1990) and that, on a weight basis it comprises well over half (68%) of the total aquaculture production, (Fig. 1) places a little different light on the vitality of finfish aquaculture. This growth is projected to continue (Nash 1988; Sandifer 1988; New 1991) and the appropriate genetic resources will be needed to underpin and enhance increased production.

Second, this industry is very diverse and multifaceted. Finfish aquaculture is conducted in many different environments, using a variety of techniques and guided by a diversity of goals. The largest quantity of fish produced on a worldwide basis is derived from freshwater aquaculture in tropical climates (FAO 1992a) and much of this production is obtained from "extensive" aquaculture, which utilizes rather low technology husbandry methods. On the other hand, one of the most rapidly growing segments of the industry is Atlantic salmon

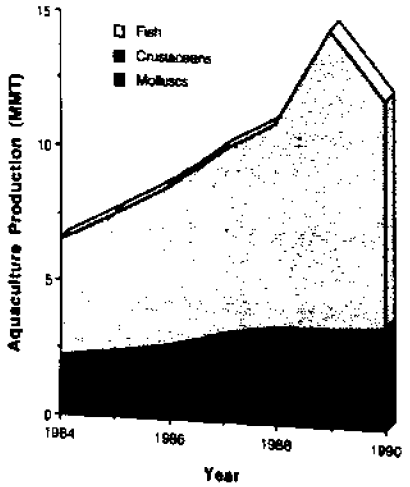

Figure 1. Worldwide annual aquaculture production (Million Metric Tons - MMT) of molluscs, crustaceans and finfish between 1984 and 1990 (FAO, 1992a).

(*Salmo salar*) culture, which are grown in cold water marine environments and utilize relatively high technology approaches. Further, while most of the production in the above two examples are for direct consumption or marketing, a large portion of Pacific salmon aquaculture is directed toward the "enhancement" of natural resources and ultimate harvest by the commercial capture or sport fisheries. Such variety in aquaculture operations mandates that the genetic resources for future finfish aquaculture must be approached with diversity in mind.

Part of the diversity seen is a reflection of the variety of species utilized in aquaculture. About 200 species are currently produced worldwide in aquaculture facilities (Nash 1987), a number which is predicted to increase in the future (New 1991). While this is a rather small sampling of the more than 20,000 fish species that are estimated to inhabit aquatic environments worldwide (Nelson 1984), it represents a much larger array of animals than is currently raised in terrestrial agriculture. It also comprises a very large genetic resource that is available for development of aquacultural stocks. On the other hand, such a broad array of animals and potential genotypes presents a formidable challenge for genetic characterization and conservation. Thus, while there is a vast genetic "reservoir" to tap for aquaculture development, defining the genetic diversity in these species and maintaining these resources will be formidable tasks.

Finally, "tracking" and maintaining finfish genetic resources are made even more difficult by multiple interests in the harvest of fish. Many species are important to the commercial capture and the sport harvest, as well as to aquaculture. Consequently, in these situations the interests of all three types

of harvest are intertwined; not only do they harvest some of the same species, but aquaculture is utilized to "enhance" species for the capture fisheries (e.g., Pacific salmon in the U.S. Pacific Northwest and Japan). Further, since aquaculture is still utilizing, for the most part, undomesticated stocks of fish, the same natural genetic resource harvested by the capture fisheries is a major source of genetic raw material for aquaculture. Therefore, all methods of harvest must be considered in making decisions regarding finfish genetic resources for the future.

With the obvious diversity and breadth of finfish aquaculture and the resources that are utilized, it is not possible to cover, in detail, how the genetic resources of individual species should be handled for the future of the industry. However, there are a number of areas where action will be beneficial for the genetic resources, irrespective of the species of fish produced or the husbandry techniques employed. Thus, it is the purpose of this report to highlight these areas, to identify their effects on the genetic resources, and to propose steps that should be taken to ensure the continuation and, perhaps, improvement of available genetic resources for the future. Hopefully the ideas presented will help catalyze more definitive action to develop methods to both make effective use of fish genetic resources and to conserve them.

Natural Resources

The first area that needs consideration with regard to genetic resources for future finfish aquaculture is the relationship of the industry and its production to natural resources. Currently, most of the industry is relying, directly or indirectly, on the genetic resources provided by the natural environment. Few truly domesticated and genetically "designed" broodstocks have been developed for commercial finfish aquaculture, so production stocks are usually derived from available natural resources. Further, when genetic problems arise, either from decreases in variability or from the consequences of inadvertent selection (Doyle 1983; Hershberger 1988), they are usually addressed by use of natural genetic variability. From a more long-term viewpoint, natural genetic resources will be the only available source of new genetic material for future stock enhancement or changes. With the current dependence of finfish aquaculture on natural genetic resources and the future importance of these resources as a source of new genetic material, more attention should be given to this area.

• Factors Affecting Natural Resources

Two factors currently have a major effect on natural genetic resources and will continue to do so unless changes occur. The most significant factor impacting finfish genetic resources is the deterioration of the environments in which fish live. The physical, chemical and biotic features of aquatic environments in this country (Reisner 1986), and around the world, are being altered at an alarming rate. These changes have already had major effects on the genetic resources of aquatic species. For example, as a result of the construction of hydroelectric dams on the Columbia River in the Pacific Northwest region of the United States, it is estimated that as many as 200 populations of Pacific salmon (*Oncorhynchus* spp.) uniquely adapted to specific freshwater environments were lost (NPPC 1987). This and other environmental changes have led to more than 100 stocks of anadromous salmonid fishes in the states of California, Oregon, Idaho and Washington being characterized as at "high

risk of extinction" (Nehlsen et al. 1991). While it is not the purpose of this paper to delve into the problems with the degradation of aquatic environments, it is imperative to emphasize that the abuse of these environments needs to be halted if finfish genetic resources are to have any future.

The second factor affecting the genetic resources of natural fish populations has been the changes in the commercial capture fishery. In the last decade the market demand for fish products increased dramatically; for example, per capita consumption in the U.S. increased nearly 25% (from 5.7 kg to 7.1 kg) between 1980 and 1990 (NRC 1992). Demand for fish products is projected to continue to increase on a worldwide basis (New 1991). The increased demand, accompanied by price increases and diminishing resources, resulted in the evolution of the capture fisheries from small, subsistence-level operations to sophisticated, highly mechanized fish capture systems. The enlarged harvesting capacity and the improved efficiency of the capture fishery have led to the rapid depletion of some fish populations and, in some cases, their complete elimination (Larkin 1977). Further, the recurring pattern of resource depletion has led to increased management regulation that utilizes increasingly selective fishing practices. The practices of over-exploitation and selective regulation of the harvest have had a demonstrable effect on salmonid genetic resources (Ricker 1981). The extent of the genetic change caused by capture fisheries can only be estimated in a few instances, since genetic data are available on a relatively small sampling of the species that are harvested. Consequently, a much more extensive genetic data base is needed to get an understanding of the natural genetic resources.

Stock Enhancement

Aquaculture has been utilized for a long time in attempts to restore/enhance the production lost from diminished or polluted environments and from overfishing (Eschmeyer 1955). For a variety of reasons the history of this use of aquaculture has shown extremely variable results (Radonski and Martin 1986). However, with improvements in technology and better scientific information aquaculture has been successful in a number of commercial capture and sport fisheries (Liao 1988; Sandifer 1988). Further, New (1991) suggests that stock enhancement will have a major role to play in meeting future fish production needs. Increased use of this type of aquaculture will present problems for the natural genetic resources if changes are not made in some operational procedures.

Inherent in attempting to raise natural populations of fish under artificial conditions and introducing them into the natural environment are a series of genetic consequences that have the potential to affect the natural genetic resources. These problems, while most extensively studied in salmonids, are not unique to a particular group of fish, nor even to fish in general (Harlan 1981). Several reviews have been published that cover the specifics of the genetic impacts that may be realized with fish (Allendorf and Ryman 1987; Hindar et al. 1991; Waples 1991). Concerns center on three basic issues shown in Table 1. While the relative importance of each of these may vary with the species, they suggest some areas that will need to be addressed for future finfish aquaculture.

Table 1. Levels of potential genetic effects on natural resources from the use of enhancement culture to restore populations (Waples 1991).

Genetic Effects of Concern from Enhancement	Causative Agents
Direct genetic effects	Hybridization, introgression
Indirect genetic effects	Altered selection regimes or reduction in population size resulting from competition, disease, or other factors.
Genetic changes to hatchery stocks	Selection, genetic drift, stock transfers

First, the genetic composition of natural resources must be more thoroughly and completely defined. Currently, only a small fraction of the species utilized for stock enhancement has been genetically characterized. Further, for the most part, only a single analytical tool has been used to define genetic variability, electrophoretic separation of genetically variable proteins, (Utter et al. 1987). More recently, electrophoretic analyses of mitochondrial and nuclear DNA have been employed (Wilson et al. 1987; Wirgin et al. 1991), but these data are not yet very extensive. Although results obtained to this point have been very informative and have yielded a wealth of previously unavailable information, additional traits should be analyzed to allow better definition of the natural genetic resources. Also, utilization of the information is problematic since the results are generally found in a wide array of journals and publications. Future plans should include the development of a genetic data management system for fish.

Second, future stock enhancement must take steps to follow sound reproductive approaches and, to the extent possible, use husbandry procedures that enhance performance in the natural environment. Because the fecundity of many finfish species is relatively high and because aquaculturists have become proficient at maximizing early survival, it has been rather common practice to use as few adults as possible for reproduction. This leads to severe and haphazard genetic changes through the process of genetic drift (Crow and Kimura 1970) and there have been numerous studies to document that this is a problem in stock enhancement (Simon et al. 1986; Allendorf and Ryman 1987; Waples and Teel 1990). In addition, husbandry practices (e.g., feeding rate, time of feeding, and rearing density), through their effect on the innate behavior of fish, can have a major impact on the survival and genetic composition of a population (Huntingford and Thorpe 1992). For example, research has shown that modification of the physical methods of presenting feed (Noakes and Grant 1992) and changing feeding times (Eriksson and Alnärä 1992) lead to increased efficiency and decreased environmental impact on the fish.

While stock enhancement will undoubtedly have an expanded role in future fish production, the lack of reproductive barriers between the cultured populations and the natural populations will mandate some operational changes to maintain the natural genetic resources. Additional genetic information will provide the base from which to assess changes. Alteration of hatchery procedures will assist in minimizing the differences between the two groups. However, sterility or

other types of barriers (e.g., geographic separation) will need to be erected to implement effective conservation of natural genetic resources.

Conservation of Natural Resources

Although there is a crucial need for conservation of fish genetic resources, the methods for management of these resources are currently inadequate and diffuse (Ryman et al. 1993). Most of the conservation efforts with fish species have been directed toward ecosystem and species management (*in situ* management). However, the harvest of commercial capture and sport fisheries still has an impact on these *in situ* systems. Management of the capture fisheries has been based on maintaining a sustainable yield, which only takes into account numbers of fish and does not consider the genetic composition of the fish harvested. Further, the concept of maintaining a sustainable yield with natural resources has come under some scrutiny (Ludwig et al. 1993).

Most of the information available for guiding conservation efforts is derived from electrophoretic separation of genetically variable proteins, as well as of mitochondrial and nuclear DNA (Ryman and Utter 1987). The major goal in these investigations has been to determine the natural population structures of the various species and assess their potential genetic relationships. The results of these analyses have shown that, in general, local populations of freshwater fishes are genetically more divergent than those of marine species, and anadromous species exhibit somewhat intermediate values (e.g., Ryman 1983; Gyllensten 1985). For example, this means geographically proximate populations of some freshwater species may be sufficiently divergent genetically to warrant consideration as separate species (e.g., Allendorf and Leary 1988), whereas populations of some marine species on different sides of the ocean may be genetically indistinguishable (e.g., Grant 1984). Fewer geographic barriers to reproductive isolation in the marine environment can undoubtedly explain a lot of these differences, but there are documented exceptions to these generalities (Jörstad et al. 1991). However, these differences in the distribution of intraspecific genetic variability have some obvious implications for the conservation of finfish genetic resources.

First, where there is a high degree of genetic divergence between populations, these units are an important source of genetic variability. Consequently, the loss of a population has a proportionately larger impact on the genetic resources of the species. Further, geographic confinement generally means smaller population sizes; reduction in numbers in small populations can lead to large losses of genetic variability. However, the magnitude of the modern harvest can have a major impact even on the genetic resources of the more numerous marine species. It would seem advisable for future *in situ* conservation efforts to develop management approaches that incorporate genetic risk analysis in their formulation and implementation.

The technology for off site (*ex situ*) management systems is developing very rapidly with fish species. Due, in part to the increasing interest from commercial aquaculture, the capability of satisfactorily propagating a wider number of species for the maintenance of living collections is becoming a reality. The guidelines are being formulated for using aquaculture for the recovery of Pacific salmon (*Oncorhynchus* spp.) listed as threatened or endangered under the U.S. Endan-

gered Species Act (Hard et al. 1992). However, obtaining a representative sampling of the genetic diversity of the population is still a problem, as is the cost of maintaining a long-term collection of these species. Contemporary research indicates that long-term storage of frozen gametes and even zygotes may be practical in the near future (Stoss 1983). However, serious constraints still exist with regard to the technologies for the storage of ova and embryos of aquatic organisms. Future conservation will, of necessity, place more emphasis on *ex situ* approaches and the use of "gene banks" to conserve genetic resources.

Use of Exotics

A final approach that should be discussed under this topic area is the transfer of populations and the introduction of exotics. This activity has a long history (about 150 years) and has been undertaken, for the most part, for man's benefit. Most often the rationale used has been to establish food or game fish (Courtenay and Robins 1975). In a review of introductions of inland species, Welcomme (1992) pointed out that 57 and 84 species have been recorded as being introduced for enhancement of sport fisheries and improvement of wild stocks, respectively. While only a few of these were successful, and fewer still have had negative impacts, the potential severity of their genetic effects when introduced into natural fish populations is too large to discount. Protection of future genetic resources will rely on stronger control of practices and should include some type of risk assessment (e.g., Kohler 1992).

Commercial finfish aquaculture also plays a role in the importation of exotics. Frequently, the argument employed for this type of activity in the U.S. is that the economy is based on exotics (Courtenay and Robins 1975). It is pointed out that virtually all of our agricultural livestock, most of our grains and vegetables, and many fruits are exotics. However, until fish have been subjected to controlled breeding for a large number of generations and they do not compete successfully without husbandry and cultivation by man, the severity of their impact on natural fish populations will be perceived to be too large to discount. Although about 117 species have been introduced into foreign inland environments for aquaculture purposes (Welcomme 1992) and some of these have been very successful (Hershberger 1991), the trend in commercial aquaculture currently seems to be directed toward exploring the potential of local species (Welcomme 1992).

Controls on the importation of exotics at any level, either state, national or international, are fragmented or ill-enforced and are based mostly on the threat of disease transmission rather than on genetic impacts. While prohibition of further introductions is unrealistic and would clearly hinder future development, greater public awareness of the perceived problems will make importation of exotics more difficult. Effective sterilization techniques will be developed for fish, as well as a larger genetic information base on which to judge the likely impact. Both of these will facilitate some use of exotics in finfish aquaculture.

Stock Development

Unlike the situation with traditional agricultural enterprises, aquacultural production is currently based on the husbandry of undomesticated stocks of fish. Consequently, the aquaculture industry is faced with the operational and genetic problems that accompany

the development of domesticated populations. The major constraints to more rapid development of stocks for commercial aquaculture are the lack of reliable estimates of genetic parameters (e.g., genetic and phenotypic variances, covariances, genetic and phenotypic correlations) for commercially important traits and the lack of designed selection programs to test their validity (USDA 1988). A number of studies have been conducted to estimate phenotypic and genotypic parameters for many quantitative traits (Gjedrem 1983; Gjerde 1986; Tave 1986). In general, these studies have demonstrated the presence of adequate genetic variability in economically important traits to realize reasonable gains through selection programs. It has also been observed that, while the phenotypic variability in many traits in fish is much higher than in other agricultural animals, the heritabilities in fish are somewhat lower (Allendorf et al. 1987). This may be a reflection of the poikilothermic nature of fish and their consequent responsiveness to the external environment. It may be that habitat and husbandry changes will have more effects on fish than have been observed with terrestrial animals.

While there has been an expansion of efforts to conduct designed breeding programs that will lead to stocks with desirable genetic traits, few of these have been of adequate duration to yield defined aquaculture stocks. Probably the most extensively developed program for this purpose is in Norway, where the aquaculture industry has supported a large breeding and selection program to develop stocks of Atlantic salmon (*Salmo salar*) for marine net-pen rearing (Gjedrem et al. 1987). There are also large programs in Israel and Hungary for the development of carp (*Cyprinus carpio*) stocks for pond rearing (Moav and Wohlfarth 1966; Bakos 1976). In the U.S., several programs with salmonids have been conducted to develop stocks for industry (Donaldson and Olson 1957; Gall and Gross 1978; Hershberger et al. 1990). However, the results from these programs are, for the most part, still too preliminary to define the fish stocks as domesticated.

Future finfish aquaculture will need to develop more efficient animals for commercial operations. It is estimated that at least 30% of the increases in rate and efficiency of protein production in agricultural animals have resulted from genetic research and comprehensive industry breeding programs (Dickerson 1970). Similar levels of change will be required for some segments of the aquaculture industry, particularly those involving high-cost inputs, to retain viability. More severe environmental constraints and increasing competition for intensive cultivation approaches (New 1991) will necessitate the use of selection and breeding approaches, as well as biotechnology, to improve the efficiency of the finfish stocks used.

Fish exhibit a number of characteristics that make them amenable to extensive biotechnological manipulation (Lewis 1988) and a number of investigations are underway to explore the transfer of genes to provide desirable traits for aquaculture (Maclean et al. 1987). In a review of the use of gene manipulation in aquaculture, Maclean and Penman (1990) highlight the variety of biotechnological approaches that can be used to develop stocks for commercial aquaculture. However, the current lack of detailed genetic information on fish species limits the potential for more extensive studies and problems of containment in an aquatic environment are formidable.

Conclusion

The scenario that unfolds from considering genetic resources for future finfish aquaculture is one of a rather minimal, although expanding, information base on the genetics of natural resources and of program development for the design and production of domesticated stocks. There is a promise and a potential for accessing large amounts of genetic variability for future programs. For finfish aquaculture to realize the increased production that, from all projections, will be needed to meet market demands, a significant portion of this genetic resource will have to be utilized in one form or another. The challenge is to develop aquaculture programs that will meet the diverse needs of the industry and, at the same time, conserve adequate genetic resources to ensure future generations will also have access to them.

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Conservation Genetics Programs for Pacific Salmon at the Washington Department of Fisheries: Living with and Learning from the Past, Looking to the Future

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Abstract

Within- and among-stock genetic diversity are fundamentally important attributes contributing to the productivity and evolutionary potential of Pacific salmon. Fish culture, harvest management, and habitat management and rehabilitation programs should be designed and executed in a manner that contributes to the maintenance of existing patterns of genetic diversity. Over the past fifteen years, the Washington Department of Fisheries has developed and implemented a number of new programs intended to promote the conservation and health of the diverse salmon resources in Washington. Key features of departmental activities as they relate to conservation genetics are: 1) generate a comprehensive inventory (identification and characterization) of all naturally spawning salmon and steelhead stocks in the state, 2) develop formal agency policies for salmon genetics and stock management and conservation, 3) review existing spawning guidelines, hatchery practices, stock transfer guidelines and fisheries management strategies to assure compliance with identified genetic (and other) objectives and policies, 4) incorporate genetic risk assessment and management as a routine component of future departmental actions, 5) pursue basic and applied research investigations regarding salmon genetics, reproductive and developmental biology, behavioral ecology and hatchery-wild stock interactions, and 6) design and implement monitoring and evaluation programs to assess the performance of specific actions and programs with respect to identified operational goals. The Department of Fisheries is working closely with the Washington Department of Wildlife, Indian tribes, other fishery management entities and volunteer groups throughout the state and the Pacific Northwest to promote the conservation and enlightened utilization of the region's fisheries resources.

Introduction

While the discussion and examples below are focused on Pacific salmon, the same general genetic concerns and many of the same problems threaten marine and freshwater finfish and shellfish species in Washington and elsewhere. Indeed, whether the situation involves hatchery production to support fisheries, harvest and habitat management activities intended to conserve native stocks, or the establishment of reserves and sanctuaries to protect stocks or entire species, programs and action plans should be based on sound genetic principles as well as relevant biologi-

cal characteristics and ecological requirements of the targeted species/communities if they are to be successful in maintaining the genetic character and health of the resource.

• **Population genetics for conservation**

Fish culture, fisheries management and the conservation of aquatic resources should be guided by four fundamental principles: 1) fish species are typically composed of multiple, differentiated gene pools or stocks; 2) native stocks are adapted to local environmental conditions; 3) within- and among-stock genetic diversity are the primary sources for ecological adaptation and evolutionary change; and 4) the overall long term productivity of any fisheries resource is maximized when the diversity and health of all component stocks are maintained.

Stocks can be defined as panmictic breeding units (populations) within a species that are reproductively isolated to a significant degree from other such units. While this concept is perhaps most firmly established for salmonid fishes, where it forms the basis for fisheries management activities and conservation efforts (Ricker 1972; Berst and Simon 1981; Nehlsen et al. 1991; Seeb et al. in press), it applies to other fish and shellfish species as well.

As the number of individuals in a population decreases, the probability of that population's extinction due to random genetic, environmental or demographic events increases. With regard to genetic effects, it is important to distinguish between short term (fewer than two generations) medium-term (three to ten generations), and long term (more than ten generations) effects (Mace and Lande 1991). There is general agreement that, in the short term, an effective population size (N_e) of at least 50 reproducing individuals per generation is adequate to avoid substantial reductions in fitness due to inbreeding depression (Franklin 1980; Frankel and Soule 1981; Nelson and Soule 1987). For medium- and long-term situations, genetic drift is a major determinant of the genetic characteristics of populations. Based on theoretical considerations, both Franklin (1980) and Lande and Barrowclough (1987) have determined that drift should have a negligible effect on the genetic characteristics of populations provided that N_e is about 500 or more. The latter authors also conclude that populations with an N_e of at least 500 can maintain nearly as much genetic variance in typical quantitative traits as an infinitely large population.

Waples (1990) has shown that the effective population size per generation for Pacific salmon is approximately equivalent to the average number of breeders per year (N_b) times the average generation length (age at reproduction) for the population. Thus, for a chinook salmon population with an average adult return age of four years, the N_e of the population would be four times the harmonic mean of the N_b values in four successive years.

While a stock's genetic vulnerability is based on N_e, this parameter is difficult or impossible to estimate with confidence for most natural populations (Hill 1981). Population biologists believe that the N_e of natural populations is almost always significantly smaller than the census size. Indeed, Nelson and Soule (1987) and others have suggested that N_e for salmonid fishes may be substantially less than the census population size due to a failure by some of the returning adults to spawn successfully, skewed sex ratios and variance in lifetime family size.

Natural stocks should be managed to: a) maintain adequate N_e to minimize inbreeding, maintain within-stock variation and retain the ancestral genetic character of the stock (= minimize genetic drift); b) maintain among-stock differences and overall diversity; and c) minimize domestication selection (reduction of fitness in the wild resulting from the different selection pressures imposed by the hatchery environment). Because increased levels of gene flow will decrease among-stock differences, it is important to avoid stock transfers and practices that increase straying rates between genetically distinct stocks.

- **Historical perspective**

There are many anthropogenic factors that have diminished and/or continue to threaten the productivity and genetic integrity of salmon stocks in Washington. For example:

> A lack of appreciation for, or disregard of, the importance of maintaining discrete, local stocks as a basis for maximizing long term productivity,

> The degradation or loss of large amounts of the freshwater habitat that originally supported salmon production (due to hydroelectric development, agriculture, forestry, mining, urbanization, etc.),

> The belief that hatchery production was an appropriate, cost effective and sustainable means to compensate for losses of natural production,

> Ineffective hatchery operations (often driven by questionable harvest management goals) such as: a) the use of non-native or "hybridized" hatchery stocks throughout large geographic areas as a means of maximizing hatchery production and flexibility while reducing costs, b) reliance on timing of return and/or spawning as the sole criterion for maintaining stock separation at hatcheries where more than one race of a given species (e.g., spring, summer and fall-run chinook) were propagated, and c) the fact that many hatchery programs provided a considerable opportunity for straying of hatchery-origin fish into natural spawning areas where interbreeding and competition had the potential to negatively impact native wild stock(s),

> Economic, social and legal pressures to provide fishery harvest opportunities that encouraged a high rate of exploitation and the proliferation and expansion of hatchery programs. The existence of numerous large hatchery programs and the ability of many hatchery stocks to withstand high exploitation rates have contributed to the harvest of wild stocks at rates that they cannot support. This overharvest has undoubtedly led to the decline or elimination of many native stocks,

> The mixed-stock nature of virtually all Pacific salmon fisheries makes stock-specific harvest management difficult or impossible,

> The fisheries themselves undoubtedly had, and continue to have a pronounced selective effect (size/age distribution, pattern of migration, timing of return, etc.) on

the genetics of many stocks (Ricker 1980a and b; Ricker and Wickett, 1980; Holtby et al. 1992).

- **Washington State Department of Fisheries Mission**

The stimulus for genetic conservation measures within the Washington Department of Fisheries (WDF) is derived from its legislative mandate. The Department is required to "...preserve, protect, perpetuate and manage the food fish and shellfish in state waters and offshore waters. The department shall conserve the food fish and shellfish resources in a manner that does not impair the resource..." (Revised Code of Washington 75.08.012).

From this legislative mandate WDF derives a custodial responsibility and commitment to incorporate resource management measures that lead to long term resource health and productivity including, presumably, a dedication to conservation of genetic resources. Although the intent for conservation of genetic resources is broadly assumed within the department, it is not directly stated in either the legislative mandate or the more recent Washington Department of Fisheries Mission Statement (1987). The mission statement stipulates, in part, the following priorities of service:

> Preserve and protect fish resources,

> Preserve and protect fish habitat,

> Manage and allocate commercial and recreational harvest of food fish,

> Meet federal court obligations with respect to Indian tribal rights,

> Enforce fisheries laws and rules,

> Improve the efficiency and effectiveness of agency management,

> Improve public understanding of fish protection and management.

Although explicit mention of genetic conservation is absent from the mission statement, the "preserve and protect" clauses seem to give adequate license to policy makers to embrace genetic conservation objectives. In practice, WDF staff have found solid policy support for hatchery genetic guidelines and several genetically- based initiatives.

The initiative for a solid genetic conservation foundation in resource management in the Pacific Northwest is a recent development. With continued technical and scientific maturation of the topic, future iterations of department policy and mission statements will likely contain specific statements of commitment to genetic conservation in the management of all resources under its jurisdiction.

- **Co-management of northwest fishery resources**

The need and desire to instill a genetic conservation premise in the management of northwest resources extends well beyond WDF. The resource is potentially impacted, both positively and negatively, by a host of participating parties. Many fund or operate salmonid and shellfish propagation facilities and/or harvest the resource. Others participate in the planning and implementation of harvest on a coast-wide basis. The entities most firmly integrated into co-management of northwest resources are the several Puget Sound, coastal and Columbia River Indian tribes, which share management responsibilities with the state.

The origins of co-management sprang from well known federal court decisions (by judges Belloni and Boldt in the late '60s and early '70s) that reaffirmed the right of treaty Indians to harvest up to 50% of the available salmon and steelhead. Although not specifically described by the federal courts, this concept evolved in response to the divisive atmosphere surrounding northwest resource management following the federal court decisions. The process has been socially and technically challenging to all co-managers involved. Co-management for development and initiation of genetic management policies means that the WDF must engage in a multilateral approach to, and regional perspective on, resource management that includes a solid foundation of genetic conservation intent. It will not be enough for the department to adopt genetic policy for which practice is limited to the state agency alone. The full support and participation of all co-managers must be enlisted. With the added incentive of recent Pacific salmon listings under the Endangered Species Act (see below), now is the time for a major move forward in gene conservation policy throughout the region.

The Departments of Fisheries and Wildlife and the treaty Indian co-managers have ultimate regulatory responsibility for resource management throughout the state of Washington. Fisheries management in Washington, however, is very complex and involves extensive interactions with numerous stakeholders and concerned parties both within Washington and beyond its borders. The range and scale of associations represented is broad, ranging from small volunteer groups and educational programs in schools to regional commissions and international treaties such as the Pacific Salmon Treaty between the United States and Canada.

The department plays a large role in the management of salmon fisheries that occur in the ocean, outside the three-mile state regulatory zone. The ocean fisheries are managed by the Pacific Fisheries Management Council representing ultimately the U.S. Secretary of Commerce. Management of ocean fisheries is critically important to the State because many stocks of salmon are impacted in major mixed-stock ocean fisheries.

- **Endangered Species Act**

The Endangered Species Act (ESA), enacted by the U.S. Congress in 1973, is undoubtedly one of the most powerful pieces of environmental legislation in the world. If a species, or distinct population segment thereof, is sufficiently reduced in numbers that extinction in its natural environment is likely, it may be declared endangered. If it is not sufficiently imperiled to be declared endangered, but is at risk of becoming endangered, it may be declared threatened. In either case, the federal government must protect it and develop measures to improve its situation. The act is controversial, criticized by some for the drastic measures that may be brought to bear to protect a species without regard for economic consequences. It is also criticized by others (e.g., Rohlf 1991) as being basically an emergency-room measure that affords too little real environmental protection. The one thing persons on both sides of the environmental fence can agree on is that management of resources under ESA protection is bureaucratically cumbersome and extremely restrictive.

Only recently has the ESA been applied to Pacific salmon, but three species in the Snake River, all of which spend some of their lives in Washington waters, have been listed. In

1991, Snake River sockeye were listed as endangered, while Snake River spring/summer chinook, and Snake River fall chinook were listed as threatened. Within the past six months, several petitions to list additional stocks of salmon and trout in the region have been submitted. The two listings for Snake River chinook salmon have had substantial impacts on WDF hatchery operations and could also have major effects on fisheries regulated by the Department, but the major impact of the ESA process has been to enlarge the scope of the department's stock conservation activities. Perhaps the most tangible indications of this impact are the department's Wild Stock Restoration Initiative and its Washington Salmon and Steelhead Stock Inventory (see below).

The specialized life history characteristics of Pacific salmon species complicated initial applications of the ESA to this group of fish. Specifically, the existence of a hierarchy of increasingly subdivided groups, ranging from species down to the level of spawning aggregations at specific geographic locations, made it unclear at what level the ESA should be applied. The National Marine Fisheries Service (NMFS), the federal agency responsible for enforcing the provisions of the ESA as they relate to anadromous fish stocks, has determined that to qualify as "distinct population segments" salmon stocks must be evolutionarily significant units (Waples 1991). Evolutionarily significant units ("ESU"s) are defined as reproductively isolated populations or population groups that represent important evolutionary components of the species.

Washington Department of Fisheries Guidelines and Policies

WDF's existing genetically-based policies and guidelines were developed in the early 1980s and persist with only minor revision. Policy development was focused almost entirely on the hatchery production of salmonids. Hatcheries and their operations are more publicly visible than the social and political nuances surrounding harvest regulation or the often subtle impacts of habitat improvement measures. The recognition and concern for genetic consequences of hatchery operations has become an extremely high profile issue in both the state and the region at this time. Furthermore, the ability to successfully apply genetic management protocols may be much greater in hatcheries than in most other aspects of resource management because the fish are in a controlled environment for an extended period of time and their reproduction can be readily manipulated.

Prior to the emergence of the stock concept (Ricker 1972), most hatchery operators and policy makers did not recognize the importance of maintaining within- and among-stock genetic diversity. For example, traditional hatchery practices routinely included massive transplants of salmon from one region of the state to another and even into and out of the state. Even now, major regions of the state and neighboring states continue to be managed as a single mega-hatchery complex to achieve fishery harvest benefits. Thus, several of the hatcheries on the Columbia River that raise "tules" (lower Columbia River mainstem and tributary spawning fall chinook salmon) freely exchange eggs and fry each season to cover shortages at some facilities with excesses from others. This practice is generally associated with the

overharvest of all but the most robust runs so that maximum short term harvest benefits can be obtained. An inevitable consequence of this management approach is a loss of among-stock diversity due to the elimination of less-productive stocks and a continued disruption of natural adaptive processes within any one hatchery or tributary.

- **Hatchery genetics manual and guidelines**

A growing recognition of the genetic risks associated with some traditional hatchery practices led the WDF Salmon Culture Division to establish genetically-based hatchery operation guidelines in the early 1980s.

A genetics primer to be used by WDF fish culturists and biologists and to serve as a basis for the subsequent development of divisional genetic guidelines was developed by Hershberger and Iwamoto (1981). This manual was distributed to all hatcheries within the WDF system and has since served to guide hatchery operations.

The Salmon Culture Division subsequently developed draft genetics policies designed to guide fish culturists and production planners toward management decisions that retained the existing within- and among-stock diversity of salmonids under their care. These documents did not lead to the establishment of formal departmental genetics policy, however, the drafts were adopted by the division as operating guidelines and have been adhered to since their development a decade ago. In fact, although no formal policy was developed, decision makers have upheld the guidelines in the face of occasional challenges from interest groups outside the department.

- **Stock transfer guidelines**

The first guideline document was simply a list of all hatcheries operated by the Washington Department of Fisheries matched with the various transfers of species and stocks that would be allowed to or from those hatcheries. These guidelines were designed to stem any further reduction of among-stock diversity, while recognizing that the status quo fishing regimes of the time depended upon some long-established stock transfers. The Stock Transfer Guidelines were written with the following goals:

> A desire to retain the remaining genetic diversity represented in our stocks (stocks being discrete breeding units),

> A recognition that certain continued transfers of fish/eggs represent the status quo,

> An intent to establish limits to transfers within the status quo so no expansion of transfers and resulting loss of among-stock diversity would occur,

> An intent to establish a salmon culture position compatible with future additional restrictions on stock transfers to allow local adaptation and an expanded array of stocks.

Since the guidelines were first written in 1982, they have become more restrictive. Some hatcheries have, at times, been required to operate below scheduled production so that the genetic conservation intent of the guidelines was not compromised.

- **Spawning guidelines**

The second guideline document (Seidel 1983) provided protocols for brood collection and spawning that would reduce or eliminate inadvertent directed selection and assure a large effective population size, i.e., control the risk of loss of within-stock diversity. The spawning guidelines provided a synopsis of the Hershberger and Iwamoto (1981) manual and described the brood collection and mating protocols for four general cases often encountered in WDF's salmon production hatcheries. These cases ranged from severely depressed runs requiring extreme approaches such as egg banks to robust runs with escapement well above production needs.

The spawning guidelines have not been comprehensively updated since they were first written. They have, however, been reviewed both internally and by geneticists from the public sector and there have been case-by-case additions and modifications to them. Although the guidelines have been serviceable, an update and expansion is needed to make them more useful.

- **Planned genetic policies of the Washington Department of Fisheries**

Interest in genetic aspects of salmon and steelhead propagation and management has increased tremendously over the past decade (Hynes et al. 1981; Fraidenburg and Lincoln 1985; Allendorf and Ryman 1987; Waples et al. 1990; Simon 1991). Perhaps the most compelling stimulus has been provided by the listing of several Snake River salmon stocks as endangered or threatened. Restoration efforts are heavily influenced by the genetic status of these stocks and the relationships between hatchery origin and naturally spawning stock components. Interest in genetic impacts of hatchery populations on wild stocks has also been amplified by the recent shift toward hatchery supplementation of wild stocks as a restoration and augmentation measure (Ryman 1991).

The relatively recent recognition of the importance of genetics and of the genetic integrity of stocks has provided an opportunity for policy development that did not exist a decade ago when the WDF Salmon Culture spawning guidelines and stock transfer guidelines were initially drafted. Taking advantage of this momentum, several department-level policy initiatives are currently underway or on the horizon.

- **WDF genetics policy**

The department's intent is to develop genetically (and ecologically) based policy that covers the entire spectrum of resource management activities, including hatchery practices, habitat protection and rehabilitation and harvest management. Progress has been sporadic and it is expected that several years might pass before a comprehensive suite of policies is in place. Genetic aspects of overall policy will be developed by starting with the most readily accomplished tasks; in this case revising the existing Salmon Culture Division spawning guidelines and the stock transfer guidelines. The ultimate goal is to have the Department's genetics policy covering not only salmon, but also the shellfish and marine fish resources that are under our stewardship. Regional co-managers are expected to contribute to the development of the genetics policy and it is anticipated that some or all of the major policy tenets will be adopted and implemented throughout the Pacific Northwest.

- **WDF stock management policy**

The development of new policies for salmonids is not limited to genetics policy alone. The genetics policy is expected to be a component of a larger stock management policy. The Stock Management Policy will also include sub-policies covering supplementation, habitat, harvest, hatchery management and wild stock management. Substantial overlap is expected among these sub-policies. The goal of the overall stock management policy is to manage stocks as an integrated natural and hatchery resource to maintain the genetic integrity and diversity of stocks and the ecological balance of the communities in which they occur. A secondary goal is to increase fish production and harvest opportunities wherever possible.

Wild Stock Restoration Initiative and Salmon and Steelhead Stock Inventory

In recognition of the importance of the diversity of fish stocks, the Washington Department of Fisheries, the Washington Department of Wildlife and Indian tribes from throughout the state have recently inventoried all naturally spawning stocks of salmon and steelhead in the state. Additionally, the co-managers are developing a program to restore those stocks that have been determined to be at depressed or critical levels. The species included in the inventory are: chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), chum salmon (*O. keta*), pink salmon (*O. gorbuscha*), sockeye salmon (*O. nerka*) and steelhead (*O. mykiss*).

The first steps in this endeavor, identifying existing stocks and determining their status, have been completed as the Salmon and Steelhead Stock Inventory (SASSI) (Washington Department of Fisheries et al. 1993). The criterion for identifying a group of fish as a discrete stock was distinctive attributes in at least one of the following: a) location of spawning, b) timing of adult return and/or spawning, c) genetic or other biological characteristics. A total of 435 discrete stocks of salmon and steelhead spawning in Washington waters are recognized in this inventory: 108 chinook stocks, 90 coho stocks, 72 chum stocks, 15 pink stocks, 9 sockeye stocks and 141 steelhead stocks. The second part of the inventory was to determine the current status of each recognized stock as healthy, depressed or critical, based on current population sizes and/or recent trends in population size. This step led to the identification of 187 healthy stocks, 122 depressed stocks and 12 critical stocks throughout the state. The status of 121 stocks could not be determined because of inadequate data.

The participants in the salmon and steelhead inventory process recognize the inventory must be a dynamic list that is responsive to new information regarding stock distinctiveness (some stocks may be added and others deleted) and stock status (as the numbers of individuals in a stock change, the status of that stock may change). The inventory also provides a basis for prioritizing subsequent stock restoration activities.

While the salmon and steelhead inventory is an important component of the current Wild Stock Restoration Initiative, it is only the first step in the process. Three other key aspects of the initiative have been identified:

> Develop and implement specific restoration plans to improve the status of critical and depressed

stocks while maintaining the status of currently healthy stocks,

> Review existing salmon culture, habitat and harvest management policies and practices and modify these or create new ones, as necessary, to achieve the goals of the initiative,

> Design and conduct comprehensive monitoring and evaluation programs that will assess the effectiveness of the restoration efforts to maintain the health and diversity of salmon resources in the state.

Table 1 lists the twelve critical stocks identified in the 1992 inventory process by region. Figure 1 indicates the basis for each determination and summarizes the restoration activities currently underway for each stock.

Genetic Risk Assessment and Management

In the last five years, the WDF Genetics Unit has become increasingly involved in the area of genetic risk management. Most of the involvement has been planning and research for genetic conservation in the Yakima/Klickitat Fishery Project (YKFP). Through our experience with the YKFP, a general conceptual framework was devel-

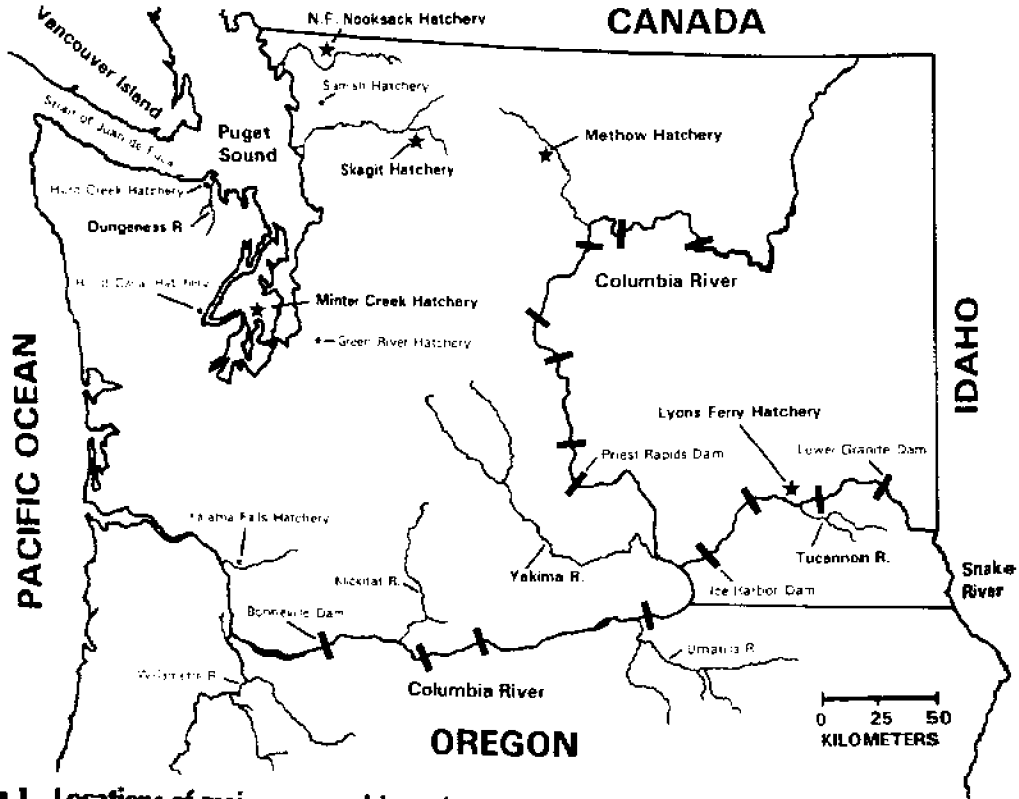

Figure 1. Locations of major geographic regions and selected river systems, hatcheries and dams in Washington State.

Table 1. Summary of the 12 critical Washington stocks identified in the 1993 Salmon and Steelhead Stock Inventory.

Region & Stock	Basis for Status Determination	Current Restoration Activities
Puget Sound		
NF Nooksack spring chinook	chronically low population size	WDF hatchery
SF Nooksack spring chinook	chronically low population size	tribal hatchery
White River spring chinook	chronically low population size	WDF captive broodstock program & tribal hatchery
Baker Lake sockeye	chronically low population size	WDF mitigation program
Deer Creek summer steelhead	long term negative trend in numbers	none
Hood Canal		
Hood Canal summer chum	chronically low population size	cooperative hatchery program
Strait of Juan de Fuca		
Dungeness chinook	chronically low population size	cooperative captive broodstock program
Discovery Bay coho	short term severe decline	none
Discovery Bay chum	short term severe decline	none
lower Dungeness pink	chronically low population size	none
Elwha pink	chronically low population size	none
Columbia River		
Asotin Creek spring chinook	chronically low population size	none

oped for managing genetic risk in hatchery projects (Fig. 2).

- **Planning**

Planning for a hatchery project of any sort should begin with an inventory of what genetic resources are present where the project will take place. The minimum required is a determination of how many stocks are present. This inventory can be accomplished by conducting an electrophoretic survey, but ideally it should include demographic and life history data as well. From this initial description of genetic resources, proposed management actions can be developed. These actions should be based in part on genetic operating guidelines, which minimally will include genetic hatchery guidelines, as well as genetic considerations of harvest rates and habitat improvements. The genetic hatchery guidelines should include measures to reduce genetic risk throughout the freshwater life history of the fish, from the time of broodstock collection until smolt release. The harvest and habitat guidelines should include measures to avoid genetically selective fisheries and habitat conditions that alter selective pressures on specific life history stages or traits.

Once management actions have been proposed, an initial qualitative genetic risk assessment can be used to evaluate their poten-

tial genetic consequences. Busack (1990) grouped genetic risks into four categories: 1) extinction; 2) loss of within-population variability (typically caused by low effective number); 3) loss of among-population variability (caused by interbreeding of distinct stocks); and 4) domestication selection (loss of fitness in the wild due to the different selection pressures imposed by the hatchery environment). Although there are many cases in which a particular genetic problem may be hard to classify, this categorization has received wide acceptance because it matches actions with their likely genetic impacts.

Although risk categorization has helped clarify discussion of risk, quantification of risk on anything more than a relative scale remains elusive. There is a serious gap between theory and practice with regard to the ability to predict impacts quantitatively. For example, decreasing a population from 500 effective breeders to 400 will reduce the amount of additive genetic variation the population can maintain (Lande and Barrowclough 1987); but the tangible consequences of this in terms of short term (and long term) stock performance are unknown.

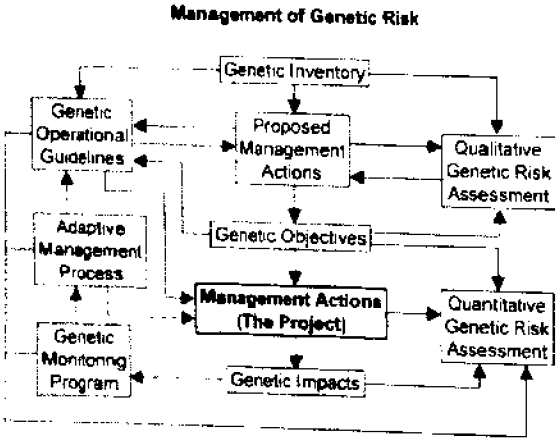

Figure 2. Genetic risk assessment and management model developed for use in Yakima/Klickitat Fisheries Project. Primary linkages in the feedback loop are indicated with dashed arrows.

The initial qualitative risk assessment will likely result in some refinement of the proposed actions in order to reduce risk. Once the proposed actions are refined, genetic objectives should be developed. This is an important step and probably the most problematic element of the entire framework. It is here that theoretical generalities about genetic risk need to be translated into measurable responses that can be detected by a monitoring program. Although the general intent may be to minimize risk, this intent must be translated into quantified, detectable, acceptable impact levels. Ideally, genetic objectives will be set for each type of risk for all stocks affected directly or indirectly by the management actions.

All activities mentioned so far can be considered part of the planning phase of project management, but there may be no clear endpoint to them. A genetic inventory may end up being more complex than expected. An unexpected stock may be found, or more detail on recognized stocks may be desired, both of which will prolong the research. As new information from the genetic inventory becomes available, genetic risk assessment needs to be updated, which may cause management actions to be further refined, and so on. Genetic risk assessment needs to be viewed as an ongoing audit of management actions. At some point, a more detailed and quantitative risk assessment needs to be developed. Whereas the qualitative assessment may have dealt with many aspects of risk in general terms, the quantitative document needs to critique the project much more carefully.

• **Implementation**

Once the project is underway, management actions will be guided by the genetic operating guidelines, genetic objectives, ongoing risk assessment and a feedback loop (dashed arrows in Fig. 2) comparing responses with acceptable genetic impacts. Without this feedback loop, there is no real management of genetic risk. The loop has two components. The first is the genetic monitoring program, which must be capable of determining with acceptable experimental power what level of genetic impact has occurred. Time frame is a vital concern here. A response may be readily measurable, but inherent variability in estimating the response may make the results unusable until several years of data are available. The second component of the loop is the process by which project managers assimilate the information from the monitoring program and take the indicated corrective action. Without a commitment by managers to such "adaptive" management, monitoring becomes just an information gathering exercise and risk is not managed.

Although developing and diagramming a conceptual framework is relatively straightforward, the real management of genetic risk is not. First, although only a few feedback connections are shown in Figure 2, in actuality, almost every component in the process feeds back into everything else. Operating guidelines, for example, may have to be designed with particular project constraints and management intents in mind. A second, and more serious problem, already mentioned, is lack of knowledge relating perceived risk to actual impact. Although not explicitly included in the risk-management diagram, research on genetic impacts, especially in the areas of outbreeding depression and domestication selection is badly needed for accurate quantification and management of genetic risk.

• **Yakima/Klickitat Fisheries Project**

This risk-management model is a product of the Washington Department of Fisheries Genetics Unit's lead role in genetic conservation planning for the Yakima/Klickitat Fishery Project (YKFP). The project is a cooperative effort involving the Washington Department of Fisheries, Washington Department of Wildlife and the Yakima Indian Nation. It is an attempt to increase salmon and steelhead production through hatchery based supplementation in two large river basins in the state (Fig. 1), while at the same time preserving the genetic integrity of the salmonid stocks present. Unlike traditional hatchery methods that are aimed simply at producing more harvestable fish, the intent of supplementation is to maintain or increase natural production. It is hoped that supplementation will play a large role in increasing salmonid production throughout the Columbia basin, but at this point it is unproven and largely untested.

Genetic concerns play an unprecedented role in YKFP. No other regional hatchery project, currently operational or planned, has dealt with genetics in such detail. The project includes several measures for reducing genetic risk including inventory of genetic resources, formal genetic risk assessment, creation and implementation of both genetic hatchery guidelines and a genetic monitoring plan and a commitment to adaptive management. The WDF Genetics Unit is responsible for all genetic inventory work, and is collaborating with a team of academic geneticists to develop draft risk assessment, monitoring, and hatchery guidelines.

Salmon culturists have had input to this process and will be involved in the review of the draft guidelines to insure that the final guidelines have broad regional applicability. Considerable progress has been made in the following areas:

> Genetic Inventory: Since 1989, spring chinook, fall chinook and summer steelhead populations in the Yakima Basin have been analyzed using protein electrophoresis. The goal of this activity was twofold: a) determine how many distinct stocks of each exist; and b) characterize each stock for future genetic monitoring in terms of allele frequency profiles, and to the extent possible, effective population sizes,

> Genetic Risk Assessment: An initial genetic risk assessment for the project was produced in 1990 (Busack 1990). It was the first document of its kind in the region. The risk assessment was basically an introduction to genetic risk and a brief qualitative description of the risks posed by hatchery operations in general and by specific elements of the YKFP. The major contribution of the document to regional genetic conservation efforts was its definition and description of four types of genetic risk. A draft version of a quantitative assessment (Currens 1993) was completed in March 1993. The new document links genetic risk assessment to other types of biological risk assessment, clarifies terminology and is a vast improvement in quantification of risk,

> Genetic Hatchery Guidelines: A draft version (Kapuscinski and Miller 1993) was completed in March 1993. This is a very comprehensive document, dealing with all aspects of hatchery operations from broodstock collection to release of juveniles,

> Genetic Monitoring Plan: A draft version is due to be completed in 1993. The intent is to have this document be a monitoring manual —a comprehensive treatment of the methods available to detect genetic impacts. Statistical power of the various methods will be determined and described in detail, as this is a major issue in any kind of monitoring.

Genetically-Based Fisheries Management

• Mixed-stock fisheries

Nearly all fisheries for Pacific salmon harvest mixtures of stocks. This is a consequence of the highly subdivided population structure of these species, their anadromous life history and the location of fisheries in non-terminal areas where stocks intermingle. Fisheries at or near the spawning grounds are economically undesirable due to the progressive decline in flesh quality as the fish approach spawning and are difficult to institute because of the highly exploited nature of the resource and the resulting competition for harvest opportunities.

The management of mixed-stock fisheries presents significant challenges to conserving these species. An individual fishery usually impacts a dynamic mixture of stocks, each with its own inherent productivity. The situ-

ation is made more difficult by the presence of numerous hatchery stocks. Because hatchery stocks can generally support higher harvest levels than their wild counterparts, there is strong economic pressure to exploit them at a maximum rate. Indeed, the demand for harvest opportunities is often in direct conflict with the stated intent of WDF harvest management policy to manage fisheries in such a way that weak stocks (i.e., those that cannot withstand high harvest rates) are protected from overharvest.

Identifying the different stocks and stock groups that are harvested in mixed-stock fisheries and estimating their contributions to catches are critical needs for optimizing these fisheries. This is to ensure their long term stability and the survival of the component stocks that support them. While there are many approaches to identifying the stocks in such mixtures, one of the most powerful and cost effective methods is genetic stock identification (GSI) (e.g., Beacham et al. 1985a, b; Milner et al. 1985; Utter et al. 1987; Shaklee et al. 1990b; Utter and Ryman 1993). This approach utilizes the naturally occurring genetic differences (detectable using electrophoresis) that distinguish many stocks, and sophisticated statistical procedures (Fournier et al. 1984; Millar 1987) to estimate stock and stock group contributions based on the analysis of one or more samples from a fishery.

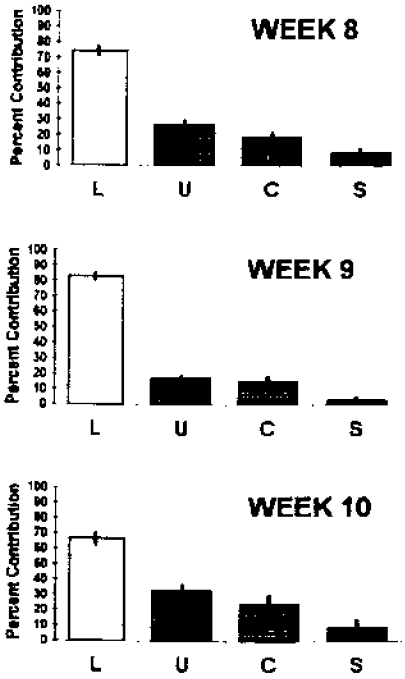

Figure 3a. Stock group contribution estimates (± 1 s.d.) for the three successive weeks of 1987 fishery.

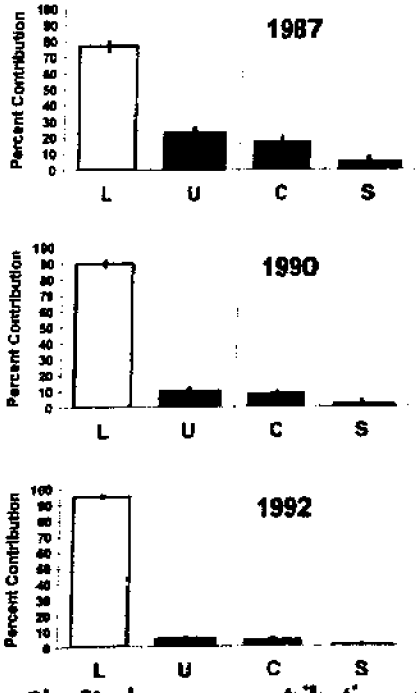

Figure 3b. Stock group contribution estimates (± 1 s.d.) for the total fishery harvest in 1987, 1990 and 1992.

L = lower river ; U = upper river;
C = mid-Columbia River; S = Snake River

- **Columbia River spring chinook gill-net fishery**

An example of the power of genetic stock identification is provided by the management of the lower Columbia River winter gill-net fishery for spring-run chinook salmon. This fishery targets the abundant hatchery stocks from the Willamette River and other lower Columbia River tributaries, but is constrained by both management intent and federal court mandate from harvesting excessive numbers of fish from both mid-Columbia and Snake River stocks (Fig. 1). The recent U.S. government listing of Snake River spring- and summer-run chinook salmon as a threatened species under the Endangered Species Act has further constrained this fishery.

The fishery was managed for many years using coded-wire tag (CWT) recoveries. However, the expense of tagging adequate numbers of hatchery fish to provide enough tagged fish in samples from the fishery and the difficulty and expense of tagging wild stocks led to the application of GSI techniques in the late 1980s. For the past several years, decisions about whether to extend or terminate the fishery after the first week of fishing have been based solely on in-season GSI estimates of upper river (mid-Columbia and Snake rivers) stock contribution. Figure 3 illustrates estimated stock-group contributions to this fishery in three different time periods in one year (Fig. 3a) and in three different years (Fig. 3b). The substantial intra- and inter-annual variation of stock group contributions to this fishery makes annual in-season monitoring a necessity. Additionally, because the GSI technique has reasonable power to estimate the presence of Snake River spring chinook stocks (Shaklee 1991), the technique provides information for evaluating harvest impacts on this ESA-protected stock. Without this information, continuation of the entire fishery could be in jeopardy. The relatively small cost of laboratory processing (approximately $14.00/fish) and high precision of the stock-group contribution estimates makes GSI a cost-effective management tool for this fishery.

Similar in-season GSI fishery estimates are conducted to optimize the management of chum salmon fisheries in Puget Sound (Baker and Bishop 1993) and to manage fisheries in British Columbia for odd-year pink salmon (PSC 1990).

- **Selective fisheries and mass marking**

Most innovative methods used to manage mixed-stock fisheries are intended to protect weak stocks from over-exploitation by limiting harvest rate to that appropriate for the weakest stock in the co-mingled aggregation. However, it is not possible to utilize hatchery production fully using existing stock ID procedures such as GSI because the hatchery or natural origin of individual fish cannot be determined at the time and location of capture. Thus, despite weak stock management intent, natural stock production goals are often not achieved. Without changes to management, it is likely there will be a continued deterioration of both mixed-stock fisheries and resource status.

It would be highly desirable if the intended harvest of robust hatchery stocks could be separated from the inadvertent harvest impacts on weaker naturally spawning (and hatchery) stocks. Mass marking of hatchery stocks shows some promise for achieving this goal. Application of visible marks to all fish produced in hatcheries (mass-marking) and selective fisheries could be an effective

instrument for achieving increased protection of weak wild and hatchery stocks by reducing their harvest in mixed-stock fisheries.

Managers must also consider the genetic implications of the increased exploitation of target hatchery stocks. The benefits gained through improved harvest access must be viewed in the context of long term genetic stability of the hatchery-based resource component. For example, it has been postulated that mean size of chinook and coho stocks harvested in British Columbia fisheries has been reduced due to the selective pressures applied by the commercial fisheries (Ricker 1980; Ricker and Wickett 1980). Additionally, the negative effects of hooking and handling mortality on the fish from the protected, unmarked stocks that would be released in such selective fisheries must also be considered.

Mass marking proposals are being actively pursued in Washington as well as Idaho, Oregon and British Columbia. Many of the proposals are aimed toward avoiding the fishery constraints posed by weak stock management, but the tool can be viewed just as easily as a potential way to reduce overall exploitation of natural stocks in order to meet escapement goals consistently and increase production, thereby reducing the risk of extinction or loss of genetic diversity.

Hatchery Operations — Selected Case Histories

Although many of WDF's fisheries policies are specifically designed to use cultured hatchery stocks to augment harvest opportunities, several of the department's newer hatchery programs are intended to enhance or supplement local native stocks. In the latter context, the hatchery operations should be designed and carried out in such a manner as to have minimal or no impact on the original genetic and biological character of the stock being cultured. Genetic evaluations are being conducted to determine whether or not the Department's hatchery operations are achieving this goal. Four specific examples of recent or ongoing studies illustrate how this is being done and what the results have been.

- **Skagit River summer chinook salmon**

The Skagit River is a major river system in northern Puget Sound (Fig. 1) that supports spring-, summer- and fall-run chinook stocks. The WDF Skagit Hatchery has, for many years, cultured all three races of chinook. The fall stock cultured at the hatchery actually originated in the Green River (in central Puget Sound) and is intended to augment fishery harvests. The spring stock in the hatchery was derived from chinook spawning in tributaries to the Skagit River and is, therefore, presumed to represent the native stock. The primary purpose of the hatchery was to increase the production of spring-run fish in the region. The summer chinook program at the hatchery was initiated to augment the numbers of fish produced naturally by the healthy wild stock in the upper river. Because the fish in this stock return as adults at a large size and in prime condition, they are highly regarded by both commercial and sport fishers. This was a major factor contributing to the initiation of the summer chinook program at the hatchery.

An ambitious and labor-intensive broodstock collecting program was conducted by WDF staff from 1975 through 1979 to obtain enough spawners to establish a hatchery

Table 2. Allele frequencies at ten informative loci in three chinook stocks propagated at the WDF Skagit River Hatchery. (year collected; N = number of fish collected). Locus and allele designations follow Shaklee et al. 1990a.

Locus & Allele	Upper Skagit River Wild Summer Chinook (1986; N =100)	Skagit Hatchery "Summer" Chinook (1986; N =102)	Skagit Hatchery Fall Chinook (1987; N =107)
sAAT-3			
*100	0.935	0.995	1.000
*90	0.065	0.005	0.000
sAAT-4			
*100	0.935	0.990	0.986
*63	0.065	0.010	0.005
*130	0.000	0.000	0.009
ADA-1			
*100	0.910	0.951	0.958
*83	0.090	0.049	0.042
sAH			
*100	0.825	0.911	0.906
*86	0.160	0.089	0.094
*116	0.015	0.000	0.000
sMEP-1			
*100	0.600	0.384	0.420
*92	0.380	0.611	0.547
*105	0.020	0.005	0.033
PGK-2			
*100	0.470	0.765	0.668
*90	0.530	0.235	0.332
PEPA			
*100	0.965	0.907	0.892
*90	0.035	0.093	0.108
PEPB-1			
*100	0.660	0.613	0.537
*130	0.340	0.387	0.463
sSOD-1			
*-100	0.730	0.623	0.570
*-260	0.255	0.328	0.388
*580	0.015	0.049	0.042
TPI-4			
*100	0.965	0.995	0.995
*104	0.035	0.005	0.000
*101	0.000	0.000	0.005

stock that would have the same (or very similar) genetic characteristics as the wild summer-run stock. The actual numbers of fish used to establish the hatchery stock totaled approximately 560 fish (ranging from 82 fish in 1975 to 142 in 1977). Since 1980, the hatchery has produced and reared large numbers of this presumed summer chinook stock. Indeed, the hatchery program was so successful that this hatchery stock was chosen as a CWT indicator stock to be used to represent the performance of wild summer chinook from northern Puget Sound in the Pacific Salmon Treaty process.

A genetic evaluation of this program was initiated in 1986 by comparing the characteristics of the summer chinook stock cultured in the hatchery, the wild upper Skagit River summer chinook stock and the fall chinook stock cultured in the hatchery. Horizontal starch-gel electrophoresis (Shaklee and Keenan 1986; Aebersold et al. 1987) was used to screen samples from each of the three groups for approximately 40 variable gene loci. The allele frequencies at several informative loci in each of these three groups of fish are shown in Table 2. Surprisingly, this analysis clearly indicated that the "summer" stock being cultured in the hatchery was significantly different ($p \leq 0.001$) from its source (the wild summer stock in the upper Skagit River). Furthermore, the hatchery "summer" stock was similar to the introduced fall stock reared at the hatchery. This analysis was repeated two years later with a second collection of the "summer" stock at the hatchery with basically the same result.

Subsequent examination of hatchery spawning records suggested that there was likely substantial (albeit unintentional) interbreeding between the fall hatchery stock and the wild summer fish brought into the hatchery to be the source for establishing a summer stock program. Because this direct genetic evaluation of the Skagit Hatchery summer chinook program showed it was not achieving its goal of propagating pure summer chinook, the hatchery stock was dropped as a CWT indicator stock for northern Puget Sound summer chinook in 1987 and the entire hatchery program for summer chinook at this hatchery is being phased out.

- **Snake River fall chinook salmon**

Legislation was passed in the mid-1970s for hatchery mitigation to compensate for fall chinook losses caused by four dams on the lower Snake River in Washington. A hatchery site was chosen at Lyons Ferry, above the lower two dams on the Snake River (Fig. 1). The fall chinook run was so low at that time, however, that a temporary hatchery operation, called an egg-bank program, was begun while the new hatchery was being built. Adult fall chinook were trapped in the Snake River, but to avoid dam passage mortalities, their progeny were reared and released at a downriver location.

The program began in 1976 and from 1977 onward adults were trapped at Ice Harbor Dam and spawned. The resulting juveniles were all marked (by fin-clipping) and were released from WDF's Kalama Falls Hatchery, which is on a Columbia River tributary below all mainstem dams (Fig. 1). Egg-bank fish began returning to Kalama Falls in 1980, and were used as broodstock along with fish trapped at Ice Harbor. A similar but much smaller operation was conducted by the U.S. Fish and Wildlife Service at two federal hatcheries in Idaho. The egg-bank program ended as the Lyons Ferry Hatchery became operational. Adults from Ice Harbor were

spawned at Lyons Ferry in 1984, and releases there began in 1985. Adults began returning to the Lyons Ferry Hatchery in 1987. Until 1990, when operations at the hatchery were changed in response to concerns raised by the petitioning and subsequent listing of Snake River fall chinook as threatened under the ESA, the Lyons Ferry broodstock consisted of adults trapped at Ice Harbor Dam and volunteers entering the hatchery itself.

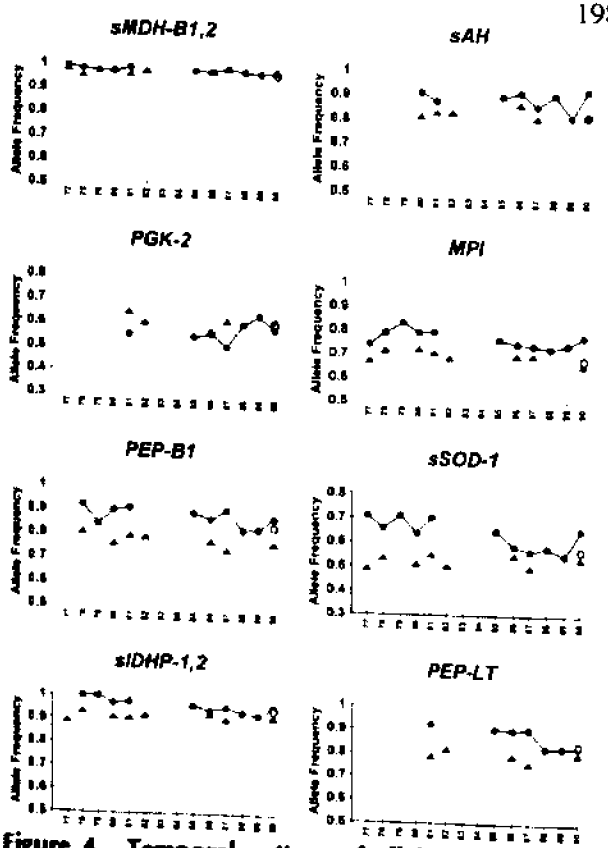

Figure 4. Temporal pattern of allele frequencies (*100) in mid-Columbia and Snake River fall chinook. Solid circles = Ice Harbor/Lyons Ferry Hatchery: pure Lyons Ferry stock. Open circle = "mixed" collection of untagged fish that returned to the Lyons Ferry Hatchery in 1990 and contained an unknown number of Umatilla Hatchery strays. Solid triangles = mid-Columbia River (Hanford Reach and Priest Rapids Hatchery stock). Collection sizes ranged from 65 to 300.

Understandably, concerns were raised about the genetic impact of the egg bank program; specifically the effect of temporarily transplanting fish several hundred miles downriver. What effects the egg-bank program had on quantitative genetic variation in the stock will never be known, but a comparison using allele frequencies at thirty loci was made between adults returning to Kalama Falls (N = 100) in 1986 and fish collected at Ice Harbor and Lyons Ferry (N = 100) in 1986. Significant differences ($p \leq 0.05$ by G-test) were found at only two loci; overall the collections were not significantly different ($p \leq 0.3$) (Seidel et al. 1988).

The intent of the Lyons Ferry Hatchery program has always been to culture the native Snake River fall chinook. However, genetic purity of the Lyons Ferry stock has been a central issue for several years, and there have been two concerns. The first was the questionable wisdom of collecting broodstock for the hatchery program at Ice Harbor Dam. Although the dam is far downstream of the remaining natural spawning grounds in the upper Snake River, fish were collected at this location because this site allowed access to more fish than any more upstream alternative due to dam passage mortalities. Nevertheless, because the dam is only about 16 kilometers above the Columbia River, there was also a possibility of trapping mid-Columbia "dip-ins" — Columbia River origin fish that had entered the Snake River, but actually would have dropped back to the Columbia River and spawned there. The second concern focused on strays in the broodstock. A low frequency of CTW strays from other hatch-

eries had always been noted at the hatchery. However, in 1989, strays from a single hatchery operation on the nearby Umatilla River in Oregon accounted for an estimated 30% of the Lyons Ferry broodstock. Native fall chinook had been extirpated from the Umatilla long ago; consequently, the stock used in this hatchery was derived from fish collected at Bonneville and Priest Rapids dams.

Unfortunately, there are no existing electrophoretic data characterizing unequivocally pure Snake River fall chinook. There are, however, electrophoretic data collected by NMFS and WDF dating back to 1977 from Ice Harbor Dam, Lyons Ferry Hatchery and the mid-Columbia. Figure 4 summarizes much of these data to display temporal trends in allele frequencies at eight genetic systems in Ice Harbor/Lyons Ferry fish and the mid-Columbia fish (including the Umatilla Hatchery fish). In years for which data from multiple collections are available, composite allele frequencies were calculated as the mean of the individual collection frequencies, with one exception: in 1990, two collections of samples were taken at Lyons Ferry, one of "known" Lyons Ferry stock (CWT-tagged fish) and one of untagged fish. Data from the untagged fish are plotted separately in Figure 4. Note that locus and allele designations throughout this report follow Shaklee et al. (1990a). Frequencies at *sMDH-B1,2** (and at *LDH-B2**; *LDH-C**; and *PEPA** — data not shown) are too similar between the two stocks to be informative. Although frequencies at *sAH** and *PGK-2** are too erratic to indicate trends, it is clear that the untagged 1990 Lyons Ferry fish are more similar to mid-Columbia fish at *sAH** than the tagged fish are. The two series are nearly parallel for *MPI** frequencies, except that the 1990 untagged Lyons Ferry collection is again more similar to the mid-Columbia collections than to the true Lyons Ferry collections. The series of *PEPB-1** frequencies appear approximately parallel, and in both the frequency of the **100* allele appears to be declining slightly. Lyons Ferry frequencies at *sSOD-1**, *sIDHP-1,2** and *PEP-LT** exhibit definite trends in the direction of the mid-Columbia series, which for each locus remains relatively stable. The frequency of the **100* allele at *sSOD-1** in the 1990 untagged Lyons Ferry collection is more similar to the 1990 mid-Columbia collection, than it is to the 1990 tagged Lyons Ferry collection.

Two time periods are of interest in examining these trends, before Umatilla straying (before 1984) and after. Four systems provide some insight into the question of whether the increasing similarity between Snake River and mid-Columbia stocks was coincident with the egg-bank program and collection of broodstock at Ice Harbor Dam or the Umatilla Hatchery straying. Three of these (*MPI**, *PEPB-1** and *sSOD-1**) exhibit little convergence in allele frequency prior to 1984. The isolocus pair *sIDHP-1,2** shows a slight convergence in 1980 and 1981. The existing data are too limited to draw strong inferences, but they provide little evidence that significant directional changes in allele frequency of the Lyons Ferry stock occurred before the time when substantial Umatilla Hatchery straying was first noted.

Although a genetic impact from mid-Columbia fish through straying and possible dip-in capture is evident, the genetic distinction between Snake and mid-Columbia fall chinook remains. A comparison of allele frequencies for the 1990 tagged Lyons Ferry and the 1990 mid-Columbia (Priest Rapids

Dam) collections by chi-square heterogeneity test is highly significant ($p \leq 0.00002$). A similar comparison of the 1990 tagged Lyons Ferry collection and the 1986 Lyons Ferry collection is not significant ($p \leq 0.71$), indicating the effect of heavy Umatilla straying in recent years could be diminished by restricting the broodstock to known Lyons Ferry fish.

In response to the high stray rate in 1989, WDF instituted two important hatchery management changes. One was to tag 100% of the 1989 Lyons Ferry brood (with CWTs) in order to exclude all of these fish from subsequent broods at the hatchery. The second was to modify broodstock collection operations for the Lyons Ferry Hatchery for 1990 and beyond. The normal broodstock collection procedure at Ice Harbor Dam and Lyons Ferry Hatchery was continued, but these fish were augmented by trapping approximately 50% of the tagged adults that reached Lower Granite Dam, the uppermost of the four dams on the Snake River and the last before the spawning grounds (Fig. 1). In addition to providing fish, this allowed monitoring of the adult run composition at the dam for the first time. The second change in management was a screening of spawners for stock origin. All CWTs were read as the fish were spawned, so that the fish could be spawned in three groups: only Lyons Ferry tagged fish, only foreign tagged fish, and all untagged fish. Progeny of untagged adults were to be used in the Lyons Ferry program only if the stray rate was deemed to be below an acceptable level. In 1990, stray levels were appreciable, so only the progeny of the Lyons Ferry tagged adults were retained for program use. Current broodstock procedures for Lyons Ferry involve use of only tagged Lyons Ferry fish in order to exclude fish of unknown origin from the gene pool. To sustain this program, 100% of the Lyons Ferry releases are now tagged. While the flow of foreign genes into the hatchery stock has been stopped by this tagging and tag reading effort, untagged strays cannot be prevented from continuing upriver to the natural spawning grounds. The Lyons Ferry Hatchery stock may well turn out to be a better representation of the original Snake River fall chinook than the natural spawners now protected under the ESA. In this regard, the Lyons Ferry Hatchery fish have recently been determined by NMFS to be part of the Snake River fall chinook "ESU."

- **Minter Creek chum salmon**

Despite a stated intent to manage chum salmon fisheries in south Puget Sound on the basis of local wild stocks, chum production at the Minter Creek Hatchery (Fig. 1) has utilized a stock that was originally derived from the Hood Canal Hatchery. In 1986, the stock being propagated at the Minter Creek Hatchery was electrophoretically characterized and determined to have a genetic profile that was basically identical to that of the Hood Canal stock. Because GSI is the primary method for estimating stock contributions to chum salmon fisheries in Puget Sound, the high degree of genetic similarity between the fish produced at the Minter Creek facility and the large numbers of chum produced at the state, tribal and federal hatcheries in Hood Canal made it impossible to estimate contributions from all south Puget Sound sources (including Minter Creek) accurately. This, in turn, made effective harvest management of south Puget Sound chum extremely difficult. Furthermore, the propagation of Hood Canal type fish in south Puget Sound and their possible straying from the facility to spawn naturally

in adjacent south Puget Sound tributaries and the use of these fish for volunteer enhancement projects in south Puget Sound clearly violated the department's goal of maintaining among-stock genetic variability.

For the reasons outlined above, the Department of Fisheries and tribal co-managers implemented a program to replace the Hood Canal original chum stock at the Minter Creek Hatchery with a native south Puget Sound stock (Elson Creek). Each year, beginning in 1988, adults returning to the Minter Creek Hatchery (presumably from the Hood Canal type stock) were removed from the system by a wipe-out fishery outside the mouth of Minter Creek and their production was replaced at the hatchery with fertilized eggs from the Elson Creek stock obtained from the Squaxin tribal facility. The eggs from Elson were taken from females throughout the run and a total of approximately 1300 females and 1300 males were spawned to provide fertilized eggs for the Minter Creek Hatchery. These practices were followed in order to meet the production goals for Minter Creek and to assure that the recipient hatchery stock (Minter Creek) would be genetically representative of the donor stock (Elson). The program proceeded in this manner for four years — the average duration of the life cycle of the Hood Canal Hatchery stock. In the fifth year (1992), because only the small fraction of fish returning as five-year-olds were of the Hood Canal Hatchery type, we simply had to identify these fish (by scale reading at the hatchery) and remove them prior to spawning to achieve complete removal of all Hood Canal Hatchery type chum from the facility. Beginning in 1993, all broods at the Minter Creek Hatchery should be the native south Puget Sound (Elson) type.

This conversion of hatchery production to a native south Puget Sound stock would not have been successful without tribal participation to furnish the fertilized eggs necessary to replace production losses resulting from elimination of the Hood Canal origin spawners. Joint state/tribal alterations in harvest management regimes were also necessary to support the program. WDF salmon culture and research staff were responsible for accomplishing the conversion at the hatchery and for identifying the nature and extent of the problem and contributing to its solution by aging the large fish returning in the final year of conversion to allow identification and removal of five-year-olds.

The situation at a small volunteer chum salmon enhancement program at Donkey Creek (also in south Puget Sound) run by the Gig Harbor Fisherman's Civic Club, closely paralleled the Minter Creek Hatchery program. The Donkey Creek program was initiated 20 years ago using fertilized eggs (Hood Canal chum stock) originally obtained from the Minter Creek facility. Although this program had been successful in establishing a run of chum back to Donkey Creek, it was inconsistent with the department's intent to use local stocks because it was founded using a foreign gene pool. Using a similar approach to that employed at the Minter Creek Hatchery, the department and the co-op replaced the potential production from all adults returning to the Donkey Creek program over the course of the last five years with fertilized eggs from the Elson stock. This remedial action should have successfully replaced the Hood Canal origin population in Donkey Creek with the more appropriate Elson stock.

- **Dungeness River chinook captive broodstock program**

The Dungeness River once supported a large, productive chinook salmon population, but in recent years the number of adults returning to spawn has decreased to approximately 200 fish per year (C. Smith, WDF; unpublished data). The depressed run size of this population threatens its long term survival due to the increased risk of a genetic bottleneck or extinction from an environmental catastrophe. In response to the critical status of Dungeness chinook, state, tribal and federal fisheries biologists and concerned citizens have joined forces in a restoration effort. The long term goal of this recovery program is to increase the number of naturally spawning fish in the river while maintaining the genetic characteristics (diversity, pattern and amount of variation) of the existing stock. However, all parties involved agreed that the critically low numbers of returning adults place this stock in such jeopardy that priority be given to increasing population size as quickly as possible.

After considering several approaches for achieving an immediate increase in fish numbers, the group decided to implement a captive broodstock program. This relatively new approach — rearing normally anadromous salmon in captivity throughout their entire life cycle — has the potential to increase population numbers dramatically in a single generation because of the high fecundity of the species (approximately 3,500 eggs per female) and the low mortalities expected from hatchery propagation. Because the design of this program was driven by concern to minimize inbreeding and genetic drift (maintain the amount and pattern of genetic variation characteristic of the natural population), an effective number of breeders (N_b) goal of 50 per year in each of four successive years was established, for an N_e of approximately 200 over the average four-year generation time of this stock.

Two concerns made achieving this goal via the traditional capture of pre-spawning adults seem unrealistic or undesirable. First, it was doubtful that the 25 pairs of adults needed for hatchery spawning to achieve the N_b goal could be obtained because of the small size, low density and protracted freshwater maturation schedule of this population. Second, there was a strong desire to retain a high level of natural production in the Dungeness River during the captive broodstock program. Removing 50 adults from the river for the captive broodstock would have removed approximately one-quarter of the natural production from the system, and would have yielded far more eggs than necessary for the program.

Because of these concerns, a novel experimental approach was devised for establishing the captive broodstock program based on the collection of fry rather than adults. The two-component approach involves the collection of pre-emergent fry by hydraulic sampling of redds (salmon nests in stream bed gravel) and the capture of post-emergent fry by electroshocking or seining in the river. The intent is to collect approximately 200 fry from each of 25 redds and up to 2500 fry from throughout the river. Redd sampling will yield representatives of a known number of essentially discrete family groups, yet will remove less than 10% of the production of each family from the river. Electroshocking/seining has the potential of capturing representatives from all families produced in the system, while also having only a small impact on the total production. These two approaches together should provide adequate

genetic representation of the natural population and yield enough fish to establish the captive broodstock program. The fish in each of the above lots will be tagged in such a way that specific crosses of individuals of known origin can be made when the fish mature.

The Dungeness chinook captive broodstock program was initiated in 1993. Fourteen family groups (N = 2,600 fish) were successfully sampled from redds and approximately 1300 post-emergent fry were obtained by electroshocking. All fish in the broodstock are presently being reared at the WDF Hurd Creek Hatchery in the lower Dungeness watershed.

Another notable feature of this captive broodstock program is that one-half of the fish will be reared to maturity in freshwater tanks (at the hatchery) and one-half in saltwater net pens (in the Strait of Juan de Fuca). This approach will allow evaluation of the relative merits of freshwater and marine captive rearing and will also minimize the risk of catastrophic failure of the program due to having all broodstock in a single facility.

The current plan calls for this captive broodstock program to be conducted for an eight-year period and monitoring and evaluation to continue for an additional four years. This will represent two generations of chinook production, will provide enough tagged fish to assess fishery impacts on the stock and should allow enough time for the limiting factor(s) responsible for the depressed status of this stock to be identified and corrective measures to be initiated. Restricting the program to two generations should also limit inadvertent domestication selection on the stock. While the captive broodstock program is expected to dramatically increase fish numbers in the short term, the long term success of Dungeness chinook restoration is entirely dependent on identifying and overcoming habitat and/or harvest management impacts that have driven the stock to its current critical state.

Hatchery Monitoring, Evaluation and Research Programs

- **Tucannon River spring chinook monitoring and evaluation**

The Tucannon River, in southeastern Washington, is a tributary of the lower Snake River. As part of the Lower Snake River Fish and Wildlife Compensation Plan, the wild spring chinook stock in this river was targeted for hatchery supplementation to provide compensation for salmon production lost because of hydroelectric development in the Snake River Basin. Because the Tucannon River spring chinook population represented one of the last wild chinook stocks in Washington with no history of significant exposure to hatchery origin fish, WDF recognized this as a unique opportunity to monitor and evaluate the effects of a new hatchery on the genetic and biological characteristics of the native stock. The Tucannon Hatchery operation was designed to incorporate the genetic principles and concerns recognized in the mid-1980s. Hatchery operations commenced in 1986, with an initial year's spawning of 48 females and 43 males, and continue (with similar numbers) to the present.

While the hatchery operations were beginning, genetic characterization of the native, pre-facility spring chinook population was initiated. In 1985, 100 outmigrating smolts from the 1983 brood were sampled from the river. In subsequent years, samples of naturally produced fish were obtained, both from

returning adults and from pre-smolts and smolts from the river. Each of these collections was electrophoretically analyzed at approximately 35 variable loci to provide a genetic characterization of the native Tucannon spring chinook stock.

Beginning in 1990, offspring from the hatchery program began returning to the system in significant numbers. Because all individuals produced in the hatchery were marked with CWTs, it was possible to separate the returning adults into either hatchery-produced or wild-origin fish. Electrophoretic analysis of returning adults and of pre-smolt/smolt collections each year has allowed monitoring of the genetic characteristics of the stock and comparison of the genetic profiles of the stock before and after artificial production was initiated.

Figure 5 shows examples of the temporal patterns in allele frequency seen at four loci in this stock. Two important patterns were evident from this analysis. First, there was significant year-to-year variation in allele frequencies at many loci. Indeed, this variation was sufficient to make overall G-tests among pairs of annual collections statistically significant in many cases. The magnitude of the inter-annual variation in allele frequencies may be a consequence of the small effective population size of this stock. Second, despite the significant annual variability, there does not seem to be any clear directional shift in allele frequency, either at

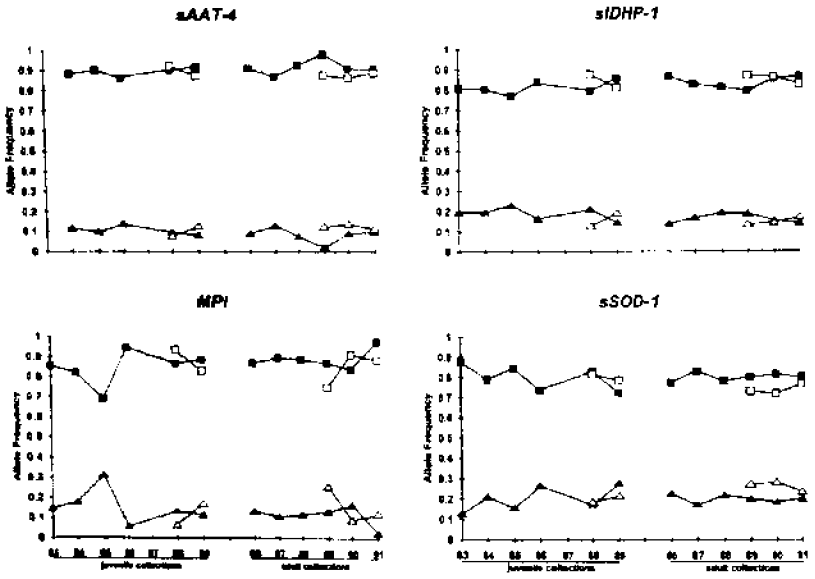

Figure 5. Allele fequency trajectories at four loci in juvenile and adult Tucannon River spring chinook before and after initiation of hatchery supplementation activities. Years shown along the x-axis at the bottom of the figure refer to the year the fish were produced (juvenile collections) or the year fish returned to spawn (adult collections). Solid symbols = frequencies for naturally produced fish; open symbols = frequencies for hatchery produced fish. Squares show the frequency of the most common allele (*100) at each locus, triangles show the frequency of the second most abundant allele at each locus. N = 50 - 100 per collection.

the four loci shown in Figure 5 or any of the 31 other variable loci being screened.

Because we have less than one complete cycle's worth of data for the stock after the hatchery operation began, it is premature to conclude that there have been no measurable changes at the loci monitored by electrophoresis. Although, as shown in Figure 5, there is no evidence of substantial directional changes. Furthermore, because electrophoresis only allows us to monitor a very small proportion of genes in the genome, there could well be substantial changes at other loci that we would never detect by the electrophoretic screening. Nevertheless, the electrophoretic monitoring represents an attempt to evaluate genetic effects of the hatchery operation. As such, this electrophoretic monitoring and evaluation is an important aspect of the supplementation effort because it has the potential to provide an early warning of genetic problems in the hatchery. However, because hatchery-produced fish are now being allowed to spawn naturally upstream (and their progeny are unmarked), our ability to distinguish between fish with and without past hatchery influence will end within the next three years and subsequent monitoring can only be done for the combined hatchery and naturally-spawning stock.

- **Hatchery conservation genetics research**

As mentioned above, there are numerous questions about conservation genetics that need to be answered for effective, rational programs to control genetic risk. Some questions can be answered experimentally and some by modelling. In cooperation with WDF's Salmon Culture Division, two small research programs at WDF hatcheries are currently underway to address some of this uncertainty.

One study, at Tucannon, is designed to evaluate the genetic impact of a single generation of hatchery rearing on performance, an opportunity created by the fact that we are still in the first cycle of returns from the hatchery. The method used is to make *inter se* matings of known hatchery (HxH) and wild (WxW) returning adults, and evaluate their progeny. Family lots are reared individually until the families are combined in rearing ponds (= "ponded"), so early performance by family can be readily evaluated. Although family identity is lost when the fish are ponded, they are ponded by treatment group (HxH or WxW) and tagged by treatment group upon release for further evaluation of group performance. Started in 1990, this study has so far revealed striking differences between hatchery and wild females in prespawning survival of adults and early survival of progeny, but the cause is unclear. Hatchery females tend to be younger and smaller than wild females, and this may account for much of the performance difference between the two groups. The data are currently being analyzed to evaluate this effect.

At the Methow Hatchery Complex (on the mid-Columbia River) the relationship between census and effective population size is being studied. Typically hatchery broodstock guidelines assume that one fish equals one effective spawner, and that the most serious departures from this are due to unequal sex ratios. In reality, probably the most important determinant of effective size in Pacific salmon is variance of family size. At Methow, full-sib families are being individually reared and marked before release. Upon their return we will be able to calculate

the variance of family size. This study was initiated in 1992 with 21 families and will be replicated in future years.

- **Future monitoring and research at WDF hatcheries**

A major issue surrounding the increasingly pervasive use of hatcheries is to what extent they domesticate the fish, making them less fit in the wild. Questions about domestication selection need to be answered, but the type of monitoring conducted in the Tucannon operation to date is unlikely to provide the information needed. The issue of domestication selection is currently being studied, but monitoring programs should be developed to evaluate changes at quantitative loci and changes in demographic profiles. This is considerably more expensive, logistically demanding and difficult to design than an electrophoresis-based monitoring program, but needs to be done.

Conclusions and Recommendations

The Washington Department of Fisheries has, like other agencies in the Pacific Northwest, conducted a number of programs and pursued policies that have almost certainly had negative impacts on the genetic integrity, productivity and survival of salmon stocks under its stewardship. Some of these effects are irreversible. However, if the department learns from past mistakes, if current practices and programs are evaluated and corrected aggressively and if enlightened policies and programs are developed and implemented in the future, it is reasonable to presume that the continuing erosion of biodiversity that threatens our fish and fishery resources can be halted or even reversed.

In conclusion, the following actions for fish management agencies are recommended:

> Establish stock management policies based on sound genetic (and ecological) principles,

> Recognize the strengths and limitations of hatchery production and ensure that it is used in appropriate situations only,

> Develop a detailed inventory of the locations, characteristics and status of all stocks, both natural and cultured,

> Implement adequate monitoring and evaluation programs for both native stocks and for hatchery programs,

> Develop an aggressive program of public education that emphasizes genetic principles, the importance of native stocks and the critical role of habitat,

> Recognize that, in the long run, there is no substitute for adequate habitat.

Acknowledgments

We gratefully acknowledge the numerous contributions of our colleagues at the Washington Department of Fisheries; for their participation in many of the specific work efforts described in this report, for the information they have provided to this overview, and for their constructive review and comments on earlier drafts of the manuscript. We are especially grateful to Anne Marshall and Steve Phelps for allowing us to summarize aspects of their unpublished results for chinook and chum; to Bob Bugert and Glen Mendel for sharing data regarding the Lyons Ferry fall chinook and Tucannon spring chinook hatchery programs, to Jim Ames, Rich Lincoln, and the countless other contributors to the SASSI process for allowing us to summarize their results; and to Marc Miller and Sewall Young for the fishery and baseline collections used in many of the studies we have summarized. We thank Robin Waples (NMFS-Seattle) for his contributions to Dungeness chinook captive broodstock planning and David Teel and others in the genetics group at the NMFS-Northwest Fisheries Science Center for allowing us to include their electrophoretic data for the 1983 brood juveniles from the Tucannon River spring chinook stock and the 1977-1982 genetic data for the Lyons Ferry fall chinook stock. Portions of the work described herein were funded by the U.S. - Pacific Salmon Treaty Act; the Anadromous Fish Act; the State of Washington General Fund; the USFWS, through the Lower Snake River Compensation Plan; and the Bonneville Power Administration, through the Yakima/Klickitat Fishery Project.

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Pacific Salmon Management and Stock Enhancement Programs in Alaska

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Abstract

In recent years stock enhancement has produced nearly 50 million salmon in Alaska annually. Genetic resource conservation has contended with two kinds of impacts; overharvesting of wild-spawned stocks and threats to diversity and fitness caused by loss of reproductive barriers between stocks. Overharvesting is exemplified by the situation in Prince William Sound where some wild-spawned salmon have been overharvested in a mixed fishery that contains ocean-ranched salmon and stocks of wild-spawned salmon. To avoid overharvesting, fishery managers can use markers to identify stocks in mixed harvests and can delay harvest until anadromous stocks are physically separate. Genetic diversity losses have been minimized by resource management policy that regulates transplantation or stock transfer and broodstock management practices.

Introduction

Nearly one-third of the world's half-billion tonne annual salmon production comes from Alaska. This is a combined product of natural spawning and stock enhancement (hatchery reared juvenile salmon released into the ocean and harvested as anadromous adults) (reviewed in ASMI, undated). In Alaska, stock enhancement of salmon is referred to as ocean-ranching or fishery enhancement, meaning that natural stocks are not objects of artificial techniques, but that local fisheries become stabilized or increase harvests from artificially-spawned stock. Protection of the genetic resources that provide this combined production is of great importance in Alaska. To understand the issues confronting resource managers and conservation scientists in Alaska today, the history of the stock enhancement program in Alaska should be reviewed.

Salmon Fisheries in Alaska

Beginning in the late 19th century, industrial salmon fishing in Alaska passed through several phases (Pennoyer 1988; Roppel 1988). Up until the 1920s, there was a proliferation of salteries, then known as canneries. During the 1920s and 1930s, there were variable, very high annual catches, reaching over 100 million fish. During the 1940s and 1950s catches declined to only a few million salmon. Spawning populations and harvests grew slowly after 1960, then rapidly after 1970. Since 1980, harvests have uniformly exceeded 100 million fish (Fig. 1).

The high catches of the 1930s were taken by a rapidly expanding fishery centered around

canneries. By 1930 over 150 canneries were packing salmon. The processing sector controlled a significant part of the catch; from the beginning, fish-packing companies owned and controlled most of the harvests. At first, simple beach seines were used to fish in the mouths of rivers, then stationary traps situated in migration corridors and power vessels were used, all owned in large part by the processing sector.

- **Harvest management and climate explain historical variation**

Early biologists recognized that it was short sighted to take all the salmon that entered a stream to continue production. An allocation of adult salmon to the spawning stream was required, and that it was short sighted to take all the salmon that entered a stream. By the 1920s a policy of restricting harvest to only half of the run was enforced by the U.S. national government. This "White Act" management was cumbersome and not responsive to variations of run strength. Weekly fishing schedules, promulgated months in advance, in the federal regulation prevented managers from adapting to variations of abundance. The result was that fishing was prohibited in the early part of the season until half of the fish had escaped to the spawning streams. Fishing was allowed only in the later part of the season. This had the effect of artificially selecting against stocks with later return timings. The naturally adaptive seasonal variation of run timing was changed (Alexandersdottir 1987). Excessive fishing, attributed to the failure of federal management efforts, was the main reason for the decline of salmon harvests during the 1940s and 1950s (Royce 1988).

The continued deterioration of the salmon harvest and lack of local control over the resource was a significant part of the political motivation toward statehood (Cooley 1963.) Won in 1959, statehood established salmon resources as common property, explicitly outlawed fish traps in favor of harvesting by individual fishermen and put in place a more responsive, locally-based fishery resource management program. The historical increase of salmon production since 1960 is in part testimony to the success of Alaska's citizen Board of Fisheries' policy of protecting spawning stocks from overfishing. This policy gradually increased escapes to the rivers until the optimum was met in each generation. Escape, in the parlance of salmon conservation, refers to the number of salmon that escape the fishery and are allowed to spawn. In managed fisheries, the manager allocates mature fish to either harvest or escape by controlling harvest activity. The historical increase in production is

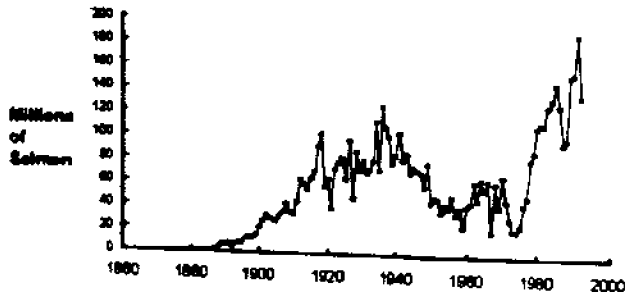

Figure 1. Annual salmon harvest in Alaska, all species, all areas. Source: Alaska Dept. of Fish and Game, Commercial Fisheries Division, March 1993.

also explained by the widely reported climatological shift toward warmer oceanic conditions that may have reached a maximum in the past five years (Hofmeister 1993; Royer 1986; Royer and Cooney 1992).

- **Stock enhancement in the 1970s**

In the early 1970s, both the long term temperature cycle (Royer 1986; Royer and Cooney 1992) and salmon production were at a minimum in Alaska (Figure 1). Despite the change in state fisheries management and the protection of spawning populations during the 1960s, production declined due to severely cold winters and cold oceanic conditions. Fisheries in Prince William Sound were entirely closed in 1972 and 1974 (Koernig and Noerenberg 1976; Simpler 1976).

Two related political changes occurred at that time; limitation of entry into the salmon fishery and creation of a modern salmon ocean ranching system. Limited entry restricted the number of fishermen who could participate in the harvest and property rights were extended to allow their participation. Salmon fishermen then became the controlling members of private, nonprofit ocean ranching corporations that have since been established in several regions of Alaska. Although they are not the sole contributors to salmon ocean ranching, these regional corporations produce most of the ocean-ranched salmon in Alaska.

Salmon ocean ranching was established based on the theory that cost-effective hatchery technology could produce salmon that are not subjected to freshwater mortality. More than 90% of the mortality in salmon cohorts occurs in freshwater and is highly variable as a result of unpredictable weather. For example, Jaenike (1993) and Hofmeister (1993) recently analyzed historical records of pink salmon production and demonstrated that in some years, severe severe winter winter temperatures apparently dessicated and killed a large proportion of incubating embryos.

Salmon ocean ranching was not intended to supplant wild-spawned production, but to remove some of the year-to-year variability of production and to provide a harvestable resource when wild-spawned salmon had to be protected from harvesting. The primary goal of salmon ocean ranching in Alaska was to provide a harvestable resource in years when natural production failed: "...artificial propagation probably is the key to doing something about stabilization of fishable stocks and that all the good management in the world may never accomplish this by itself..." (Koernig and Noerenberg 1976). Koernig and Noerenberg qualify as architects of salmon ocean ranching system in Prince William Sound and in Alaska; Koernig resource is a fisherman from Cordova, and Noerenberg was a pioneer management biologist in Prince William Sound and former Commissioner of Alaska's Department of Fish and Game.

A secondary goal, accomplished in the regional private nonprofit corporations, was to take local action to stabilize the fishing industry in order to capture wealth from the salmon resource in the local community. This was an extension of the political impetus that drove Alaska to statehood. The goal was to reduce the economic hegemony of the large packing companies, to put the harvest in individual fishermens hands, and remove resource management authority from the federal to the local level. What could not be accomplished by the state or national government was accomplished in the local community.

Protection of the productivity of wild-spawned salmon was a central part of the local management vision. It had become clear that wild-spawned production was not sustaining the industry, and that both the resource and the local industry were failing. Stock enhancement was sought as a means to sustain both the industry and the natural resource.

Other regional salmon ranching corporations in Alaska are similar to the corporations established in Prince William Sound, which served as a model. Their operations are not directly supported by public monies, as are most salmon hatcheries in other states. These private nonprofit corporations are supported by the fishermen, both by a monetary assessment based on the value of their individual landings and by a "cost recovery" harvest of salmon. Salmon taken for cost recovery are allowed to escape the common property harvest and enter a terminal area near each hatchery where the corporation harvests them.

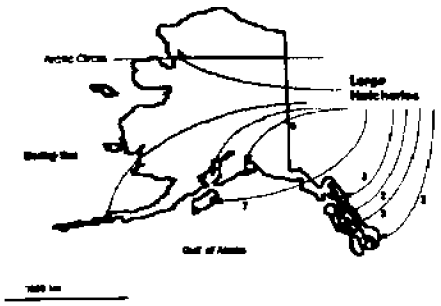

Figure 2. Large salmon hatcheries in Alaska (capacity above 20 million embryos). Arrows denoting more than one facility in a local region are labeled with the pertinent number of hatcheries.

In less than 20 years the Alaska salmon ocean ranching program has increased its annual production to nearly 50 million adult salmon, weighing more than 50 thousand tonnes (FRED 1990, 1991, 1992). Employment derived from this production was estimated to generate over $100 million in yearly personal income in Alaska (FRED 1991). The Alaska ocean ranching program is an important part of the small United States marine aquaculture industry (NRC 1992). There are about 36 productive salmon hatcheries in Alaska releasing over 1.7 billion fry and smolts each year (FRED 1991; Fig. 2). It rivals the annual production of the Japanese chum salmon ocean ranching industry, the world's largest, which produces near 100 thousand tonnes a year (Japan Fisheries Association 1991).

Genetic Resource Management

Overharvesting wild-spawned salmon stocks, induced by harvest of enhanced stocks, is the most severe threat to genetic diversity and productivity connected to Alaska's salmon ocean ranching industry. This was recognized in the history of salmon enhancement and has continued to be recognized as a difficult problem (Helle 1976, 1981; Geiger et al. 1992; Eggers et al. in press; Geiger et al. in press). Embryos of wild-spawned stocks undergo greater mortality (more than 90%) in freshwater, therefore, more spawners are required to produce the next generation. If wild-spawned stocks and hatchery stocks are mixed together and become indistinguishable in the fishery, wild-spawned stocks are vulnerable to excessive harvest. This risk threatens entire population genomes.

Other threats to genetic diversity and fitness of salmon stocks have been associated with

hatchery production. These threats have been reviewed in the context of Alaska's resources (Allendorf et al. 1992; Helle 1976, 1981). They include the deterioration of reproduction barriers, loss of genetic variability between stocks (transplantation, straying) and loss of genetic variability as a consequence of domestication selection.

- **Protection from overharvesting salmon**

The pink salmon fishery in Prince William Sound illustrates measures that could be taken to minimize the effects of overharvesting. Fishery managers in the Sound believe that overfishing has occurred and is a clear danger to pink salmon. Their analysis of the 1992 harvest found that a very small number of salmon had spawned. Fewer salmon had spawned than in the catastrophic years 1972 and 1974, when fisheries were closed to protect spawners (Geiger et al. 1992).

Four large hatcheries produce pink salmon in Prince William Sound, releasing 600 million fry each spring. These fish mix with wild-spawned salmon and leave the Sound during their first summer. In a short period of mid-summer weeks, surviving hatchery- and wild-spawned adults migrate through narrow passages in the southwest entrance to the Sound (Fig. 3) and return to the hatcheries. Their abundance is only roughly predictable. The challenge for managers is to regulate the fishery for the protection of wild-spawning stocks without knowing whether the salmon wild-spawning are abundant.

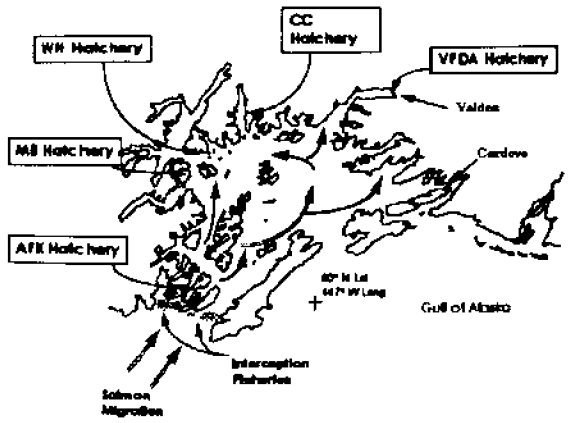

Figure 3. Anadromous salmon migration paths and locations of large salmon hatcheries in Prince William Sound, Alaska. AFK Hatchery: A.F. Koernig Hatchery; MB: Main Bay; WN: Wallace Noernberg; CC: Cannery Creek; VFDA: Valley Fisheries Development Association. Each is a pink salmon hatchery releasing more than 100 million fry each year, except MB, which is a sockeye salmon facility. Interception fisheries in the southwest entrance of Prince William Sound harvest mixtures of salmon migration to hatcheries and to wild spawning streams.

Since 1980, as the stock enhancement effort has increased, the fishery has become focused on the narrow entrances to the Sound where the salmon are concentrated (Fig. 3) (Geiger et al. 1992). The primary objective of the managers is to ensure adequate escapement of adult salmon to the wild-spawning streams. Management strategy has been to monitor both escapement and catch, and to use that information to regulate fishing efforts (Geiger et al. 1992). Escapement is monitored by flying over the short, steep streams and estimating the numbers of spawners in the streams. Catch is monitored by a system of enforced reporting at the time of first sale, when fishermen deliver their daily catch. If abundance is low as the salmon enter the Sound, managers strive to restrain

fishing efforts, thereby protecting the stocks from overfishing.

Catch is the most useful early indicator of abundance. The entrance of salmon into the Sound and the primary fishing effort occur before the fish are fully mature, and before they enter the streams. However, catch is a biased indicator of the abundance of wild-spawned salmon when hatchery-bound salmon are present in the run. Managers are faced with a quandary because each component of the salmon run is unpredictable. A large catch may result from abundant hatchery salmon even though there are a few wild-spawned fish. In this case, if the manager permits open fishing, wild-spawned fish would be over-fished, apparently the case in 1992. In this case, if the manager does not know whether wild-spawned salmon are abundant and closes fishing, a valuable harvest would be lost.

In theory, there are solutions to this quandary. Managers can restrain fishing in the narrow entrances to the Sound and permit fishing only after the salmon have approached their natural stream, also called the "terminal area". By that time the hatchery-spawned and wild-spawned salmon have largely separated from one another because the hatchery terminal area is geographically separate from the wild spawners' terminal area. The cost associated with this alternative may be severe. Anadromous salmon rapidly lose value as they leave the ocean and approach their spawning ground, because body fat is converted to gametes or metabolized, and secondary sex characteristics distort their shapes and darken their skins. Technological aids are also possible. Salmon as small as 0.25g, from different stocks, can be artificially marked or tagged (e.g., with coded micro wires) (Thrower and Smoker 1984). A sample of salmon in the fishery can, therefore, be proportionately identified and managers can judge whether a stock is in danger of overfishing. This technology has been demonstrated in the management of pink salmon fisheries in Prince William Sound. Managers there believe tagging will be necessary if serious overfishing is to be avoided in the near future (Peckham 1992; Geiger et al. 1992).

However, tagging technologies are expensive and only a small sample of hatchery production can be tagged (in recent practice in Prince William Sound 1/1000). It costs as much as $.20 to tag each of several hundred thousand fry and as much as $20 to detect, recover, decode and tabulate information from each of several thousand recovered adults. However, few tagged fish can be recovered and inferences drawn from these small samples are inherently imprecise.

Mass-marking procedures have been proposed and may be applied in the future. Informational marks can be induced in the microstructure of salmon otoliths by manipulations of the temperature of incubation of embryos (Volk et al. 1990). This technology has been successfully applied at reasonable cost and at production scale in a large pink salmon hatchery (Munk et al. In Press).

- **Threats to diversity and fitness**

The productivity of Alaska's salmon resources is preserved within the huge genetic diversity between five species and between hundreds, even thousands, of different stocks of salmon in each species. These stocks are each adapted to a local environment that can, in principle, be defined by physical characteristics (water temperature, spawning substrate size, etc.), temporal characteristics, and biological characteristics

(predators, prey, etc.). This diversity is naturally maintained by reproductive barriers between stocks, i.e., by the remarkable tendency for salmon to home to their natal stream for spawning.

Viewed within the strict constructs of conservation biology theory, salmon ocean ranching presents other threats to the genetic diversity of salmon in Alaska. Consider a scenario in which a large hatchery with a broodstock of tens of thousands of fish is situated near a typical array of wild-spawning stocks, each numbering a few hundred to several thousand spawners. The prediction is that fish straying from the large hatchery broodstock will mix with the wild-spawning stocks. The wild-spawning stocks can be harmed by the mixture of reproductive fish from a different stock, especially from a hatchery stock that has its ancestral origin in a different environment and has experienced domestication selection. Straying, even though small in relation to the hatchery broodstock, would be large relative to the size of the wild-spawned stocks. The result could be the loss of locally-adapted alleles or the disruption of co-adapted genomes in the wild-spawned stocks.

There is a continuing debate in Alaska on these issues. To a large degree these threats to genetic diversity, fitness and resource productivity are only conjecture. Hard evidence of losses is not common. Those charged with biological conservation, who must act conservatively, are pitted against those charged with improving the near term productivity and value of the resource and who view these threats as only hypothetical.

There is some evidence in support of the conservationist view, however, surveys of neutral allele frequencies have revealed there is significant variation between different Alaskan stocks, although on the scale of large regions, the variation is subtle (McGregor 1982; Gharrett et al. 1988). Quantitative genetic analysis in anadromous pink salmon has revealed significant additive genetic variability of adaptive traits like the seasonal timing of spawning migration (Smoker et al. In Press). Experimentation has suggested the potential for disruption of coadapted genomes (Gharret and Smoker 1991).

Other important evidence for the conservationist view is lacking and little is known about the dynamics of homing and straying. Surprising evidence of widespread straying in both wild-spawned and hatchery stocks of pink salmon in western Prince William Sound has emerged from a large project studying the effects of the 1989 oil spill. In this project, hundreds of thousands of coded wire tags were placed in fry emigrating from seven streams and in fry emigrating from four hatcheries. Tags were recovered from adults returning both to hatcheries and to the seven streams (Sharp 1992). Interpretation of even large studies like Sharp's are complicated by uncertainties about the effects of the tags themselves, the reproductive fitness of observed strays and about the historical effect of recent geological history (western Prince William Sound spawning grounds experienced severe tectonic disruption in a 1964 earthquake).

Little is known about the structure of adaptive variation, particularly on fine temporal and geographic scales. Studies of pink salmon in Auke Creek in Juneau, Alaska, provide evidence that adaptively important traits like the seasonal timing of spawning migration, development rate of embryos, emergence timing of fry and selection of spawning habitat all have a basis in genetic

variability (Gharrett and Smoker, In Press a, b). This infrastructure may be typical of wild-spawned stocks, but is not well characterized and is not recognized in resource management policy.

Policy and Practice in Alaska

Public policy in Alaska aims to "do no harm," and to be conservative in the face of uncertainty. The Alaska Department of Fish and Game has regulatory control over salmon ocean ranching. A permit is required for transportation of salmon from one place to another or for the release of salmon from a hatchery into natural waters. A qualified geneticist must review each application for a permit. The Department's genetics policy was written in 1985 (ADFG 1985) and specifies that: salmon cannot be brought to Alaska from outside its boundaries, salmon cannot be transplanted between major faunal regions, and transplantations within a region are permissible only over short distances if phenotypic characters of the donor are appropriate to the receiving site. The policy also seeks to protect wild-spawning stocks by establishing sanctuaries and prohibiting purposeful planting of domesticated fish into indigenous stocks; maintaining genetic diversity in hatchery broodstocks by setting minimum effective population size standards for founder stocks and broodstocks; and by prohibiting overt artificial selection.

The policy has produced both broad successes and some failures. Long distance and casual transplantations have been eliminated and inbreeding in hatchery broodstocks is now very rare or nonexistent. However, regional genetic sanctuaries have not been established and the diversity of broodstocks in some regional ocean ranching programs is small. For example, in the large 600-million-fry pink salmon program in Prince William Sound, there are only two ancestrally separate broodstocks in each of the odd and even year lines. Overt artificial selection is avoided in hatchery broodstocks, but some practices may invite genetic change. Whatever shortcomings the policy has had, adherence to the policy will continue to go a long way toward conservation of salmon genetic resources in Alaska (Allendorf et al. 1992).

Conclusion

Management of the salmon ocean ranching program in Alaska has always been based on protection of the diversity of wild-spawned stocks. Early statements by policy makers and resource managers reveal a concern for protection of genetic resources: "The guideline is this; natural stocks must not be affected adversely by hatchery operations" (FRED 1980). This policy is in marked contrast to those of salmon hatchery programs in Japan, where chum salmon are exclusively produced in hatcheries and wild-spawning is only accidental, or in the U.S. Pacific Northwest where hatcheries were built in mitigation for habitat destroyed by hydroelectric and other development. Whether Alaska can be successful in practicing large scale ocean ranching without loss of natural salmon variability and productivity is still debated.

In Prince William Sound, a large center for salmon ocean ranching, conservation theory and resource development practice come into particular conflict. The public-servant resource managers from Alaska Fish and Game are unabashed in expressing their con-

viction that large scale ocean ranching is seriously threatening wild stock productivity. They report that in 1992 wild stocks were seriously overharvested (Geiger et al. 1992). Indeed, the recent production of tens of millions of pink salmon adults from more than half a billion fry each year is a far cry from the early plan of 3 million adults from 200 million fry each year (Koernig and Noerenberg 1976). If there were only 3 million enhanced salmon per year, the manager's harvest quandary would be more tractable. However, in the modern market, a resource of three million salmon would not support a regional industry.

Some conservation scientists have attributed this rapid development in Prince William Sound to "greed" (Hilborn 1992; Ludwig et al. 1993). The use of this derogatory term is unfortunate, ignorant of history and naive of the economic theory of free markets. The roots of Alaskan ocean ranching are an effort to stabilize and enable local industry, and not to abandon salmon resources to careless harvesting by large outside industrial firms.

From the historical point of view, ocean ranching is a success. As Prince William Sound managers point out, without salmon ocean ranching there would have been no harvestable salmon resource in three of the past five years (Geiger et al. 1992). Perhaps the truth is in the early view that wild-spawned production in Prince William Sound is too unreliable to sustain an annual harvest (Koernig and Noerenberg 1976).

Arguably the trend toward local resource management has been successful in conserving most of the wild-spawned salmon resources and their productivity, even though it is a continuing and difficult process. The trend toward economic self-determination began with Alaska's statehood a generation ago, with the control of the large packing companies eliminated. This trend has continued in the development of large new supplies of salmon by the nonprofit salmon ranching corporations, which are controlled by the fishermen. These new sources of salmon are a new force in the market and are leading to the development of new products and markets. Ocean ranching has had a dynamic effect on the industry.

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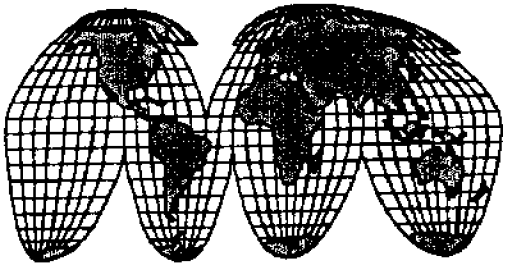

National Fish Breeding Programs in the Philippines

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Abstract

During the last five years, there have been several research programs on tilapia genetics in the Philippines. The focus of this research has been on the genetic improvement for growth in tilapia. The application of the results of these research efforts through a national breeding program is important to sustain a high level of productivity for tilapia. These research efforts were developed through international collaborations and now provide the framework to initiate a national tilapia breeding program. The establishment of this program is important for disseminating information and for providing organized channels to produce and distribute tilapia fingerlings.

Introduction

Fish play a significant role in providing animal protein in the Filipino diet and in generating livelihood to improve the income of fish farmers. Considerable development in fish production has taken place through aquaculture. This sector of the fisheries industry contributed 26.6% to the country's total fish production in 1991 (BFAR 1991).

Among cultured fish, milkfish (*Chanos chanos*) and tilapia are two important components of the aquaculture industry. Milkfish has become the main species cultivated in brackish water, while tilapia has become popular in freshwater ponds, fish pens and cages. The tilapia production from aquaculture grew from 30,772 metric tons in 1983 to 76,570 metric tons in 1991, contributing 79.5% to the total tilapia production (Bureau of Agricultural Statistics). This increase in production is a clear indication of the potential tilapia have to expand freshwater aquaculture.

History of Tilapia Culture

Tilapia farming began with the Mossambique tilapia (*Oreochromis mossambicus*) introduced in the Philippines in 1950 (Guerrero 1985). But because of the undesirable characteristics of the species, notably its precocious maturity, the culture of *O. mossambicus* did not expand. Instead, *O. mossambicus* is often regarded as a pest in milkfish ponds.

Starting in 1972, the introduction of Nile tilapia (*Oreochromis niloticus*) from various origins (Table 1) changed the outlook of the fish farmers toward the tilapia. Consequently, the tilapia aquaculture industry in the Philippines has grown. This species was preferred for culture because of its faster growth, lighter color, tolerance to a wide range of environmental conditions, disease

Table 1. Tilapia introductions in the Philippines from 1950 - 1982 (Guerrero 1985; Guerrero and Tayamen 1988): 1988 - 1989 (Eknath et al. 1993).

Species (Origin)	Year	Source	Agency
Oreochromis mossambicus	1950	Thailand	BFAR[a]
O. homorum x *O. mossambicus*	1971	Singapore	PS[b]
O. niloticus (Uganda)	1972	Israel	LLDA[c]
O. niloticus (Egypt)	1972	Thailand	BFAR
Tilapia zilli	1973(?)	Taiwan (?)	?
O. aureus	1977	USA	CLSU[c]
O. niloticus (Ghana)	1977	Israel	CLSU[c]
O. niloticus (Ghana)	1977	Singapore	BFAR
O. aureus (Israel)	1977	Singapore	BFAR
O. aureus (Israel)	1978	Singapore	SEAFDEC[d]
O. niloticus (Ghana)	1979	Singapore	SEAFDEC
O. aureus (Ghana)	1979	Israel	CLSU/ICLARM
O. niloticus (Ghana)	1979	Israel	CLSU/ICLARM
O. aureus (Israel)	1982	Israel	PS
O. niloticus (Ghana)	1982	Israel	PS
O. niloticus (Egypt)	1988	Egypt	ICLARM
O. niloticus (Ghana)	1988	Ghana	ICLARM
O. niloticus (Senegal)	1988	Senegal	ICLARM
O. niloticus (Egypt)	1989	Egypt	ICLARM
O. niloticus (Kenya)	1989	Kenya	ICLARM

[a]Bureau of Fisheries and Aquatic Resources
[b]Private Sector
[c]Central Luzon State University
[d]Southeast Asian Fisheries Development Center

resistance and increased consumer acceptance. Today, *O. niloticus* has become the most widely used tilapia species for aquaculture practices ranging from backyard ponds to commercial pond or cage culture. Also, because of the relative ease of culture, the technology for the tilapia culture is within the capability and resources of small-scale fish farmers.

Basis for Tilapia Genetic Improvement

One major constraint affecting further expansion of the tilapia industry was the poor quality of tilapia fingerlings used in production systems (Recometa 1985; Smith et al. 1985). Electrophoretic studies indicated widespread introgression of genes from *O. mossambicus* (Taniguchi et al. 1985; Macaranas et al. 1986). Moreover, founder and bottleneck effects (Pullin and Capili 1988), and inbreeding as a consequence of poor stock management, may have caused genetic

deterioration resulting in reduced performance of farmed tilapias. These problems prompted some government institutions, like the Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU) and the National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR), to start research on the genetic improvement of tilapia in collaboration with national and international institutions.

Current Research on Tilapia Genetic Improvement

The Genetic Improvement of Farmed Tilapia (GIFT) is a collaborative research project that started in 1988. The project is co-financed by the Asian Development Bank (ADB) and the United Nations Development Programme/Division for Global and Interregional Programmes (UNDO/DGIP). The GIFT project is being executed by the International Center for Living Aquatic Resources Management (ICLARM) in cooperation with NFFTRC/BFAR, FAC/CLSU, Marine Science Institute (UP/MSI) and the Institute of Aquaculture Research in Norway (AKVAFORSK). The GIFT project's approach is to bring diverse tilapia germplasm from Africa to Asia where the species are already farmed. Collected wild stocks of Nile tilapia from Egypt, Ghana, Kenya and Senegal were imported to the Philippines. The primary objective of the project is to evaluate the growth of these strains along with the farmed strains (Israel, Singapore, Taiwan and Thailand) to build a base population and to initiate a genetic improvement program to increase the production of tilapia. The results indicated highly significant differences among the growth performance of the eight strains. With the exception of the Ghana strain, the newly introduced African wild strains performed as well as, or better, than the most widely farmed Asian strains (Eknath et al. 1993).

Significant milestones have been achieved by the project, including the establishment of a broad genetic base population and an appropriate breeding strategy to begin a selection program. Eknath et al. (1991) reported that the mean growth rate of the GIFT project's base population was at least 20-30% higher than the most widely cultured commercial strain of tilapia in the Philippines. Also, the Tilapia Germplasm Reference Collection initiated by the GIFT project, will be a source of germplasm for further strategic research.

A related selective breeding effort at the Freshwater Aquaculture Center, funded by the International Development Research Center (IDRC), has also achieved significant results in testing within-family selection as a procedure for improving the growth rate of Nile tilapia. The response was measured by comparing the growth performance of the eighth selected generation and a second generation random-bred control that was maintained along with the selection program. The selected fish were from 8 to 37% heavier than the control line (Bolivar et al. in press). This response was consistent with the results in subsequent selected generations.

The research program, Genetic Manipulations for the Improvement of Tilapia, is investigating the use of genetic manipulation to produce all-male producing broodstock. This work is being conducted at FAC/CLSU in collaboration with the University College of Swansea, UK, and is supported by the Overseas Development Administration.

This research is focused on the sex determination mechanism in different strains of Nile tilapia. The technique for *O. niloticus* is based on the production of large numbers of YY supermales, which will yield all-male progeny when crossed with normal females (Mair and Little 1991).

These collaborative genetics programs in the Philippines are not only generating improved strains and important information, but are also providing training and experience that will strengthen the manpower capability in the field of genetics, and establishing necessary facilities to set and run breeding programs.

National Fish Breeding Program

The only national fish breeding program in the Philippines is for milkfish or "bangus". However, the launching of the National Bangus Breeding Program in 1981 (Lopez et al. 1986) was not in the context of genetic improvement, but to intensify fry production to ensure availability of fry and fingerlings for growout operations. So even for the most commercially important aquaculture species, no national breeding program has been developed.

At present, the National Freshwater Fisheries Technology Research Center (NFFTRC), a national tilapia broodstock center, is working to increase tilapia production and distribution of good quality tilapia for hatcheries and grow-out operations (Tayamen 1988). The center has concentrated on distributing the *O. niloticus* "Israel" strain, because its growth is faster than the other strains available. The center's distribution of the "Israel" strain has been effective particularly in Central Luzon, which is the major tilapia producing region in the Philippines.

Self-sustaining Breeding Program

To fully attain the objective of increased fish production, significant achievements in tilapia genetics improvement must be maintained and applied by way of a national breeding program. There is a strong need for such a program to ensure the continuity of efforts to improve the culture performance of tilapia stocks. The steps applied in the GIFT project to develop a breeding program were described by Eknath et al. (1991). The improved fish developed in the breeding program will soon be ready for dissemination. The national breeding program is very important because effective dissemination of genetic gain is possible only when there are organized channels for the production and distribution of tilapia fingerlings.

The establishment of a national fish breeding program in the Philippines is envisioned to commence following the experience of the GIFT project. The project is providing the experience of how a national breeding program can be built. At present, the national institutions involved in this endeavor, the BFAR/NFFTRC and FAC/CLSU, are formulating strategies for a self-sustaining fish breeding program where a breeding nucleus will be established and research outreach stations will be organized to serve as multipliers for the effective commercial distribution of improved tilapia stocks.

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A Review of Fish Genetic Research and Conservation Issues in Taiwan

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Abstract

In 1991, fish production from aquaculture was about 290,000 tons. There are currently about 60 cultured food fishes and an undetermined number of imported ornamental fishes in Taiwan. In the past three decades, aquaculture research in Taiwan has focused mainly on breeding, fry production, feed, nutrition and pond management. Genetic research has been fragmented. The most prominent success in genetic techniques has been the hybridization of tilapias. Very little research has been conducted on selection for growth in fishes. However, some hatchery operators have performed growth selection by choosing spawners with faster growth rates and better body conformation. No systematic breeding lines for mating have been established in these hatcheries because it is labor intensive, requires more facilities than most hatcheries have available and most operators have an inadequate knowledge of the procedure. Inbreeding depression occurs inadvertently when only a few spawners are used as broodstock, especially if the fry are produced through artificial fertilization. In marine fishes, inbreeding depression is often not a problem due to their long breeding period and random mating behavior in captivity. Endemic fish resources have been depleted due to pollution of natural habitats, overfishing and strong competition for habitat with exotic fishes, such as tilapias. Stock enhancement and some conservation policies have been carried out for some marine fishes, the Japanese eel (*Anguilla japonica*), brook masou salmon (*Oncorhynchus masou formosanum*) and sweetfish (*Plecoglossus altivelis*). This review discusses research and practices on selective breeding, chromosome manipulation, biotechnology/genetic engineering, conservation of fishes and related problems in Taiwan.

Introduction

Aquaculture has centuries of history in Taiwan. Due to depletion of fisheries resources its importance has increased in recent years. The custom of buying live fish has paved the way for aquaculture products to compete with imported fishes. The popularity of sport fishing in ponds has also provided a vast market demand for aquaculture. All these have emphasized the need for species diversification in aquaculture. Aside from endemic fishes, more exotic species are needed for aquafarmers to meet consumers' preference. The food fishes currently cultured in Taiwan are listed in Tables 1a - 1c.

In Taiwan, genetic research in aquaculture has been fragmented and lags far behind

agriculture and livestock production. This can be attributed to a number of reasons.

> Due to its importance and profitability, fry production is the first priority in the development of aquaculture. Improving breeding and larval rearing techniques are primary concerns of researchers and aquafarmers,

> Availability of a variety of fish species for aquaculture provides more topics for researchers and wider selection of fry production for fish farmers,

> Selection programs are labor intensive, time consuming and require facilities and specialized researchers.

The only prominent success in genetic research in Taiwan has been the hybridization of tilapias.

Stock enhancement efforts and effective conservation policies are needed to protect fishery resources in Taiwan. Most endemic freshwater fishes are facing extinction due to the serious destruction of habitat, overfishing and competition with exotic species for habitat. Overfishing and polluted estuaries also endanger the survival of marine fish resources. Several organizations in Taiwan, such as the "Society of Stream," have already been established to protect and recruit wild fish stocks. Hopefully, efforts like this one will become a trend.

Selective Breeding

• Hybridization

Hybridization is conducted mainly to produce monosex offspring, sterility and polyploidy, and to increase hybrid vigor. Finfish hybrids produced in Taiwan are listed in Table 2.

• Tilapias

Hybridization often occurs among mouth-breeding tilapias. In Taiwan, the main mouth-breeding tilapias are *Oreochromis niloticus, O. aureus, O. hornorum, O. mossambicus*, white tilapia and red tilapia. Morphological studies have been conducted on these tilapias by Tsay et al. (1992).

Hybridization of tilapias has been successful in improving the growth rate, sex ratio, cold tolerance, body size and salinity tolerance (Kuo 1969; Hu and Yu 1977). Monosex culture of male tilapias is more profitable because males grow faster than females.

In Taiwan, the first cultured tilapia was *O. mossambicus*. Despite its high meat quality, it has undesirable traits, such as precocity, low growth rate and condition factor [(body weight/body length, cm^3) x 100)], deep black body, small body size and poor tolerance to low water temperatures. The successful hybridization between the *O. mossambicus x O. niloticus* was first carried out by Mr. Ho Kuo, the former director of the Lukang Branch of the Taiwan Fisheries Research Institute (Kuo 1969). He found that body color, body size, growth rate, condition factor and cold tolerance of hybrids are related more to the paternal parent.

Hybridizing *O. niloticus* and *O. aureus* is also popular (Hu and Yu 1977). Due to its better growth rate, large body size, and high male:female ratio in the offspring, the *O. niloticus x O. aureus* hybrid has replaced the *O. mossambicus x O. niloticus* hybrid. A comparison of attributes between these two species and their hybrid is shown in Table 3. *O. niloticus* has a better growth rate, large

Table 1a. Principal finfish culture species in Taiwan and their breeding methods. Freshwater Species.

Scientific Name	Common Name	Broodstock/Seed Source	Breeding Method
Anguilla japonica	Japanese eel	WF	1/M
Aristichthys nobilis	Bighead carp	SC	AF
Bidyanus bidyanus	Silver perch	SC	AF or IS
Boulengerochromis microlepis	King fish	SC	NS
Carassius auratus	Crucian carp	SC	NS
Channa maculata	Snakehead	SC	AF
Cichlasoma managuense	Freshwater grouper	SC	NS
Cirrhina molitorella	Mud carp	SC	AF
Clarias fuscus	Walking catfish	SC	AF
Ctenopharyngodon idellus	Grass carp	SC	AF
Culter erythropterus	White fish	SC	AF or IS
Cyprinus carpio	Common carp	SC	AF
Hypophthalmichthys molitrix	Silver carp	SC	AF
Megalobrama amblycephala	Wu-chang fish	SC	AF
Micropterus salmoides	Large mouth bass	SC	NS
Misgurnus anguillicaudatus	Pond loach	SC	AF
Pangasius sutchi	Thailand catfish	SC	AF
Parasilurus asotus	Chinese catfish	SC	AF
Plecoglossus altivelis	Sweetfish	SC	AF
Salmo gairdneri	Rainbow trout	IE	1/M
Siniperca chuatsi	Kuei Fa bass	SC	AF or IS
Morone saxatilis x M. chrysops	Striped bass hybrid	IF	1/M
Oreochromis aureus	Blue tilapia	SC	NS
Oreochromis niloticus	Nile tilapia	SC	NS

Broodstock/seed source: **SC**: Spawners in captivity. **IE**: Imported eggs. **IF**: Imported fry. **WS**: Wild spawners. **WF**: Wild fry. **1/M**: No fry production in Taiwan.
Breeding method: **AF**: Artificial fertilization. **IS**: Induced spawning (spawners are released into the pond or tank after hormonal treatment). **NS**: Natural spawning.

Table 1b. Principal finfish culture species in Taiwan and their breeding methods. Freshwater or Saline Species.

Scientific Name	Common Name	Broodstock/Seed Source	Breeding Method
Acanthopagrus latus	Yellow fin sea bream	SC	IS
Acanthopagrus schlegeli	Black sea bream	SC	NS
Chanos chanos	Milkfish	SC	NS
Glossogobius giuris	Flat-head goby	SC	NS
Lateolabrax japonicus	Japanese sea bass	WS or SC	IS or AF
Lates calcarifer	Giant perch	SC	NS
Liza macrolepis	Large scale liza	WF	1/M
Mugil cephalus	Grey mullet	SC	IS
Oreochromis mossambicus	Mozambique tilapia	SC	NS
Oreochromis niloticus x *O. aureus*	(Hybrid)	SC	NS
Oreochromis - red	Red tilapia	SC	NS

Broodstock/seed source: **SC**: Spawners in captivity. **IE**: Imported eggs. **IF**: Imported fry. **WS**: Wild spawners. **WF**: Wild fry. **1/M**: No fry production in Taiwan.
Breeding method: **AF**: Artificial fertilization. **IS**: Induced spawning (spawners are released into the pond or tank after hormonal treatment). **NS**: Natural spawning.

Table 1c. Principal finfish culture species in Taiwan and their breeding methods. Saline Species.

Scientific Name	Common Name	Broodstock/Seed Source	Breeding Method
Boleophthalmus pectinirostris	Mudskipper	WF	$\frac{1}{M}$
Epinephelus malabaricus	Black spotted grouper	WS or SC	NS or IS
E. suillus	Red spotted grouper	SC	NS or IS
E. tauvina	Estuary grouper	IF	$\frac{1}{M}$
E. akaara	Red grouper	IF	$\frac{1}{M}$
E. fuscoguttatus	Brown marbled grouper	IF	$\frac{1}{M}$
Lutjanus argentimaculatus	Red snapper	SC	IS
Nibea japonica	Grey croaker	WS	AF
Pagrus major	Red sea bream	SC	NS
Plectropomus leopardus	Blue-spotted grouper	SC or WS	NS or AF
Plectorhinchus cinctus	Yellow spotted grunt	WS	AF
Pomadasys hasta	Silver grunt	SC	IS
Rachycentron canadum	Canadian sergeant fish	WS	IS
Scatophagus argus	Spotted butter fish	WF	$\frac{1}{M}$
Sciaenops ocellatus	Red drum	SC	NS
Seriola dumerili	Greater yellowtail	SC	IS
Siganus canaliculatus	Rabbit fish	WF	$\frac{1}{M}$
S. fuscescens	Dusky spinefoot	WF	$\frac{1}{M}$
S. guttatus	Golden spinefoot	WF	$\frac{1}{M}$
Sillago sihama	Sand borer	SC	NS
Sparus sarba	Goldlined sea bream	SC	NS or IS
Takifugu rubripes	Tiger puffer	IE	$\frac{1}{M}$
Terapon jarbua	Tigerfish	WF	$\frac{1}{M}$
Trachinotus falcatus	Permit fish	SC	IS
T. blochii	Snubnose dart	SC	IS

Broodstock/seed source: SC: Spawners in captivity. IE: Imported eggs. IF: Imported fry. WS: Wild spawners. WF: Wild fry.
Breeding method: AF: Artificial fertilization. IS: Induced spawning (spawners are released into the pond or tank after hormonal treatment). NS: Natural spawning.

Table 2. Hybridization of fish in Taiwan.

Female Parent	Male Parent	References
Oreochromis aureus	*O. hornorum*	Yu and Lay, 1985
O. aureus	*O. mossambicus*	Yu and Lay, 1985
O. aureus	*O. mossambicus*	Hu and Yu, 1977; Yu and Lay 1985
O. hornorum	*O. aureus*	Yu and Lay, 1985
O. hornorum	*O. mossambicus*	Yu and Lay, 1985
O. hornorum	*O. niloticus*	Yu and Lay, 1985
O. mossambicus	*O. aureus*	Hu and Yu, 1977; Yu and Lay 1985
O. mossambicus	*O. hornorum*	Yu and Lay, 1985
O. mossambicus	*O. niloticus*	Kuo, 1969; Kuo, 1978; Yu and Lay, 1985
O. niloticus	*O. aureus*	Hu and Yu, 1977; Yu and Lay, 1985
O. niloticus	*O. hornorum*	Yu and Lay, 1985
O. niloticus	*O. mossambicus*	Kuo, 1969; Yu and Lay, 1985
Ctenopharyngodon idella	*Megalobrama amblycephala*	Tang and Huang, 1989
Pagrus major	*Acanthopagrus schlegeli*	Lin (unpublished data)
Cichlasoma citrinellum	*Cichlasoma synspilus*	Personal communication

body size and longer body shape. However, it can easily lose its scales when handled, which results in red lesions, and thus loses its appeal to consumers in the live fish market. Although the growth performance of *O. aureus* is far less than *O. niloticus*, their scales are firmly attached.

Currently, two private tilapia hatcheries share an estimated 90% of the fry market in Taiwan. They use hybridization techniques and growth selection to improve the sex ratio, growth rate and body conformation. Body conformation in tilapias is an important trait for selection. A "good" body conformation will allow a larger size fillet.

The Japanese market prefers frozen fish fillets for *sashimi* (raw fish). The suitable body size of tilapias for fillet processing is between 1.2-1.5 kg. These tilapias are cultured in water with a salinity range of 3-18 ppt. To produce such a body weight in tilapias and improve the profitability of cultured tilapias,

Table 3. Comparison of important attributes between *O. niloticus*, *O. aureus* and their hybrid.

Attributes	*O. niloticus*	*O. aureus*	Hybrid (*O. niloticus* Female x *O. aureus* Male)
Growth Rate	Higher	Lower	Highest (more males)
Scale	Easily shed	Firmly attached	Firmly attached
Slime	Few	Slimy	Intermediate
Sex Ratios	About 1:1	About 1:1	About 85%
Head Size[1]	Large	Medium	Small
Body Shape	Long	Intermediate	Round

[1] In relation to body size

O. niloticus x *O. aureus* hybrids must be produced (Personal communication, 1993).

- **Grass Carp x Wu-chang Fish**

Grass carp (*Ctenopharyngodon idella*) and Wu-chang fish, also known as blunt snout bream (*Megalobrama amblycephala*) are important freshwater fishes. A comparison of their attributes are listed in Table 4. Both species have their own defects and merits for aquaculture. To correct the defects between these two species, hybridization between female grass carp and male Wu-chang fish was carried out (Tang and Huang 1989; Tang et al. 1990). Fertilization rates were as high as 95% and the hatching time was about 30 hours at 21-24°C for both grass carp and the hybrid (Tang et al. 1990). During a culture period of 17 months, grass carp and the hybrid showed almost the same increase in body length. Survival rates of grass carp and the hybrid were 98.5% and 97.0%, respectively.

Although the external appearance of the hybrid shows a closer resemblance to grass carp, the hybrid's morphological characteristics and biochemical traits were intermediate between that of the parental species. The hybrid's efficiency of feeding on aquatic plants was intermediate between the two pure species. Also, sterility was found in the hybrid. Sterile fish are suitable for release into reservoirs and lakes to prevent overpopulation. Fertilization did not succeed in the reciprocal hybridization of female Wu-chang fish and male grass carp. Although the grass carp x Wu-chang hybrid has good traits for aquaculture, its similar appearance to the low-priced grass carp may be an obstacle to its culture development in Taiwan.

- **Other Fishes**

In the Penghu Branch of the Taiwan Fisheries Research Institute, a cross between red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) has been carried out (Lin, unpublished data). Their hybrids were found to be viable and studies are continuing.

In ornamental fishes, the hybrid blood parrot fish has been a prominent success in Taiwan.

Table 4. Comparison of important attributes between grass carp, *Ctenopharyngodon idella*, and Wu-chang fish, *Megalobrama amblycephala*, and their hybrids.

Attributes	Grass Carp	Wu-chang Fish	Hybrid (*C. idella* Female x *M. amblycephalus* Male)
Feeding Habit	Strong herbivore	Weaker herbivore	Intermediate
Growth Rate	High	Low	Close to grass carp
Boniness	Slight	High	Slight
Body Size	Big	Small	Close to Grass carp
Disease Resistance	Lower	Higher	Intermediate

Blood parrot is a hybrid between *Cichlasoma citrinellum* x *Cichlasoma synspilus* and its reciprocal cross. This hybrid was accidentally discovered by an ornamental fish fancier. Value of the blood parrot depends on body color, which should be reddish, and head shape, which should be parrot-like. Its red body color can be improved by using appropriate feed. Fish with these valuable traits are selected from the offspring. The percentage of high value blood parrot fish in offspring has been inconsistent, and varies from 100% in one batch to a very low percentage in others (Personal communication, 1993).

Selection for High Growth Rate

Traits available for finfish selection are growth rate, age at maturation, body coloration, temperature tolerance, disease resistance, food conversion efficiency and meat quality. Growth rate is the most important trait for improvement of fish production through selection. Large genetic variance and high fecundity in fishes make strong selection possible. In Taiwan, growth selection has been carried out in tilapias and loach.

- **Tilapias**

Only a few studies on growth selection have been conducted in Taiwan. Some hatchery operators have performed growth selection by choosing spawners with phenotypes for faster growth rate and better body conformation.

Huang and Liao (1990) reported that there was little response for growth rate using bidirectional mass selection for high and low body weight in *O. niloticus*. Realized heritabilities of body weight for both the high and low lines were 0.08 and 0.03, respectively. This lack of response to selection was probably due to depleted genetic variability in the breeding stocks. Only fifty-six fingerlings were introduced from Japan in 1966 (Liao and Chen 1983), all obtained from the same fish farm in Japan. Inbreeding or genetic drift may have reduced the genetic variation in the population.

- **Loach**

Loach, *Misgurnus* spp., is widely distributed in temperate freshwater areas, especially in mainland China. Hybridization has been found among various species. The endemic male loach is smaller than that of the female

and the growth rate usually decreases after maturity. The average body weight and length of endemic adults is only about 50g and 15cm, respectively.

Successful selection for growth rate was carried out in a private hatchery (Su 1991). One giant male was found among the reared loach and was used as a breeding spawner. Through four generations of selection, growth rate and body size of both sexes was significantly improved. A body weight of 200g and body length of 30cm was achieved. Although body size and growth rate improved, inbreeding depression was found after several generations. Evidence of this was in the increasing incidence of golden loach and poor survival rate in early development. This was attributed to the small number of spawners used for breeding and the use of artificial fertilization. However, the giant loach produced were found by consumers to be less tasty than the smaller endemic loach.

Selection for Body Color in Red Tilapia

Red tilapia was first found on a private farm in 1968 and it was concluded that this red tilapia was a mutant albino from *O. mossambicus*. *O. niloticus* was crossed with these mutants to improve growth rate (Kuo 1978). Through yearly selective breeding, the hybrid acquired a glorious reddish color. This was in contrast to the original red tilapia that had large black spots on the skin. Three types of progeny were produced: black, red and white. Survival rate of the white tilapia was quite low due to an abnormality of the swim bladder.

Huang et al. (1988) reported that the body coloration of red tilapia was inherited as a single gene with incomplete dominance. The different colored F_1 progeny from red tilapia, crossed with *O. hornorum*, *O. aureus* and *O. niloticus* gave very different sex ratios. One hundred percent male red tilapia progeny were produced using a female red tilapia crossed with male *O. hornorum* x *O. aureus* hybrid. The other colored progeny gave 88.4-91.6% males (Kuo and Tsay 1984). The growth rate of the red tilapia progeny improved when red tilapia was crossed with *O. hornorum*, *O. niloticus* and *O. aureus* in a three-way cross and reciprocal cross (Kuo and Tsay 1984, 1985, 1986, 1987, 1988, 1989). The expression of pigmentation of red tilapia varied with age and was affected by the environment. To fix the coloration of red tilapia selective breeding of red tilapia needs further study.

Chromosome Manipulation

- **Polyploidy**

Triploidy is considered valuable in aquaculture because it provides functional sterility. Functional sterility occurs when the pairing of chromosomes during Meiosis I is impeded by the presence of three homologous chromosomes, resulting in the uneven or aborted separation of homologous chromosomes. Sterile fish are advantageous in aquaculture because fish population size in the pond can be controlled and non-indignous species can be introduced, which is a benefit to ecological concerns.

Chang and Liao (1993) reported 100% triploidy in blue tilapia (*O. aureus*) using heat shock treatment three minutes after fertilization. About 20% of the second polar body was released about five minutes after fertilization. It was completely released after seven minutes when the embryos were incubated at 30°C. The growth rate of the

triploids was not significantly different from the diploid fish after a twenty-four-week growth test. However, the gonadosomatic index of triploids was significantly different from twenty-four-week-old diploid fish. The difference in growth rate between male and female triploids was lower than in male and female diploids. Through a histological study, some testis developed. Ovaries of triploid fish are like threads and have very few mature eggs. A histological study showed that most oocytes are oogonium (Chang et al. 1993).

There have been several studies in Taiwan that have induced triploidy in loach (*Misgurnus anguillicaudatus*) (Chao et al. 1986; Chao et al. 1993). One hundred percent triploid loach can be produced by cold shock of 1°C, lasting for 30-40 minutes and starting 5 minutes after fertilization. In common carp (*Cyprinus carpio*), 66.6, 80.4, 86.2 and 86.6% triploids were produced in the groups treated at 1°C for 30 minutes starting 1-3 minutes after fertilization.

- **Gynogenesis**

Inducing gynogenesis is a special form of parthenogenesis in which diploid eggs are activated by degenerated spermatozoa. It is suggested that this technique will become a method for rapid development of inbred broodstock. In Taiwan, gynogenesis has been induced in rainbow trout (*Salmo gairdneri*) (Liu et al. 1993) and loach (Chao and Liao 1990).

- **Biotechnology/Genetic Engineering**

Microinjection and electroporation are the most common methods used to produce transgenic fish. In a study on loach by Tseng et al. (1992), the cDNA of salmon growth hormone was carried by sperm after electroporation and fertilization of the eggs. After examining 180 fifteen-day old fry with a radiated DNA fragment, results showed a 40% positive reaction. Transgenic fish can be successfully obtained by this method. This is especially true for fishes with high fecundity and hard and opaque egg membranes, where observation of the nuclear position is hampered.

Conservation

- **Cryopreservation**

Cryopreservation is a convenient way to preserve the gene pool of males for long periods of time. However, the preservation of oocytes by cryopreservation has not been established. In Taiwan, several studies on cryopreservation have been conducted (Chao 1982, 1991; Chao and Liao 1987; Chao et al. 1986, 1987, 1992) (Table 5).

Stock Enhancement and Conservation Issues

Massive releases of hatchery-reared fry into the wild is often practiced to increase fishery resources. In Taiwan, which is facing a depletion of fishery resources, the study and practice of stock enhancement has been carried out for species such as, Japanese eel (*Anguilla japonica*), grey mullet (*Mugil cephalus*), black sea bream (*Acanthopagrus schlegeli*), brook masou salmon (*Oncorhynchus masou formosanum*) and sweetfish (also known as ayu) (*Plecoglossus altivelis*). The recovery rates of these release programs, however, have been very low, making any evaluation inconclusive.

- **Eel**

Hatchery-reared eels have not been successfully produced in captivity to date. Eel seed is still entirely supplied from wild-caught

Table 5. Status of research on cryopreservation of fish sperm in Taiwan (Chao 1991.)

Species	Experiments on				Proof of Motility After Preservation		Fertility Test in		Fertilization Rate (%)[1]	
	S.C	E.S.	O.C	F.R.	S. T.	L. T.	L	H	S.F.M.	C.F.M.
Mugil cephalus	•	•	•	•	•	•	•	•	64.9	23.6
									38.9	47.4
									48.9	38.4
Acanthopagrus schlegeli	•	•	•	•	•	•	•	•	91.5	96.0
									77.4	62.3
									99.0	98.0
									71.4	84.5
Epinephelus malabaricus	•	•	•	•	•	•	•	•	95.7	93.8
									93.2	65.6
									85.0	65.6
									93.3	91.9
									84.6	91.9
Oreochromis aureus	•	•	•	•	•					
O. mossambicus	•	•	•	•	•	•				
O. niloticus	•	•	•	•	•					
O. niloticus x *O. aureus*	•	•	•	•	•	•	•		72.7	85.7
O. sp. (red tilapia)	•	•	•	•	•	•	•		93.4	90.0
Tilapia zillii	•	•	•	•		•	•			
Chanos chanos	•	•	•	•	•					
Siganus oramin	•	•	•	•	•	•				
Plecoglossus altivelis	•	•	•	•	•					
Lateolabrax japonicus	•	•	•	•	•					
Misgurnus anguillicaudatus	•	•	•	•						
Boleophthalmus chinensis	•	•	•		•					
Micropteru salmoides	•	•	•	•						

[1]Only samples with good results are shown.

S.C. = Sperm characteristics; E.S. = Extender selection; O.C. = Optimal cryoprotectant; F.R. = Freezing rate; S.T. = Short term; L.T. = Long term; L = Laboratory; H = Hatchery; S.F.M. = Satisfactory frozen milt; C.F.M. = Control fresh milt.

Table 6. Quantity of adult Japanese eel, *(Anguilla japonica)*, released near its assumed spawning ground in Taiwan, 1976 - 1991[1] (from Liao et al. 1993).

Year	Kg[2]	No.[3]
1976	1508	3393
1978	1548	3484
1979	500	1125
1981	2000	4500
1982	300	675
1983	1000	2290
1986	1000	2250
1987	1200	1440
1988	1200	2050
1989	1200	1474
1990	2700	3857
1991	1748	3905

[1]No releasing was conducted in 1977, 1980, 1984, and 1985.
[2]Total body weight.
[3]Number of adult eel.

elvers or fry. Demand for seed is high due to the depletion and fluctuation of wild fry resources. Since 1976, several tons of eel have been released near the assumed spawning ground to increase eel fry resources (Liao et al. 1993). In recent years, hormonal pellets have been implanted into the released eels. The number of eels released in the past fifteen years is listed in Table 6. Although there is no scientific data to support the effectiveness or efficiency of eel stock enhancement, fishermen and fry collectors have acknowledged the program's significance and effectiveness--they felt that the fry supply has increased after the release of adult eel (Personal communication, 1993).

- **Grey Mullet**

Grey mullet migrates annually from December to January to spawn in southwestern Taiwan. However, the availability of grey mullet stock is not assured because its maturity performance is quite different in captivity. The migratory strain matures at three years, while the local strain matures at two years of age. The migratory route in the whole life cycle of grey mullet is unknown. Thus, thousands of tagged juvenile mullet have been released in Dapong Bay in southwestern Taiwan to investigate its migratory route (Kuo, unpublished data).

- **Black Sea Bream**

Black sea bream (*Acanthopagrus schlegeli*) is a coastal marine fish with a limited migration. Due to its limited migration stock enhancement may be useful in increasing the available stocks of this species. Thousands of tagged hatchery-produced fry have already been released to study its migratory route (Kuo, unpublished data).

- **Brook Masou Salmon**

Brook masou salmon (*Oncorhynchus masou formosanum*) is an endemic coldwater fish that inhabits the Tachia River in the western part of central Taiwan, where the water temperature is below 16°C. It is closely

related to *O. masou*, which is distributed in northern Japan. Brook masou salmon has generated interest mainly because of its applicability to the study of biogeography and because overfishing has lead to the serious depletion of its resources. The hatchery production of brook masou salmon has been carried out since 1985 (Yu et al. 1987). The government and private sector have joined in releasing cultured fry to recruit its resources. Evaluation of the effectiveness of these releases has been difficult because the brook masou salmon is a main source of foodfish among the aborigines who live within the release areas.

• **Sweetfish**

Sweetfish (*Plecoglossus altivelis*) is a migratory coldwater fish distributed in eastern Asia. In Taiwan, it inhabits rivers in the western part of central to northern Taiwan (Chen 1986; Hsiao and Mak 1978). Sweetfish spawn in brackishwater areas during winter and the fry migrate upstream in freshwater areas to grow. In Japan and Taiwan, it is widely cultured and popular as foodfish for its tasty meat. Due to overfishing and pollution of its habitat, its natural population has been extinct in Taiwan since 1967.

In 1981, the Japanese Fishing Society presented one million fertilized eggs to the Taiwan Fisheries Research Institute through its Chupei Branch. About 50,000 fry survived, which were subsequently released into the wild (Peng et al. 1982). Since then, several stock enhancement programs had been undertaken by some government agencies, religious groups and the Taiwanese Fishing Society. Sweetfish has now been reported in some rivers and dams (Shen 1992, 1993; Suzuki 1993). Shen (1992, 1993) reported that sweetfish was found to be breeding and comprising 2.0-2.8% of the total fish population within the Feitsui Dam in northern Taiwan. The quick recovery of the sweetfish population in several dams in Taiwan has been attributed by Suzuki (1993) to the abundant minerals, plankton, and protozoa and higher winter water temperatures in the dams. Sweetfish has now become landlocked in Taiwan, as in the case of brook masou salmon.

Problems

• **Inbreeding**

Due to the high fecundity of most fishes, only a few spawners with superior traits are used as breeders. Inbreeding may occur due to the breeding method adopted during artificial fertilization. Thus, inbreeding depression in aquaculture has become a common phenomenon. Fry produced from a small number of breeders poses the obvious risk of reducing the total amount of genetic diversity.

In freshwater fish, inbreeding depression can be serious because most fry are produced by artificial fertilization and are from a small number of breeders. In addition, exotic cultured fish are usually imported in small numbers, thus, the broodstock are also susceptible to inbreeding depression. In most marine fishes, however, inbreeding depression is not serious because natural spawning can be performed in captivity. Most marine fishes mate randomly and breed for many years and can, therefore, minimize the inbreeding level of succeeding generations. For example, milkfish spawners are reared from wild fry. Moreover, their spawning period can last more than ten years from breeding age and they mate at random. Schom and Bailey (1986) suggested that inbreeding can be

minimized if forty to fifty spawners are chosen at random in each generation. For the commercial production of marine fry in Taiwan, more than fifty spawners are usually stocked in breeding ponds.

Introduction of Exotic Fishes

Due to the limited variety of endemic freshwater fish species and for economic reasons several species have been intentionally introduced into Taiwan for aquaculture.

- **Tilapias**

Tilapias are the most well-known of the exotic species in Taiwan. The annual production of tilapias reached a record 100,000 tons in 1986 (Liao and Shyu 1992). Tilapias have provided the main source of cheap and readily available animal protein for the Taiwanese since World War II. However, due to their prolificacy, territorial behavior and strong resistance to pollution, tilapias have become the dominant fish to survive in rivers, reservoirs, drainage systems, estuaries and saline lagoons. Estuaries are the nursery of most marine fishes in their early developmental stages. Due to tilapias' carnivorous feeding habits as juveniles, the existence of abundant tilapias in these habitats endangers both endemic freshwater and marine fishes. For example, Dapong Bay, which is located in southwestern Taiwan, abounds with tilapias even when it becomes hypersaline during the dry season. These tilapias are mostly *O. mossambicus* and its hybrids. The existence of tilapia stocks may pose a serious threat to the fish resources in the bay.

The introduction of exotic fish has been getting much attention in recent years, particularly due to its detrimental effect on endemic fishes. This issue has created a dilemma for those governments interested in introducing exotic fishes for increasing fish production and conservation. Tilapias have been viewed by some countries as a nuisance, noxious and feral, but the worldwide spread of tilapias is still proceeding at a rapid pace. For example, despite the severe penalties for the introduction of exotic fishes in Australia, tilapias have been reported to be spreading widely in Australia after being introduced by the ornamental aquaria fish trade (Mather and Arthington 1991). The depletion of milkfish resources in the saline lagoon of Buada in Nauru has been attributed to the invasion of tilapias (unpublished official communication between the government of Taiwan and Nauru). In Taiwan, the introduction of tilapias is intentionally conducted by the government and private hatcheries. Although tilapias have become the most successfully cultured exotic species in Taiwan, overproduction and the general perception that animal waste is being used for tilapia feed has depressed prices. Smaller tilapias harvested from drainage and culture ponds have become the main feed for cultured high value marine fishes, particularly grouper.

- **Grouper**

The culture of grouper has become popular in Taiwan in recent years. Two species, *Epinephelus malabaricus* and *E. suillus*, are preferred by growout farmers because they have attributes that are suited for aquaculture. However, the current fry supply is inadequate to meet the demands of growout farms. This is due to the lack of established larval rearing techniques. Thus, grouper fry are imported from Thailand, the Philippines, Indonesia and Sri Lanka. The species of these imported fry are difficult to identify. Besides the possibility of introducing diseases into Taiwan, some fry could be se-

lected as broodstock by hatcheries and hybridized in ponds with local species by induced spawning.

• **Other Exotic Fishes**

Other exotic fishes, such as largemouth bass (*Micropterus salmoides*), hybrid striped bass (*Morone saxatilis* x *M. chrysops*) and silver perch (*Bidyanus bidyanus*) have important roles in freshwater culture in Taiwan. These exotic freshwater fishes were introduced into Taiwan for their fast growth, good appearance, high meat quality and ability to feed at lower temperatures. In winter, growth is retarded in most endemic fishes due to lower water temperatures. Some species, such as milkfish (*Chanos chanos*) and permit fish (*Trachinotus falcatus*), cannot tolerate the low water temperatures during winter, particularly below 14°C. These exotic fish are also preferred in sport fishing ponds.

Due to economic reasons depleted and polluted freshwater fauna, the introduction of high value freshwater fishes has been undertaken. In fact, most of the cultured freshwater fishes in Taiwan are exotic fishes that have become domesticated. The decision to introduce marine fishes, however, should be made judiciously due to the existence of endemic species and their unpolluted habitat. There is the potential for invasion of exotic marine species to have a detrimental effect on the endemic fish populations.

• **Ornamental Fishes**

The ornamental fish trade in Taiwan has grown considerably within the past two decades. A variety of ornamental fish has been imported from Africa, Latin America and Southeast Asia. Some ornamental fishes have shown potential as food fish, such as freshwater grouper (*Cichlasoma managuense*), an endemic fish of Central America. This grouper is now cultured widely in southern Taiwan, particularly in Pingtung County.

Although the majority of ornamental fishes in the world are bred in hatcheries, a significant number may still be caught from the wild. An active ornamental fish trade may also pose a threat to the wild stock of the exporting countries. On the other hand, those species with strong, aggressive and territorial behavior can endanger the survival of endemic fishes in the importing countries. In Taiwan, exotic tropical ornamental fishes have not yet been reported to inhabit rivers due to the lower water temperature during winter. However, for the sake of aquatic animal conservation, not only in Taiwan but also in other countries, laws and regulations including severe punitive measures to control the trade of ornamental fish are needed.

Conclusion

Genetic research, aquaculture and conservation are interrelated. For example, hybridization is used to highlight traits, such as hybrid vigor and special external features, and are important to the profitability of aquaculture. Selection of available traits has potential in aquaculture due to the large genetic variance and high fecundity of fishes.

Hybridization for the purpose of increasing profitability in aquaculture can also endanger natural fish populations if the hybrids inadvertently escape into the wild. With selective breeding, a well-selected fish will have

a narrow genetic variation, which is unsuitable for stock enhancement. Stock enhancement by releasing hatchery-reared fry to replenish extinct or depleted wild fish stocks may also lead to genetic drift if fry are produced from only a few spawners. There is a need for long-term investigation of genetic variation in wild stock after stock enhancement is undertaken.

Advances in transportation and communication technology in the late 20th century have made activities between countries commonplace. Fast and convenient transportation coupled with fast and efficient communications have virtually "shrunk" the world, opening new horizons to activities such as the expansion of trade of one country's endemic aquatic animals to other countries. In addition, overfishing and pollution has threatened wild fish stocks of many countries. Conservation of endemic and extinct fishes has thus, become a major concern of these countries. Laws regulating exportation of threatened endemic stocks should be enacted, which should be coupled with a strong enforcement policy. To recover wild stock resources, an emerging and important trend is stock enhancement, as the Taiwanese experience in the recovery of sweetfish stock has exemplified. Any enhancement program, however, should be undertaken judiciously. Before it can be undertaken, for example, techniques for broodstock management, artificial propagation and nursery management should first be well established. Furthermore, the impact of hatchery-reared fry on wild stock populations should be assessed, particularly its impact on the gene pool. A stable wild fish stock gene pool is an invaluable broodstock resource for aquaculture, not only for the generations of today but also for the generations of tomorrow.

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A Review of the Fish Breeding Research and Practices in Indonesia

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Abstract

Common carp (*Cyprinus carpio*) is the most popular cultured freshwater fish in Indonesia, especially in West Java. However, due to an assumed lack of knowledge about broodstock management and selection procedures, the current seed stock raised by fish breeders tends to grow slowly. Common carp was introduced to Indonesia several hundred years ago, but prior to that time, fish breeding had been practiced by farmers. Some farmers selected broodstock from private stocks and, though selection techniques were not well known, still grew improved fish. It has been reported that the growth rate of cultured common carp in Indonesia tends to decrease from time to time, possibly as a result of inbred traditional farm stock. Therefore, the Research Institute for Freshwater Fisheries (RIFF), in collaborative selection programs with International Development Research Centre of Canada (IDRC-Canada), has implemented a genetics project to solve the inbreeding problem. RIFF also conducts selective breeding experiments to improve other species such as tilapia and freshwater giant prawn.

Introduction

Aquaculture is becoming an increasingly important source of protein for the Indonesian population. Fish are highly nutritious, considered a traditional food and very efficient converters of feed into high quality protein. Development of sustainable aquaculture to produce the necessary quality and quantity of seed is urgently needed.

In Indonesia, there are more than 650 freshwater fish species. These are predominantly cyprinids, which account for 196 (Schuster 1950), and there are about 10 freshwater species usually bred and cultured in ponds for consumption. The latter include common carp (*Cyprinus carpio*), Java carp (*Puntius gonionotus*), mata merah (*Puntius orphroides*), giant gouramy (*Osphronemus gouramy*), kissing gouramy (*Helostoma temminckii*), Java tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), nilem (*Osteochilus hasselti*), snakeskin (*Trichogaster pectoralis*) and walking catfish (*Clarias batrachus*).

In six freshwater species, river eel (*Anguilla bicolor*), freshwater eel (*Fluta alba*), patin catfish (*Pangasius pangasius*), jelawat carp (*Leptobarbus hoeveni*), sleeper gobies (*Oxyeleotris marmorata*) and kapiek (*Puntius belinka*), the seed is commonly obtained from wild stocks.

Some species will breed in ponds (i.e., sleeper gobies, jelawat carp and patin catfish) following the use of the hypophysation technique or induced spawning (injecting pituitary hormone). Before this technique was used, seedstock were collected from domesticated ponds and the wild.

Three introduced fish species, Thai catfish (*Pangasius sutchi*), grass carp (*Ctenopharyngodon idellus*) and silver carp (*Hypophthalmychthys molitrix*), have been successfully bred by injecting pituitary hormones.

Other species found and caught in open water are salted or sold as ornamental fish. These include Asian barb (*Puntius spp.*), snakehead (*Ophiocephalus* spp.), glass catfish (*Kryptopterus spp.*), jambal catfish (*Pangasius spp., Wallago spp., Macrones spp.*), toman (*Ophiocephalus micropeltus*) and knife fish (*Notopterus spp.*) (Suseno and Djajadiredja 1980).

Besides food fish, there are many indigenous and exotic species of ornamental fish (Soejanto 1967). *Botia* spp., *Resbora* spp. and *Bubis* spp. are commonly caught from open water. Imported exotic species include discus (*Symphysoda* spp.), oscar (*Astronotus ocellatus*) and menfish (*Pterophylum scalare*). Some are bred in ponds, while for others, fish breeders try to select for color, shape, improved performance and market value. (Directorate General of Fisheries 1989).

Problems with Traditional Culture Techniques

Very often fish exhibit low growth rate, early maturity, poor survival rate, low resistance to stress and diseases and early spawning. Common carp for instance, which normally mature around 10 to 22 months (Hardjamulia and Suseno 1976) have been observed to mature at 7 to 9 months of age (Ardiwinata 1953). Early spawning may interfere with normal fish growth because when fish spawn at small sizes, the average growth rate of the offspring will be less than their parents.

Dharma et al. (1986) had observed some examples of common carp in certain areas in West Java and reported that high inbreeding rates occurred because the farmers did not have a good understanding of broodstock management. Farmers only had a small number of broodstock, usually less than 10 pairs. This situation is very serious because the effective breeding number (N_e) will be small and the inbreeding rate will be high (Kincaid 1983).

In the case of Nile tilapia (*Oreochromis niloticus*), it is hard to maintain a pure line. It crosses easily with Java tilapia (*Oreochromis mossambicus*) and the hybrids are found in most culture ponds. The growth rate of the hybrids is lower than is seen in Nile tilapia.

Genetic Improvement Project Strategy and Institutional Arrangements

Genetic improvement is a powerful means to increase productivity, growth and survival rates. Improved breeds exhibit increased growth and survival. The project involves several types of activities, such as the collection and evaluation of local strains, manipulation of chromosomes through induced gynogenesis and polyploidy, hybridization, genetic engineering and importing pure lines of fish (Fig. 1). The objective of all these activities is to obtain a high quality of broodstock and to produce superior seed.

The Research Institute for Freshwater Fisheries (RIFF) is the primary institution implementing aquaculture genetic projects. The project is funded by the Government of Indonesia (GOI) and the International Development Research Centre of Canada (IDRC-Canada). RIFF is responsible for the production of parent stock, improvement of breed-

ing techniques, and for the development of fish breeding and selection, hybridization and gynogenesis. High quality broodstock and fish breeding techniques will be disseminated from RIFF to the Freshwater Aquaculture Development Centre in Sukabumi, West Java, where parent stocks will be produced and breeding and selection techniques will be verified. Verified breeding techniques and high quality broodstock will be disseminated to provincial hatchery centres, local or regional hatchery units and to farmers' hatcheries. Improved seed stock produced by the provincial and regional hatcheries may also be used for restocking programs.

Research Activities in Genetic Aquaculture

A pioneering collaborative research project on genetics in aquaculture is currently being funded by IDRC-Canada and the GOI, and is being executed by RIFF through the Freshwater Aquaculture Development Centre. Since 1985, the primary objective of the project has been to improve common carp breeds through collection, evaluation and selection of local strains. The same objectives have been applied to tilapia since 1990.

Interspecies and intraspecies hybridization have been conducted on common carp strains such as Majalaya, Taiwan and Sinyonya (Table 1) (Suseno et al. 1980; Suseno et al. 1986) and manipulation of the chromosomes through induced gynogenesis and polyploidy has also been initiated. Both hybridization and gynogenesis activities are funded solely by the GOI (Gustiano et al. 1987).

Two pure lines of *Oreochromis niloticus* and red tilapia (called red NIFI) were introduced from Thailand in 1989 and the GOI now conducts experiments using these fish species. An evaluation of some characteristics of Nile tilapia is as follows:

In 1989, two strains of tilapia were imported from Thailand, red tilapia (called red NIFI strain) and black Nile tilapia (called Chitralada strain). Both strains were compared with three local strains of Nile tilapias from Bogor, Sukabumi and Cianjur areas. The result showed that red NIFI has a better growth rate than the others (Widiati and Sudarto 1992). The fish are now being evaluated, and are expected to improve local tilapia populations in most Indonesian fresh water bodies that have already decreased perform-

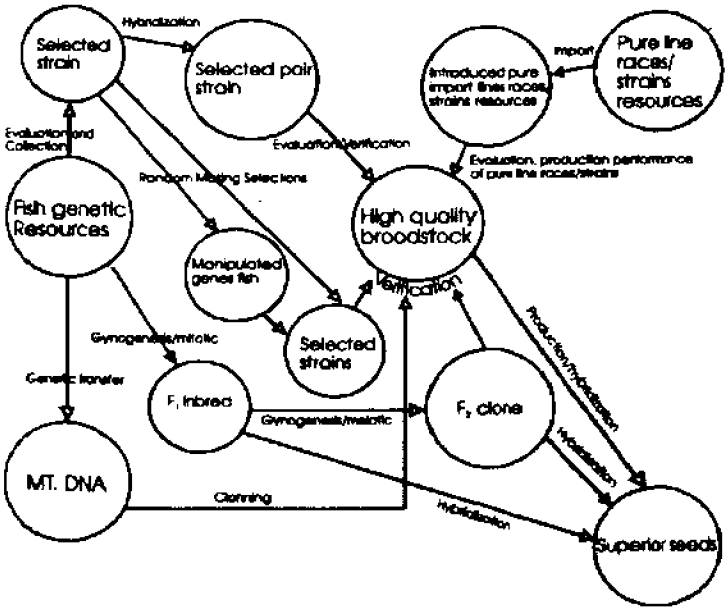

Figure 1. Network of Aquaculture Genetics.

Table 1. Intra- and interspecific hybridization of freshwater cultivated fish.

No.	Races/strains crossed		The best hybrid (in terms of growth)	Source
1.	Intraspecific crosses of common carp (*C. carpio*):			
	Majalaya	x Taiwan	Majalaya (male) x Taiwan (female)	Suseno et al. 1980
		x Sukabumi		
		x Bogor		
	Punten	x Taiwan	Taiwan (male) x Punten (female)	Suseno et al. 1983
	Sinyonya	x Majalaya	Majalaya (male) x Sinyonya (female)	Suseno et al. 1986
2.	Interspecific crosses:			
	Common carp	x Java carp	Java carp (male) x common carp (female)	Gustiano et al. 1988

ances due to introgression of Java tilapia (*Oreochromis mossambicus*). Through this activity it is expected that new improved breeds of *Oreochromis niloticus* will be produced. Selection of red tilapia was started in 1993 to produce red strains, which have better growth rates and will be used for export purposes.

Interspecific and intraspecific hybridization of common carp has been performed on several races to produce hybrids that have the best growth rates. The best rate was found in a cross of Majalaya (male) x Taiwan (female) (Susenso 1980). Two strains of punten and Taiwan common carp were also hybridized and the best growth rate was seen in Taiwan (male) x Punten (female) (Suseno et al. 1983). Of the local races (Sinyonya x Majalaya) that were hybridized, Majalaya (male) x Sunyonya (female) showed the best growth rate (Suseno et al. 1986).

Interspecific hybridization was also conducted using common carp and Java carp. The best growth rate was found in a cross of Java carp (male) x common carp (female) (Gustiano et al. 1988).

Results of Genetic Experiments

Preliminary results of growth rates of different common carp reared under controlled conditions in Jatiluhur reservoir varied significantly. Biomass also differed significantly between different strains (Sudarto et al. 1990).

- **Morphological differences in various strains of geographically isolated Indonesian common carp (Nugroho et al. 1990):**
  - > Characteristics selected for Indonesian common carp include growth rate, morphology, effect of brood stock management, various color and body conformations and selection intensity in rice fields. The results showed that there was no significant differences between strains, but there were differences due to the sex of the Indonesian carp.

- **Genetic differences in various strains of common carp and the effect of brood-stock management (Matricia 1990):**

  > Growth rate and body conformation (shape) or truss morphometrics of different strains of common carp were compared under field condition on 14 farms in West Java, East Java, West Sumatra and North Sumatra. Statistical analysis of growth rates showed a significant difference between farms: Strains from West Sumatra have the fastest growth rate. Statistical analysis of morphological differences in various strains of common carp indicated that body shape, in terms of body depth over length, varies significantly between strains. This result suggests that morphological characters may be considered as genetic markers for genetic improvement of common carp in Indonesia.

- **Selection intensity on common carp (*Cyprinus carpio*) in rice-fish ponds was affected by differences in color of common carp:**

  > The results showed that there was a significant difference between the two color types, where the dark type (green and blue) survived better than the light type (red and yellow). Due to this finding the farmers were instructed to use dark colored common carp to increase their harvest in rice-fish ponds where many predators are present (Sudarto 1991).

- **Selection of local common carp collected from various isolated areas in Indonesia (Nugroho et al. 1991):**

  > In this experiment, the different strains were ranked hatching rate, survival rate, growth rate and disease resistance during the first growth stage (3-5 cm in size). The first four top-ranked strains were Sutisna-Kuningan, Cianjur-Wildan, Rajadanu-Kuningan and Yogyakarta.

- **Study of pigment cell and color inheritance in common carp:**

  > Identification of genetic color polymorphism on Indonesian common carp was conducted (Gustiano 1991). Indonesian common carp has eight pure color types: white, red, orange, yellow, green, blue, black and gray. Black carp have two belly colors: white and yellow. Each color also has its own characteristic shape, abundance and intensity of chromatophores.

  > Melanophore composition content and inheritance on green and blue common carp was examined. Melanophores of native green common carp are more dendritic and less intense than those found in blue carp. These melanophore characteristics can be modified to differ from the native fry, the progeny of green x yellow female and blue male x yellow female (Gustiano 1990).

  > Color inheritance of common carp: Ontogenic studies were conducted on fry that were bred from eight color variants of Indonesian carp. Early in the first stage, one to three months, most of the fry showed two colors (bright or white, and dark or blue color). By the age of four months the colors changed to be-

come permanent. The brightly colored carp became white and yellow while the darker colored carp became blue and green (Gustiano 1990).

Conclusion

For wild species whose natural populations are decreasing, (Kristanto et al. 1992, paper in press) domestication has been in operation since 1990 (Sumastri et al. 1992, paper in press) and has produced fry through induced spawning. In the case of jelawat carp, the experiment was conducted in farm conditions. Selected farmers and extension officers worked together on the project and traditional farmers were encouraged to exchange their traditional breeding practices for new technologies. On the other hand, the success of breeding keli catfish in captivity is expected to improve the number of this species in nature because farmers will no longer collect fish from the wild.

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A Review of the Fish Breeding Research and Practices in Vietnam

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Abstract

In 1992, Vietnam produced over 350,000 tons of fish from aquaculture, of which a considerable part was freshwater fish production. Despite this success, Vietnamese fish culture is now facing many problems, especially around the deterioration of genetic quality in a number of cultivated fish species. The first genetic improvement programs of cultured fish species were done on carp and tilapia. Success has only been achieved on the commercial hybridization of common carp, however, positive results from long-term selective breeding programs were also obtained. Presently, three stocks of fifth generation selected hybrid common carp are being maintained. Experiments were performed on one stock to determine the realized heritability of body weight. They showed that mass individual selection could be effective. A selective breeding program for silver carp has not been done. The first step will be to obtain pure lines of Vietnamese and Chinese silver carps that have been uncontrollably mixed since the 1960s.

Introduction

Over 56,000 ha in small ponds and lakes, 394,000 ha in man-made lakes and reservoirs, and a part of 544,000 ha of rice fields are used for freshwater fish culture in Vietnam. According to Ministry of Fisheries, data reports 351,260 tons of fish were produced by aquaculture in 1992. A considerable portion was obtained from freshwater fish culture. The important cultured fish species in Vietnam are Asiatic carps, which include common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix* and *H. harmandi*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), mud carp (*Cirrhinus molitorella*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), puntius (*Puntius gonionotus*) and some species of *Clarias*, *Pangasius* and tilapia (*Oreochromis mossambicus* and *O. niloticus*). Total annual fish seed production is 4 billion larvae, fry and fingerlings. This production has fulfilled the demand for stocking in Vietnam.

Culture fisheries in Vietnam are now facing many difficulties, including feed and infectious disease problems. The genetic quality of a number of cultivated fish species has been deteriorating since the late 1960s. It was therefore necessary to carry out fish breeding research programs to enhance aquaculture productivity. In the 1970s, genetic research focused on hybridization for commercial production. Selective breeding research programs for cultivated fish species, such as common carp, silver carp and tilapia have been conducted since the 1980s.

Commercial Hybridization

- **Preliminary research programs on interspecific crosses of carp and tilapia**

Early crosses between female bighead and male silver carps revealed that hybrid viability in the fry and fingerling stages of the hybrid was better than in pure silver carp. However, the growth rate of these hybrids in all the experiments was less than that of silver carp. It was concluded that this hybrid is not profitable for culture (Thien and Tuong 1983).

The tilapia, *Oreochromis mossambicus* and *O. niloticus*, were introduced to Vietnam in 1951 and 1973, respectively. They became a popular species to culture due to their adaptability and ability to thrive in different bodies of water. Since they breed naturally throughout the year in warm water, it is difficult for the fish farmers to control tilapia populations in ponds. High stocking densities and inadequate food supplies in ponds have resulted in low growth rates, small marketable size, low production yield, and low market value.

In 1978, an experiment comparing the growth rate of two species of tilapia revealed that the Nile tilapia grew nearly twice as fast as the mossambica tilapia. The hybrids obtained by crossing these species grew moderately in comparison with their pure sibs (Fig. 1). The hybrid obtained from a cross between female Nile tilapia and male mossambica tilapia grew better than the hybrid obtained from a reciprocal cross.

The sex ratio of the hybrid populations was interesting. In the case of crossing female *O. niloticus* x male *O. mossambicus*, 71% - 80% of the hybrids were males. In a reciprocal cross (female *O. mossambicus* x male *O. niloticus*), only 27% - 32% of the hybrids were male (Thien 1983). Unfortunately, the reasons for these results were not clear. For fish culture in Vietnam it was considered better to use the pure Nile tilapia or its hybrid by crossing female Nile tilapia x male mossambica tilapia rather than using *O. mossambicus*.

- **Intraspecific crossing of common carp**

In Vietnam, eight varieties of local common carp were investigated, of which white carp - a variety with high viability - is the most popular (Trong 1983). However, white carp and the other varieties of Vietnamese common carp exhibited slow growth rates and early maturity. Attempts to determine the effectiveness of heterosis by crossing these varieties were not successful. Hungarian mirror carp was introduced to Vietnam in 1970 and scale carp was introduced in 1975. The Hungarian carp showed fast growth and proper maturity, but was susceptible to disease and possessed low viability.

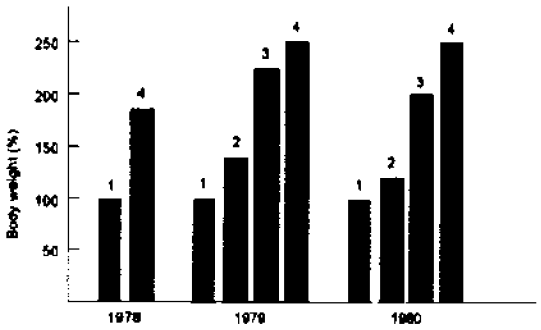

Figure 1. Comparison of growth between *Oreochromis mossambicus* (1), *O. niloticus* (4) and their hybrids: ♀ *O. mossambicus* x ♂ *O. niloticus* (2) and ♀ *O. niloticus* x ♂ *O. mossambicus*.

Table 1. Survival percentage[1] of fry and fingerlings of Vietnamese common carp (V), Hungarian common carp (H) and their reciprocal hybrids (VH, HV).

Stage	Crossing	1974	1975	1976
Fry	V	51.6 ± 0.21	--	71.2 ± 0.23
	VH	61.6 ± 0.20	70.0 ± 0.17	80.0 ± 0.16
	HV	60.4 ± 0.20	44.3 ± 0.21	78.0 ± 0.17
	H	22.3 ± 0.18	40.0 ± 0.20	37.6 ± 0.24
Fingerlings	V	85.9 ± 9.4	--	78.3
	VH	94.9 ± 1.90	76.2 ± 2.9	90.0 ±3.3
	HV	81.4 ± 7.5	76.7 ± 2.3	73.0 ± 11.3
	H	45.7 ± 5.2	38.6 ± 2.4	46.3

[1]The mean of three repeated times (ponds) in each year (VH - ♀ Vietnamese x ♂ Hungarian)

The first hybrid generation (F_1) cross between Vietnamese white carp and Hungarian carp possessed the best characteristics from their parents, i.e., high survival rate, fast growth and nice appearance (i.e., big body and small head). The percentage of fry and fingerling F_1 hybrids that survived was much higher than that of Hungarian carp (Table 1, Fig. 2). The survival of Vietnamese carp was also higher than Hungarian carp and similar to the hybrids.

Hybrid carp grew much faster than Vietnamese carp in both the mixed culture (same ponds) and monoculture (separate ponds) (Fig. 3). In general, the growth rate of Hungarian carp was considered high, but in most experiments the growth rates of the hybrids were higher (Fig. 4).

The best productivity from our research program was obtained from raising hybrid carp. As a result, over 10 million hybrid carp larvae, fry and fingerlings have been provided to farmers annually, considerably augmenting the cultured carp production in Vietnam. However, due to improper breeding management, the base stocks of common carp were gradually losing their purity, thus decreasing the effectiveness of crossing for hybrids.

Selective Breeding of Common Carp

Since 1981, research programs have focused on selection of common carp with the intention of creating a fish breed with stable genetic qualities. In the first phase (1981 - 1985), the program focused on the assessment of initial materials for selection (Thien et al. 1987). To bring together a number of positive qualities from different hybrid varieties and to improve the genetic variability,

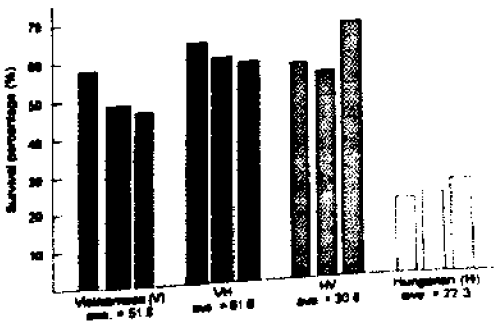

Figure 2. Survival percentage of fry from Vietnamese (V) and Hungarian (H) common carp and their hybrids.

Table 2. Representative data on the mass individual selection of the hybrid stocks of common carp.

Year and generation	Stocks	Total number of fish	Body weight, g	Indices collected through selection		
				Severity V, %	Intensity i=S/δ	Differential S,g
1986 F_1	♀ H x m (YxV)	400	162 ± 6	12.5	2.77	99
	♀ V x m (YxH)	400	178 ± 4	12.5	1.66	84
	♀ Y x m (HxV)	1720	187 ± 8	7.5	1.94	82
1988 F_2	♀ H x m (YxV)	248	152 ± 7	10.1	1.76	117
	♀ V x m (YxH)	258	104 ± 5	9.7	2.03	177
	♀ Y x m (HxV)	253	148 ± 9	9.9	1.60	164
1989 F_3	♀ H x m (YxV)	75	149 ± 8	33.3	1.25	52
	♀ V x m (YxH)	243	155 ± 12	32.9	0.80	62
	♀ Y x m (HxV)	74	310 ± 16	33.8	0.77	41
1991 F_4	♀ H x m (YxV)	200	260 ± 6	20.0	1.26	74
	♀ V x m (YxH)	209	197 ± 5	19.1	1.75	124
	♀ Y x m (HxV)	189	299 ± 6	25.9	1.24	47

V-Vietnamese, H-Hungarian and Y-Indonesian Yellow common carp.

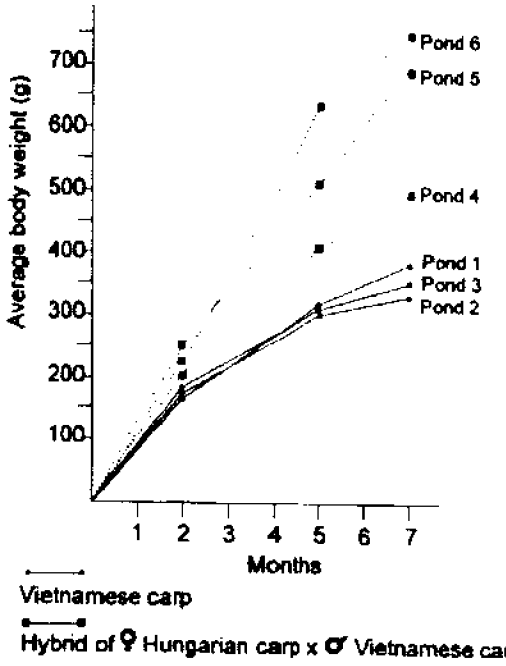

Figure 3a. Growth of Vietnamese common carp and a hybrid of Hungarian carp and Vietnamese carp by monoculture in separate ponds.

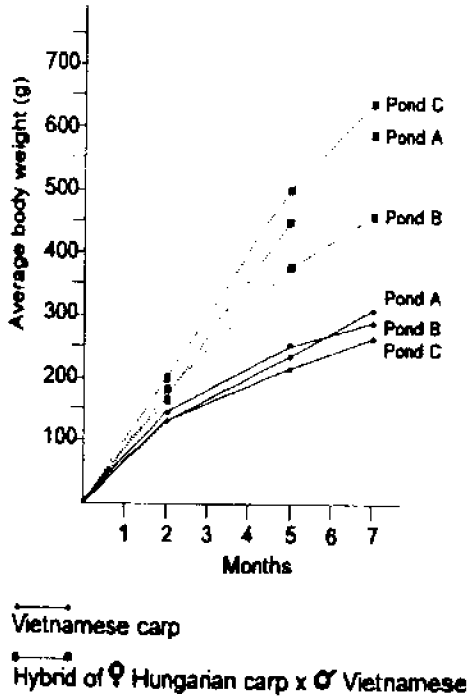

Figure 3b. Growth of Vietnamese common carp and a hybrid of Hungarian carp and Vietnamese carp by mixed culture.

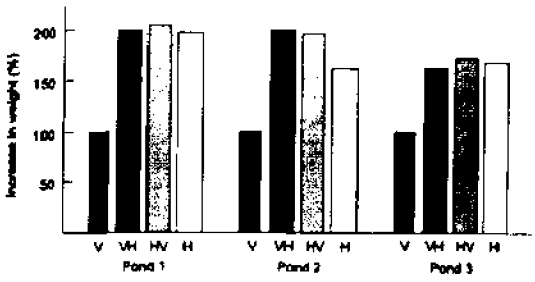

Figure 4. Increase in weight of Vietnamese carp (V), Hungarian carp (H) and their hybrids (VH and HV). Mixed culture in three growout ponds.

the Vietnamese white carp, the Hungarian scale carp and the Indonesian yellow carp were crossed. It was found that in the F_1 generation the main characteristics of hybrids depended on the rate of heredity from the pure varieties. However, in the F_3 and F_4 generation the differences in growth and morphology were no longer striking among the three hybrid stocks. This may have been caused by selection for the same traits through several generations.

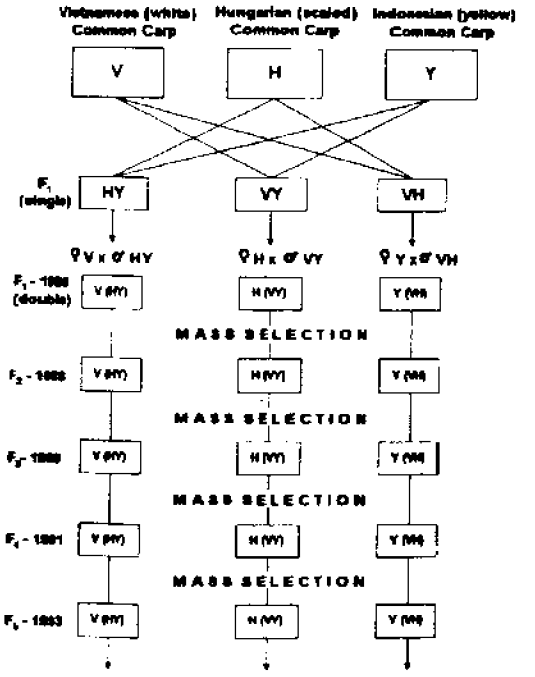

Figure 5. Mass selection of hybrid common carp.

In the second phase of the program (1986 - 1995) mass individual selection has been carried out among the three hybrid stocks over six generations (Fig. 5). Due to a limited number of ponds, the number of experimental fish in each stock was limited. Even though the scale of selection is small (Table 2), the indices obtained proved to be acceptable.

Preliminary assessment of selection effectiveness was made by determining the realized heritability of body weight (Fig. 6). Before mass selection was done, a randomly collected control population was kept. Another group was collected by selecting for body weight (experimental group). The offspring of the two groups were reared in the same ponds to a marketable size. In the 1988 experiments, the results were analyzed and adjusted according to the methodology of Wohlfarth and Moav (1972), because of the difference in body weight between two groups of fingerlings when stocked. The realized heritability (h^2) of body weight was 0.2 to 0.29 for hybrid common carps from a cross of female Hungarian x male (Vietnamese x Indonesian) (Table 3). These data showed that research on the effectiveness of mass individual selection with the hybrids could be accepted.

Experiment in Restoration of the Purity of Vietnamese Silver Carp

A native species of silver carp (*Hypophthalmichthys harmandi*) was investigated in Vietnam. In 1956, the Chinese silver carp

Table 3. Heritability (h^2) of the body weight of hybrid common carp ♀ Hungarian x ♂ (Vietnamese x Yellow).

Year (Beginning)	Parent's bodyweight, g		Offsprings' body weight, g		Heritability h^2 = R/S
	Contr. Stock	Selected stock	Contr. stock	Selected stock	
1987	162 ± 6	261 ± 9	180 ± 4	209 ± 6	0.29
1988	218 ± 10	312 ± 21	316+	335+	0.20
1991	246 ± 5	334 ± 9	+	+	+

+ Before adjustment it was 286 ± g and 365 ± 9 respectively,
++ The data will be received at the end of 1993.

(*H. molitrix*) was introduced. Due to the improper management of the pure stocks during artificial spawning, the two species of silver carp were mixed. Crossed uncontrolled crossing occurred. The undesirable silver carp hybrids showed slow growth rate and early maturity.

The first step of the silver carp selection program aimed to restore the pure populations of these two species. Based on the morphological and biochemical indices, the two stocks of Vietnamese and Chinese silver carp were identified (Thien and Tien 1988). The second step of the selective breeding program was to improve the purity and genetic qualities of the identified stocks.

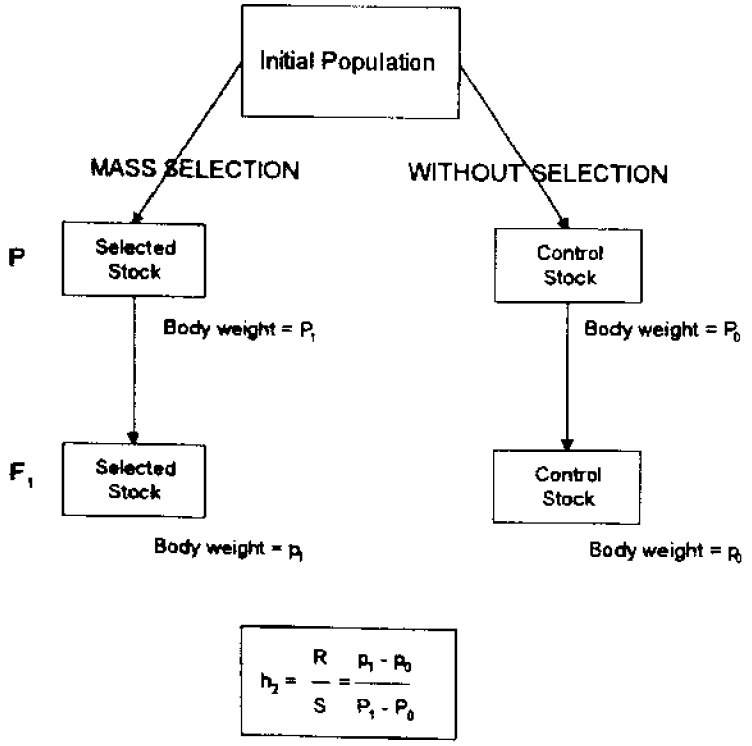

Figure 6. Determining realized heritability (h^2) of body weight.

Conclusion

A brief review of the fish breeding research and practices in Vietnam showed that despite small and scattered research programs, some initial success was achieved that enhanced aquaculture productivity. Selection of common carp and silver carp will continue in the future. The selective breeding of Indian major carps will also be conducted and will initially focus on selection of mrigal.

A proposal for International Center for Living Aquatic Resources Managment (ICLARM) support on genetic improvement of farmed tilapia was submitted and will be approved in the near future. Enlargement of research programs on the genetic structure of fish populations, karyotypes, gynogenesis and chromosome manipulation will also occur.

Acknowledgements

We are grateful to the International Foundation for Science for supporting our research program on common carp selection in Vietnam (Grant A - 0456).

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A Review of Fish Breeding Programs and Conservation Issues in Thailand

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Abstract

In the past four decades, the aquaculture industry in Thailand has emphasized improving both seed supplies and aquaculture techniques. Six freshwater and two coastal species have been successfully cultivated in hatcheries and induced spawning by hormone injection has been developed to control the reproduction of hatchery stocks. Little attention has been given to the improvement of stock quality. The Cooperative Program of Aquaculture Genetic Network in Asia, which is supported by the International Development Research Center (IDRC), started a program in 1982 that was aimed to genetically improve broodstock quality. The objective of the program was to develop improved strains of economically important species, such as tilapia, walking catfish, common carp and Java carp through selective breeding and broodstock management. These species were raised under farm conditions with the goal of improving growth rate and disease resistance, and a number of selection procedures have been developed to improve growth rate of economically important species. Several improved strains have been achieved after seven years of this program: two improved strains of Nile tilapia (*Oreochromis niloticus*), Chitralada and NIFI strains; one strain of the red tilapia; and one strain of the walking catfish (*Clarias macrocephalus*). Selection programs for other species, such as the Java carp (*Puntius gonionotus*) and the seabass (*Lates calcarifer*), are now being conducted. Population genetics and genetic manipulation have been studied under the European Economic Community (EES) grant since 1990. The project, which continues until 1994, concerns three species; the Nile tilapia, Java carp and walking catfish. In this paper selective breeding programs of economically important species are reviewed and genetic conservation of genetically improved species are also discussed.

Introduction

The aquaculture industry provides an important source of animal protein for the people of Thailand. In 1990, aquaculture contributed 297,000 tons of freshwater and marine fish. This was 61% more than the production in 1980 (Thai Department of Fisheries 1990). Thirty-five percent of the total production (103,800 tons) was from freshwater aquaculture and 65% (193,200 tons) was from coastal aquaculture. During the past several years, the Department of Fisheries (DOF) has placed more emphasis on improving aquaculture production in terms of increasing seed supply and improving aquaculture techniques. Little attention has been given to improving broodstock quality. This has resulted in the deterioration of economically important traits; decreased growth rate, small maturation size and low disease resistance. These problems are seen in common carp (*Cyprinus carpio*), walking catfish (*Clarias* spp.) and tilapia (*Oreochromis niloticus*).

To counteract these problems, the aquaculture genetics program in Thailand, supported by the International Development Research Center (IDRC) under the Network Genetic Project, was started in 1982 where both aquaculture genetic research and staff training have been conduced. The most worthwhile outcome of this project has been the formation of an aquaculture genetic research group and the establishment of the National Aquaculture Genetic Research Institute (NAGRI). In addition, there are four regional research centers that serve to develop broodstock, maintain stocks of genetically-cataloged fishes, and release improved strains to other fisheries stations and farmers.

NAGRI has been involved in joint research with many countries. At present, the institute is supported by two major sources of international funding the IDRC and the EEC. The purpose of the Thai/IDRC project is to develop domesticated strains of economically important species such as tilapia, catfish and common carp. The project started in 1982 and will be completed in May 1993. Dalhousie University in Canada has collaborated with the research program and NAGRI staff has been trained in aquaculture genetics during the program. The Thai/EEC project focuses on two main research areas; population genetics and genetic manipulation. The project started in 1990 and will end in 1994. Since 1982, the Department of Aquaculture at Kasetsart University has studied genetic improvement of economically important species. This work has been supported by the Thai government.

Freshwater fish species

There are six important freshwater species in Thailand: Nile tilapia (*Oreochromis niloticus*), walking catfish (*Clarias* spp.), Java carp (*Puntius gonionotus*), striped catfish (*Pangasius sutchi*) and sepat Siam (*Trichogaster pectoralis*). Breeding programs for these species have been carried out in fifty freshwater fisheries stations around the country. Each station keeps its own stocks and the seed produced is distributed to fish farms in the stations own region. In addition, the stations are responsible for restocking the species in natural waters and village ponds each year. In 1989, the target for restocking freshwater species around the country was 75 million fry. Each year this number tends to increase 10% (Suraswadi 1988).

Selective breeding for genetic improvement has been developed in three species; Nile tilapia, walking catfish and Java carp.

- **Tilapia (*Oreochromis* sp.)**

Selective breeding of tilapia for genetic improvement has been developed under the Thai/IDRC project. This project involves selection methods for increasing growth rate using within-family selection, size-specific mass selection and indirect selection or selection for age at maturation (Uraiwan 1993). There are three improved strains.

Two lines of the Chitralada strain of Nile tilapia were selected. The Chitralada strain was introduced from Japan in 1965. The first line was selected for its high growth rate by within-family selection. The second line was selected for age at maturation. Indirect selection was developed to find the selection method that best accommodates broodstock management practices on fish farms. The indirect selection experiment illustrated that selection for early maturation can improve growth rate, therefore, this type of selection can be applied under farm conditions. After three generations of these selection experi-

ments, the fish in the selected line were compared to the fish in the control line. The results indicated that fish in the selected lines grew an average 36% faster than those in the control line by within-family selection, and 11% faster than the control line by indirect selection (Uraiwan 1990).

One selected line of the NIFI strain of Nile tilapia was modified by mass selection or size-specific selection. The selected strains were distributed to test their performance in Pitsanulok Fishery Development Research Center in the north of Thailand. The strain evaluation experiments have been conducted in both government and private fish farms. The testing will be completed by the end of 1993 (Uraiwan et al. 1993).

In 1968, the Thai red tilapia was found at the Ubonratchathani Fisheries Development Center (Tangtongpirod et al. 1982). Red tilapia were selected for high growth rate using a size-specific selection technique for six generations (Jarimopad 1989). Under farm conditions in the northeast of Thailand the growth rate of the selected red tilapia are now being compared with the local strain.

In collaboration with the ASEA-EES Aquaculture Development and Coordination Programme (AADCP), NAGRI has recently developed genetic manipulation and sex reversal techniques for the Nile and red tilapia. The project began in 1990 and will continue through 1994. Triploid fingerling of the Nile tilapia will be used for an experimental intensive culture system at Nakhornsawan Province, and experiment on monosex male red tilapia has been developed in Chachoengsao Coastal Aquaculture Development Center in Chachoengsao Province.

- **Walking catfish (*Clarias macrocephalus*)**

There are three species of walking catfish in Thailand: *C. macrocephalus*, *C. batrachus* and *C. gariepinus*. A number of selective breeding programs have been developed for these species.

Mass selection for increasing growth rate of *C. macrocephalus* has been conducted since 1986 under the Thai/IDRC project. After three generations of selection, fish in the selected line were 11.8% heavier and 2.35% longer than those of the control line (Jarimopad et al. 1989).

Aeromonas hydrophila is one of the most serious disease problems in Thailand. Strain selection for resistance to *A. hydrophila* has been conducted since 1987 at the Department of Aquaculture, Faculty of Fisheries, Kasetsart University (Na-Nakorn and Lekhaanantakun 1992b). Growth rate and resistance to *A. hydrophila* for five different strains and their hybrids of *C. macrocephalus* have been compared. After one generation of selection for disease resistance, a slight improvement for resistance to *A. hydrophila* was observed with the heritability estimated at 0.17 (Na-Nakorn, personal communication).

To improve growth rate and increase disease resistance, chromosome manipulation and gynogenesis have been developed in *C. macrocephalus*. Unfortunately, the triploid *C. macrocephalus* showed slower growth and lower survival than the diploid (Na-Nakorn and Lekhaanantakun 1992a). Gynogenesis of *C. macrocephalus* is now being investigated at the Kasetsart University, Department of Aquaculture.

The hybrid of the female *C. macrocephalus* and male *C. gariepinus* is a commercial success and is now preferred by farmers. This is because the hybrid grows faster than

the pure *C. macrocephalus* (Nukwan et al. 1990).

- **Java carp (*Puntius gonionotus*)**

The selective breeding program for Java carp has been developed over four decades and has emphasized induced spawning by hormone injection and increased growth rates by optimum management. However, the genetic improvement program just started in 1992. Female carp are known to grow faster than males. Therefore, the objective of the early genetics program was to produce cultured monosex females. Roongratri et al. (1992) produced all female fingerlings using gynogenesis. The gynogenetic offspring had a survival rate of 61%. These offspring were sex-reversed to produce sex-reversed-males, which in turn produced all-female stock.

Na-Nakorn and Legrand (1992) induced triploid carp by cold shock. They produced 90-96% triploid carp by treating the fertilized eggs at 15°C. A comparison of growth rates for the diploid and triploid Java carp is still being conducted. A new approach for genetic improvement of Java carp is on-farm-selection, which will be addressed in the section on genetic conservation.

Coastal fish species

The economically important coastal fish species are seabass (*Lates calcarifer*) and grouper (*Epinephelus tauvina*). Similar to the freshwater species, there are seven coastal fisheries stations that are responsible for developing culture techniques and producing seed supply. Although the complete life-cycle of these species has been controlled, coastal farmers still prefer to obtain their stock from natural sources because offspring from hatchery stock have lower survival rates than those in the wild. Therefore, genetic improvement programs for this species have not been developed. Most of the earlier research emphasized spawning and rearing techniques. The Coastal Aquaculture Division, Department of Fisheries has developed culture techniques for these species.

- **Seabass (*Lates calcarifer*)**

The seabass has been cultivated in Thailand for over 40 years. In 1973, the first successful spawning of the seabass took place at the Songkhla Coast Fisheries Station (Chomdat and Pucharean 1979). Spawning and rearing techniques have also been extended to the private sector. At present, this species has become one of the economically important coastal species in Thailand.

The fish can be reared in earthen pond and cages and the optimum stocking densities are one individual/m^2 in earthen ponds, and 4-6 individuals/m^2 in cages (Wongsomnik and Maneewong 1976.) The seabass culture is now facing problems of slow growth and lack of natural broodstock. Therefore, selective breeding for improving growth rate is under consideration by the Department of Fisheries. The main goal of this program is to convince farmers to use hatchery seed supplies. Strain selection will be the first step in developing a selective breeding program. The government fisheries stations will then propagate these selected strains for the private hatcheries.

Genetic Conservation

- **Background**

Two aspects of aquaculture development in Thailand that will soon be competing or interfering with each other are genetic improvement and conservation of genetic bio-

diversity. Both activities are urgently required to meet Thailand's rural population needs for food over the short and long-term.

The need for genetically improved aquaculture broodstock is essential. Natural populations of aquatic animals are fished to the point of extinction and aquaculture continues to supply an increasing proportion of the protein requirements for rural people. The value of genetic conservation in aquaculture (as opposed to genetic progress) has been recognized in Thailand.

Maintaining adequate stocks, with their original genetic diversity, is in the long-term interest of countries like Thailand. A national genetic conservation program can be integrated with the genetic improvement program under the responsibility of an institute like NAGRI. A proposal for such a program has been drawn up and submitted for internal and international funding.

- **Program objectives**
  - > To establish genetic improvement and conservation in aquaculture as economically self-sustaining activities within a rural economy,
  - > To determine the overall rate of genetic improvement that can be attained by a farmer-operated, for-profit, aquaculture venture,
  - > To analyze the steady-state level of genetic conservation or genetic erosion that result from a market driven balance between gene flow among farms and regions. To improve the genetic base that leads to genetic improvement and to the differentiation of local breeds.
- **What to expect from a successful outcome**
  - > A number of fisheries stations and individual farmers will participate in the program by developing improved aquaculture seed (fry for sale to other farmers in their areas),
  - > Local farmers benefit because their productivity increases from using genetically improved stock,
  - > The genetic (geographical) heterogeneity of the breeds will be preserved or possibly enhanced by the local improvement process,
  - > NAGRI and its satellite stations will provide broodstock management training and advice to farmers.

Conclusion

Genetic improvement studies conducted in Thailand for the past decade have emphasized economically important freshwater species. A number of these studies have aimed to improve growth and disease resistance because of their importance to farmers. There has been relatively little work on marine species because the culture technology in this area is not well developed and farmers still prefer to collect seed from the wild. However, government fisheries stations hope to resolve the culture problems and begin propagating selected strains in private hatcheries.

The National Aquaculture Genetic Research Institute's genetic conservation program is concerned with maintaining the genetic diversity of local species in Thailand. The program aims to integrate two aspects of aquaculture development in Thailand, genetic improvement and genetic diversity. The goal is to promote aquaculture as a self-sustaining activity, which is important within a rural economy.

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A Review of Traditional Fish Selective Breeding Research and Practices in China with Emphasis on the Use of Genetic Markers

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Abstract

This paper reviews traditional selective breeding of fish research in China. It contains information on traditional genetic improvement, including intraspecific hybridization of common carp. Examples of common carp breeding are discussed, which include Red carp 8305 that are bred by combining traditional selective breeding, genetic markers and gynogenesis. This paper emphasizes the use of genetic markers for selective breeding of fish and gives some new results from work completed during the past five years. Genetic markers such as scale and color patterns, antigens, isozyme and nonzymic protein alleles and report genes are included. Gynogenesis, androgenesis and somatic cell breeding are emphasized.

Introduction

China has the longest history of fish culture and fish breeding. There are over a dozen varieties of common carp (*Cyprinus carpio*) and several dozen varieties of goldfish (*Carassius auratus*). The formation and stabilization of these fish varieties took several hundred years. In aquaculture, one simple way to obtain high growth rate and hybrid vigor is to cross two pure lines or two varieties. Among cultured fish species in China, only common carp are used for this purpose. Only first generation hybrids can be used for culture. Every year, hybrid seed is produced by crossing two different varieties. However, these parent varieties are easily contaminated, or even destroyed by fertile hybrids that breed with wild stocks. Therefore, keeping parental varieties from contamination and using genetic markers to identify germplasm is essential.

Without any knowledge of genetics, fish farmers of the past have generally carried out fish selection using only phenotypes. No doubt, this prolonged fish breeding progress. Thanks to the accumulation of knowledge on fish genetics and the achievements of modern biology, fish breeders can now make use of both traditional selective breeding and modern breeding techniques. This will hopefully accelerate the development of fish breeding methods in China.

Traditional Genetic Improvement

• Intraspecific hybridizations

Increasing the productivity of cultured fish may be achieved by increasing the growth rate. In the aquaculture industry, it is advantageous to commercially culture first generation hybrids with heterosis. Heterosis typically occurs in intraspecific hybridizations. However, except for the decorative goldfish

varieties (*Carassius auratus*) and the common carp varieties (*Cyprinus carpio*), no other freshwater fish variety has been commercially hybridized in China. There are over a dozen varieties of common carp in China and more than ten crosses have been made between them since 1970. From these crosses, five combinations showed obvious hybrid vigor (Table 1). From the five combinations, a hybrid between Xingguo red carp (female) and scattered scaled mirror carp (male), named harvest carp, appeared to be the most distinct. For both fingerlings and adults, the average individual increase in harvest carp weight was 50-60% greater than the average of its parents, and 100% greater than wild common carp. Harvest carp is now reared in several areas of China as a new stock.

All five hybrid carp showed a higher growth rate, had a higher recapture rate and were less susceptible to disease (Subgroup of Carp Studies, Inst. Hydrobiol. 1975). Presently, these hybrid carp have been popularized throughout China. They are stocked in ponds, small lakes and net-cages in reservoirs throughout China.

Production and cultivation of unisexual hybrids is also advantageous because female common carp grow much more rapidly than males. On the other hand, male tilapia grow much more rapidly than female tilapia.

The all-female gynogenetic offspring of mirror carp were employed for artificial sex reversal. The phenotypic male (XX) was crossed with red common carp (XX) and all-female hybrid offspring were obtained. Female common carp mature one year later and grow 20% faster than the males in the first and second culture years (Wu et al. 1990). All-female hybrid carp have now become a widely cultivated fish throughout China and better productivity results have been obtained (Wu et al. 1991a).

Male tilapia were employed for artificial sex reversal using estrogen. The phenotypic female was then crossed with a normal male and 75% male offspring were obtained. At the same time, some supermales (YY) were detected in these offspring (Yang et al. 1980). If a supermale is crossed with a normal female, all male tilapia will be obtained. However, it is a lot of work to make a test cross for every sex reversed individual.

- **Interspecific hybridizations**

From over 100 combinations of interspecific hybridizations, there were only five combinations between genera that resulted in valuable and fertile (or semi-fertile) offspring (Table 2).

Nevertheless, the hybrid progeny from a cross between crucian carp and wild common wild carp has been cultured because of its accelerated growth rate. The hybrid between *Megalobrama amblycephala* and *Parabramis pekinesis* is resistant to lower oxygen content in the water. Also, the hybrid between *Cirrhinus molitrella* and *Sinilabeo decorus* was found to have an improved resistance to low temperatures. A tetraploid hybrid was detected in the cross between grass carp (*Ctenopharyngodon idellus*) and common carp, and a triploid hybrid appeared in the backcross. This triploid hybrid seemed resistant to GCRV (Grass Carp Reovirus).

Examples of Common Carp Breeding

Some examples of common carp breeding in recent decades are presented here. There are over a dozen common carp varieties in China, but it has not been possible to trace

the origin and breeding method for all of them.

- **Red purse carp**

Red purse carp is a short and deep bodied fish that differs from big-belly carp by its deformed caudal vertebrae. Though it has been cultivated for over 300 years, pure broodstock was rarely available 30 years ago. Beginning in 1969, a selection program was carried out. Using a heterozygous broodstock, selection for red color, body form and growth rate was carried out four times using summer and winter fingerlings, yearlings and adults. Undesirable individuals were eliminated in each generation. After six generations, the red purse carp was characterized by pure red coloration, short and deep body and a faster growth rate (Jiang and Wu 1989).

- **Xingguo red carp**

The Xingguo red carp was established by mass (individual) selection. By 1984, the Xingguo red carp had also passed through six generations of selection. Body form, red coloration and rapid growth rate were the main criteria for selection. The Xingguo red carp variety exhibits red coloration and rapid growth rate (Jiang and Wu 1989).

- **Red carp 8305**

By means of gamma or ultraviolet rays, the sperm of related species (*Carassius auratus* or *Megalobrama amblycephala*) were irradiated and used as an activation agent. The matured eggs of Xingguo red carp were then successfully induced to become gynogenetic eggs. After gynogenesis was induced twice, some individuals were artificially reversed into "physiological males." After using growth rate as a selection criterion, and testing two generations of progeny from three gynogenetic lines, a pure line of Red carp (Red carp 8305) was established by mating a "physiological male" with its sister (Wu et al. 1991b).

- **Jian carp**

The Jian carp was bred by selecting for a hybrid between the Red purse carp and wild Yuanjiang carp. A series of combined breeding techniques was used including fish family selection, interstock hybridization and genome engineering. After six generations of directed selection, Jian carp has stable inheritance characteristics. The Jian carp is an improved variety with many advantages, including rapid growth rate and a high feed conversion rate (Zhang, personal communication). Nowadays, it is widely cultured in China.

Use of Genetic Markers for Selective Breeding of Fish

In general, phenotypic traits are heterozygous. By segregating the offspring, it is very difficult to rid the individuals of undesired characteristics and to reach the target trait. Phenotypic differences are often controlled by different genes. Any particular phenotypic expression that can be used to detect recombinant genotypes or genes is called a genetic marker.

Hybrid carp have played an important role in increasing aquaculture productivity throughout China. Unfortunately, hybridization of broodstock was not controlled and the pure or initial stocks became "contaminated" in several fish hatcheries. This led to segregation and decreased hybrid vigor. Frequently, fish farmers determine the offspring qualities of hybrid carp by the use of genetic markers.

• **Scale and color patterns**

The inheritance of scale and color patterns have been studied intensively in carp. Results indicate that the scale-covering in carp is controlled by two pairs of genes, *A-a, N-n,* and body coloration by two other pairs of genes, *R-r, B-b*. The scattered scale-covering is a recessive trait controlled by *aa* and *nn* genes. The red body coloration controlled by *rr* and *bb* is another recessive trait (Subgroup of Carp Study 1975; Wu et al. 1980).

If any scattered scale pattern or red coloration appears in the hybrid offspring it suggests that the hybrid parents were contaminated. In one study, a new recombinant form with scattered scale-covering and red body color was produced from the hybrid offspring of mirror carp and red carp. This hybrid can be employed for backcrossing to the hybrid parents and eliminates heterozygous maternal or paternal characteristics (Wu et al. 1980).

» Gynogenesis

Embryos of carp varieties with red body color (*rr, bb*) are transparent without any pigment, except in the eyes. When any of these loci are heterozygous, pigments will occur on the embryo. This change is easily detected with the naked eye before the hatching stage. The sperm of other varieties of carp or other related species with *R* or *B* genes were irradiated by gamma rays or ultraviolet rays and were used to activate the eggs of red carp. The gynogenetic embryos are easy to distinguish from the hybrid embryos because of their lack of pigmentation and transparent appearance. Hence, by combining gynogenesis with functional sex reversal, a new pure line of Red carp 8305 was developed and established (Wu et al. 1981).

» Androgenesis

Red carp sperm and common carp eggs (*R, B*) have been used in a gene marker study using androgenesis. Because the expression of pigmentation is controlled by dominant genes (*R* and *B*), the androgenic embryos that lack pigment are easy to identify (Ye et al. 1990).

» Gene transfer

Genetic markers have also been used in gene transfer studies. Using microinjection and sperm as a vector, Liu and his colleagues successfully conducted a total DNA mediated gene transfer in red carp (Liu et al. 1991). Total DNA of crucian carp liver was isolated and transferred into the fertilized eggs of red carp by microinjection or sperm absorption. By means of pigmentation on the embryos, the color gene transfer was easy to detect. The results of experiments showed that the pigmentation transfer rate was 0.69-1.94%. This result indicated that the total DNA transfer might be a potential method in breeding for resistance to low temperature and diseases.

• **Antigens**

In studies of genetic variability in fishes, considerable attention has been paid to the analysis of blood groups. Antigen reactions were used in early studies on fish population genetics. The first report on the results of a study on the specificity of red blood cell antigens from different varieties (or strains) of common carp was made by Tong et al. (1987). They demonstrated that the allotypic antigens of red blood cells of common carp were particular to a given carp variety and can be considered as one of the markers for identification of fish varieties.

Recently, red blood cell antigen polymorphism was demonstrated in red crucian carp

(*Carassius auratus* var.). The analyses of phenotypes in adult fish and their offsprings indicated that this antigen system, nominated as S system, contained two dominant antigen factors, S^1 and S^2 as well as a zero factor, S^0. Thus, individuals tested had four phenotypes, S^1, S^1 S^2, S^2 and S^0 (Tong and Wu 1990).

It is generally accepted that Major Histocompatibility Complex (MHC) is another important genetic marker used to identify pure lines. The MHC with high polymorphism is closely related to tissue transplantation. Scale transplantation between different individuals is a simple and effective method of assaying for the MHC loci. The grafts between individuals of different MHC genes were rejected within two weeks (temperature ranging 21-28° C). However, if genes controlling MHC are homogeneous, the survival rate of the graft will be high. In the new pure line of Red carp 8305, the survival rate of scale transplantation between different individuals was over 87% (Wu et al. 1991b). Using a spleen graft test, Zhu conducted a genetic analysis of three different gynogenetic clones of crucian carp (*Carassius auratus gibelio*). The experimental results showed that grafts between individuals of different clones were rejected within seven to fourteen days, but the survival rate of grafts between different individuals of the same clone was up to 97% (Zhu 1990).

• Isozymes and nonzymic proteins

Biochemical genetics techniques have led to a new stage in fish population genetic studies. These techniques can be used for analyzing genetic differences between individuals within a given fish species. The electrophoretic separation of serum proteins and liver esterases on polyacrylamide or starch gel was carried out for natural gynogenetic crucian carp (*Carassius auratus gibelio*). It was found that the serum proteins and liver esterases of this fish assumed four different phenotypes (Zhu and Jiang 1987). All progeny of a given maternal crucian carp possessed the same phenotype as their mother. So these four clones were named as A,B,C and D-clone, respectively. At the same time, grafts between different individuals of the same clone survived (97%), but grafts between different clones were rejected (Zhu 1990). Moreover, it was found that the growth capacity of these clones was diverse. Their growth differences could be as high as 50%. This is very important for the selective breeding of crucian carp. Presently, the D-clone of crucian carp is selected as a good culture clone.

There are seven alleles of transferrin (A, B, C, D, E, F, G) in the transferrin locus. G-locus and I-locus of esterase were also found to be polymorphic in the common carp population. Examination of these loci showed that the alleles do not differ between different individuals of the Red carp 8305 and no heterozygous individuals have been found (Wu et al. 1991b).

• Report genes

A report gene can easily be identified through selection of corresponding drugs or reagents. This kind of gene is quite useful for the selection of cell lines. If a report gene is connected in some way with a trait that is hard to detect, then the existence of this trait can be verified by assaying its relevant report gene.

The grass carp (*Ctenopharyngodon idellus*) is a well known cultured fish because of its large size, quick growth and herbivorous feeding habits. On the other hand, it is also

notorious for its susceptibility to grass carp Reovirus (GCRV) infections. This kind of disease is very serious and can destroy a fish farm. Selecting a line of grass carp that is resistant to GCRV is important to the Chinese fish breeder. Fortunately, a fish cell line that is Actinomycin D-resistant was isolated. Moreover, this resistant cell line is not susceptible to GCRV (Wang et al. 1989). Cultured fish cells are able to develop into normal fish when placed into a mature fish egg (Chen et al. 1986). Also, nuclear transplantation techniques and electric fusion between cell nucleus and unfertilized eggs were developed (Liu et al. 1988; Yi et al. 1988; Chen et al. 1990). The donor nucleus could promote the recipient egg to cleave and develop into a normal individual. The genotype of the resultant fish resembled that of the donor cell nucleus. Therefore, the nucleus of the selected fish cell line can certainly serve as a donor in fish selection and breeding.

Conclusion

Before the 1980s, Chinese fish geneticists and breeders made an effort to hybridize among varieties or between different species, using body color and growth rate as selection criteria. Recently, fish geneticists and breeders in China combined traditional selective breeding with gynogenesis and androgenesis. This has accelerated the development of some new fish strains. Today, some fish geneticists are concentrating on the use of genetic markers. It is expected that in the near future, the use of genetic markers and other modern techniques will make selective breeding of fish more effective.

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A Review of Modern Fish Breeding Research and Practices in China

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Abstract

Chinese fish breeding scientists have devoted great effort to applying biotechnology to fish breeding. They have already established two fish cell strains and have successfully obtained gynogenetic fish, cell-fusion fish and nuclear-transplanted fish through various modern breeding techniques. Progress has also been made toward the production of: (1) vaccines for the haemorrhagic disease of grass carp, (2) the clone of a growth hormone gene and an antifreeze protein gene cDNA and, (3) transgenic fish. Moreover, a breakthrough has also been made in the techniques of sex manipulation of tilapia and artificial induction of polyploidy. All these achievements have proven that biotechnology has an effective application in fish breeding, thus opening up new directions for breeding practices in China.

Introduction

Fish breeding is an essential component of aquaculture production. The proper selection and cultivation of improved breeds is considered one of the most important means for increasing fish production. China has a long history of fish culture, but until the 1960s little progress had been made in the study of genetic improvement of farmed fishes. Although cross-breeding research was performed from late 1960 to early 1970, it was performed spontaneously, without any prior planning. Fish breeding research developed rapidly in the early 1970s after two national conferences on the basic theory and practice of selection of improved breeds of farmed fishes were held. By 1986, biotechnology had developed and was practiced in fish breeding. Based on traditional selective breeding modern biotechnology of cell engineering and gene manipulation, research on fish genetics and breeding in China entered a new stage.

Traditional selective breeding has been practiced as an effective means of fish breeding for many years in China. The major progress achieved includes the heterosis utilization of common carp hybrid F_1 (Ma 1976; Liu 1979), and the breeding of Hebao red carp (*Cyprinus carpio wuyuanenesis)* (Guo 1983). However, traditional breeding practices have limits; they take a very long time. A minimum of 10 years and sometimes hundreds of years may be needed to develop a new breed. Over the past ten years, modern fish breeding technology has developed rapidly and has the potential to be widely practiced in China. When modern breeding technology is properly combined with traditional techniques, the genetic improvement of a particular fish breed will accelerate. A new and more desirable breed could be developed in a relatively short period of time.

Cell Culture in Fishes

Research on fish cell culture was initiated in the 1970s in China. The major objective of cell culture research in aquaculture at that time was to solve some of the difficult problems that could not be solved by traditional breeding technology. At present, cell culture is used to study the prevention and treatment of haemorrhatic disease of grass carp (*Ctenopharyngodon idellus*). Grass carp is an economically important farmed fish in China that is susceptible to haemorrhagic disease. Its survival rate is only 10-15% from fry to food fish. If the disease is effectively controlled and the survival rate rises over 30%, the national production of fish will greatly increase. In recent years, vaccines prepared from the body fluid of diseased fish have been commonly used to prevent and treat haemorrhagic disease in grass carp. However, this vaccine has low potency and an unstable quality and therefore, cannot meet the demand of large-scale fish production.

Through the techniques of cell culture, the first cell strain (ZC-7901) and substrain (ZC-7901s) from the snout tissue cells of grass carp were established in 1979 (Zhang et al. 1981). As a result, a haemorrhagic virus (Reovirus) of grass carp with a high purity and strong pathogenicity was obtained through the culture of the cell strain. The cell vaccine supplied for the experiment is now manufactured on a small scale and provides good results in immunity experiments. Such cell strains have been passed over generations and still have a high activity and stability. Since then, another cell strain (CZK) cultured from grass carp kidney tissue was developed in the Yangtze River Fisheries Research Institute (Zuo et al. 1986). The establishment of these two cell strains have filled gaps in the field of fish cell culture in China and have provided a new technique and means for fish virus and breeding study. An experiment on the industrial production of the cell vaccine is still underway. It is expected that a product will be available for sale to fish culturists in three to five years.

Meanwhile, more research is being conducted to establish a nucleic acid probe, and new diagnostic techniques are being developed to accurately identify haemorrhagic disease of grass carp and other diseases at an early stage.

Nuclear Transplantation in Fishes

Nuclear transplantation involves transplanting the nucleus of a cell into the enucleated cytoplasm of another cell. It is also known as nucleo-cytoplasm hybridization. The objective of this research is to study the relationship between the cell nucleus and cytoplasm during the process of embryonic development and to understand the basic theory of fish genetics, development and cell division. Nuclear transplantation in the economically-important Chinese carp was first conducted in the early 1970s and successfully achieved when a hybrid fish with normal growth was obtained from the combination of the nucleus of common carp (*Cyprinus carpio*) and cytoplasm of crucian carp (*Carassius auratus*).

The experimental results showed that part of the nuclear-transplanted fish had inherited some features from the common carp and crucian carp and some fish were in the intermediate state (Table 1). This revealed that both the nucleus and cytoplasm had some effect on the expression of genetic information. The sperm from the hybrid also possessed normal capabilities of fertilization and

Table 1. Comparison of some morphological characteristics of common carp, crucian carp and their hybrid fish from common carp nucleus and crucian carp cytoplasm.

Type of Fish	Morphological characteristics			
	Pair of barbels	Pharyngeal teeth	No. of scales on lateral line	No. of vertebrae
Common carp	2	Molar-shaped, 5 teeth in 3 rows, 3-1-1	35-38 majority 36	4 + 32 to 4 + 36
Crucian carp	none	Wedge-shaped, 4 teeth in one row	28 - 30 majority 28	4 + 26 to 4 + 30
Hybrid carp	2	Molar - shaped 5 teeth in 3 rows similar to carp but in a few cases irregular	32 - 36 majority 34	4 + 26 to 4 + 30

produced offspring. But the hybrid between common carp and crucian carp by sexual hybridization usually showed poor development of sperm and could not produce offspring. It was concluded that by using nuclear transplantation, the difficulty in hybridizing distantly-related fish could be overcome and a new technique for fish breeding could eventually be developed.

In China, nuclear-transplanted fishes obtained thus far from different genera, subfamilies and order. They include hybrids between common carp nucleus and crucian carp cytoplasm (CyCa) (Yan et al. 1980); crucian carp nucleus and common carp cytoplasm (CaCy) (Yan et al. 1984); hybrids between the combination of grass carp nucleus and blunt snout bream (*Megalobrama amblycephala*) cytoplasm (CtMe) (Yan et al. 1985); and hybrids between tilapia nucleus and loach (*Misgurnus anguillicaudatus*) cytoplasm (OrMi) (Yan et al. 1991). All these nuclear-transplanted hybrids, except the last example, show normal growth, development and reproduction. For example, artificial breeding is performed on CyCa hybrid fish to produce a large quantity of F_3 offspring that have a higher growth rate (about 30%) than their maternal and paternal lines. Now that the F_3 hybrids have stable genetic characters, they are commonly cultured by Chinese farmers on a large scale. During 1985-1988, Pan (1989) hybridized CyCa hybrid F_2 (male) (i.e., nuclear-transplanted fish) and scattered scale mirror carp (female) and successfully obtained F_1 offspring, which were called Ying carp. The F_1 hybrid was especially superior in body weight increment. Their growth rate was about 47% and 67% higher, and the average population weight gain was 109% and 140% higher than those of their parental line and the nuclear-transplanted fish, respectively. The individual body weight gain and the population weight increment of two-year-old fish was 60% and 75.1% higher compared to their parental line. These examples describe typical aquaculture production in China. In addition, many Chinese scientists have achieved some progress in transplanting cultured cells or body cells into enucleated fish eggs. It is hoped that based on the nuclear transplanta-

tion technique some new fish breeds with good economic characters and high resistance to disease or coldness will be developed.

Gynogenesis in Fishes

To induce gynogenesis, normal fish eggs are stimulated by inactivated sperm that have been treated with UV lights. The eggs are then treated with either temperature shock or hydrostatic pressure to produce diploid female chromosomes. Induced gynogenesis is of significant importance to aquaculture because it helps produce monosex fish to increase production, and it helps develop a pure line in a relatively short period of time. The pure line can greatly shorten the breeding time and improve the breeding efficiency. For example, the purity of grass carp produced after successive gynogenesis for two generations (about ten years), is equivalent to that of the eighth to tenth generation (forty to fifty years) using traditional breeding techniques.

Artificially inducing gynogenesis in fishes started in the late 1970s in China. The progeny of crucian carp developed through allogynogenesis are called allogynogenetic crucian carp. The application of allogynogenesis to fish breeding is considered a great achievement. Gynogenetic fishes including crucian carp, common carp, grass carp and silver carp have been produced in succession; however, they are have not yet been reared for commercial production.

Among the gynogenetic fishes, allogynogenetic crucian carp is significant in fish production. Allogynogenetic crucian carp is a triploid offspring derived from artificial fertilization of the triploid fengzheng crucian carp (*Carassius auratus gibelio*), as the maternal line and Xingguo red carp (*Cyprinus carpio* red breed), as the paternal line (Jiang et al. 1983). This is a special model of breeding in the course of the development of allogynogenetic crucian carp.

The sperm of Xingguo red carp did not form a sperm pronucleus or fuse with the egg pronucleus. The allogynogenetic crucian carp inherited the "good" characters from the maternal line and from the paternal line exhibited superiority growth. This revealed that heterologous sperm not only gave a gynogenetic stimulus to the ova of crucian carp, but could produce some effect on the gynogenetic offspring. The allogynogenetic offspring do not have segregative phenomena and their growth was about one to two times higher than that of normal crucian carp. Moreover, it was 34.7% higher than that of the maternal line. At present, allogynogenetic crucian carp is widely cultured in more than twenty provinces and cities in China. This indicates that the gynogenetic fish have reached a practical stage for commercial production.

Induced Polyploidy in Fishes

Artificially inducing polyploidy produces sterile triploid fish. These sterile fish control overpopulation, show accelerated growth, an extended life span and produce improved meat quality. Research on artificially inducing polyploidy in fishes was initiated in China in the mid-1970s. Since then, triploid silver carp, grass carp, common carp and crucian carp have been successfully produced. The triploid common carp was induced from a diploid hybrid, which was a result of a cross between Xingguo red carp (female) and scattered mirror carp (male). At five-months-old the triploid hybrids were

much larger in size than the corresponding diploid ones (Wu et al. 1975).

Wu et al. (1981) reported that a cross between Xingguo red carp (female) and grass carp (male) produced tetraploid grass carp. In 1983, they back-crossed the tetraploid grass carp with the diploid grass carp and obtained the back-crossed triploid grass carp. The tentative results have shown that resistance to haemorrhagic disease was considerably improved in the second generation produced by the triploid grass carp. This experiment is still continuing. Although studies on inducing polyploidy in fishes have been carried out for about 20 years, it has not yet been applied to commercial production because of its slow progress. Therefore, in the future, more effort will be placed on how to increase the survival rate of triploid fish and improve techniques of inducing chromosome doubling to obtain larger numbers of tetraploid fish. These tetraploid fish are hybridized with diploid fish for the production of triploid fish.

Sex Manipulation in Fishes

Growth is highly variable between male and female fishes. In general, the male tilapia grows faster than the female and conversely, the female common carp grows faster than the male. Accordingly, sex control and manipulation in fishes has great significance in aquaculture. Yamamoto (1975) started research on the artificial sex manipulation and reversal in fish in the 1950s. Research was initiated in the late 1970s in China and the research priority was sex control and sex reversal of tilapia. Yang et al. (1980) proposed the use of a three-line combination for obtaining genetic all-male tilapia mossambica (*Oreochromis mossambicus*) and they produced six hundred supermale (YY) tilapia mossambica. Furthermore, they also obtained all-male tilapia mossambica by combining the male homozygous line and the prime line (YY x XX). Then, the supermale (YY) tilapia mossambica was crossed with tilapia nilotica (*O. niloticus*) to produce all-male hybrid offspring. Experimental results revealed that all-male tilapia can remarkably increase fish production. The production of all-male tilapia mossambica and the hybrid between tilapia mossambica and tilapia nilotica was 58.8% and 39-47% higher, respectively, than that of their parental lines. The all-male hybrid tilapia are now extensively cultured with high economic efficiency in some provinces and cities in China.

Although the culture of all-male tilapia has a great effect on the increase of fish production, there still exist some technical problems. For example:

- It is relatively inconvenient to produce supermales (YY),
- It is difficult to reverse the supermale fish (YY) into a functional female,
- There is no easy and practical method available to precisely identify between the genetic and the functional female or between the genetic and the functional male.

At present, many trials are being conducted to solve these problems to further increase tilapia production in China.

Cell Fusion in Fishes

Cell fusion is also called cell hybridization. The research priority on cell fusion in China

is to fuse the blastula cell and the ripe egg cell with an electric fusion meter. The cell-fusion fish produced thus far (those from the blastula and egg of the same species), include loach, common carp and crucian carp. Electric cell fusion is more commonly adopted because of its simple operation. All the experimental parameters can be set up in advance and precisely controlled during treatment. The experiments can then be repeatedly carried out under the same conditions. Research on cell fusion is a new topic in China.

The success of electric fusion between the blastula cell and the ripe egg cell has revealed that adult fish could possibly be produced from the combination of a blastula cell and a body cell, thus showing another new method for fish breeding. However, many technical issues need to be resolved such as the effect of multi-cell fusion on the fused fish, control of cell fusion one to one between the blastula cell and egg cell, and the effect of the nucleus of the receptive egg on the fusion fish.

Gene Transfer in Fishes

Palmiter (1982) successfully transferred the rat growth hormone gene into the fertilized eggs of mice. The transgenic mice grew to twice the size of their non-transgenic siblings. Such research findings have further encouraged studies on gene transfer for animal breeding. Because fish are characterized by high fecundity, they are excellent experimental material for gene transfer research. It is also, possible to obtain synchronic eggs by controlling their fertilization and development in vitro. Therefore, some progressive achievements have been made on transgenic fishes (Chourrout et al. 1986; Fletcher et al. 1988; Brem et al. 1988; Maclean et al. 1986; Ozato et al. 1986; Dunham et al. 1987; Zhu et al. 1986 and Chen et al. 1990). Some labs have already developed transgenic fish and a few have been shown to express the protein product (a product of gene expression) (Chen et al. 1989).

The study on gene transfer in fishes was started early in China and much progress has been achieved. Zhu et al. (1985) (*Carassius auratus)* obtained transgenic goldfish and the they were twice as big the non-transgenic fish. Zhu et al. (1986) also succeeded in transferring the human growth hormone (hGH) gene into the fertilized eggs of the loach and found that in 135 days, the growth rate of a transgenic loach was 3-4.6 times higher than the control group (non-transgenic). Thereby, it showed the biological effects of integration and expression of hGH gene in the receptive fish. Zhu et al. (1989) transferred MT-hGH gene by microinjection into the fertilized eggs of common carp, crucian carp and loach. In over 50% of the eggs the foreign genes became integrated and became transgenic fish. Of these, about half expressed the foreign genes and showed a variation in growth rate. Through sexual reproduction, the transgenic fish transmitted the foreign genes to their offspring, which also possessed the ability to express the hGH. Xia et al. (1992) introduced the linealized DNA fragments of the recombination of DNA molecule of hGH gene with the mouse promoter MT-1 gene into the fertilized eggs of blunt snout bream and common carp. This was done with microinjection and living experimental fish. Using the dot blot, southern, northern, and radioactive immunity measurement methods, it was found that the foreign genes showed a proper integration, transcription and enhanced growth effect. The foreign genes could be genetically trans-

mitted into the next generation with an enhancement of growth by the sexual cells.

Although much progress has been achieved on the study of transgenic fish, the technology is still in the experimental stage. However, the following problems need to be solved before putting it into practice.

At present, the gene used in most of the labs is hGH gene and the promoter is mouse metallothionein-1 gene (MT-1). The recombinant DNA is not ideal as a target gene because consumers are often reluctant to accept a fish with the hGH gene. Therefore, it is necessary to clone the fish growth hormone and promoter genes because they contain the DNA sequence of the fish itself, and are more easily integrated and expressed in the receptive fish.

The method of monosex development should be perfected to accelerate the creation of a new strain with good characters. After the foreign gene is transferred into the fertilized eggs, the gene is randomly integrated, forming transgenic fish of all forms. The segregation of foreign genes usually takes place in the population of offspring produced by mating the female and male transgenic fish. In this case, it is rather difficult to develop a new breed by the normal breeding techniques. In order to culture a desirable new breed with fast growth and high genetic stability, gynogenesis must be applied for two successive generations and sex reversal must be used for the individual transgenic female with the best integration and expression of the foreign gene. The cycle of establishing a pure line could then be shortened.

Isozymes in Fishes

Since the 1970s, studies on fish isozymes have attracted more interest from the Chinese fish breeding scientists. Isozymes have been widely used in the following areas:

- **Identify fish species**

Xia et al. (1985) used serum esterase and serum protein as genetic markers to identify tilapia aurea (*O. aureus*), tilapia nilotica and their hybrids. This was done to avoid species contamination in farmed tilapia. This research is important for conservation of pure strains.

- **Establish germplasm criteria**

Isozymes are used as marker to study the germplasm criteria of the major farmed fishes, thus providing the necessary information for genetic conservation of broodstock and for setting up pure strain farms. This study is also important to the development and use of natural broodstock resources (Chou et al. 1991).

- **Identify genetic structure of broodstock populations**

This is significant for genetic conservation and for management of fishery resources (Li et al. 1990).

Although Chinese scientists have paid increasing attention to studies of fish isoenzymes, the analysis of zymograms depends only on the mobility and stain color of enzyme bands. It was not until the mid-1980s that LDH-A_4, LDH-B_4 and LDH-C_4 were purified from the white skeletal muscle, heart and liver tissues of grass carp, respectively. Their corresponding antibodies were also prepared from rabbits immunized with purified LDH isozyme (Xia et al. 1990; Xue et al. 1991; Wu et al. 1993). The experimental results revealed that these antibodies did not have species specificity and could perform an immunochemical reaction with the same antigen of other fish species.

Therefore, the immunochemical reaction between the antibody and antigen could be used to precisely analyze the patterns of LDH isozymes and the subunit composition of grass carp, blunt snout bream, silver carp, common carp, crucian carp, tilapia aurea and mandarin fish (*Siniperca chuatsi*). The antibody of LDH-A_4 was also used to analyze the differential gene expression for lactate dehydrogenase of mandarin fish (Xia et al. 1992). In China, immunology has been applied to the study of fish isozymes, thus further promoting development of fish isoenzyme research.

Prospects

Research on the application of modern technology for fish breeding began later in China than in other parts of the world. However, rapid progress has been achieved in the areas of nuclear transplantation, transgenic fish, induced polyploidy, cell fusion, artificial induction of gynogenesis, isozyme and sex control. Based on the progress reviewed above and conditions specific to China, future research priorities will focus on the following:

> Isolate and clone the fish growth hormone gene, anti-coldness protein gene and other target genes and transfer them into fertilized eggs for development of a new fish breed with faster growth and higher resistance.

> Continue the study of fish cell culture to establishing cell strains for multiple purposes. Cell fusion will then be performed on different cultured cells to produce superior individual fish.

> Control the genome of fish, improve the techniques of gynogenesis and induced polyploidy, thus purposefully create larger numbers of polyploid and gynogenetic fish.

> Strengthen the prevention and treatment of fish disease. Establish industrial production of a cell vaccine to increase the survival of grass carp. Develop techniques for isolating and identifying the virus, and for detecting those pathogens causing serious diseases.

> Improve the techniques of sex control, especially reversing the supermale tilapia (YY) into the functional female (YY) for obtaining all-male tilapia, which are cultured in commercial production.

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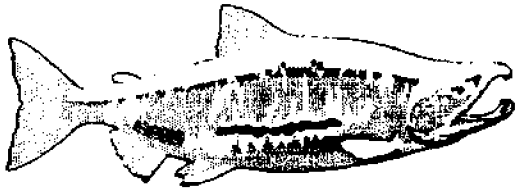
Part III: Discussion Group Summaries



Discussion Group Summaries

Introduction

Six informal round-table discussion groups were conducted during the selective breeding conference. These sessions provided an opportunity for participants to discuss and share information on the critical issues of selective breeding for aquaculture and stock enhancement. Conference participants served as moderators and developed the framework for their discussion group session.

Three of the discussion groups examined selective breeding from the aquaculture production perspective and two groups considered the stock enhancement perspective. The interaction between selection and conservation of genetic resources was considered throughout the discussions.

The last discussion session was devoted to developing two sets of guidelines for selective breeding; one for aquaculture and the other for stock enhancement. Following the conference, these guidelines were further refined and developed by Graham Gall, Trygve Gjedrem, Kenneth Leber and William Smoker.

Aquaculture Production and Conservation

Priorities for Genetic Improvement of Cultured Fish Stocks

This discussion group focused on the initiation of genetic stock improvement programs for cultured fish stocks. Genetic improvement programs have been developed in some countries but in others, industry resistance persists. Several institutional breeding programs released selected fish for trials on private farms under conditions familiar to the farmers. Following the trials, farmers in many countries were willing to purchase the selected fish and support the selective breeding program. Successful genetic improvement programs for fish, as well as models from other animal industries, were discussed. The moderators for this session were Trygve Gjedrem and James Parsons.

- **United States**

In the United States, there are no industry-based genetic improvement programs for fish, as in other animal industries. Although research programs have shown that selective breeding can improve fish stocks' performance, it has been difficult to implement programs in the aquaculture industry.

From 1940-1960, university and the U.S. Department of Agriculture (USDA) research station programs were involved in selective breeding for chickens. However, in the 1960s, the chicken breeding program was taken over by private industry and the majority of government-supported selection research efforts ended. Since the 1950s, the USDA and university research stations have been involved in dairy cattle breeding. These programs are paid for largely by private industry and jointly managed. Genetic improvement programs for pigs, sheep and beef

cattle have received government and university attention since the 1930s, but are not integrated with private industry programs. Finally, research units or breeding stations have well defined processes of developing improved plant strains before they are distributed to the commercial producers.

Private research efforts have demonstrated improvements in rainbow trout stocks. However, with few exceptions, current industry trout stock improvement programs do not use up-to-date technology and are thus inefficient.

Genetic improvement programs for channel catfish have not made a strong impact on the industry because program applications have not been transferred throughout industry. Many farmers still use fish strains they developed themselves.

Why is aquaculture lagging behind other animal breeding programs? Possible reasons include: (1) resistance to community improvement programs for fish stocks by independent farmers, (2) the mechanisms for developing and distributing improved genetic material throughout the industry are not in place, (3) lack of funding continuity in research programs, and (4) absence of industry-based genetic improvement programs.

- **Norway**

In Norway, genetic improvement programs for fish have been cooperative efforts between industry and government research institutes. When programs for Atlantic salmon and rainbow trout were initiated, initial results convinced the farmers that genetic improvement was important. Since the 1980s, the fish farmers' association has been operating such a program; however, it is a cooperative effort, and research institutes are still involved in certain aspects of the program.

In Norway, as well as the rest of Europe, farmers' cooperatives and private companies have been very important in the development of selective breeding programs for other farm animals. The resistance to starting breeding programs for fish in other countries is difficult to explain, especially since research has shown there is a much greater potential for significant improvements in fish performance as compared to other farm animals.

AKVAFORSK, a nonprofit research institute in Norway, exemplifies successful initiation of a selective breeding program for fish. Because of Norway's successful experience with breeding farm animals, breeders recognized a similar potential in salmon and trout. AKVAFORSK began operation in the 1970s when the government research institute initiated a breeding program. Base fish populations were established and the institute began a selection program. At first the farmers were not convinced by the research results. But when the selectively-bred fish were placed on their farms and they saw improved performance, farmers began to support the program. In the 1980s, a national breeding program was started and utilized AKVAFORSK improved stocks. Improvements have focused on growth rate, age at maturation and disease resistance. Now the breeding program is entirely supported by the private sector and the program's costs are built into the salmonid egg price.

- **Thailand**

In Thailand, selective breeding research on fish has been primarily conducted with tilapia. As in Norway, the research institute found the farmers were disinterested in obtaining genetically-improved tilapia until

they observed improvements in growth and performance on their own farms. There is very little interaction between government and industry in this program. At this time, tilapia breeding programs are supported by the Thai government, with assistance from donor countries.

- **Israel**

The breeding research model in Israel is different from those in Norway and Thailand. The best tilapia breeding research is conducted by communal farms or kibbutzim. The kibbutz develops improved strains and sells them to the farmers. Competition is keen, and the farmers are very interested in obtaining the best-performing tilapia. Carp cross-breeding programs have been conducted for many years in Israel. Hybrids are being produced by crossing different carp strains.

- **Indonesia**

Breeding research in Indonesia has been conducted with carp. Base populations have been collected and selective breeding programs have been initiated at research institutes. However, once the selected fish are transferred to private farms, breeding protocols are ignored and farmers often cross the improved strains with their own fish. It is, therefore, difficult to obtain reliable results about performance of the improved stocks.

- **China**

Selective breeding in China is conducted at research institutes and financed by the government. Though cross-breeding is common in the country, it wasn't until 1978 that private industry was allowed to develop and today, every time an improved fish strain is developed, the farmers want to obtain the fish. Thousands of private aquaculture farms are in operation, but few make enough money to support genetics research. Therefore, the research is supported by the government. The Ministry of Agriculture receives funding from several government agencies to support selective breeding research. Scientific awards are given out to the best researchers, consequently providing the primary incentive for researchers to develop improved strains of fish. The availability of financial support for breeding research varies among research institutes; some are well funded while others struggle for livelihood.

- **Taiwan**

In Taiwan, selective breeding research at the universities and research institutes is focused on disease-resistance and improving cold-tolerance of species. Very few private farmers practice selective breeding, but those who do focus on improving growth rates. Some tilapia hatcheries have been successful in improving growth rates, but only the hatcheries that produce the fastest growing tilapia are able to compete and stay in business. Because there is competition between hatcheries to produce quality fry, genetic improvement will be important to fish farmers in the future.

- **Vietnam**

The biggest problem in Vietnam is the declining performance in cultured fish. Reasons for this decrease may include inbreeding depression, uncontrolled crossing between species and selection of smaller-sized fish as broodstock. In the 1970s, the Vietnamese government funded a selective breeding program, but because the farmers would not purchase the improved larvae, the program distributed them free of charge. About ten years later the selective breeding

program began to show some positive results and farmers personally observed improved fish performance. The demand for selectively bred fish increased and now the stocks are sold for higher prices than other fry and fingerlings.

- **Philippines**

A collaborative effort, The Genetic Improvement of Farmed Tilapias (GIFT project), has recently been initiated in the Philippines. Because the country lacks a national breeding program and a fish farmers' association, a government agency disseminates breeding information throughout the country via satellite research stations. The GIFT strategy is to (1) show the farmers that the "improved fish" are better than fish they are currently raising, (2) initiate a national tilapia breeding program, and (3) make the genetic resources available to the private farms because small farms lack the resources to start or operate a breeding program. The national breeding project is designed to be managed by national institutions and to generate research funds through the sale of fingerlings. The goal is that one day farmers will be able to run the program themselves. The GIFT project is modeled after the Norwegian selective breeding program (see discussion of AKVAFORSK) and has as its mission, the development of breeding programs in Asian countries.

- **Japan**

The situation in Japan is somewhat different than in other countries. There are two types of government-operated institutions that produce fish in each prefecture; one produces stocks for release or stock enhancement and the other produces fish for aquaculture operations. Non-selective breeding is practiced in the government stock enhancement hatcheries where they produce large numbers of offspring, using as many parents as possible. For example, the Japanese government presently releases 16-20 million red sea bream offspring and 10 million chum salmon per year.

There are also many private hatcheries that produce fish for aquaculture. The type(s) of selective breeding for aquaculture used in those hatcheries is not well known. When operators are asked about their techniques, they say it is a trade secret, but researchers suggest it may be just poor record keeping.

To date, very little selective breeding research has been done in Japan. However, the government fisheries department recently took an interest in selective breeding research and is preparing to launch a large-scale research project. In 1992, a ten-year breeding research plan was initiated with an annual budget of $2 million. The program will focus on breeding sea bream, abalone, flounder, salmon and most other species that are cultured in Japan. The problem is that the plan is very grand, and Japanese researchers and technicians are not well trained in genetics. Therefore, prior to initiating the research program, a large-scale educational effort must be implemented.

- **Singapore**

In Singapore, ornamental fish farms are small family-run businesses, which are unable to contribute to selective breeding research and development efforts. Extensive research has been conducted on selective breeding of ornamentals, but it all has been funded by the government.

Guppies exemplify the potential of selective breeding for improving fish performance. Selective breeding has been conducted for approximately 40 years with guppies, and

because they are able to produce about three generations per year, breeders have produced about 120 generations of selected guppies. This has resulted in a ten-fold increase in the size and weight of male guppies, as compared with the wild-type guppies. These results demonstrate selective breeding potential and its impact on fish production.

• Conclusions

To create a successful selective breeding program, there must be an on-site demonstration (data or photos are insufficient) that allows farmers to directly observe and compare improved fish in comparison to their own stock. This has been done successfully in Vietnam, Thailand, the Philippines, China and Norway.

Although conference participants thought the first-hand-observation approach might convince U.S. fish farmers, specifically salmonid farmers, of the merits of selective breeding, some participants suggested the difficulty might be in the length of time needed to observe economic results. The farmers' limited technical understanding of selective breeding also presents an obstacle. When comparing time elements of selective breeding improvements to those of improved feed, the feed industry can demonstrate an immediate improvement. The same rapid results are observed with the introduction of a new vaccine. But the general perception, even among many of the workshop participants, is that the results of genetic improvement may not be seen for many years.

A U.S. farmer/workshop participant believed the problem with initiating a genetic improvement program in the United States is that U.S. farmers are faced with many barriers to success (regulations, limited capital, etc.). These barriers consume all of the farmers' energy and resources, leaving little time to consider genetic improvement. The same barriers limit the availability of U.S. funding for selective breeding research because research monies are committed to other areas such as nutrition.

Both Dr. Gjedrem and Dr. Gall noted that it does not take ten to fifteen years to see selective breeding results. Dr. Hershberger's research has shown that selective breeding of coho salmon can result in an average gain of 10% per generation. Because selection works in a step-wise fashion, improvements are seen in every generation. This is not the case with cross-breeding or hybridization. Cross-breeding takes approximately five generations to achieve a positive result, and the resulting crossbred stock is not itself amenable to further improvement. Many farmers have made the mistake of investing in hybridization or cross-breeding research, instead of selective breeding, and they have been dissatisfied with the results.

Genetic Conservation Issues Related to Aquaculture

The purpose of this discussion group was to consider conservation of genetic variability for aquaculture production. Many approaches are possible, therefore, conservation and preservation decisions must be handled on a country by country basis. Examples were cited from Indonesia, Vietnam, China and Taiwan of programs that have been established to conserve the genetic diversity of aquatic and terrestrial species. Some countries have determined which species to focus on and how to maintain the genetic material or germplasm, while others are still developing their criteria. Modera-

tors for this session were William Wolters and Sifa Li.

The first step in genetic conservation is to determine which species, and varieties within a species, will be conserved. If only the species important to aquaculture production are considered, they need to be identified and ranked in order of importance. Another approach is to preserve a few individuals from a variety of species. Genetic conservation of all species and their varieties may be unrealistic, but such an approach would provide greater conservation of genetic variability.

Identification of genetic resources for selected species is the second step in genetic conservation. An inventory of the populations or species must be conducted and a determination of the population size and numbers of varieties must be established.

Some researchers have expressed concern about selective breeding and its potential to decrease the genetic variability in cultured species. However, in some agricultural crops that have a long history of culture, this has not been the case. For example, the poultry industry was concerned about losing genetic variability in chickens and turkeys. Yet, despite many years of selection, significant loss in genetic variability has not been documented. Variability has been further maintained by commercial producers and hobbyists.

Figure 1. Current Conservation Methods

- DNA or cell banks
- cryopreservation of gametes (especially sperm)
- establishing stock centers or "fish parks"
- hatchery maintenance of broodstock
- maintaining stocks in nature

Individual countries must also determine how to use the safest or most secure approaches to maintain the genetic variability of conserved species (Fig. 1). The maintenance of live fish, for example, may not be feasible.

There is a high degree of risk in maintaining species in stock-centers. Care must be taken to prevent gene loss and contamination from other varieties or species, to maintain genetic differences and to minimize domestication. The funding required to maintain genetic material can change as priorities within a country or government change.

The potential of breeding programs to revitalize endangered species was also discussed. This has been done with terrestrial and aquatic species, such as the American bison and American alligator.

Conservation Issues Related to Biotechnology/Genetic Engineering

The purpose of this discussion group was to consider the conservation issues related to biotechnology and genetic engineering of fish. The first half of the discussion considered the benefits and risks associated with biotechnology. The second half focused on steps to consider in the decision-making process regarding the field testing and use of genetically-modified organisms (GMOs). The moderators for this discussion group were Eric Hallerman and Chingjiang Wu.

• Benefits and Risks

One of the benefits posed by biotechnology is aimed at gene pool conservation. Three techniques that may prove useful in gene pool conservation are cryopreservation, an-

drogenesis and chimera production. Cryopreservation of sperm can effectively create a stored gene pool, androgenesis will allow subsequent regeneration of those sperm into complete living animals and chimera production is used to generate complete animals from cryopreserved embryonic cells. Although fish embryos cannot yet be cryopreserved, fish cells can. Cells from the early embryonic cleavage stages can be injected into the blastocysts of developing eggs and become incorporated into a newly forming embryo. In the future, cryopreservation of raw DNA may be a mechanism to conserve gene pools; however, at this time we are unable to regenerate animals from raw DNA. The San Diego Zoo in California has set up a storage facility and is presently cryopreserving the DNA of many different types of organisms. This material will be held at the zoo until the technology is developed.

Another potential benefit of biotechnology is to relieve the commercial fishing pressure on wild stocks. Through biotechnology sterile fish can be produced, which can then be harvested by commercial fishermen. With few exceptions, these fish are unable to reproduce and, therefore, pose little threat to the wild stocks, although behavioral and ecological impacts of the released fish have not been examined.

There are some risks posed by biotechnology. Both ecological and genetic interactions could occur between wild and genetically modified fishes. The ecological interactions could occur through competition, predation and habitat alteration due to the presence of genetically modified fish. Crosses between wild and genetically modified fish could effect the fitness of the wild fish. The result could span between two extremes, where at one end of the spectrum there is a genetic improvement in the stock and at the other end the genetic stock could become less viable. There is also the concern that these genetically modified fish could potentially affect the evolution of wild stocks.

Another risk from biotechnology is that it is often seen as a technical fix and may not address the real problem affecting the fishery. For example, biotechnology does not address the problem of decreased habitat quality or over-exploitation. It is possible, though, to rephrase this risk as a benefit, rather than a deficit. Biotechnology may enable compensation for decreases in habitat quality or over-exploitation through increased fisheries production. An appropriate balance between conservation and utilization of resources needs to be achieved.

- **Decision making process for the use of genetically-modified organisms**

Before genetically-modified organisms find general use, the risks need to be assessed. It is possible to use a model system or an experimental mesocosm (a simulation of the natural environment) to assess the types and magnitudes of risk. The closer the simulation to natural conditions, the better the test. The mesocosm could even be a small, isolated, manageable natural system. It may be possible to include conspecifics and other elements of the relevant aquatic community, along with the genetically modified fish. By monitoring the results, looking for perturbations and determining if the aquatic system has the resiliency to deal with those perturbations, it may be possible to assess the risks associated with genetically-modified organisms. There are problems with scale, replication and cost.

The best way to look at the decision making process is to consider it as a step-wise process of risk assessment. It should start with a simple mesocosm and if the results show a negligible risk, then the assessment can move to a more complex or larger-scale test. There are time constraints on this process, though. The risk assessment must be done expeditiously so that it has academic and industry support. Also, the experiments to evaluate the risk need to be strong tests. In other words, the genetic manipulations have to be great enough to generate an impact; if no impact is observed as a result of the manipulation, then the evidence supporting no impact is stronger. Similarly, the test must be well designed so that presence or absence of impact is regarded as credible.

Once the risks have been identified, they need to be managed. A decision making process for management of risks is outlined in Gregory (1992). Gregory identified a six-step process in decision making (Fig. 2).

The key stakeholders in aquatic biotechnology include the developers of genetically-modified organisms, aquaculturists, environmentalists, regulators and society as a whole. The technical and managerial alternatives must be clearly identified in order to consider the options. The consequences of each alternative must be identified (i.e., enhanced aquaculture profitability, displacement of local genetic stock). The likelihood of both good and bad consequences should be outlined. Estimating likelihood of particular consequences is the most difficult step to accomplish. An approximation using a computer model might be the first step. The likelihood of increased aquaculture profitability due to use of genetically-modified organisms would be high, but the likelihood of ecological risk may be lower than with non-modified organisms, if the modified organisms are sterile. The reactions of the stakeholders to the various consequences of use of genetically-modified organisms need to be considered and weighed against one another. Finally, as the consequences are linked to one another, all stakeholders need to be included in the decision making process.

Figure 2. Six-step Decision Making Process

- Identify key stakeholders who will be impacted
- Determine possible technical and managerial alternatives
- Identify consequences of each alternative
- Estimate likelihood of these consequences
- Determine the stakeholders' values regarding consequences
- Link values to the consequences of the alternatives

A better understanding of the usual complications in risk management will improve the decision-making process. Gregory (1992) identified the complications listed below:

> Zero risk is an illusion.

> Risk decisions involve conflicting objectives.

> Risk decisions involve statistical rather than individual effects.

> Risks to life must be traded off against other considerations.

> The analysis of risk is never objective.

Risk management is a process and the outcome varies with the society. What works best in one location or country may not work in another.

Reference:

Gregory, R. 1992. A Decision Framework for Managing the Risks of Deliberate Releases of Genetic Materials. *In*: A. Rosenfield and R. Mann, Eds. Dispersal of Living Organisms into Aquatic Ecosystems. Maryland Sea Grant. pages 421-434.

Guidelines for a Selective Breeding Program to Improve Fish Performance

These guidelines outline a general approach to the development of selective breeding programs for improved fish performance. However, each selection program must be designed for specific species, production system, breeding goals and community of farmers involved in fish production.

It must be emphasized that an effective selection program requires a dedicated commitment to long-range permanent improvement of production efficiency. Because substantial physical and financial resources may be required, industry representatives must find the program acceptable and beneficial.

Experience has shown that during the early years of a breeding program, new and innovative selection programs require strong leadership and resource support from a governmental and/or institutional organization. One mechanism that insures a successful long-term selection program is the formation of a breeders' cooperative. Initially, it can provide orderly advice for the development of the selection program and later take over responsibility for the selection program.

These guidelines were initially developed by the discussion group session moderators (Table 1). The guidelines were expanded and revised during a subsequent discussion session and later significantly expanded by Graham Gall. The moderators for this discussion group were Graham Gall and Supattra Uraiwan.

Table 1. Guidelines for a Selective Breeding Program to Improve Fish Performance

- Establish a sound support base and a well developed mission
- Assess potential production system, set market objectives and evaluate available stocks
- Choose a selection method and mating system
- Define a rearing and testing system
- Develop protocols for data collection
- Establish data collection method

- **Guidelines**

» Establish a sound support base and a well developed mission.

Fish farmers can provide valuable ideas and focus to a selection program. Therefore, its design and development should involve farmers at the earliest possible stage. However, based on genetic knowledge of the species and its performance characteristics, extensive scientific evaluation must be conducted to ensure that the program's goals are achievable. The evaluation requires a thorough study of the scientific literature and an interpretation of available data by qualified quantitative geneticists.

The scientific evaluation should review

> the biology of the species,

- the biological nature of characteristics potentially affecting the economics of production,
- the degree of heredity (heritability estimates) for all potentially important characters,
- possible correlations between performance characters,
- the relative importance of all identified characters to the economics of production, and
- the potentially limited genetic material required for the program.

Genetic material may be limited by importation regulations, current numbers of stocks used by farmers and the domestication level of available stocks.

The selective breeding program should include replicate selection lines (broodstocks). An unselected control line should be included if it involves a new species, a new approach or a demonstration and feasibility research effort. Replication is important for monitoring selection responses, providing genetic information to improve the selection program and for validating results. The unselected control line will identify changes in performance due to environmental fluctuations that might otherwise be interpreted as genetic changes.

» Assess potential production system, set market objectives and evaluate available stocks.

It is extremely important that industry production systems and market objectives be carefully examined for both current operations and anticipated future changes. This information is essential for defining sound breeding objectives. Program goals must be specifically designed to define the characteristics (performance traits) that will be the targets of selection and to improve performance and economic gain for the industry.

Improved growth rate is the fish breeder's most commonly pursued goal, and discussion of this performance trait exemplifies why breeding goals must be specifically defined. Growth rate can be described in a number of ways, but the only genetically and biologically meaningful definition is "the change in body size observed over a specified growing period." For example, the trait for carp growth rate could be defined as weight gain seen during the second summer of rearing. This would require that both the beginning and ending dates be specified, with growth calculated as the gain in weight of individual fish (or families of fish) over this specified time interval. Body weight at a specified time or size should not be used as a measure of growth rate. Rather, since larger body size can be achieved through changes in growth rate at various points during the life of the fish, body weight is simply the body size at the specified time. For example, larger tilapia could be produced by faster growth during the juvenile stage, with little change in growth rate during later stages – just prior to market size. Thus, selection for body weight will be effective in improving growth only for those cases in which selection will reduce the time required to grow fish to market size. In these cases, either the life-cycle stage at which changes in growth occur is not important in the production system, or available scientific information indicates that changes in growth with greatest economic impacts can be expected during the stage of production with greatest economic impact.

Once breeding goals have been defined for the production system and market objectives identified, all available genetic material in the form of stocks or strains, should be evaluated for its appropriateness to the breeding goals. It is important to begin the selection program with a stock that meets the industry's performance objectives as closely as possible. Following evaluation of performance, the most productive stock(s) are chosen to form the initial broodstock (referred to as the base populations).

It is possible to design a simple selection program using only a single base population or a more complex program using two or more populations. With the latter system, the objective is usually to improve parallel lines (broodstocks) for different performance characters with the intent of combining the lines for production through cross-breeding.

If a single stock is chosen, then a relatively large sample of individuals should be obtained to form the base population. The size of the initial sample should be sufficient to ensure a broad sampling of the genetic material possessed by the donor stock.

If more than one stock is chosen to form the base population, the stocks should be crossed in all combinations and then interbred as a closed population for one or two generations prior to initiating selection. This process ensures that genetic material contributed by each stock is mixed before the selection of desirable individuals.

» Choose a selection method and mating system.

The operation of a selection program involves two steps per generation: (1) selecting superior individuals to parent the next generation (referred to as the **selection method**); and (2) mating the selected individuals to produce the next generation of individuals (referred to as the **mating system**). The first step defines the way the fish will be ranked in terms of performance. The second step determines the method used to produce eggs (seed). The mating system can (1) be part of the production system, in which case all production is from the eggs of selected parents; or (2) produce only eggs that will form the next generation of selected broodstock. In the second case, eggs for production must be obtained through a multiplication step, which can include the cross-breeding of selected broodstocks.

There are several different selection methods that can be used, and the decision between them is based on which approach is expected to provide the most efficient response. Briefly stated, the choices available to fish breeders include:

> **Mass Selection** - selection of individuals based solely on the performance of the individual fish,

> **Family Selection** - selection of families based on the average performance of all family members,

> **Within-Family Selection** - selection of individuals within families based on the performance of individual fish relative to the average performance of their family members,

> **Combined Selection** - a specific combination of family and within-family selection, and

> **Breeding Value Selection** - selection based on estimated true breed-

ing value of individuals obtained from a linear models statistical procedure.

All selection methods (except mass selection) require that the breeding population be pedigreed (individual fish marked for identification). Although mass selection can be performed without pedigree data, the lack of information upon which to judge the ancestry of individual fish will result in a slower selection response and may produce a response rate that is not economically viable.

Superior (selected) individuals must be mated to produce the progeny for the next generation of improved broodstock. A number of mating systems might be used, including random mating, assortative mating and cross-breeding. Random mating is the most popular system. Selected males are mated randomly with selected females. This can be done by mating the fish in single pairs and producing full-sib families. Assortative mating involves mating the best selected males with the best selected females in rank order. This method is desirable when a large number of broodstock are used to provide both the future selected progeny and seed for future production. Selected future progeny are taken from matings among the best individuals. Production eggs are obtained from the lower ranking fish.

To enhance the effectiveness of the selection method, regardless of whether mating is random or assortative, it is generally desirable to establish families of both half- and full-sibs within the selected broodstock. In these cases, the families are produced by mating each male to more than one female, usually at random. If there is concern about the rate of inbreeding or the desire to prevent inbreeding in early generations of selected parents and their progeny, the random mating method can be modified to eliminate matings among half- and full-sibs.

The cross-breeding method of mating is used to produce eggs whenever it is desirable to combine genetic material from more than one selected broodstock source. Selection methods that produce special lines for specific performance traits in separate broodstocks must use cross-breeding for seed production. However, cross-breeding cannot be used to produce future generations of the selected broodstocks. For example, in one line a selection program could be set up to select for improved body size at a specific age, and in a parallel line selection could be for improved survival. Eggs for production are then obtained by crossing males from one selected line with females from the second selected line. However, the pure lines (selected broodstocks) must be maintained by mating selected males and females within each line.

» Define a rearing and testing system.

Rearing and testing systems will determine the kind of data used to rank fish performance. The rearing system should reflect industry use; however, it is often more desirable to rear fish at the research station and at test stations located on cooperating farms. Rearing at the research station will provide detailed information on broodstock performance for traits of direct interest for selection, as well as on other traits being monitored. The use of test stations ensures that broodstock are regularly tested for selected traits of interest under farm conditions. The information from the two sources can be used to rank the fish for selection.

Defining rearing and testing systems involves identifying the types of rearing containers (i.e., tanks, raceways, net pens),

rearing densities, seasons or stages of life-cycle for the test (i.e., complete life cycle, final stage growth, first and second maturation) and length of test periods (i.e., full summer growout, 120-day test period, through first maturation). The test also determines the number of fish tested, including total number of individuals and families. In addition, the test design must include the number of fish to be selected.

The intensity of selection will be determined by the total number of fish tested relative to the number selected. The total number of fish tested will be determined by the capacity of the rearing and testing system, while the number of fish selected will be determined primarily by the number of females required to provide the appropriate number of eggs. To ensure that selected individuals in seasonal spawning species (i.e., salmon, trout) of both sexes mature at the same time during the spawning season, it may be necessary to select a larger number of individuals than needed to meet egg requirements. However, this should be avoided if possible.

» Develop protocols for data collection.

Performance traits must be precisely described and defined, relative to when and how performance will be measured. The description defines the specific genes, or genotypes, that control performance traits. To evaluate the full effects of the selection program on overall net performance of broodstock, protocols should be developed for traits of interest in the selection program (traits used to rank fish for selection) and monitored traits.

To achieve a proper trait description, data collection protocols must properly reflect the performance traits as defined for production systems and market objectives. For example, if the selection program goal was to increase body size of salmon at market time, setting up a protocol to measure body weight at the end of a two-year growout period would define a different performance trait than a protocol to measure body length at the end of a two-year growout period. Similarly, a protocol to measure body weight at the end of one year would define a third performance trait. The most appropriate measure of body size will depend on the objectives of the program. If the salmon are sold (marketed) and valued by weight, then measuring body weight would define a trait more reflective of the product to be marketed. Conversely, a protocol based on body length would be more appropriate for a market in which value is based on body length.

Deciding when measurements should be taken (e.g., at the end of one or two years) depends on the market and the genetic correlation among traits. If the stock requires two years to reach market size, then measuring body size at market time directly reflects the production objective. Improving market size at two years, based on measuring body weight at one year, would be successful if there was a high genetic correlation between body weight at the two ages. Shortening the data collection period may reduce the time required to complete the selection of superior individuals, thus reducing the cost of rearing.

The data collection system should not only identify and measure performance for the trait under selection, but also monitor economically significant performance traits not under selection. These non-selected traits should be carefully defined and data collected at the appropriate interval as an essential part of monitoring the overall effects of selection. One or more of these traits may

negatively change as a correlated response to selection. For example, selection for larger body weight at a specific age or time could result in correlated changes in age at first maturity. If these changes become economically significant, the selection program should be modified to prevent the continued accumulation of detrimental effects on net performance of the stock--possibly by including additional traits as selection criteria.

» Establish data collection method.

Establishment of an effective and efficient data recording method is one aspect of selection programs that is often neglected. Key design aspects of the data collection method include identification of fish, maintenance of pedigree information, ease of access to data used in making selection decisions, computer maintenance of data files and compatibility of data type and format with data analysis programs.

Fish identification is particularly important in evaluating results and identifying necessary modifications to the selection program, because it is possible for genetic correlations between traits to change as selection advances. For example, selection to improve two-year body weight based on performance at one year could initially be effective due to a high positive genetic correlation. However, because selection changes gene frequencies, it is possible that the correlation between the two traits could deteriorate to an unacceptable level after a few generations.

A large amount of data will be generated as a selection program progresses. This information should not be lost because of poor data recording and storage methodologies. Data can be used to estimate genetic parameters specific to the program's broodstock and potentially reveal facts not available in the scientific literature. In addition, parameter estimates for these data will be more applicable to the broodstock under selection than estimates contained in published literature. Finally, the genetic information will be valuable for monitoring selection progress and defining specific and essential modifications to the selection program.

» Design a regular evaluation procedure.

The program should be monitored frequently during the initial phases. In fact, following the order of activities given in these guidelines, it may be necessary to undertake one or more iterations of the program design even before selection is initiated. It is often difficult to anticipate each step in advance.

Once selection is initiated, changes should not be undertaken until at least two generations of selection have been completed, unless a major problem is identified. Short-term responses may reflect random chance events. When modification appears necessary, the program should be evaluated by reviewing each activity outlined in these guidelines. It is also important to identify how modification decisions will be made, particularly with regard to involving the fish farmers who are associated with the program.

Stock Enhancement and Conservation

Genetic Conservation Issues Related to Stock Enhancement

This discussion group focused on genetic conservation issues related to stock enhancement. Stock enhancement programs have been developed and implemented in many countries around the world. The group approached this topic by: (1) citing examples of stock enhancement programs, (2) discussing the different approaches to stock enhancement and (3) discussing the purpose of stock enhancement. The moderators for this discussion group were James Shaklee and Kenneth Leber.

After hearing a few examples, it became clear that the definition for enhancement varies from place to place. The moderators, therefore, suggested the following definition of stock enhancement: *An increase in the number of fish in the natural environment by an artificial means*. Enhancement was further defined in the session to address only enhancement of native or endemic species. However, a number of enhancement programs for exotic species were discussed.

- **Examples of Stock Enhancement Programs**

In the southern United States, several states near the Gulf of Mexico have initiated stock enhancement programs to increase the catch of red drum (*Sciaenops ocellata*) by recreational fishermen. Hatcheries have been addressing the public's concern about the limited numbers of broodstock used to produce the fry that are released into the Gulf.

Dr. Taniguchi discussed several stock enhancement efforts underway for many different species of fishes, crustaceans and molluscs in Japan. One of the species with many millions of fish released over the years is red sea bream (*Pagrus major*). Until recently, very little data have been collected on the impact of these enhancement programs. A positive impact resulting from the red sea bream enhancement program in Kagoshima Bay, was recently documented. Scallop enhancement in Hokkaido was also discussed. Scallop seedstock were released and the natural production increased significantly. In fact, the natural production increased so much that scallop seedstock are no longer released in the area. There are also successful large-scale enhancement efforts for ayu (*Plecoglossus altivelis*) underway, which are publicly supported by fishermen in Japan.

Many species of ornamental fish are collected and sold in the southeast Asian aquarium trade. In Singapore and elsewhere, there are efforts underway to develop captive breeding technology for several ornamental fishes. The goal is to relieve the harvest pressure on wild stocks by producing fish for the aquarium trade in captive breeding programs.

Overfishing in Vietnam's Red River has significantly reduced fish populations, and despite enhancement efforts to replenish Red River fishes such as silver carp, bighead carp, mud carp and tilapia, the populations have not increased. This is probably because the size and numbers of released fish were too small. Enhancement efforts in reservoirs with the same species have been more successful.

In China, there are several kinds of enhancement efforts underway: hatchery release programs to meet increased fishery demands; programs to overcome reproduction

dispersal barriers posed by dams; programs to introduce new species to lakes, rivers and bays; and programs to enhance production from land-based aquaculture. A carp enhancement program was started on the Yangtze River because of concerns about isolating potential breeding populations by dams. The Yangtze River enhancement program involves collection of wild broodstock and hatchery production of fry, which are released into the river. Many fishes, including Chinese carps and sturgeon, are released to meet fishery demands. A number of exotics have been introduced into China's rivers, reservoirs and streams, including icefish in Taihu Lake and smelt fish in northern China. Enhanced production goals for aquaculture include increased production through selection of the best spawners and increased low temperature tolerance of a species through genetic manipulation.

In Thailand, there is a stock enhancement program for economically important species, including shrimp and fish. About 75 million fry of all species were produced and released in 1989. Thailand has regulations to limit catches of gravid fish or shrimp during the spawning season. There is also an enhancement effort to produce and release penaeid shrimp because the natural penaeid populations are declining.

In Indonesia, tilapia and carp have been introduced and released for a variety of reasons. There have been indirect enhancement effects where cage-cultured fish escape and reproduce in lakes or bays.

An Oceanic Institute research program in Hawaii has been investigating the impacts of stock enhancement on mullet *(Mugil cephalus)* populations. The goal is to evaluate the effectiveness of enhancing endemic stocks of this species. Results have shown that enhancement can significantly increase coastal mullet populations and that carrying capacities can support the increased production.

Enhancement efforts in Norway began in the 1800s with salmonids and cod. Large numbers of small fish were released in the 1930s, but when the impact of the enhancement program was examined, the Norwegian government stopped the releases. More recently (1980s), an inshore cod broodstock population was used to produce fry for release. The 15-20 gram cod (*Gadus morhua*) were released inshore and recapture data showed that the majority of the fish stayed within 2 km of the release point. This demonstrated that enhancement can increase the coastal cod populations.

- **Approaches to Stock Enhancement**

The stock enhancement examples discussed in this section show that two different approaches have been used for fishes around the world. The first approach is the intentional relocation of fish stocks from one region to enhance stocks in another region. The second is to collect local broodstock from a particular location for enhancement purposes and to release their progeny into the source region. The genetic risk to the natural stocks will vary depending on which approach is used. The intentional movement of fish between regions has the potential to change the genetic characteristics of the local population. Further discussion is needed to determine which is the best approach.

The land-locked ayu enhancement programs in Japan have involved the movement of stocks between different regions and habitats. Dr. Taniguchi indicated that the results have been very positive; populations have

increased and adapted to their new environment.

Concerns about the impact of domestication selection, or the decreased fitness of wild populations because of exposure to the hatchery environment, may be overestimated. Assuming that the genetic resources are properly managed, several generations in the hatchery may have a minimal impact on the overall gene frequency. The genetic impact could be more significant, however, if a new genotype was brought in from another region to enhance a local population. The genetic risks and benefits of each approach should be evaluated before a decision is made.

- **Purpose of Stock Enhancement**

Stock enhancement programs have been implemented in response to reduced fish populations that resulted from environmental changes and overfishing.

In response to environmental or habitat changes (e.g., construction of hydroelectric plants), stock enhancement can be effective. In many cases, it appears that the local gene pool is able to deal with these environmental changes and that given enough time, adaptation occurs.

Overfishing may cause significant losses of genetic variation. Ricker (1981) has presented data suggesting that fishing efforts have caused dramatic changes in the average size and age of Pacific salmon populations. A fishery can affect both within-population variability and among-population variability.

It also was suggested that stock enhancement could create fisheries or new populations where they do not presently exist. Preserves for wild stocks could then be established in locations away from the fisheries. In southeastern Alaska, for example, the chinook salmon fishery was overfished and in the 1980s, a stock enhancement program was established to produce and release hatchery fish to replace the wild-stock fishery. In this case, the enhancement program was protecting against the loss of among-stock genetic variability.

Stock enhancement was discussed as a means of complementing wild production and stabilizing fisheries. Alaska's Prince William Sound and Iceland were cited as examples. The specifics on the Prince William Sound sea ranching program are presented in the paper by William Smoker (this volume). A private sea ranching program for selectively-bred Atlantic salmon is being developed in Iceland. It is hoped that this selection program will increase maximum sustainable yields of wild Atlantic salmon stocks in Iceland.

Reference:

Ricker, W.E. 1981. Changes in the average size and average age of Pacific salmon. *Can. J. Fish. Aquat. Sci.* 38:1636-1656

Aquaculture and Wild Stock Interaction

This discussion session focused on the interactions between cultured and wild stocks. The discussion began with a call for examples of specific genetic effects of cultured stocks on wild populations. The moderators noted at the outset that direct evidence for a negative impact of cultured stocks on wild populations is limited and concerns about impact may be based largely on hypothetical situations. The moderators for this discussion group were William Smoker and Nobuhiko Taniguchi.

- **Examples of Impact of Cultured Stocks on Wild Stocks**

One potential example of a negative impact is a centralized Scandinavian facility that cultured brown trout (*Salmo trutta*), collected from a single source, and then stocked them in freshwater systems throughout the country. It was suggested that the wild stocks were overwhelmed by cultured stocks, and that a subsequent population crash may have resulted from the enhancement effort.

In China, cultured hybrid carp have been stocked directly in natural systems or have escaped from cages in well developed aquaculture areas. Consequently, many wild carp populations have been destroyed. As a result of the 1991 Yangtze River flood, many carp escaped. Though their impact is unknown today, wild common carp populations are found only in a few areas of China (northeast portion of the Yellow River and in some Mongolian lakes).

The introduction of icefish in Mongolian lakes of China demonstrates a negative impact on an entire ecosystem. Although icefish populations increased following its introduction, other fish and shrimp populations in the lakes decreased. A negative impact on the ecosystem has not yet been documented for grass carp introduction, but some researchers believe it may have a negative impact in the future.

In Thailand, a population decrease of a native species, *Clarias batrachus*, was observed following the release/escape of a hybrid catfish (*C. macrocephalus* X African walking catfish).

It was suggested that the discussion be confined to changes in genetic structure of populations, instead of indulging in examples of ecological competition for food or space. The moderator(s) stated that the loss of an entire gene pool is a change in the genetic structure of a population.

Genetic interactions between cultured and wild Atlantic salmon (*Salmo salar*) stocks in Norway is a point of concern for aquaculturists. A few years ago there were many salmon escapes from floating net pens due to winter storms. Later, sick fish were found in the rivers; however, there was no direct evidence linking the disease problem to the escaped cultured stocks. There has also been a problem with the parasite, *Gyrodactylus salaris*, and although there is some evidence of its introduction through cultured fish, it is also possible that the parasite was present in the natural system before the escapes. In response to these incidences, Norway has adopted farm construction regulations to guard against the escape of cultured fish.

Due to intentional introductions or unintentional escapes of fish, there are several examples of genetic structure changes in local stocks. In Ireland, there is direct genetic evidence that escaped Atlantic salmon have successfully reproduced with local stocks. The introduced genes have been found in the local population. In Texas and the rest of the southern United States, there are introduced large- and/or smallmouth bass (*Micropterus*) populations that have hybridized with local stocks. In some locations, the hybrids are less fit, and in others, they are more fit than the non-hybridized stocks. In Colorado, the local gene pool has been affected by the introduction of rainbow (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarki*). Both intra- and interspecific variation have been observed. In these three cases, negative impacts have not necessarily been documented, but there is evidence to indicate

changes in the gene pools. Some conference participants noted that an increase in the gene pool variation is not necessarily negative.

Clarias batrachus was introduced to the Philippines in the 1980s and similar to Thailand, the native species, *C. macrocephalus*, has almost disappeared. Now African catfish are being farmed and although there are no reports of escapes, it is expected that it will happen eventually. A well documented release involves *Oreochromis mossambica* (the so-called native species of tilapia) and *O. nilotica* (the introduced species). Initially farmers were pleased with the performance of *O. nilotica*, but introgression was later documented when the species hybridized with *O. mossambica*.

In Vietnam, there is a big problem with interspecific hybridization between Vietnamese silver carp and Chinese silver carp. The hybrids, not the pure native silver carp, have been found in the major river systems. It is possible that the Vietnamese silver carp genetic resource has been lost.

Red sea bream have been cultured in Japan for many years. Breeding guidelines, which recommended that a large number of parent fish be used for seed production, were developed in 1980. Genetic variations of hatchery and wild populations were later monitored from 24 locations, where a 10% loss in the genetic variability of the hatchery populations was recorded. In Japan, this rate of loss may be acceptable.

- **Is Genetic Change Always Bad?**

After reviewing the participants' comments about genetic interaction, Graham Gall noted that two extreme examples were presented: James Shaklee cited trout and bass hybridization in the United States as documented genetic structural changes in the local stocks. Shaklee noted that although fitness changes have not been measured, if wild stocks are fit, then changes in genetic structure will decrease fitness; Trygve Gjedrem cited genetic interactions between Norway's single highly-selected cultured Atlantic salmon and wild Atlantic salmon stocks. The genetic variability between these two stocks ranges from 1-6%. Gjedrem believes that a constant level of escapes over a long period will eventually reduce the genetic variation of the wild population and that escapes should be avoided. He also believes that it is difficult to change the gene frequency of wild populations and that natural selection will limit the impact of cultured escapees on wild populations. However, some change is not necessarily negative.

Because 75% of Norway's farmed fish production comes from one genetic stock, participants suggested that producers might be courting disaster. The necessary genetic variability to survive potential disasters, such as disease or environmental changes, may not be available. Gjedrem countered by saying that Norway has enough strain diversity to respond to change. Eric Hallerman, however, noted that researchers don't know how much of a genetic load this population can withstand.

Graham Gall concluded that to evaluate the impact of cultured fish on wild stocks, the public and fishery managers must be persuaded to conduct experiments. Though this puts wild populations at risk, some risk is required to prevent future disaster.

Guidelines for Genetic Resource Management in Stock Enhancement Programs

These guidelines outline the steps involved in establishing a stock enhancement program that includes genetic resource management as a priority. Stock enhancement programs are defined here as programs that obtain broodstock or juveniles directly from existing wild population(s) of a target species for the purpose of releasing the progeny back into the wild to supplement or replenish existing population(s).

The objectives of stock enhancement programs should be clearly defined and understood prior to full-scale implementation. The group decided that the introduction of exotic species, transfer of stocks and hatchery-release programs to mitigate the effects of habitat loss should not be considered "enhancement" for the purposes of these guidelines.

The genetic structure of wild stocks targeted for enhancement should be identified and managed according to objectives of the enhancement program. In the interest of both production aquaculture and conservation, effort must be made to maintain genetic diversity.

The following principles should be considered before a stock enhancement program is implemented:

Any selective manipulation of stock abundances can result in a genetic change in the population. Stock enhancement efforts will effect the genetics of natural stocks if gene flow occurs between cultured and wild stocks. This could be due in part to random drift effects that result from a small number of parents in the captive stocks. Founder effects can result in the loss of rare alleles from the cultured stocks or an increase in rare alleles in the wild stocks. When stock transfers occur, genes adaptive at one location may result in reduced fitness at another. For example, the timing of spawning migrations may not coincide with environmental conditions required for spawning in the new location.

Likewise, selective removal of individuals from a population through fishing can also affect population fitness. For example, frequencies of alleles controlling rapid growth could be reduced in a stock as a consequence of size-selective fishing or genetic variability in habitat selection can also be modified by site-selective fishing. Thus, the genetic consequences of exploitation must be considered when planning and evaluating genetic goals and objectives of stock enhancement.

Stock enhancement must consider gene conservation. Loss of genetic variability in wild stocks can have two important consequences: (1) long-term loss of fitness and production in natural populations, and (2) short-term loss of genetic resources for domestication in enhancement and aquaculture. Careful protocols can reduce the loss of genetic resources needed for both wild and farmed stocks to adapt to environmental changes.

Monitoring and evaluation are essential for long-term success of stock enhancement. Genetic monitoring includes an initial inventory of wild stock genetic resources and periodic characterization to evaluate any changes in gene frequencies following hatchery releases.

- **Guidelines**

These guidelines were initially developed during a workshop discussion group and later significantly expanded by Kenneth Leber, with input from William Smoker. Mod-

Table 2. Guidelines for Developing a Genetic Management Plan for the Target Species.

¤ Define the status of the target stock
¤ Define the harvest and genetic goals of the enhancement program
¤ Identify and quantify genetic risks and consequences
¤ Define an enhancement strategy
¤ Implement a monitoring and evaluation program
¤ Outline research needs and objectives
¤ Develop a feedback mechanism

erators for this discussion group were Graham Gall and Supattra Uraiwan.

There are three sets of guidelines included: Guidelines for Developing a Genetic Management Plan for the Target Species (Table 2), Guidelines for Sampling Hatchery Broodstock Material from Natural Populations (Table 3) and Guidelines for Management and Operation of the Hatchery and Production System (Table 4). For a more detailed overview of hatchery guidelines for genetic concepts pertaining to enhancement, broodstock collection, spawning, rearing and release procedures, see Kapuscinski and Miller (1993).

- **Guidelines for Developing a Genetic Management Plan for the Target Species**

» Define the status of the target stock

Inventory the population genetic structure (e.g., by biochemical assays) and determine whether subpopulations exist within the ecosystem affected by enhancement.

» Define the harvest and genetic goals of the enhancement program

For example, is the goal strictly to keep existing natural genetic variability intact? Or is the goal to provide marketable fish? Identify the desired balance of hatchery and wild stocks (e.g., 50:50 or 90:10). The former requires a careful breeding plan that avoids selection, the latter requires isolation from wild-stock resources.

» Identify and quantify genetic risks and consequences

If gene flow from hatchery to wild stocks is anticipated, then wild stock allele frequencies could change. Since the array of allele frequencies in the wild stock is adaptive, changes could result in decreased fitness of the population.

» Define an enhancement strategy

Release strategies can affect the impact of enhancement efforts on the genetic structure of a population. Release strategies include factors such as size at release and release time and location. Studies have shown that post-release survival is often directly related to release size. The goal is to release the smallest size that is biologically and economically feasible in order to minimize unintentional genetic selection in the hatchery.

Choice of release site can be a critical factor in controlling the impact of gene flow from hatchery to wild stocks. Using release site to isolate breeding stocks can have at least two significant impacts on a wild stocks: (1) it can greatly reduce the probability of gene flow from hatchery to wild fish, and (2) it can reduce the potential for over harvest of wild stocks through "mixed-stock harvest."

Harvest levels are generally regulated based on stock abundance. When abundances are

high, larger catches are allowed and when abundances fall below a certain level required to maintain maximum sustainable yield, the fishery may be closed. When hatchery and wild stocks are mixed, allowable harvest rates are generally increased to take advantage of the greater yields possible through the harvest of hatchery fish. This can lead to unintentional overfishing of the wild stock. In the extreme case, abundances of wild broodstock could be reduced to levels well below what is needed to maintain genetic diversity and/or stock survival.

One mechanism to conserve genetic diversity of endangered or severely depleted stocks is to target a fishery on an isolated hatchery stock and thereby reduce harvest pressure on the wild stock. This requires an understanding of dispersal patterns and migration behavior of hatchery stocks in the wild and may not be applicable to wide ranging marine stocks. This approach has the greatest potential as a management tool for species with low dispersal rates that return to a specific breeding habitat or at a specific time of year.

» Implement a monitoring and evaluation program

Several steps are needed to evaluate enhancement effects and monitor gene flow from hatchery stocks to wild populations.

> Determine wild stock genetic variability prior to release of hatchery stocks. This information will provide a baseline for comparison after a stock enhancement program is implemented. Recent advances in isozyme and DNA marker methodologies provide basic tools for characterizing population genetic structure.

> Evaluate the genetic structure of the breeding population that will be used to propagate fish for release. Comparison of allele frequencies in the hatchery with those of the wild stock will guide decisions about 'effective population size' and collection sites for the hatchery broodstock.

> Evaluate the genetic structure of the F_1 generation produced for release into the wild. This will allow assessment of whether spawning or rearing protocols have led to detectable genetic changes. This might also reveal whether selection in the hatchery has made a significant impact on the genetic structure of the progeny to be released.

> Conduct periodic monitoring of the genetic structure of the wild stocks that may experience gene flow from hatchery stocks. This will provide a feedback mechanism, subsequent to the initiation of a stock enhancement program, which can be used to refine or alter hatchery practices in order to achieve conservation of wild stock genetics.

» Outline research needs and objectives

Research activities should include an evaluation of the critical uncertainties related to genetic impact of gene flow between hatchery and wild stocks. Marking systems used to identify hatchery fish can be used to evaluate impacts of various hatchery or release variables and provide a constant source of new information about the impact of hatchery releases. Coded-wire tags provide an effective mechanism for evaluating assumptions about survival of hatchery fish and

Table 3. Guidelines for Sampling Hatchery Broodstock from Natural Populations

- ¤ Sample the full range of phenotypic diversity within the managed unit
- ¤ Have a geneticist review the sampling plan

subsequent mixing or isolation of hatchery and wild stocks. Genetic markers, however, can be used to identify offspring from hatchery releases and evaluate the extent of interbreeding between hatchery and wild stocks.

» Develop a feedback mechanism

The feedback loop between the monitoring and assessment activities and the hatchery management activities is a key management requirement that allows enhancement programs to adapt to changing environmental and enhancement effects. Objectives and management strategies should be refocused to incorporate new information as research results show what works and what doesn't work among the various enhancement tactics used to manage genetic impacts.

- **Guidelines for Sampling Hatchery Broodstock from Natural Populations**

» Sample the full range of phenotypic diversity within the managed unit

This approach should be followed whenever the goal of the enhancement program is to supplement or rebuild an existing stock. If the strategy is to enhance harvest yields with an isolated cultured stock, sampling strategy may be designed to cover a subset of the full phenotype range. This may require selecting individuals either from very early or from very late returns of migrant spawners, in order to isolate hatchery and wild stocks based on the timing of spawning migrations.

> In a systematic format, sample randomly from the entire donor population.

> Sample to achieve an effective population size of at least 200 (100 females and 100 males).

» Have a geneticist review the sampling plan

Sampling strategies include: (a) sampling a single domesticated broodstock in a localized region, in cases where the objective is long-term fisheries enhancement (e.g., ocean ranching of free ranging hatchery fish), (b) frequent sampling of wild populations, in cases where conservation is a priority. This strategy increases the probability of including rare alleles in the captive broodstock.

- **Guidelines for management and operation of the hatchery and production system**

» Avoid inbreeding, except when desired

To avoid inbreeding, the appropriate captive broodstock population size must be determined and the pedigree information for the captive broodstock must be maintained and used to guide spawning practices.

» Maintain adequate effective population size to minimize random genetic change

» Avoid domestication of stocks

Domestication of stocks should be avoided if conservation is a priority and if restoration of stocks is a goal. Concerns include selection in culture systems and effects of the physical environment.

» Consider the use of genetic markers

Genetic markers will enable the identification of the "enhanced product." Examples

where this strategy is being developed or has been used are cod enhancement in Norway, red drum enhancement in Texas, U.S.A. and Pacific salmon enhancement in Alaska and Washington, U.S.A.

» Use selective breeding only when captive culture or ocean ranching of domesticated stocks is the goal and when the consequences are well understood

It should be recognized that the use of domesticated or selectively bred stocks could lead to a substantial decline of wild stock abundances as a result of interbreeding of wild and hatchery fish. Reproductive isolation of ocean-ranched and wild stocks should be a goal in the management of ocean-ranched production systems.

» Monitor and respond to gene flow from hatchery to wild stocks

If conservation is the goal, then gene flow from hatchery to wild populations must be monitored. Corrective measures are needed if the genetic structure of hatchery stocks are significantly different from wild stocks and there is gene flow from hatchery to wild stocks.

» Minimize genetic selection in the hatchery

Selection can be avoided by reviewing hatchery-management and operational practices that pertain to culling excess production, restricting reproductive period, mating scheme/mating system control and distributing pedigreed individuals randomly throughout the rearing facility. Just because you may have 150 broodstock in the spawning system doesn't ensure they all have an equal genetic contribution to progeny. To remove bias and increase likelihood of equal contribution from each parent, isolate spawning females into separate spawning tanks and use an equal number of fertilized eggs from each female.

Table 4. Guidelines for Management and Operation of Hatchery and Production System.

- Avoid inbreeding, except when desired
- Avoid domestication of stocks
- Maintain adequate effective population size to minimize random genetic change
- Consider the use of genetic markers
- Use selective breeding only when appropriate
- Monitor and respond to gene flow from hatchery to wild stocks
- Minimize genetic selection in the hatchery
- Evaluate how to improve survival and behavior under natural conditions

» Evaluate how to improve survival and behavior under natural conditions

Reference:

Kapuscinski, A.R. and L.M. Miller. 1993. Genetic Hatchery Guidelines for the Yakima/Klickitat Fisheries Project. Public Review Draft Report. 75 pp.

Appendices

Appendix I: Workshop Participants

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Appendix II: Workshop Agenda

Monday, May 3, 1993

8:15 am	**Dr. Paul Bienfang** The Oceanic Institute	*Introduction and Welcome*
8:30 am	**Dr. Trygve Gjedrem** Agricultural University of Norway	*International Selective Breeding Programs: Constraints and Future Prospects*
9:15 am	**Mr. Su-Lean Chang** Taiwan Fisheries Research Institute	*A Review of Fish Genetic Research and Conservation Issues in Taiwan*
10:15 am	**Mr. Sudarto** Research Institute for Freshwater Fisheries - Indonesia	*A Review of Fish Breeding Research and Practices in Indonesia*
11:00 am	**Dr. Violet Phang** National University of Singapore	*Breeding Programs for Ornamental Fish Production in Asia*
11:45 am	**Dr. Tran Mai Thien** Research Institute for Aquaculture in Vietnam	*A Review of Fish Breeding Research and Practices in Vietnam*
2:00 pm	Discussion Group A	*Priorities for Stock Improvement*
3:30 pm	Discussion Group B	*Genetic Conservation Issues Related to Aquaculture*

Tuesday, May 4, 1993

9:00 am	**Dr. William Hershberger** University of Washington	*Genetic Resources for Future Finfish Aquaculture*
9:45 am	**Mr. Sifa Li** Shanghai Fisheries University - People's Republic of China	*A Review of Genetic Conservation and Practices in China*
10:45 am	**Dr. William Smoker** University of Alaska	*Management of Pacific Salmon and Artificial Enhancement Programs*
11:30 am	**Dr. James Shaklee** Washington Department of Fisheries	*Genetic Conservation Programs for Washington State Salmon: Enhancement and Management*
2:00 pm	Discussion Group C	*Aquaculture and Wild Stock Interaction*
3:30 pm	Discussion Group D	*Genetic Conservation Issues Related to Stock Enhancement*

Wednesday, May 5, 1993

9:00 am	**Dr. Graham Gall** University of California	*Knowledge Base and the Development of Fish Breeding and Conservation Programs*
9:45 am	**Dr. Supattra Uraiwan** National Aquaculture Genetics Research Institute - Thailand	*A Review of Thailand's Fish Breeding Programs and Conservation Issues*
10:45 am	**Mr. Nobuhiko Taniguchi** Kochi University - Japan	*Use of Chromosome Manipulated Fish in Aquaculture in Japan*
11:30 am	**Mrs. Remedios Bolivar** Central Luzon State University in the Philippines	*National Fish Breeding Programs and Conservation Issues in the Philippines*

Thursday, May 6, 1993

9:00 am	**Dr. William Wolters** USDA/ARS Catfish Genetics Research Unit - Mississippi	*Channel Catfish Breeding and Selection Programs: Constraints and Future Prospects*
9:45 am	**Mr. Chingjiang Wu** Institute of Hydrobiology, Academia Sinica - People's Republic of China	*A Review of Traditional Fish Breeding Research and Practices in China with Emphasis on the Use of Genetic Markers*
10:45 am	**Mr. Dequan Xia** Freshwater Fisheries Research Institute People's Republic of China	*Review of Modern Fish Breeding Research and Practices in China*
11:30 am	**Dr. Eric Hallerman** Virginia Polytechnic Institute & State University	*Public Policies Regulating the Use of Transgenic Fish: Current and Future Needs*
2:00 pm	Discussion Group E	*Conservation Issues Related to Biotechnology/ Genetic Engineering*
3:30 pm	Discussion Group F	*Guidelines for Genetic Resource Management in Aquaculture Selection and Stock Enhancement*

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